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The Leeuwenhorst
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16-21 july 1995

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Conference participants who joined the barbecue at the Institute for Systematics and Population Biology, University of Amsterdam, 18 July 1995 (photo M. van Couwelaar).

Contents

Foreword

The present volume contains a majority of the papers presented at the Sixth International Conference on Coelenterate Biology held at the "Leeuwenhorst" Conference Centre, Noordwijkerhout, The Netherlands from 16 to 21 July, 1995.

Although the number of participants was relatively small, only 130 scientists from 27 countries, those present entered into many enthusiastic discussions which covered both main line developments and specialised details of recent coelenterate research. The papers and posters here published demonstrate the advances made in coelenterate biology over the five years since the Southampton conference, and my sincere thanks are due to J.C. den Hartog for his painstaking editorial work.

Review papers by invited speakers emphasize progress made in the fields of taxonomy, molecular biology, reproduction, behaviour, symbiosis, medical research and biodiversity, and highlight some of the problems involved in the conservation of coelenterates.

The more detailed studies presented in contributed papers and posters give a good overview of the application of modern theories and techniques in classical taxonomy, the application of physics in biogeography, and of the chaos theory in ecology, physiology and biogeography to quote just some examples.

Unfortunately, a number of good scientists from developing and Third World countries who intended to submit challenging contributions were prevented from doing so either by a lack of funding, or as a result of inadequate anticipation by their international counterparts.

However, in spite of various practical barriers and a universal scarcity of finances, our science is gradually making advances. On the other hand, in many areas world-wide, coelenterates themselves and their habitats show a trend of decline and degradation, observed perhaps most strikingly in tropical coral reefs.

Discussion during the conference revealed that some subdisciplines, notably taxonomy, are at present under threat due to reorganisation in some institutes, and reduction of funding in others. Support for research was requested from participants at the conference, and recognition of this problem sought.

Some coelenterate populations increase dramatically as a result of eutrophication caused by Man's activities, while others decrease just as dramatically as a result of tourism and pollution. The remorseless exploitation of Nature by Man and the inherent pollution which results are alarming. However, as the exploitation of natural resources and pollution of our biosphere go hand in hand with an ever-increasing world population, it is Man's task to integrate all the findings of scientific research, even in our, perhaps modest, discipline of coelenterate biology, to help suppress these ill effects as much as possible. It was gratifying that this theme received the full attention of the conference, and it was concluded that the infrastructure of sciences which includes Coelenterata is at present far from adequate. Recent reorganisations in various institutions in several countries have excaberated the situation, even though world-wide problems of economy, conservation, recreation and medical topics that demand optimal organization and funding are linked with a knowledge of coelenterates. These issues were intensively debated during the conference by participants from all the main subdisciplines of coelenterate research. As a result, the fol-

lowing four main recommendations, resulting from views expressed during the plenary session of the conference on Friday 21 July, were put forward:

1. Considering the dramatic decrease of taxonomic knowledge in the world, education and training in taxonomy should be stimulated. In addition, special attention should be paid to community or assemblage structure and life-history studies.

2. Specific research relating coelenterates inhabiting inland neritic and freshwater habitats to conservation management should be encouraged internationally. This is needed in order to generate adequate policies to save the disjunct gene pools of these taxa, which are small and therefore endangered.

3. Taxonomic data bases, such as that provided by the Expert Centre of Taxonomic Indentification (ETI) should be used as standardising tools to facilitate easy access of taxonomic data, and should be supported internationally.

4. The Seventh International Conference on Coelenterate Biology should be held within six years from now.

Our main effort must be directed towards the continued incorporation of main theories and techniques into recognized disciplines together with the reciprocal incorporation of different coelenterate studies into research programmes of worldwide social and economic interest. We hope that by the time of the next ICCB, some progress will have been made in this respect.

I wish to express my thanks to all the participants of the conference for generating such a pleasant atmosphere in which many stimulating contacts were made. Considerable efforts by a number of people were directly responsible for its success. In particular I wish to thank the Comité d'Honneur (J. Bouillon, R.G. Hughes, J. van der Land, G.O. Mackie, P. Tardent and W. Vervoort), the Scientific Advisory Committee (R.P.M. Bak, P.F.S. Cornelius and B.W. Hoeksema) and my fellow members of the Organising Committee (R.M.L. Ates, J. Bleeker, J.C. den Hartog, J.H.M. Kouwenberg, A.C. Pierrot-Bults, P.H. Schalk, R.W.M. van Soest, H.W. van der Veer and J. Vermeulen). I speak for us all when I extend a special thank-you to Dr. A.C. Pierrot-Bults for her optimal organization of the adminstration and finances of the conference. Special thanks are also due to Else Pierrot and Bertus van Tuyl for their spontaneous help.

For sponsoring the conference we are grateful to the Royal Netherlands Academy of Sciences (KNAW), Amsterdam; the Zoological Museum, University of Amsterdam; the National Museum of Natural History (NNM), Leiden; the United Nations Educational, Scientific and Cultural Organisation (UNESCO), Paris; the International Union of Biological Sciences (IUBS), Paris; and the Expert Centre for Taxonomic Identification (ETI), Amsterdam.

S. van der Spoel
Chairman, Organizing Committee
Institute of Systematics and Population Studies (ISP)
University of Amsterdam

Editorial Preface

More than two years have passed since the 6th International Conference on Coelenterate Biology was held at the "Leeuwenhorst" Conference Centre in Noordwijkerhout, The Netherlands. If I could have anticipated the magnitude of the task that would be involved in editing the proceedings of that conference, I might have thought twice before volunteering to act as main editor. I am all too aware that the process of reviewing and editing has taken far too long, and that some of the authors and participants waiting for the volume may have become impatient.

It will do no harm to explain the reasons for the delay. In the first place, to safeguard the quality of the conference volume, the organizing committee decided, with some hesitation, that all papers should be peer reviewed. Having agreed on this procedure, the majority of papers were reviewed by at least two external referees. I am thankful for the efforts of all these referees, although, admittedly, there was great variation in the quality and usefulness of their reports! Many of them thoroughly, or even meticulously, reviewed the papers entrusted to them and helped in a constructive way by improving both language and contents. Others restricted themselves to brief comments, not always constructive, but still helpful to at least the editor. For this reason, and because it is also the task of the editor to evaluate the opinions and comments of reviewers, I have, strictly speaking, not acted only as editor but also reviewed many papers myself. For papers on subjects beyond my competence, I naturally had to rely mainly on the judgement of the external reviewers.

The character of proceedings of conferences and symposia should not be compared to that of high quality journals, which can afford to select on quality alone. I suspect that I may not be alone in considering that the proceedings of international conferences should reflect, and be informative about, research being done worldwide on the subjects or themes covered, and should not as a matter of course include only papers of prime quality. This applies also to our own discipline concerning various aspects of coelenterate biology.

Therefore, to capture as much as possible of what was presented at the conference, it has been my intention from the outset to have most of the submitted papers published. I have also tried to ensure that all participating nationalities are represented. This has resulted in several papers, which might have been rejected in other circumstances, having been given special attention to make them fit for publication; often a time-consuming activity.

Possibly some relatively modest but uncontroversial papers have been accepted, while one or two papers dealing with more important scientific questions were rejected because they were regarded scientifically unsound.

Although the page limit was six published pages per article, and ten for invited speakers, it became clear that strict adherence to this was not feasible. Most contributors exceeded this limit, so I have not generally insisted on shortening their papers. Consequently, I may owe an apology to those authors who modestly kept themselves to the page limit, as some of them might also have liked a few more pages.

The length of time taken by the reviewing process has further strenghtened my

opinion that this procedure was not truly worth the effort of either reviewers or editor. It is true that the quality of most of the papers has improved. But at what cost? First, the editor had to trouble a host of potential reviewers with the request to evaluate and, where possible, improve one or more papers. True enough most of them cooperated generously, but it seems unlikely that any of them was really happy with the request.

Then there was the sometimes substantial but necessary correspondence with the authors. I perforce mailed, faxed or e-mailed more than 400 letters, which generated a similar number of replies. Fortunately, authors generally had patience with me, although I kept harassing them with questions and suggestions for change.

The delay in printing surely has compromised the novelty of some of the papers. Therefore, I am inclined to advise the editor(s) of the proceedings of the next conference not to seek perfectionism but to turn to the "quick and dirty" approach, thus ensuring that the conference volume is available within a reasonable time. The result may not be very different!

Not being a native English speaker, and despite many English speaking reviewers having helped to improve the English of the papers entrusted to them, I am well aware that the volume definitely suffers in places from what might be called Dutch English. After all it would have been unwise and unfair to continually try the patience of reviewers with questions about language or to ask them to fully revise purely stylistic aspects of papers.

I am grateful to all the reviewers, but I would like especially to mention a few who did more than could reasonably have been expected of them. First, Professor Wim Vervoort, former Director of the Rijksmuseum van Natuurlijke Historie (RMNH), now Nationaal Natuurhistorisch Museum (NNM) at Leiden, and my predecessor as Curator of the Coelenterate Section. Others who have been of great support are Dr Dale R. Calder, Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto, my friend Paul F.S. Cornelius, The Natural History Museum, London, and Professor Siebrecht van der Spoel, Institute for Systematics and Population Biology, University of Amsterdam. Last but not least, I have much appreciated the great support, moral and practical, of my friend Leen P. van Ofwegen. He not only helped me with good advice, but he also took care of the lay-out of the present volume and of all the desk-top publishing work involved in it, including most of the preparation of the indexes. In this capacity, he often acted more or less as a shadow-editor. It was a blow that, just when the end of our sometimes seemingly Sisyphean labour was in sight, in December 1996, he broke a leg, which caused more than five months of extra delay.

An alphabetic list of the other colleagues who acted as reviewer or who gave advice, is given below.

Dr C. van Achterberg, NNM, Leiden; Dr P. Alderslade, Darwin, Australia; Professor M. Arai, Pacific Biological Station, Nanaimo, Canada; Mr R.M.L. Ates, Zaandam, The Netherlands; Professor R.P.M. Bak, Netherlands Institute for Sea Research (NIOZ), Texel, The Netherlands; Dr B. Buddemeier, Kansas Geological Survey, University of Kansas, Lawrence, Kansas; Professor J.W. Burnett, Department of Dermatology, University of Maryland, Baltimore; Dr P.S. Davies, Division of Environmental

and Evolutionary Biology, IBLS, University of Glasgow, Scotland; Professor Z. Dubinsky, Department of Life Sciences, Bar Ilan University, Ramat Gan, Israel; Dr I.M. Dutton, Coastal Resources Management Project, Jakarta; Professor Dr D.G. Fautin, Department of Systematics and Ecology and Natural History Museum, University of Kansas, Lawrence, Kansas; Dr P. Fenner, International Consortium for Jellyfish Stings, North Mackay, Queensland; Drs C.H.J.M. Fransen, NNM, Leiden; Dr. M. Grasshoff, Naturmuseum und Forschungsinstitut Senckenberg, Frankfurt; Professor C.J.P. Grimmelikhuijzen, Department of Cell Biology and Anatomy, Copenhagen; Professor C. Hand, Bodega Marine Laboratory, Bodega Bay, California; Professor T. Heeger, Marine Biology Section, University of San Carlos, Cebu City, Cebu, Philippines; Dr P.J. Herring, Southampton Oceanography Centre, University of Southampton, UK; Dr. B.W. Hoeksema, Programme Buginesia WOTRO-UNHAS, Ujung Pandang, Indonesia; Dr. M. Hoogmoed, NNM, Leiden; Dr. R.H. Karlson, Ecology & Evolutionary Biology Program Department of Biology, University of Delaware, Newark, Delaware, USA; Dr. I.D. McFarlane, Department of Biological Sciences, University of Hull, UK; Professor G.O. Mackie, Biology Department, University of Victoria, Victoria, BC, Canada; Dr G. Mapstone, Brookwood, Surrey, England; Dr A.C. Marques, São Paulo, Brazil; Dr. D.J. Miller, Department of Biochemistry and Molecular Biology, James Cook University of North Queensland, Townsville, Queensland; Dr D.M. Opresko, Oak Ridge National Laboratory, Oak Ridge, Tennessee; Professor D.C. Potts, Division of Natural Sciences, University of California, Santa Cruz; Mr F. Reinhardt, General Inspection Service, Ministery of Agriculture Nature-management and Fisheries, The Hague; Professor J. Ryland, School of Biological Sciences, University of Wales, Swansea; Dr K.P. Sebens, Department of Zoology, University of Maryland; Dr R.W.M. van Soest, Institute for Systematics and Population Biology, University of Amsterdam; Professor D.B. Spangenberg, Department of Pathology, Eastern Virginia Medical School, Norfolk, VA, USA; Dr A. Svoboda, Ruhr-universität, Facultät für Biologie, Bochum, Germany; Professor P. Tardent, Department of Zoology, University of Zurich; and Dr. R.B. Williams, Tring, Herts, England.

I have not given the present volume a specific title. In accordance with the former conference in 1989 at Southampton, the papers presented in 1995 at Noordwijkerhout were not restricted to selected topics, but dealt with most aspects of recent coelenterate research. I presume and expect that future conferences will go on in this way; our relatively small group of coelenterologists can hardly afford otherwise. As a consequence it seems increasingly meaningless to give special but more or less similar titles to the volumes of what might be called this series.

Finally, in spite of all the help I have had, readers will undoubtedly find shortcomings in this volume. Of course I have to take full responsibility for these. Nevertheless, especially because contributors and conference participants had to wait so long for these proceedings to appear, I hope that they will be satisfied with the ultimate result.

J.C. den Hartog
Editor
NNM, Leiden

References to the major taxonomic groups
(References may be included in more than one group)

GENERAL

Arai: 1; Davies: 123; Grasshoff: 195; Grimmelikhuijzen: 215; Hoeksema: 253; Miller et al.: 345

ANTHOZOA

Actiniaria: Ates: 11; Barra et al.: 39; Chintiroglou & Simsiridou: 101; Fautin: 151; Fukui: 163; Greenwood & Yunes: 209; Harland & Davies: 233; Hinde: 239; Hudman & McFarlane: 265; Migné & Davoult: 321; Mihalik & Brooks: 337.

Antipatharia: Grange: 185; Miller & Grange: 353.

General: Chadwick-Furman: 91; Fautin: 151.

Octocorallia: Brito et al.: 63; Migné & Davoult: 321; Reinicke: 393; Schleyer et al.: 429; Williams: 497.

Ptychodactiaria: Dayton et al.: 135.

Scleractinia: Bak & Meesters: 27; Best: 47; Buddemeier et al.: 71; Delvoye: 143; Karlson & Cornell: 287; Migotto: 329; Ritchie et al.: 403; Schleyer et al.: 429.

Zoanthidea: Migotto: 329; Ryland; 423.

HYDROZOA

General: Boero et al.: 53; Sheiko & Stepanjants: 437; Stepanjants et al.: 455;

Hydroida/Hydromedusae: Boero et al.: 53; Calder: 85; Galliot et al.: 167 (*Hydra*); Goswami & Dey: 177 (Limnomedusae); Grohmann et al.: 227; Kubota: 295; Marfenin: 315; Nogueira et al.: 365; Orlov: 371; Pierobon et al.: 385 (*Hydra*); Rossi et al.: 409; Rosso & Marques: 415; Suárez-Morales et al.: 465; Taddei-Ferretti et al.: 473; Yamashita et al.: 511.

Siphonophora: Lakkis & Zeidane: 301.

Trachylina: Mackie & Singla: 307.

SCYPHOZOA

General: Burnett et al.: 77; Cornelius: 109; Sheiko & Stepanjants: 437.

Coronatae: Jarms: 271.

Cubomedusae: Avian et al.: 21; Ueno et al.: 491.

Rhizostomeae: Jensch & Hofmann: 279; Othman et al.: 379.

Semaeostomeae: Spangenberg et al.: 447; Toyokawa et al.: 483.

Stauromedusae: Hirano: 247.

References to some tentative major themes
(References may be included in more than one theme)

Behaviour: Dayton et al.: 135; Kubota: 295; Mackie & Singla: 307; Orlov: 371; Spangenberg et al.: 447; Taddei-Ferretti et al.: 473; Toyokawa et al.: 483; Yamashita et al.: 511.

Biochemistry/Toxicology: Burnett et al.: 77; Greenwood & Yunes: 209; Othman et al.: 379.

Biodiversity/Species assemblages: Bak & Meesters: 27; Grohmann et al.: 227; Karlson & Cornell: 287; Lakkis & Zeidane: 301; Noqueira et al.: 365; Rosso & Marques: 415; Sheiko & Stepanjants: 437; Suárez-Morales et al.: 465.

Biogeography and distribution: Calder: 85; Dayton et al.: 135; Hirano: 247; Lakkis & Zeidane: 301; Sheiko & Stepanjants: 437; Stepanjants et al.: 455.

Biorhythms: Kubota: 295; Taddei-Ferretti et al: 473; Ueno et al.: 491.

Bleaching: Migotto: 329.

Conservation: Best: 47; Chadwick-Furman: 91; Hoeksema: 253.

Coral reefs: Bak & Meesters: 27; Best: 47; Buddemeier et al.: 71; Chadwick-Furman: 91; Hoeksema: 253; Karlson & Cornell: 287; Reinicke: 393; Schleyer et al.: 429.

Development, growth and regeneration: Galliot et al.: 167; Grange: 185; Marfenin: 315; Ritchie et al.: 403; Rossi et al.: 409; Spangenberg et al.: 447; Ueno et al.: 491.

Ecology: Bak & Meesters: 27; Boero et al.: 53; Buddemeier et al.: 71; Goswami & Dey: 177; Orlov: 371; Reinicke: 393.

Electrical conduction/Neuro-physiology: Barra et al.: 39; Grimmelikhuijzen & Leviev: 215; Hudman & McFarlane: 265; Mackie & Singla: 307; Pierobon et al.: 385; Taddei-Ferretti et al.: 473.

Endosymbiosis: Buddemeier et al.: 71; Davies: 123; Delvoye: 143; Harland & Davies: 233; Hinde: 239; Ritchie et al.: 403.

Evolution/Phylogeny: Boero et al.: 53; Buddemeier et al.: 71; Grasshoff: 195; Miller et al.: 345; Williams: 497.

Faunistics: Goswami & Dey: 177; Grohmann et al.: 227; Lakkis & Zeidane: 301; Nogueira et al.: 365; Rosso & Marques: 415; Sheiko & Stepanjants: 437; Suárez-Morales et al.: 465.

Titles of contributions
(Arranged in alphabetic order according to author)

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Coelenterates in pelagic food webs

M.N. Arai

Arai, M.N. Coelenterates in pelagic food webs.
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 1-9, fig. 1.
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Key words: Feeding; dissolved organic material (DOM); predation; growth; metabolism.

Abstract: In the last two decades there has been much progress in understanding of coelenterate predation on copepods and larval fish. In order to obtain a balanced view of the contribution of coelenterates to pelagic food webs, further knowledge of predation on coelenterates and of other sources of nutrients for coelenterates is needed. Coelenterates obtain nutrients from other animal groups and from dissolved organic material. The assumptions involved in prediction of feeding rates from metabolic measurements presently make these predictions highly speculative.

Introduction

During the last two decades there has been much work on coelenterate feeding on arthropods and larval fish, to the neglect of other aspects of the biology of pelagic coelenterates.

The result is an unbalanced view of the position of coelenterates in pelagic food webs. In this short review I want to emphasize some of the areas which I believe need to be addressed in future to give us a more balanced view; particularly predation on, and alternative sources of nutrients for, coelenterates.

I also want to consider the prediction of feeding rates. In recent years there have been good papers in which feeding rates are derived from the numbers of coelenterate predators, their gut contents and digestion rates. However there have also been more speculative predictions of feeding rates based on metabolic and growth rates of the coelenterates, and I will discuss some of the assumptions involved in these speculations.

The paper considers the pelagic coelenterates including the hydrozoan and scyphozoan medusae, siphonophores and ctenophores. In the limited space available I will only mention an idiosyncratic selection of the many papers on the biology of these groups.

Predation

Pelagic coelenterates are eaten by a wide variety of predators. The best documented are other coelenterates and vertebrates. Purcell (1991: 335) summarized 26 species of scyphomedusae and hydromedusae known to consume other coelenterates. Species of the ctenophore genus *Beroe* also feed primarily on gelatinous prey such as other ctenophores and salps (Reeve & Walter, 1978: 265; Matsumoto & Harbison, 1993: 283). Other invertebrate predators include chaetognaths, arthropods, and gastropods (Arai, 1988: 1921).

Of vertebrate predation, that by fish was reviewed by Arai (1988: 1913) and by Ates (1988: 29). Examples of commercially fished species which utilize coelenterates are spiny dogfish *Squalus acanthias* Linnaeus, 1758, chum salmon *Oncorhynchus keta* (Walbaum, 1792), chub mackerel *Scomber japonicus* Houttuyn, 1782, and Atlantic mackerel *Scomber scombrus* Linnaeus, 1758. Other vertebrates which eat pelagic coelenterates include sea turtles, seabirds and humans (Ates, 1991: 305). However only the fish are present in sufficient numbers to be likely to have a significant impact on coelenterate populations.

In general we probably have a fairly good qualitative view of possible predators large enough to consume adult coelenterates. There is a need for more knowledge of the predators of larval stages.

There is also very little quantitative data yet. Lacking macroscopic hard parts coelenterates rapidly turn to mush and disappear unless stomachs of predators are examined immediately after catching them. Nevertheless some data on stomach contents have been obtained. A table in Arai (1988: 1915) summarizes stomach content data by weight or volume for 11 wild caught fish species eating coelenterates. Similar stomach content data are available for coelenterate predators such as the scyphozoan *Phacellophora camtschatica* Brandt, 1835 (see Strand & Hamner, 1988: 409). Unfortunately there are as yet no measurements of the rates at which coelenterates are digested by fish or coelenterate predators. It is therefore impossible to calculate feeding rates from stomach contents even when the latter data are available. This is one gap in our knowledge which would be fairly easy to fill.

An alternative method of estimating predation is examination of rates of predation in the laboratory, and then attempts to extrapolate to the field conditions. For example, in the laboratory consumption rates of the ctenophore *Mnemiopsis leidyi* (A. Agassiz, 1865) by butterfish *Peprilus triacanthus* (Peck, 1804) range between 4 ml/gm dry wt/hr to 184 ml/gm dry wt/hr, depending on fish size and feeding history (Oviatt & Kremer, 1977: 236). In Narragansett Bay, Rhode Island, the estimated resulting rate of removal would be approximately 5% per day of the total number of *M. leidyi* present in late summer. Calculations of this type based on laboratory data are likely to be less accurate than those based on field data.

Intake

Much more is known about predation by, rather than predation on, coelenterates. There is now an extensive literature on diets and feeding rates of pelagic coelenterates feeding on macroscopic animals, both in the field and in the laboratory. Data on coelenterates feeding on fish have been reviewed by Alvarino (1985: 12), Purcell (1985: 739), Arai (1988: 1918), and Bailey & Houde (1989: 5). Feeding on other plankton by siphonophores was reviewed by Mackie et al. (1987: 223), by hydromedusae by Purcell & Mills (1988: 463) and Arai (1992: 100), by scyphomedusae by Arai (in press), and by ctenophores by Reeve & Walter (1978: 249).

Most work has been done on adult coelenterates. Many have a broad range of prey and there have been a number of papers on selectivity (Green et al., 1986: 1493; Purcell & Mills, 1988: 463; Madin, 1988: 413; Costello & Colin, 1994: 327; Sullivan et al., 1994: 335).

Some coelenterates also utilize microzooplankton. *Aurelia aurita* (Linnaeus, 1758) and *Mnemiopsis leidyi* feed on the larger aloricate ciliates rather than on tintinnids (Stoecker et al., 1987: 901; Stoecker et al., 1987: 667; Båmstedt, 1990: 215). It is probable that a number of other coelenterates, particularly lobate ctenophores, will be found to feed on protozoa or other microzooplankton.

Another possible source of nutrients is dissolved organic material (DOM). Glucose or amino acids labelled with ^{14}C are taken up by Cnidaria including scyphomedusae such as *Aurelia aurita* ephyrae, hydromedusae such as *Tiaropsis multicirrata* (Sars, 1835) and siphonophores (Shick, 1975: 126; Erokhin, 1980: 3; Ferguson, 1988: 1227; Wilkerson & Kremer, 1992: 237). The labelled DOM is utilized; it is incorporated into complex organic compounds of the coelenterates and labelled carbon dioxide is released during metabolism. Uptake occurs even in oligotrophic areas such as the Sargasso Sea (Ferguson, 1988: 1227). However, Cnidaria may also release organic nitrogen to the environment (Costello, 1991: 119). Further measurements are needed to evaluate net influx or loss of free amino acids, and hence the importance of this source. DOM may be especially important in situations where eutrophication increases its concentration.

Symbionts are present in only a few pelagic coelenterates including the hydrozoan *Velella velella* (Linnaeus, 1758), the coronate scyphozoan *Linuche unguiculata* (Schwartz, 1758) and several of the rhizostome scyphozoa. In these species net translocation from the symbionts may be an important source of nutrients (Kremer et al., 1990: 609).

Prediction of feeding rates

In the last fifteen years, there has been a lot of progress in measuring feeding rates in the field (Arai, 1988: 1918; Bailey & Houde, 1989: 37). Calculations of predation rates require data on gut contents, digestion rates, and population densities of the predator. Evaluation of the effects on the prey requires additional data on the population size, or better the production rate, of the prey. There are also a number of studies where field data on predator and prey abundance have been combined with predator consumption rates measured in the laboratory. Laboratory feeding rates may, however, differ from those in the field for a number of reasons including container size, and absence of alternative prey.

In addition there have been predictions of feeding rates from measurements of growth and respiration and calculations using the energy or carbon budget approach. To date these predictions are speculative but they are interesting because they highlight gaps in our knowledge. Discussions of some of the necessary assumptions are included in recent budgets for the hydrozoan medusa *Cladonema californicum* Hyman, 1947, by Costello (1991: 119), for the scyphozoan *Aurelia aurita*, by Schneider (1989b: 17), and Olesen et al. (1994: 9), and for the ctenophore *Mnemiopsis mccradyi* Mayer, 1900, by Kremer & Reeve (1989: 553). Fig. 1 summarizes the energy budget of non-symbiotic heterotrophic organisms.

I have already discussed intake including macroscopic animal prey, microorganisms and dissolved organic material. This intake is balanced by output, metabolism and accumulation of organic material.

FOOD (ANIMAL PREY + microorganisms)
+
Dissolved organic material
=
Food wastes
+
Excretion (NH_3 + other nitrogenous products)
+
RESPIRATION (maintenance + active)
+
Anaerobic metabolism
+
SOMATIC GROWTH
+
Other production (mucus + reproduction)

Fig. 1. Intake and output of energy by non-symbiotic heterotrophic organisms.

The output includes both wastes, which are not assimilated from the food, and metabolic products. The food wastes vary with the diet. Macroscopic faeces may be formed from such rejected items as fish skeletons and arthropod exoskeletons. However smaller particles and minipellets, down to 3 to 50 μm in diameter, are also ejected by hydromedusae (Arai & Chan, 1989: 609; Gowing & Silver, 1985: 395). These may all be accompanied by mucus. The resulting complex is difficult to measure, particularly with relation to the smaller particles.

The few quantitative studies of assimilation have so far concentrated on copepod diets. Siphonophores fed copepod prey egest coherent pellets each containing remains of a single copepod. Purcell (1983: 257) found high assimilation efficiencies of 90% or greater for four species of siphonophores. Anninsky (1988: 64) found that assimilation of copepods by *Aurelia aurita* varied from 39 to 86% depending primarily on feeding intensity. Similarly the assimilation efficiency of *Mnemiopsis mccradyi* is greatly decreased at high copepod densities (Reeve et al., 1989: 535). There is a need for investigation of assimilation with varied diets and feeding rates.

Studies of excretion of metabolic products have concentrated on nitrogenous substances. Ammonia excretion has been measured for a variety of pelagic coelenterates under various feeding regimes (Schneider, 1990: 219; Matsakis, 1992: 55; Arai, in press; Nemazie et al., 1993: 451). However there may also be release of substantial amounts of amino acids and of urea (Kremer, 1982: 149; Båmstedt, 1985: 607). For

example, the dissolved organic nitrogen released by *Mnemiopsis mccradyi* eating copepods is approximately a third of the total nitrogen release (Kremer & Reeve, 1989: 558). These measurements also need to be extended to other species and diets.

Respiration as a measure of aerobic metabolism has been investigated for a larger number of pelagic coelenterates than excretion. The factors affecting rates of oxygen consumption, such as size, environmental conditions, swimming and feeding activity, and metabolic substrates, have been extensively described (Kremer et al., 1986: 403; Arai, 1986: 188; Larson, 1987: 93; Percy, 1988: 61; Schneider, 1992: 377; Arai, in press).

Interpretation of these respiratory rates as a measure of aerobic metabolism depends on assumptions about the substrates utilized. For carbon budgets the respiratory quotient, i.e. the ratio of carbon dioxide production to oxygen utilization, varies from 0.72 to 1.0 depending on the substrates utilized. During starvation coelenterates such as *Aequorea victoria* (Murbach & Shearer, 1902) and *Pleurobrachia pileus* (O.F. Müller, 1776) decrease in size with little change in composition (Hoeger, 1983: 251; Arai et al., 1989: 289). This implies that in starvation protein, lipid, and carbohydrate are broken down in the same ratio as their occurrence in the body. However the protein contribution to metabolism may still vary depending on the extent to which the free amino acids are degraded and on whether the final excretory product is urea or ammonia. After feeding the substrates would vary with the composition of the prey.

Anaerobic metabolism is often assumed to be unimportant to pelagic coelenterates, since they are normally present in well oxygenated water, and enzymes of glycolysis and the Krebs cycle are present. However, some of these animals can also survive in oxygen depleted water such as that produced under eutrophic conditions. Hydrozoa, Ctenophora and Scyphozoa (including *Aurelia aurita* planulae) have been observed in water of less than 0.5 ml $O_2 l^{-1}$ (Smedstad, 1972: 120; Davis, 1975: 2319; Vinogradov et al., 1985: 98). Most dramatically Bayly (1986: 199) found the hydromedusa *Rathkea lizzioides* O'Sullivan, 1984, extending 10 cm down into the hydrogen sulphide containing anoxic layer of an antarctic lagoon.

Very little is yet known about the relevant chemical reactions that may occur in anaerobiosis. Lactate dehydrogenase is present in a number of hydrozoan and scyphozoan medusae, particularly in the swimming muscle (Thuesen & Childress, 1994: 84). By comparison with other animals, lactate is probably only temporarily produced during sustained swimming bouts and then further metabolized. However the enzyme phosphoenolpyruvate carboxykinase is also present in scyphistomae of *A. aurita* and *Chrysaora quinquecirrha* (Desor, 1848) (see Lin & Zubkoff, 1977: 303). This indicates that an alternative anaerobic pathway may be present in scyphozoa in which succinate is produced from phosphoenolpyruvate. There have been no measurements of accumulation or excretion of succinate, or of the fatty acid proprionate which in some other invertebrates is produced from succinate and excreted. It is unlikely that succinate production is important in well oxygenated water. However, it should be investigated in eutrophic conditions.

Somatic growth of a number of coelenterates has been calculated from laboratory experiments or from change in biomass of field populations with time (Larson, 1986b: 89; Reeve et al., 1989: 535; Kremer & Reeve, 1989: 553; Costello, 1991: 119). During periods of rapid growth with adequate food supply interpretation of field

measurements is fairly straightforward (Brewer, 1989: 277; Schneider, 1989a: 507; Lebedeva & Shushkina, 1991: 314; Olesen et al., 1994: 9). However at other times, calculation of production of coelenterates is complicated by the ability of individuals to survive starvation by degrowth. From biomass reduction alone it is difficult to distinguish between the effects on the population of low production, and the effects of predation during which the production rate remains high. This distinction is important because that portion of produced organic material which is lost through predation, must be included in the production rate in order to balance the equation.

Mortality may be due to a number of factors. I have already discussed the need to get better data on mortality due to predation. Other possible sources of mortality are parasites, lack of an adequate food supply, and senility, all of which may interact (Mills, 1993: 194). It is probable that for most species mortality is highest in the earliest stages, when the biomass is still low. It is lower during the rapid growth phase, and then again high following reproduction (Brewer, 1989: 272).

There has been little examination of parasites with reference either to mortality, or to the burden of regeneration and repair which must also be included in somatic growth. Parasites of pelagic coelenterates include protozoa, larval actinians, trematode and cestode larvae, nematodes, and hyperiid amphipods (Lauckner, 1980a: 167; Lauckner, 1980b: 239; Théodoridès, 1989: 118). As long as they are able to feed, hosts are capable of extensive regeneration of portions damaged by parasite grazing. Mills (1993: 194) found that the grazing damage to the hydromedusae *Aequorea victoria* (Murbach & Shearer, 1902) and *Mitrocoma cellularia* (Agassiz, 1865) from hyperiid amphipods and anemone larvae increases in the autumn as food levels drop. However, if the medusae are fed in the laboratory they are still capable of regeneration and repair.

Mucus is produced by all of the pelagic coelenterates, but there are no quantitative measurements of mucus production. It is not known how much of this mucus is lost to the external medium and how much recycled. For example *Aurelia aurita* can feed by trapping small organisms in external mucus. However the mucus is moved with the food by ciliary currents to marginal food pouches and eventually into the gastric pouches (Southward, 1955: 201). Mucus is certainly lost when ejected with faeces or sexual products, but in practice that amount may be measured with the faeces or reproductive products.

To date the few measurements of reproductive production have concentrated on release of ova (Larson, 1986a: 995). For ctenophores such as *Mnemiopsis mccradyi*, the rate of egg production is very sensitive to food supply. Although very large numbers may be produced, the carbon expended is always less than that used for somatic growth (Reeve et al., 1989: 535). In the case of scyphozoa such as *Aurelia aurita* where the zygote is brooded, release of planulae has been measured (Schneider, 1988: 295). However, there is a need for measurements of sperm production. In hermaphroditic ctenophores release may be minimal. In the Cnidaria there may be a greater production especially if sperm are bound into sperm strings as in *Aurelia aurita* (Hamner et al., 1994: 353).

Summarizing, the many sources of error in present carbon and energy budgets make prediction of feeding rates using them highly speculative. However, they do demonstrate that we need to know a great deal more about the chemical processes

underlying the flow of organic material in these animals as a basis for understanding their ecology. We also need further examination of the amounts and sources of mortality at various stages in the life cycle.

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Gastropods carrying actinians

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Key words: Actiniaria; Gastropoda; hermit crabs; symbiosis; review; behaviour; distribution; evolution.

Abstract: The occurrence of gastropod molluscs (Gastropoda) carrying actinians (Actiniaria) is reviewed. More than 30 species-pair combinations have been recorded. About 10 actinian species are known to be carried by living gastropods more or less regularly. Aspects of specificity, behaviour and the distribution of such associations are discussed in relation to a previous hypothesis concerning the ancestry of the better known hermit crab\gastropod shell\actinian symbiosis.

Introduction

The potential suitability of a gastropod as a partner in a symbiosis with actinians has been stressed by several authors (e.g. Ross, 1967: 310). In clinging to the shell of a living gastropod actinians were proposed to gain: a suitable substrate in an otherwise unsuitable environment (Riemann-Zürneck, 1994), and easy transport and movement (Balss, 1924; Ross, 1974a; Arnaud, 1976) supposedly leading to an enhanced food supply (Riemann-Zürneck, 1980, 1994; Hain, 1990; Whorff, 1991) including the procurement of metabolic waste products of the gastropod (Riemann-Zürneck, 1994). The benefits for the gastropod to be derived from an actinian symbiont were proposed to be camouflage and protection provided by the nematocyst armature of the actinian (Arnaud, 1976; Hain, 1990; Riemann- Zürneck, 1994).

Much information is available on the symbiosis between Hermit crabs and one or more Actinians using the same vacated Shell of a gastropod (**HSA**) (see Ross, 1974b, for a review). As to the origin of HSA symbiosis several authors (Balss, 1924; Carlgren, 1928b; Ross, 1974a) have expressed their opinion that the shell-mounting behaviour of certain actinians may have developed from an association between actinians and molluscs. Few studies (Hand, 1975; Ross & Kikuchi, 1976) have been published on the symbiosis between Gastropods and Actinians (**GA**).

Review of gastropods carrying actinians

As a possible stimulus for further study a review of information gathered from the literature on GA symbiosis is here presented in two tables and a map.

Table 1. A preliminary review of associations between gastropods and actinians as reported in the literature arranged in the order of the gastropod family.

gastropod species	actinian species	source	map
family **Buccinidae**			
Austrofusus glans (Röding, 1798)	*Calliactis conchicola* Parry, 1952	Hand, 1975	(1)

Austrofusus glans	*Paracalliactis rosea* Hand, 1975	Ross, 1974b[1]	(2)
Buccinum undatum Linnaeus, 1758	*Hormathia digitata* (O.F. Müller, 1776)	Fabricius, 1797; Lütken, 1861; Norman, 1869	(3)
Buccinum undatum	*Calliactis parasitica* (Couch, 1838)	Ates, pers.obs.[2]	(4)
Buccinanops cochlidium (Dillwyn, 1817) [= *Bullia cochlidia* (Dillwyn, 1817)?]	*Phlyctenanthus australis* Carlgren, 1949[3]	Pastorino, 1993	(5)
Colus spec.	*Hormathia nodosa* (Fabricius, 1780)	Moore et al., 1994[1]	(6)
Colus gracilis (Da Costa, 1778)	*Hormathia digitata*	Norman, 1869; Gravier, 1922, as *Sipho glaber*	(6)
Cominella spec.	*Tealianthus incertus* Carlgren, 1927	Carlgren, 1927[1]	(7)
Japeuthria ferrea (Reeve, 1847)	*Hormathia andersoni* Haddon, 1888	Song, 1992	(8)
Neptunea antiqua (Linnaeus, 1758)	*Hormathia digitata*	Sars, 1850; Lütken, 1861; Norman, 1869; Gravier, 1922; Pearce & Thorson, 1967	(9)
Sipho curtus (Jeffreys, 1867)	*Allantactis parasitica* Danielssen, 1890	Danielssen, 1890; Kwietniewski, 1898; Gravier, 1918, 1922; Davenport, 1955; Dales, 1957	(10)
Neptunea despecta (Linnaeus, 1758)	*Hormathia digitata*	Pax, 1922	(11)
Siphonalia filosa (Adams, 1863)	*Hormathianthus* spec.	Ross & Kikuchi, 1976	(12)
family **Xenophoridae**			
Xenophora digitata Von Martens, 1878	*Calliactis brevicornis* (Studer, 1878)	Studer, 1878; Carlgren, 1928a	(13)
Xenophora longleyi Bartsch, 1931	Actiniaria spec.	Whorff, 1991	(14)
family **Cerithiidae**			
Cerithium spec.	*Paranthus sociatus* Uchida, 1940	Uchida, 1940[4]	(15)
family **Aporrhaidae**			
Aporrhais spec.	*Sagartiogeton undatus*[5] (O.F. Müller, 1788)	Chintiroglou et al., 1992	(16)
family **Melongenidae**			
Melongena melongena (Linnaeus, 1758)	*Calliactis tricolor* (Le Sueur, 1817)	Duerden, 1902	(17)
family **Nassariidae**			
Hinia festiva (Powys, 1835)	*Paranthus sociatus*	Ross & Kikuchi, 1976	(18)
Nassarius spec.	*Paraiptasia radiata* (Stimpson, 1855)	England, 1992	(19)
Nassarius cf. *deshayesiana* (Issel, 1866)	unidentified	Moolenbeek, 1995[6]	(20)
Niotha livescens (Philippi, 1849)	*Paranthus sociatus*	Ross & Kikuchi, 1976	(21)

family **Fasciolariidae**			
Fasciolaria tulipa (Linnaeus, 1758)	*Calliactis tricolor* Cutress & Ross, 1969	Duerden, 1902	(22)
Fusinus perplexus (Adams, 1863)	*Hormathia andersoni*	Song, 1992	(23)
family **Potamididae**			
Velacumantus australis (Quoy & Gaimard, 1831)	unidentified	Walker, 1970[7,1]	(24)
family **Cassidae**			
Cassidaria spec.	*Sagartiogeton undatus*[5]	Chintiroglou et al., 1992	(25)
family **Turritellidae**			
Turritella spec.	*Anthothoe panamensis* (Verrill, 1869) or *A. carcinophila* (Verrill, 1869)	Carlgren, 1951 Pickens, 1996[8]	(26)
family **Naticidae**			
Polinices spec. and other species	*Anthothoe panamensis* or *A. carcinophila*	Pickens, 1996[8]	(26)
family **Volutidae**			
Harpovoluta charcoti (Lamy, 1910)	*Isosicyonis alba* (Studer, 1878)	Arnaud, 1976; Hain, 1990	(27)
Provocator corderoi (Carcelles, 1947)	*Isosicyonis alba*	Riemann-Zürneck, 1980, 1986	(28)
Provocator spec.	*Isosicyonis alba*	Fautin, 1984	(29)
Scaphella georgiana? (Clench, 1946)	*Calliactis tricolor*	Carlgren & Hedgpeth, 1952	(30)
family **Muricidae**			
Murex spec.	*Calliactis parasitica*	Krumbach, 1914; Balss, 1924; Ross, 1974a	(31)
Bolinus brandaris (Linnaeus, 1758) (= *Murex brandaris*)	*Calliactis parasitica*	Stachowitsch, 1977	(32)
Hexaplex trunculus (Linnaeus, 1758) (= *Trunculariopsis trunculus*)	*Calliactis parasitica*	Stachowitsch, 1977	(33)
Thais haemostoma (Linnaeus, 1767)	*Aiptasia diaphana* (Rapp, 1829)	Fainzilber, 1982[1]	(34)
Hexaplex trunculus	*Aiptasia diaphana*	Fainzilber, 1982[1]	(35)
Hexaplex spec.	*Calliactis parasitica*	Ates, pers. obs.[9]	(36)
unidentified	*Allantactis parasitica*	Riemann-Zürneck, 1994	(10)
unidentified	*Hormathianthus tuberculatus* Carlgren, 1943	Carlgren, 1943[10]	(23)
unidentified	*Hormathia digitata*	Ross, 1974a; Riemann-Zürneck, 1994	(6)
unidentified	*Calliactis polypus*	Cutress, in Ross & Sutton, 1968	(37)
unidentified	*Bunodactis chrysobathys* Parry, 1951	Parry, 1951[11,1]	(38)

Notes relating to table 1 and 2:

[1] In view of the limited number of reported occurrences of this association, the possibility exists that the relationship between the gastropod and the actinian is temporal and/or does not go beyond the mollusc offering a suitable substrate. Reinvestigation would be useful.

[2] In August 1976 I found one specimen of *B. undatum* carrying three specimens of *C. parasitica* in a tidal stream in the Golfe de Morbihan (Brittany, France).

[3] Originally described from south-west Australia.
[4] This is possibly the association studied by Ross & Kikuchi (1976).
[5] In the rest of its vast distributional range *S. undatus* has not been reported to be associated with living gastropods. In the Netherlands, England, Scotland and France this species shows no preference for substrates containing calcium carbonate (personal observation).
[6] In March 1995 Mr Rob Moolenbeek (personal communication) noted conspicuous actinians measuring about 0.5 cm on *Nassarius* cf. *deshayesiana* in the vicinity of Masirah Island, Oman.
[7] In a collection of 394 gastropods, 2% carried one to four actinians.
[8] According to a letter by Dr P.E. Pickens (Tucson, Arizona), dated 23 April, 1996, specimens of *Anthothoe carcinophila* were found occurring on gastropods, mostly *Polinices* and *Turritella*, on two locations in the northern Gulf of California. The actinian was identified by Prof. C.E. Cutress who also stated that the use of the name *A. panamensis* by Carlgren (1951: 432) for the same species is based on a misidentification.
[9] In July 1984 I found two specimens of *C. parasitica* on two specimens of *Hexaplex* spec. in the Ria de Arosa, south of La Toja (Spain), at a depth of about 50 cm below low tide level. In July 1990, in the same area, I found ten unidentified gastropods of at least two species (50 specimens examined), carrying one or two specimens of *C. parasitica*.
[10] Song (1992) held *Hormathianthus tuberculatus* conspecific with *H. andersoni*.
[11] Though "usually attached to encrusting bottom forms such as tunicates, etc." (Parry, 1951: 117), the caption of fig. 8 (Parry, 1951: 107), depicting an actinian on a living gastropod, reads: "*Bunodactis chrysobathys*". Hand (1975) does not mention the occurrence of *B. chrysobathys* on gastropods.

Table 2. Review of the actinian species reported more than once to be carried by a living gastropod, including some details derived from the literature concerning the nature of their symbiosis.

actinian species	number of known gastropod host species	known to occur on other objects?	tendency to monopolize the gastropod shell substrate?	ability to remount gastropod in experiments?
family **Hormathiidae**				
Allantactis parasitica	unknown[12]	yes[14]	yes	unknown
Calliactis brevicornis	1	no	unknown	unknown
Calliactis conchicola	1	unknown	unknown	yes
Calliactis parasitica	4	yes	no	unknown
Calliactis tricolor	3	yes	unknown	unknown
Hormathia digitata	4[13]	yes	no	yes
Hormathianthus spec.	3	yes	no	yes
family **Actinostolidae**				
Paranthus sociatus	3	no	no	yes, but settling success limited
family **Aiptasiidae**				
Paraiptasia radiata	1	no	no	unknown
family **Actiniidae**				
Isosicyonis alba	2	no	yes	unknown
Phlyctenanthus australis	1	yes	yes	unknown
family **Sagartiidae**				
Anthothoe panamensis or *A. carcinophila*[8]	>4	no	no	unknown

Notes relating to table 2:
[12] Danielssen (1890: 23) and Kwietniewski (1898: 123) refer to a single host species, whereas Carlgren (1942: 34) noted species of *Sipho, Neptunea* and other gastropods as hosts.

[13] Pearce & Thorson (1967) noted *H. digitata* in the Skagerrak on *N. antiqua* to the exclusion of any other substrate. See, however, table 1 for sources referring to other parts of the distribution range of the actinian species.

[14] The records of Gravier (1918, 1922) of *A. parasitica* on stones and on mud bottoms may need confirmation.

Discussion

General

Considering the proposed benefits (see introduction) the established number of records of GA symbiosis (table 1) is low. So, either these benefits are not what they seem to be, or there are considerable barriers or high costs in becoming involved in this type of symbiosis. Locally GA symbiosis may be ecologically important (Hain, 1990; Riemann-Zürneck, 1994), but to what extent is unknown. More knowledge about GA symbiosis will also be useful when studying the ancestry of the HSA. Balss (1924: 778), Carlgren (1928b: 170) and Ross (1974a: 121-123) argued that HSA symbioses originate from associations between gastropods and actinians. The outline of the events leading to the more abundant HSA has been elaborately discussed by Ross (1974a: 121-123) who addressed several questions undermining his own hypothesis. The present review warrants a renewed discussion of aspects of specificity, behaviour and distribution.

Specificity

Some 60 species-pairs of hermit crabs/actinians are recorded in the literature (Ates, in preparation). *Calliactis parasitica* is recorded with at least six hermit crab species, *C. polypus* with at least ten and *C. tricolor* with three. Even the classic example of an obligate symbiont, *Adamsia palliata* (O.F. Müller, 1776), is known to occur with at least three hermit crab species (Ates, 1995). The provisional pattern can be identified that those actinians involved in a partnership with hermit crabs have more hosts than those involved in a partnership with gastropods (see column 1 in table 2), i.e. the latter seems to be more specific than the former. A discussion of the possible relation between specificity and primitiveness of these types of symbiosis is premature. The costs of, or the barriers to, acquiring an actinian symbiont may be higher for a gastropod than for a hermit crab.

A study of the degree of the mutual dependence of the partners in a GA symbiosis may start by obtaining answers to the questions in table 2. Only a few of the associations mentioned in table 1 have been studied with the aim of obtaining information as to possible interactions between the two partners. The information shown in table 2 is therefore no doubt incomplete. Other criteria important to interpret the degree of mutual dependence of the partners in a GA symbiosis will come to light when making a detailed study of the pair in a living condition. Because of the inaccessibility of their habitats, the obligateness of the symbiosis with gastropods would be hard to prove for actinian species like *Isosicyonis alba* (see Riemann-Zürneck, 1980; 1986) or *Allantactis parasitica* (see Riemann-Zürneck, 1994) i.e. their absence from other substrates is not easily established.

Behavioural aspects

Those partnerships recorded only once or those in which the identification of the actinian is doubtful (see table 1) are not included in table 2.

The following examples suffice to demonstrate that there are degrees of intimacy between the partners involved in a GA symbiosis.

Mud-bottom living gastropods, from which trematode cercariae emerge, offer a suitable habitat for actinians preying on these cercariae (Walker, 1970). Considering that only 2% of the gastropod *Velacumantus australis* carried one of more actinians, the adaptive significance of this association seems to be negligible. In this example of a GA symbiosis no behavioural adaptation from either side is to be expected (except perhaps with relation to the preference of the actinian larva when settling on the gastropod shell) and the distribution of the actinians on the shell will probably be at random. If the actinian prefers a certain location on the gastropod shell, as in *Hormathia digitata* (personal observation), behavioural adaptations on its part seem to be imperative. It seems inconceivable how a gastropod would be physically capable of controlling the location of the actinian on its shell, contrary to a hermit crab.

In some of the known cases of GA symbiosis one or both partners seem to have undergone morphological modifications, like the relatively thin shell of *Provocator corderoi* and *Harpovoluta charcoti*, and *Isosicyonis alba* covering it completely. The monopolization of the shell surface by the actinian may provide the gastropod the best protection against predators.

The so-called shell-factor has been demonstrated to be functional in some HSA symbioses. In the GA symbiosis this factor may similarly play a role in uniting the partners, but in relation with living gastropods it has only been shown to be present in some species of *Calliactis*, for instance in *C. tricolor* (see Cutress & Ross, 1969) and perhaps in *C. polypus* (see Ross & Sutton, 1968). What triggers the association of the other gastropod and actinian species in table 2 is unknown. *Hormathianthus* spec. successfully resettled on living *Siphonalia filosa*, its regular host, whereas success of resettling on empty *Siphonalia*-shells and on living gastropods of other species was limited (Ross & Kikuchi, 1976: 43-44). Possibly the behaviour of the gastropod is more important in initializing the symbiosis than hitherto realized from aquarium experiments (Hand, 1975: 512, last paragraph; Ross & Kikuchi, 1976: 45). Without the cooperation of the gastropod, in approaching and remaining stationary near its future partner, the initiation of the symbiosis in situ may hardly be feasible, unless the actinian colonizes the gastropod shell as a larva. In the latter case the ability of the adult actinian to remount the gastropod in experiments could be absent.

Zoogeographical aspects

The distribution of the known GA symbioses in shallow water is concentrated in four areas: the north-eastern Atlantic, the Caribbean, Japan and New Zealand (fig. 1). In water deeper than about 200 m three locations are apparent: the western Caribbean, western Africa and south western Atlantic including the neighbouring Antarctic. This distribution pattern is extremely patchy. Relatively well surveyed areas may be over-represented.

Although the distribution of GA symbioses is probably incompletely known, it

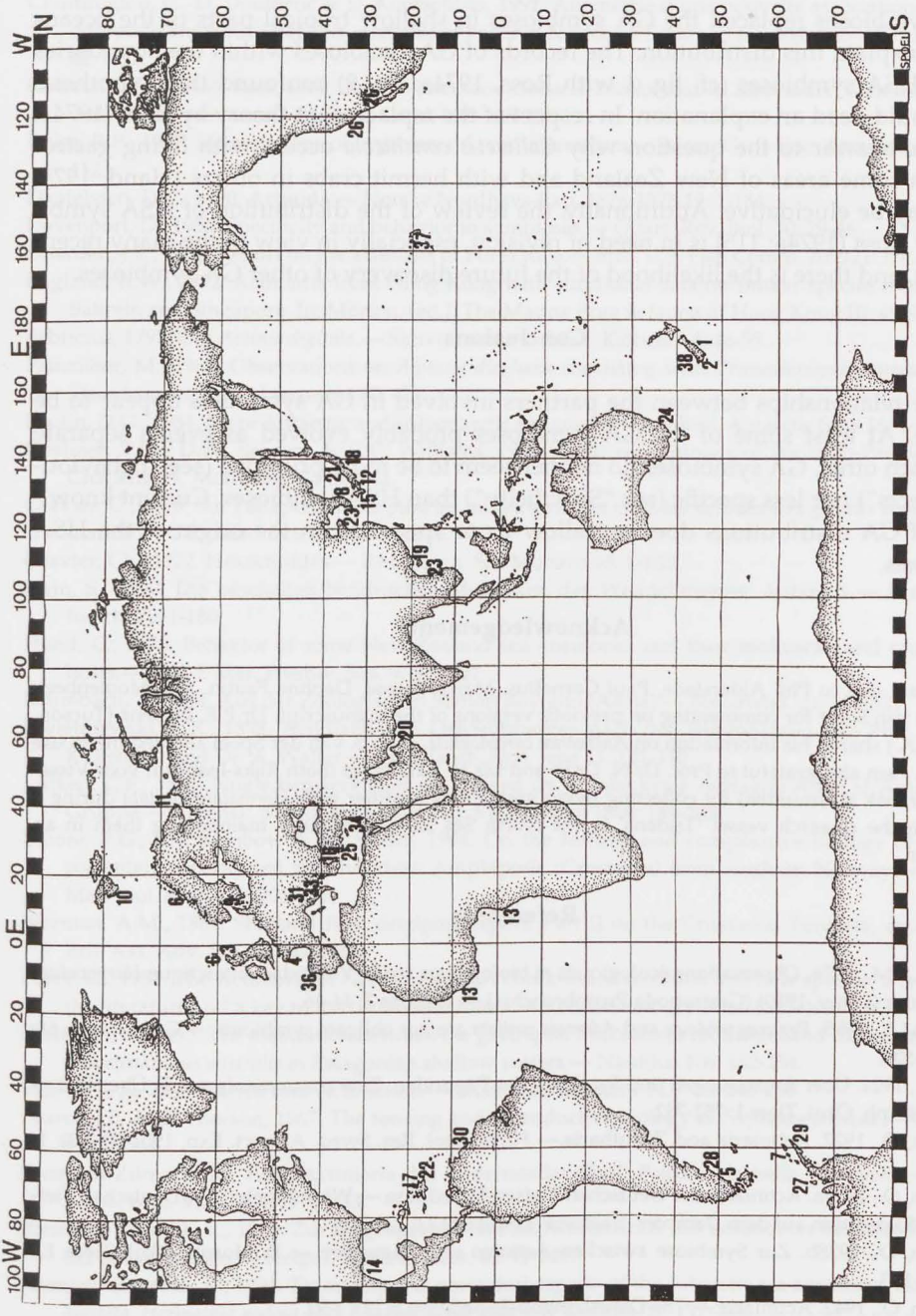

Fig. 1. Geographical distribution of gastropods carrying actinians.

seems clear that it does not follow a latitudinal richness pattern (cf. Vermeij, 1983: 324). According to Ross (1974a: fig. 2) HSA symbioses occur mainly in shallow tropical parts of the oceans. The conjecture by Ross (1974a) that at some time in the past HSA symbioses replaced the GA symbioses in shallow tropical parts of the oceans would explain this distribution. The records of GA symbioses within the boundaries of the HSA symbioses (cf. fig. 1 with Ross, 1974a: fig. 2) confound this hypothesis and would need an explanation. In respect of the replacement theory by Ross (1974a: 123) the answer to the question why *Calliactis conchicola* occurs with living gastropods in some areas of New Zealand and with hermit crabs in others (Hand, 1975: 509) may be elucidative. Additionally, the review of the distribution of HSA symbioses by Ross (1974a: 115) is in need of revision, especially in view of the many recent records, and there is the likelihood of the future discovery of other GA symbioses.

Conclusions

The relationships between the partners involved in GA symbioses appear to be diverse. At least some of the GA symbioses probably evolved as events separate from each other. GA symbioses do neither seem to be more primitive (see "Behavioural aspects") nor less specific (see "Specificity") than HSA symbioses. Current knowledge of GA distributions does not allow us to speculate on the origin of the HSA symbiosis.

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Note added in proof:

Gosliner et al. (1996) published a picture (no. 445) taken in the Philippines showing *Strombus bulla* (Röding, 1798) carrying four or more actinians on its shell.

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The nematocysts of *Carybdea marsupialis* Linnaeus, 1758 (Cubozoa)

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Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 21-26, figs 1-2, tab. 1.
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Key words: Cubozoa; *Carybdea marsupialis*; nematocysts.

Abstract: Nematocysts of the cubozoan *Carybdea marsupialis* Linnaeus, 1758, were examined. Three categories were observed: atrichous isorhizic haplonemes, heterotrichous microbasic euryteles and holotrichous isorhizic haplonemes. Some characteristics in the tubule of the euryteles seem to be peculiar to the cubozoans.

Introduction

The discovery of *Carybdea marsupialis* Linnaeus, 1758, near the Italian coasts of the central and north Adriatic Sea in 1985 provided an opportunity to study both its cnidom and its toxicity (Rottini et al., 1995). The present study provides information on the morphology of the nematocysts of the species.

Specimens of *C. marsupialis* were collected in the harbour of Cesenatico (north Adriatic Sea) from 1989 to 1994 in the period July-September. The method for nematocysts purification from the tentacles, morphometric measurements on unfixed nematocysts (Interference contrast light microscopy micrographs, LM) and fixation for the scanning electron microscopy (SEM) are described in Avian et al. (1995).

Results

Nematocyst types

The cnidom of *Carybdea marsupialis* contains three nematocyst types: atrichous isorhizic haploneme, heterotrichous microbasic eurytele and holotrichous isorhizic haploneme (hereafter referred to as atrich, eurytele and holotrich, respectively). Their morphometric data are summarized in table 1.

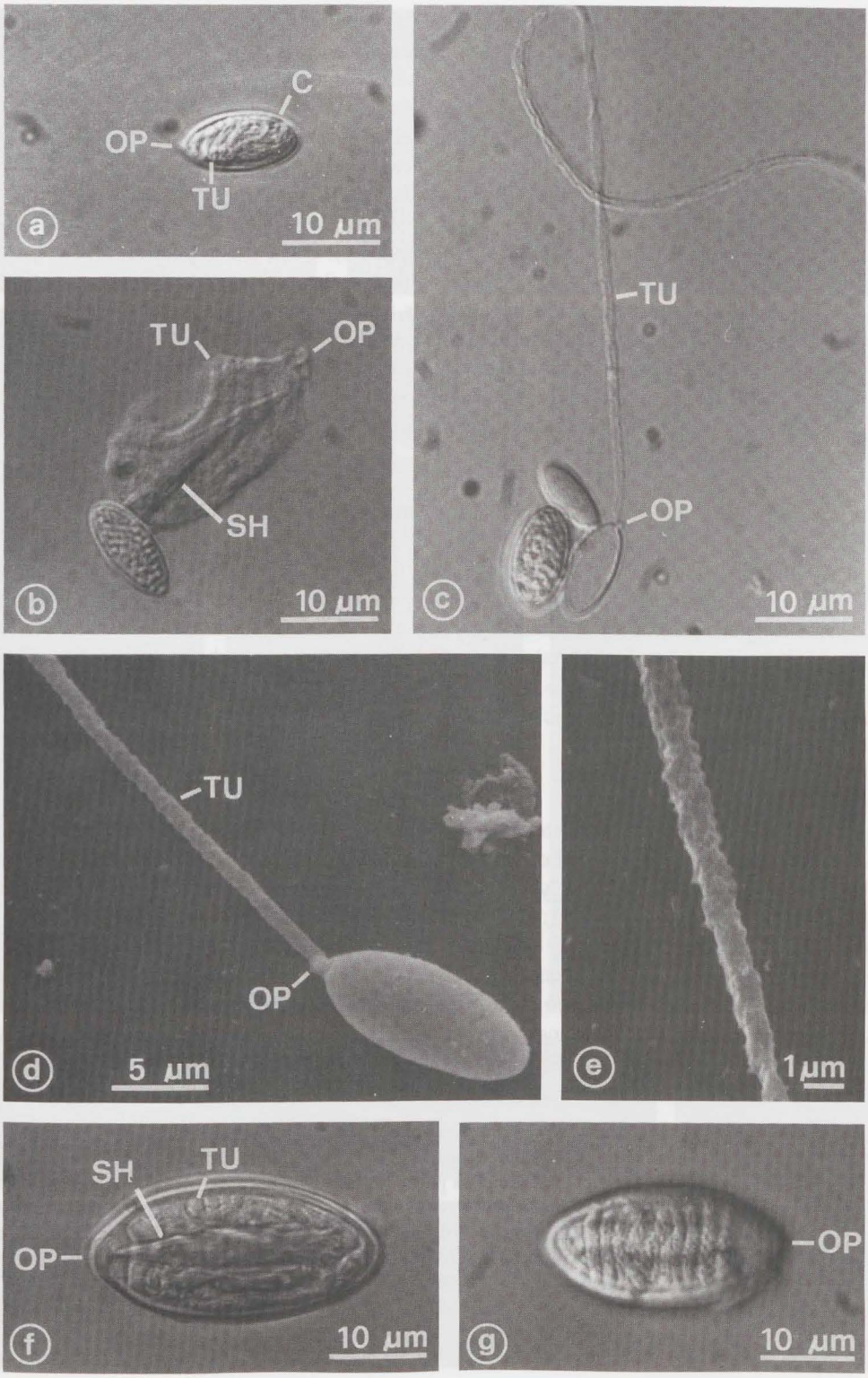
The most abundant type, the atrich, is the smallest. The capsule is ellipsoid, widest between mid-length and the opercular pole (figs 1a-d). The uneverted tubule is isorhizic, arranged helically in the opercular half of the capsule, with irregular coils in the abopercular half of the capsule (fig. 1a). When everted, under LM it exhibits a smooth surface (fig. 1c). Higher SEM magnifications show three prominent, helical coils, rough but not spined (figs 1d-e).

The secondmost frequent category, the eurytele, includes the largest nematocysts with an ellipsoidal capsule (figs 1f-g; 2a-b). The uneverted tubule consists of a well defined shaft that lies in the central main axis of the capsule; from its end the distal

Table 1. *Carybdea marsupialis*: morphometric parameters of nematocysts before and after discharge. Linear measurements are in µm, volume is in µm³. Tubule measurements were performed on discharged nematocysts. $\bar{X}$ = mean; SD = standard deviation; n = number of counts; P = probability; W/L ratio = width/length ratio; n.s. = not significant; * = the length of the spines measured on SEM micrographs; ** = Student's t test performed on the proximal and distal diameter of the shaft.

	Undischarged				Discharged				Student's		
	$\bar{X}$	SD	Min	Max	$\bar{X}$	SD	Min	Max	n	t	P
Atrichous isorhizic haplonemes											
Capsule											
Length	12.86	1.88	8.99	18.05	12.00	1.80	5.92	15.07	107	1.91	<0.03
Diameter	6.64	1.19	4.29	9.88	5.96	0.96	2.96	7.45	107	2.57	<0.006
volume	612.61	297.47	182.03	1685.58	473.60	168.63	54.32	814.30	107	2.36	<0.01
W/L ratio	0.52	0.05	0.42	0.63	0.50	0.05	0.39	0.63	107	1.50	n.s.
Tubule											
Length			277.26	28.66	210.27	341.69					
Diameter			0.86	0.19	0.65	1.12					
Heterotrichous microbasic eurytele											
Capsule											
Length	31.37	5.29	17.02	42.26	28.89	3.45	21.35	36.11	104	2.44	<0.008
Diameter	16.86	2.86	12.11	23.99	15.12	1.63	12.14	19.00	104	3.33	<0.001
volume	9476.81	5696.20	1602.17	36953.38	6830.93	2188.87	2897.43	11688.46	104	2.83	<0.003
W/L ratio	0.54	0.09	0.45	0.96	0.53	0.04	0.46	0.61	104	1.22	n.s.
Shaft											
Length	23.46	3.29	15.25	31.94	25.24	3.62	17.35	31.22	104	2.01	<0.02
Prox. diam.					4.18	0.73	3.12	5.58	30	7.68**	<0.001
Dist. diam.					6.40	0.90	4.30	7.77	30		
spines*											
L. max					10.89	1.65	8.01	12.91	20		
L. min					7.87	1.38	5.50	8.94	20		
n/coil					23.67	4.72	17.00	29.00	20		
Tubule											
Length					965.72	252.42	554.00	1510.00	20		
Diameter					2.72	0.67	2.10	3.66	20		
spines*											
Length					0.70	0.11	0.62	0.77	38		
n/coil					22.00	2.83	20.00	24.00	10		
Holotrichous isorhizic haplonemes											
Capsule											
Length	20.72	2.55	15.11	24.94	16.81	3.62	9.68	21.81	67	5.19	<0.001
Diameter	19.05	2.37	13.79	22.86	14.40	2.84	8.71	17.89	67	7.28	<0.001
Volume	4461.31	1472.78	1647.84	7448.01	2380.00	1154.30	451.25	4290.84	67	6.23	<0.001
W/L ratio	0.92	0.04	0.86	0.98	0.86	0.06	0.75	0.95	67	5.63	<0.001
Tubule											
Length					736.75	181.58	289.12	938.00	12		
Diameter					1.89	0.36	1.63	2.42	12		
spines*											
Length					0.40	0.08	0.30	0.50	10		
n/coil					19.25	0.96	18.00	20.00	10		

Fig. 1. *Carybdea marsupialis*, LM and SEM micrographs of nematocysts. **a.** Undischarged atrich. **b.** Undischarged atrich (bottom); in the center, an undischarged eurytele with the capsule wall partially collapsed. **c.** Two undischarged atrichs (left) and one fully discharged (center). **d.** Discharged atrich. **e.** Discharged atrich, detail of an everted tubule; proximal part of the tubule at the bottom. **f.** Undischarged eurytele; focus on the shaft. **g.** Undischarged eurytele; focus on the lateral tubular coils.
C = capsule; OP = operculum; SH = shaft; TU = tubule.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tubule arises, forming one or two ascending coils that reach the beginning of the shaft. The following portion of the tubule is arranged in a series of five to six concentric coils, not around the shaft, but placed laterally, just beneath the lateral wall of the capsule (figs 1b, f-g; 2a). The everted tubule consists of a shaft enlarged at its distal half, with three series of flat, triangular, lamellar spines decreasing in length distally. These series are arranged helically, but the pitch of the spiral is very slight. The proximal, longer spines are arranged downwards, pointing towards the capsule, whilst the distal smaller spines are arranged upwards (figs 2d-e). The tubule exhibits a smooth proximal portion of 10-20 m long (figs 2d-e), and a distal portion armed with three series of small spines angled downwards (fig. 2g).

The least common type, the holotrich, is a nematocyst with a subspherical capsule (fig. 2c). The uneverted tubule is generally arranged in some ordered coils beneath the operculum, and a mass of irregular coils in the abopercular area (fig. 2c). The everted tubule exhibits three series of small and short triangular spines, with their tip slightly tilted downwards. The tubule is isodiametric for about the first third or half of its length.

Nematocyst distribution and relative abundance

Tentacles (nematocysts in rings): atrichs 63.3%; euryteles 35.5%; holotrichs 1.2%. Pedalia (nematocysts grouped in warts): euryteles more abundant than (>) holotrichs, atrichs very rare. Exumbrella (larger warts): euryteles > holotrichs > atrichs (uncommon). Velarium (smaller warts): euryteles > holotrichs > atrichs (uncommon). Oral lappets (no warts): euryteles > holotrichs > atrichs (uncommon). Gastric cirri: euryteles > holotrichs (uncommon); atrichs absent.

Discussion

Of the three types of nematocysts observed in *Carybdea marsupialis*, the atrichs are similar to those reported from other Cubozoa and from scyphomedusae such as *Pelagia noctiluca* (Forskål, 1775), *Cyanea capillata* (Linnaeus, 1758), and *Rhizostoma pulmo* (Macri, 1778)(Avian et al., 1991; Heeger et al., 1992).

The holotrichs also closely resemble those found in several scyphozoans, again including *P. noctiluca*, *C. capillata* and *R. pulmo* (Avian et al., 1991; Heeger et al., 1992). The only distinctive difference is the degree of spiralization of the series of spines, slighter in *C. marsupialis*. A nematocyst which, in undischarged condition, closely resembles this type was detected by Southcott (1967) in *Carukia barnesi* Southcott,

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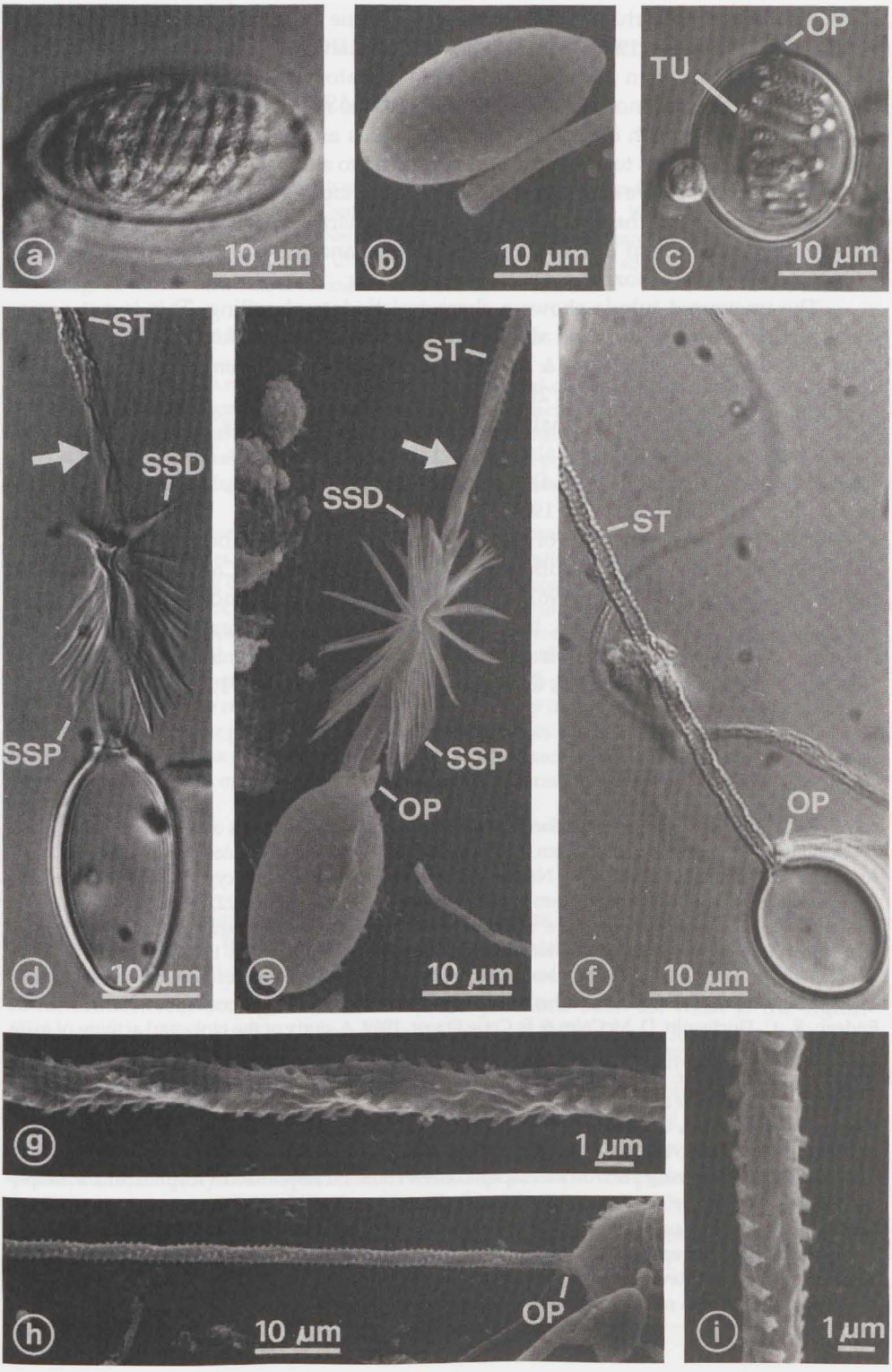
Fig. 2. *Carybdea marsupialis*, LM and SEM micrographs of nematocysts. **a.** Undischarged eurytele; plane of focus on the lateral tubular coils. **b.** Undischarged eurytele. **c.** Undischarged holotrich. **d.** Discharged eurytele; arrow indicates the proximal spineless portion of the everted tubule. **e.** Discharged eurytele; on the bottom the distal portion of an atrichous everted tubule. Arrow indicates the proximal spineless portion of the everted tubule. **f.** Discharged holotrich. **g.** Discharged eurytele, detail of the everted tubule, median portion, origin of the tubule on the left. **h.** Discharged holotrich. **i.** Discharged holotrich, detail of the everted tubule, origin of the tubule on the left.
OP = operculum; SH = shaft; SSP = proximal spines of the shaft; SSD = distal spines of the shaft; ST = tubular spines; TU = tubule.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1967. Termed as anisorhiza, it is probably the same type. As previously observed (Calder, 1974; Östman, 1991; Heeger et al., 1992; Avian et al., 1991), the terms isorhiza and anisorhiza are often applied to the same nematocyst, depending on the importance given to the common diameter reduction at the more distal portion of the everted tubule. The holotrich of *C. marsupialis* maintains an isodiametric condition of the tubule over $^1/_3$ - $^1/_2$ of its total length, and according to a revised terminology proposed by Avian et al. (1995), we consider it to be a holotrichous isorhiza.

On the other hand the eurytele exhibits some characters easily detectable by light microscopy, and present in both the Carybdeidae and the Chirodropidae, that may prove typical of the cubozoans. These characters are:

– The uneverted tubule shows a characteristic lateral coiling. This is not a species-specific character; it occurs also in other cubozoans, like *Chiropsalmus quadrumanus* (F. Müller, 1859) (cf. Calder & Peters, 1975: fig. 1d) and *Chironex fleckeri* Southcott, 1956 (cf. Endean et al., 1969: fig. 2f).

– The distal spines of the shaft are strongly angled upwards, much more than in scyphozoans. This character is also present in other cubozoans, like *C. quadrumanus* (cf. Calder & Peters, 1975: fig. 1d), *Carukia barnesi* (cf. Southcott, 1967: plate 7, fig. 1), and *C. fleckeri* (cf. Endean et al., 1969: fig. 2f).

– The most proximal part of the tubule is completely smooth. This again is a character found also in other cubozoans like *C. quadrumanus* (Calder & Peters, 1975: fig. 1d), *C. barnesi* (Southcott, 1967: Plate 7, fig. 1), *C. fleckeri* (Endean et al., 1969: fig. 2d, g), but not in scyphozoans, like *Pelagia noctiluca, Cyanea capillata, Aurelia aurita* (Linnaeus, 1758), *Rhizostoma pulmo* or *Rhopilema nomadica* Galil, Spanier & Ferguson, 1990 (cf. Heeger & Möller, 1987; Östman, 1991; Avian et al., 1991; Heeger et al., 1992; Avian et al., 1995).

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Coral diversity, populations and ecosystem functioning[1]

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Key words: Biodiversity; ecological strategies; keystone species; functional groups.

Abstract: In view of the world-wide degradation of coral reef communities and loss of biodiversity, reef science is facing urgent questions such as: what is the effect of biodiversity on the functioning of coral reefs in terms of sustainability of communities, biogeochemical processes, etc. Reef degradation is connected with human impact which is directly acting on populations, raising questions such as: what is our understanding of coral population-ecosystem interaction, and what is the significance of individual species?
To explore some of these questions, we will consider the concepts of keystone species, functional groups and key processes in the context of coral reef communities. Thought experiments show differences in function among coral species. We can look for functional relationships between species and various key processes such as reef calcification and coral recruitment. Key processes must be identified because the significance of species is different in different processes. Functional groups are groups of species with similar effects in ecosystem processes. If such groups are identified, biodiversity within groups as well as among multiple groups must be considered. Keystone species, with an indispensable role in the community, may emerge most clearly in stressed or marginal environments. This paper illustrates our present position in reef science, shows that there is only an incomplete interpretation of such notions currently possible in reef ecology, and shows that we cannot answer basic questions such as: how much alpha biodiversity must be conserved to keep a Caribbean coral reef ecosystem functioning?

Introduction

At recent reef science meetings much concern has been shown about the status of coral reefs, because they are reported to be declining world-wide (e.g. Wilkinson, 1993; Ginsburg & Glynn, 1994). In the framework of ongoing changes in coral reef communities, reef ecologists are faced with questions such as: what is the significance of coral biodiversity? How suitable is the biological species concept in coral taxonomy? What is the meaning of coral population biology in relation to coral reef functioning? Such questions lead to a relatively simple conclusion: We need a better understanding of the significance and the roles of individual coral species.

This is clearly demonstrated by the fact that we do not know the answer to the question: how many coral species are needed to perform the processes necessary for the functioning of a coral reef? A conceptual model addressing this question is illustrated in a simple diagram (fig. 1) which shows the relation between diversity (in this paper synonymous with species richness) and an arbitrary index of ecosystem functions. There are four relationships feasible:

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[1] This is NIOZ publication 3129.

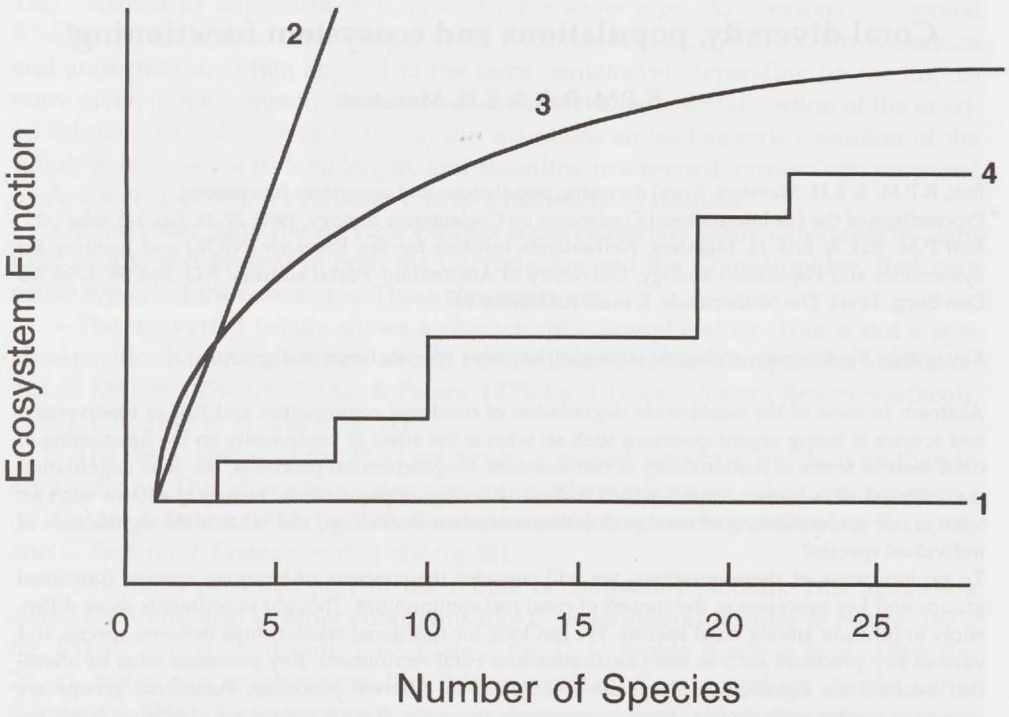

Fig. 1. Models of possible relationships between species richness and ecosystem function (modified after Vitousek & Hooper, 1994). 1. Number of species does not influence ecosystem function. 2. All new species add to ecosystem function, resulting in a linear relation between species number and ecosystem function. 3. Increasing number of species relates strongly to ecosystem function initially but this effect wears off: an asymptotic relationship. These three models differ essentially from the last. 4. Some species have an effect on ecosystem function, others have none; there is no simple relationship between species abundance and ecosystem function.

1. The number of species does not influence ecosystem function.

2. Any new species introduced in the system adds to ecosystem function and the result is a linear relation between species number and ecosystem function.

3. Initially there is an increase in ecosystem function with increasing numbers of species but this effect wears off: we find an asymptotic relationship. In these three models there are no differences in ecosystem function between the species. The species are indistinguishable in their ecosystem function. This is an essential difference with the fourth type of relationship.

4. Some species have an effect on ecosystem function while other species have little or none. In this model there is no simple relationship between species abundance and ecosystem function.

A reef perspective

There is an immediately obvious question concerning reef ecology when the importance of species richness is considered: There are some 100 coral species on any

Indonesian coral reef site; are they all needed for the reef to exist or to function? As a parameter of ecosystem function we can take whole reef calcification. We do know that the occurrence of only a few coral species is enough to allow rapidly growing reefs to exist in the Caribbean. An example is the reef cored at Alacran (fig. 2; Macintyre et al., 1977) where only four or five coral species, *Acropora cervicornis* (Lamarck, 1916), *Montastrea annularis* (Ellis & Solander, 1786), *Porites porites* (Pallas, 1766) and *Diploria* spec. were sufficient to build one of the most rapidly growing reefs known. Such comparisons force us to ask: What is the significance of an individual species? Are all species equal? Do we need all species? Do we adhere to the "rivet" hypothesis (all species contribute to the integrity of the system -as rivets in an aircraft, Ehrlich & Ehrlich, 1981; Lawton & Brown, 1994), or to the "redundant species" hypothesis - which maintains that species richness is irrelevant? The example of Alacran reef appears to point to an alternative to these two extreme points of view. Some species, such as *Acropora cervicornis*, were important. Others, such as *Favia fragum* (Esper, 1795), apparently were not. The ecosystem function of reef accumulation appears to be a modification of the model 4 relationship (fig. 1).

In comparing Indonesian and Caribbean reefs we used reef accretion as the parameter for ecosystem function. Accretion was determined by the accumulation rate of a few coral species, a function of species-specific colony growth. This immediately points to another necessary focus in our discussion, the process concerned, i.e. the specific aspect of ecosystem function. The species mentioned may have been sufficient in terms of reef calcification and accumulation rates of Alacran reef, but what about processes of coral reef maintenance such as resistance to disturbance or catastrophes, and essential reef community processes such as generation of structure and substratum? Clearly we need to identify the processes of importance in the functioning of coral reef ecosystems. To be able to look into this we need to have definitions. Coral reefs are the product of a coral reef community which can be defined as: an assemblage of living corals and their associated organisms that has the potential for reef production (Buddemeier & Hopley, 1988). With the realisation that community-level processes are driving forces in coral reef function it is clear that we need to go beyond biochemical cycles, beyond the transfer of material and energy. At this level key processes will emerge, defined in terms of the biology of populations and we need to consider the role of individual species.

Parallel problems are recognised and similar questions are raised by ecologists active in the study of other ecosystems (e.g., Carleton Ray, 1988; Lawton & Brown, 1994; Steneck & Dethier, 1994; Vitousek & Hooper, 1994). These studies have generated useful concepts such as keystone processes, species functional groups and keystone species. We will briefly examine these concepts in relation to coral community ecology.

Some input data from reefs

What are ecosystem key processes in general and in coral reefs? First to mind come general ecosystem processes such as primary production, secondary production and decomposition; these can also be identified on coral reefs (e.g., Dubinsky, 1990). On reefs, zooxanthellate coral species are important in primary production, but most coral primary production is self consumed. Less is known about the role of

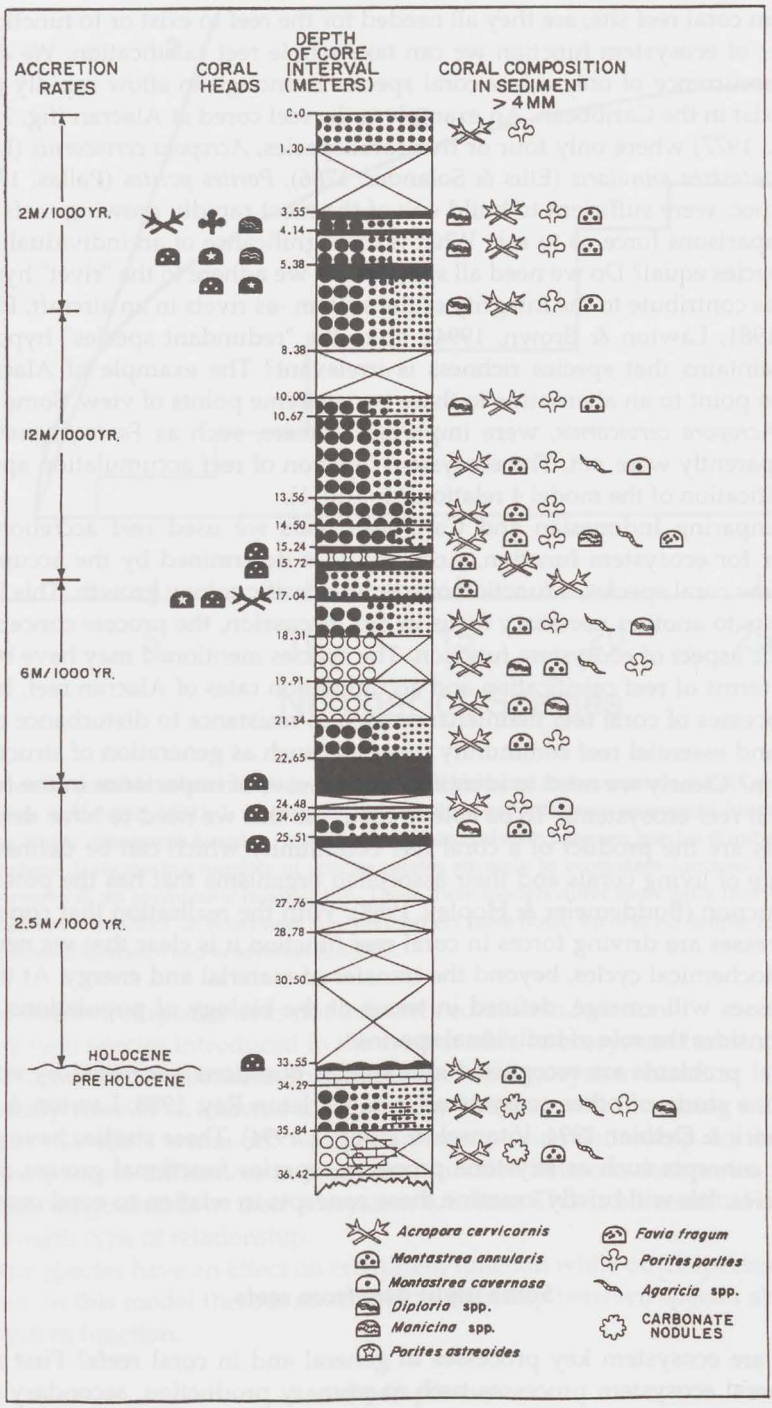

Fig. 2. Holocene coral reef accumulation rates and species composition in a Caribbean coral community; Alacran Reef, Mexico. (Modified after Macintyre et al., 1977).

corals in secondary production, at least about the qualitative aspects, but many corals are predators as well as prey to other organisms. There is a coupling of coral trophic biology with reef microbial processes and corals are linked to processes of decomposition, but again, the quantitative role of corals here is virtually unknown (but see Sorokin, 1993). As far as typical coral reef processes are concerned we know that corals are involved in community calcification, hard substratum generation and 3-dimensional structure formation. Are these key processes, and what is the function that species play in relation to a particular keystone process? Helpful here is the identification of functional groups. Functional groups are defined as: groups of species that bear a certain set of common structural and/or process features (Körner, 1994), or as groups of species with similar impacts on ecosystem processes (Hobbie et al., 1994). In coral reefs possible examples are the groups of species responsible for bottom structural relief, for mucus boundary layer production or for the provision of new hard substrata.

Functional groups are assemblages of species carrying out a similar task, but there is another approach possible to study the role of species. This is to consider: are there species of keystone importance in the reef communities, and if so, can they be identified? Keystone species have been defined as species which determine "the integrity and unaltered persistence of the community through time" (Paine, 1969) or species whose removal leads to still further loss of species from the community (Roughgarden, 1986). The original notion of a keystone species was that of a predator but there are different kinds of keystone species (e.g., Bond, 1994). A possibly good example for a Caribbean reef would be the large structural, rapidly calcifying *Acropora palmata* (Lamarck, 1816). These considerations lead us to examine differences between coral species and to see if they are easily grouped into functional groups or identified as keystone species.

Are corals easily grouped in functional groups with very different characteristics linked to different community processes? Are there differences, linked to community processes, so extreme between corals that some species could be a non-contributing "accidental", and another species of key importance and the sine qua non for a reef community? The answers to such questions are found in the ecological strategies of coral species. As examples of such characteristics we take colony partial mortality, abundance of juveniles, population size/frequency distribution and coral linear growth. These characteristics are relevant because they always translate into ecosystem functions such as substratum complexity and maintenance of biomass or calcification.

Partial mortality on colony surfaces is a function of regeneration capacity of colony surface damage (lesions). This is a characteristic differing sharply among corals. Figure 3 shows the regeneration rates of superficial lesions in the colony surface of eight species of Caribbean corals (Meesters & Bak, 1993, ms). Lesions decrease in size exponentially. The slope of the regression line of log size vs time shows significant variation among species. This variation in species-specific regeneration capacity is reflected in the occurrence of partial mortality on the reef (Meesters et al., 1996; in press). Because regeneration rates differ, so do location and extend of partial mortality on the reef.

Diversification in ecological strategy is also demonstrated by the enormous difference in abundance of juveniles of coral species over a reef in Curaçao (table 1).

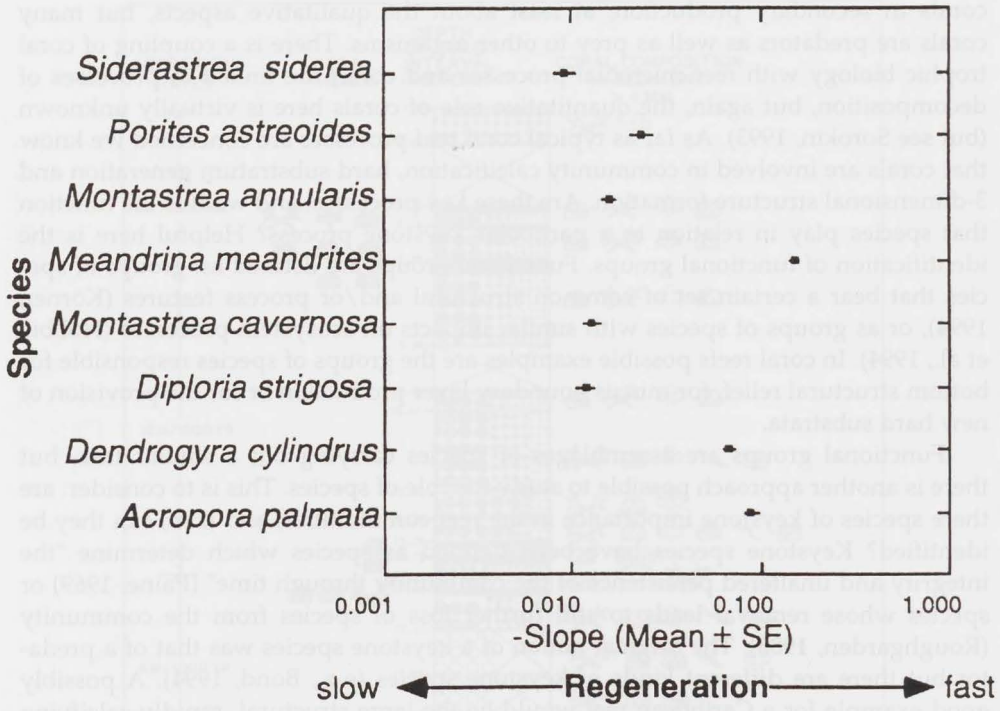

Fig. 3. Regeneration rates of lesions (initial size 160-180 mm^2) in colony surfaces of 8 Caribbean coral species. Lesions decrease in size exponentially during the regeneration process (Lesion size = $S_0 \times 10^{(slope \times days)}$. The slope of the regression line shows significant variation between species.

Such abundances are related to life history characteristics such as reproductive effort, tissue maintenance, skeletal growth and mortality. These variables are also expressed in another demographic characteristic: population size/frequency distribution. Figure 4 shows the difference between the species *Diploria strigosa* (Dana, 1846) and *Montastrea faveolata* (Ellis & Solander, 1786). The populations differ in mean size, in abundance of small colonies as well as in total abundance and colonies of *M. faveolata* get very much larger, (nota bene: abscissa is logarithmic scale). Linear growth of the carbonate skeleton (extension rate) is another related ecological characteristic which differs greatly between species (table 2).

Some ecological concepts on the reef

The next question is: in view of such differences and others, can we discern groups of species with similar impacts on ecosystem processes? Coral calcification is a well studied typical reef ecosystem process and should supply a good example. We can rank species calcification on an arbitrary scale from 1 to 50 for a list of 18 scleractinians (for species see table 1), taking into account species specific calcification rates and coral cover (impact is calcification × abundance; data Curaçao, Bak, unpublished). Figure 5 shows that there are roughly three functional groups, with high

impact, little impact and no impact, respectively, on reef community calcification. The high impact group includes important species in calcification: *Madracis mirabilis* (Duchassaing & Michelotti, 1860) (no 3), *Acropora palmata* (no 5), and *Montastrea annularis* (no 12). The low impact functional group includes less abundant corals with low to medium calcification rates such as the mussid genera *Scolymia* (no 16) and *Mycetophyllia* (no 17).

When functional groups perform community tasks that are indispensable for the continued persistence of the community they will contain the potential keystone species of the community. In our example: a certain level of community calcification is a basic requirement for a coral reef system. When the composition of the community is changed, through temporal or spatial variation, only one species may perform this crucial task. In polluted areas in Curaçao *Acropora palmata* is no longer present and the prominence of *Montastrea annularis* is much reduced. *Madracis mirabilis* is probably the keystone species in the key process of calcification at such localities. Keystone species emerge depending on the composition of the community. In marginal environments, when other members of functional groups drop out, keystone species must emerge. It should be interesting to explore these concepts southward along the reefs of West Australia or into the Persian Gulf.

Table 1. Relative abundance of juvenile corals (maximal diameter < 40 mm) as percentage of total number of juvenile corals (n = 360) over the reef in Curaçao (depth 3 to 37 m, *Agaricia* excluded. (Data after Bak & Engel, 1979).

1	*Stephanocoenia michelinii*	8.6
2	*Madracis decactis*	3.6
3	*Madracis mirabilis*	0.6
4	*Madracis pharensis*	2.5
5	*Acropora palmata*	0.6
6	*Leptoseris cucullata*	23.9
7	*Siderastrea siderea*	1.4
8	*Porites astreoides*	20.8
9	*Porites porites*	1.4
10	*Diploria strigosa*	0.8
11	*Colpophyllia natans*	1.7
12	*Montastrea annularis* spp. complex	0.8
13	*Montastrea cavernosa*	1.1
14	*Meandrina meandrites*	16.1
15	*Dichocoenia stellaris*	0.3
16	*Mussa* spp./*Scolymia* spp.	0.6
17	*Mycetophyllia ferox*	0.3
18	*Eusmilia fastigiata*	10.8
19	*Millepora* spp.	3.9
20	unidentified	0.3

We can try to complete our understanding of the role of coral species by adding other properties, in addition to calcification, to species character lists. In a thought experiment we can supply the total list of Curaçao stony corals (see Bak, 1975) with a ranked importance for four properties that are related to key reef processes:

1. Calcification.

2. Hard substratum generation (this is in terms of newly created bare surface area but necessarily speculative because there is uncertainty about the mortality rate of some species).

3. Coral mucus production (important in linking reef benthos to water column microbial parameters).

4. Production of recruiting juvenile colonies.

Table 2. Linear growth (skeletal extension in mm $year^{-1}$) in some Caribbean scleractinian coral species. (Sources: Bak, 1976; Bak & Criens, 1981; Bak, 1983; Gladfelter et al., 1978; Van Veghel & Bosscher, 1995).

Madracis mirabilis	17
Acropora palmata	47-99
Acropora cervicornis	71
Acropora prolifera	59-81
Agaricia agaricites	24
Porites astreoides	3-4
Montastrea franksi	4-6
Montastrea annularis	11-13
Montastrea faveolata	9-13
Montastrea cavernosa	3

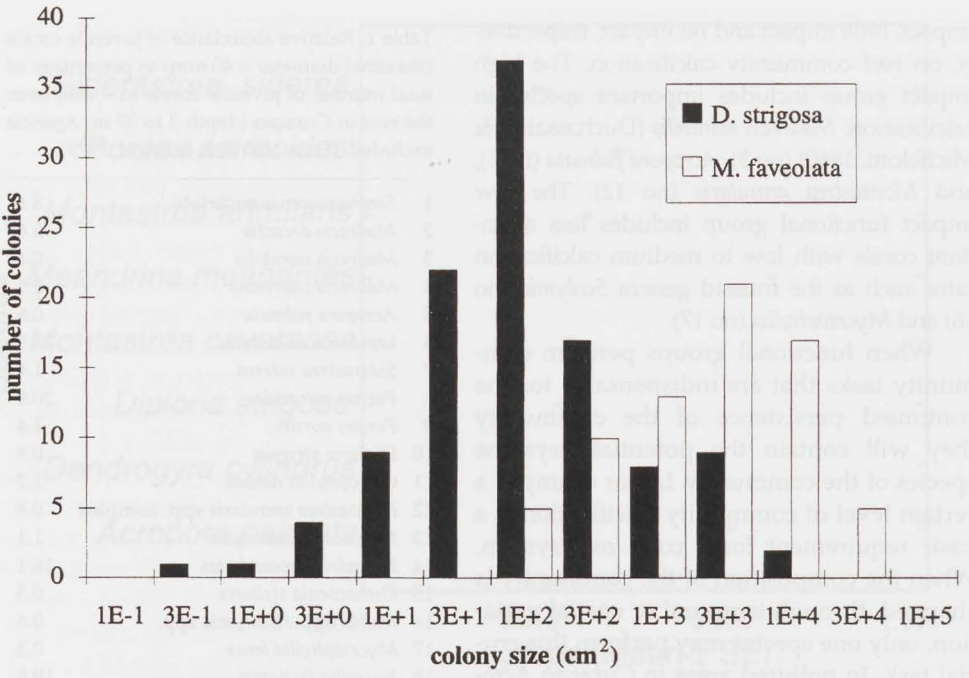

Fig. 4. Size/frequency distributions of *Diploria strigosa* and *Montastrea faveolata* populations at the leeward reef terrace (depth 7 m) in Curaçao. Note logarithmic abscissa.

The point is: can we use such characteristics to identify broader functional groups that cover not one, but a number of important, potentially key processes, in a coral community? On the other hand: are there species so outstanding that they account for most of a particular coral community process (keystone species)?

Multidimensional scaling (MDS; Systat, 1992) is an ordination technique used to study the similarity or dissimilarity of objects. The objects, in our case the various coral species, are positioned in space in such a way that the distances between objects are representative of similarity and dissimilarity between species. When the input in MDS consisted of available data on calcification, substrate generation, mucus production and density of recruits for all 53 scleractinian species listed for Curaçao (Bak, 1975), the result was overlapping groups of points in a large cluster. This is because there are large gaps in the input data set and consequently many species are not separated. When such species are excluded and we use only species for which at least some data are available, the technique is useful (fig. 6). There are clear distinctions as well as groupings. There is a large distance between *Acropora palmata*, a species characterised by rapid calcification and small numbers of recruits, and *Agaricia humilis*, a species negligible in reef calcification but common as small recruiting corals. A small cluster (fig. 6) suggests a number of species of high similarity, a possible functional group. Not all similarity here does result from true similarity in ecological characteristics. The cluster includes species such as *Meandrina meandrites* (Linnaeus, 1758), *Colpophyllia natans* (Houttuyn, 1772) and *Eusmilia fastigiata* (Pallas, 1766), as well as *Stephanocoenia michelinii* (Milne Edwards & Haime, 1848), *Dichocoe-*

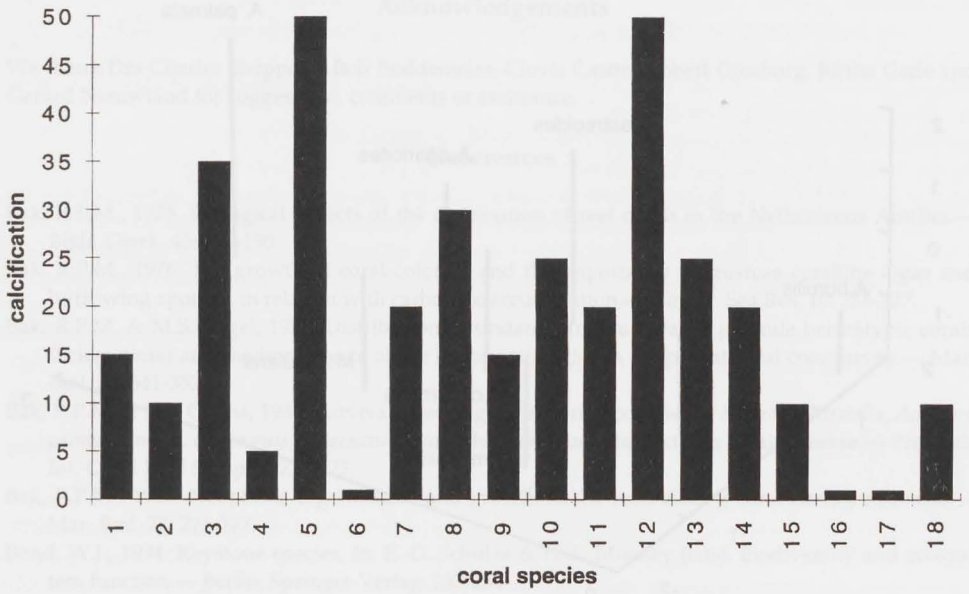

Fig. 5. Comparative contributions (relative units) to community calcification of 18 Caribbean coral species (listed in table 1).

nia stellaris (Milne Edwards & Haime, 1848) and *Agaricia grahamae* Wells, 1973. These are very different corals in some aspects, overlapping in this cluster because we do not know the input factors for the analysis with sufficient precision.

The MDS exercise with these few species primarily shows that the more we know of species the more distinguished they appear. The identification of functional groups is limited by the data set used, but a larger data set could potentially identify species groupings. Also, a study of a comprehensive set of coral community data analysed along an environmental gradient could produce very interesting results. The role of species in changing habitats could possibly be predicted moving the MDS model from central to marginal reef areas. Such analyses could parallel descriptive studies of coral communities along coral reef gradients and help to understand the emergence of keystone species in the community.

We would like to answer the question: How many and which coral species are needed to keep a Caribbean reef system functioning? But there are few published experiments to test the effect of biodiversity on clearly defined functions. Most of the information is circumstantial and concerns only one process (e.g. the Alacran reef accretion study). It appears that a specific process can be performed by only a few species. But there are many complications. What sort of Caribbean reef are we talking about? We can assume that essential coral reef community processes are performed in all Caribbean reefs. However, the role of specific species may not be the same in different reefs. Species composition along the Florida reef tract is different from that in the southern Caribbean. *Diploria* species become very prominent at the northern margin of Florida reefs. These corals are also abundant in stressed environ-

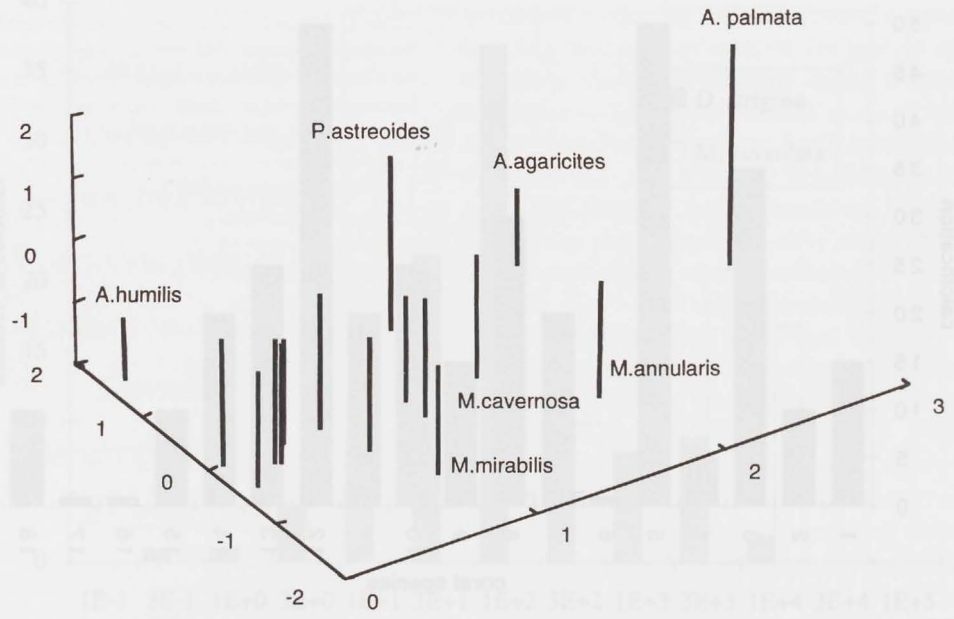

Fig. 6. Multidimensional scaling of selected Caribbean corals. 3-D plot of the analysis using selected coral species. Axes represent dissimilarity between species and are not defined by model.

ments in Curaçao. Does this point to a keystone role of *Diploria* that is usually hidden in high diversity reefs? We must conclude that there is not much data on possible changes in the function of species in the reef system. This precludes any conclusion on the importance of species diversity per se.

This uncertainty concerning the function of species is not limited to spatial and geographical variation. In change through time, on a scale relevant from the geological point of view, again the role of individual species asks for our attention. Do species change in their function through time? Are changing roles the result of changing associations? Are insignificant role players of to-day cast for a leading role to-morrow? A possible example of such a species or species group could be *Mussismilia*, important as reef builder in Brazil to-day but seemingly present in a more insignificant position through the geological record in Europe and other ancient reefal environments. Such possible changes in the significance of species are of interest in view of coral reef global change scenarios.

There are three obvious areas to explore: community changes in stressed environments, marginal reef habitats, and the palaeobiological perspective. These studies will have to shed light on the role of individual species and the possible emergence, through space or time, of keystone species. To understand partioning of function in reef processes between the diverse coral species we need more data on coral population biology and ecological strategies. Such basic biological data could be used, e.g. in multivariate analyses, to identify coral functional groups.

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Control of discharge of acontial nematocysts in *Aiptasia diaphana* (Rapp, 1829)

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Barra, P.F.A., G. Musci & A. Salleo. Control of discharge of acontial nematocysts in *Aiptasia diaphana* (Rapp, 1829).
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Key words: Nematocytes; nematocysts; discharge; acontia; isolation; NO; *Aiptasia diaphana*.

Abstract: nematocysts contained in nematocytes isolated from acontia of *Aiptasia diaphana* (Rapp, 1829) through a nonenzymatic method based on treatment with SCN^- in the presence of low Ca^{2+}, are not discharged by depolarization induced by either by electric stimuli or high $[K^+]_o$. This is true whether the nematocytes are isolated enzymatically from tentacles or non-enzymatically from acontia. On the other hand, depolarization is effective in inducing nematocyst discharge in nematocytes partially protruding from the acontial tissue. On the basis of this result, the unresponsiveness of completely isolated nematocytes is ascribed to the lack of signalling through a diffusible transmitter rather than to impairment of transduction mechanisms due to isolation method, as shown by their responsiveness to exogenous nitric oxide. Since at present NO is the only effective stimulus for inducing nematocyst discharge in isolated nematocytes, it is hypothesized that endogenous NO could play the role of transmitter in the control of discharge in in situ nematocytes.

Introduction

The control mechanisms of nematocyst discharge are still largely unknown. Recent results obtained in anthozoan tentacles suggest that nematocytes are effectors of a multicellular system including nematocytes and supporting cells, termed CSCC (Cnidocyte-Supporting cells Complex) (Watson & Hessinger, 1989: 1589). In particular, the chemoreceptive sites for *N*-acetylated sugars and various amino compounds are located in supporting cells while the mechanoreceptive function is performed by the ciliary structure located in the nematocyte. It has been proposed (Watson & Hessinger, 1989: 1589) that supporting cells, once activated by chemical stimuli, induce an elongation of the cnidocil, thereby modulating the response frequency of the mechanoreceptor to lower frequencies, matching those of the prey. Mire-Thibodeaux & Watson (1994: 282) observed that sensitizers induce elongation of the kinocilia of sensory cells rather than those of nematocytes. In any case, such a model requires a cell-to-cell signalling, whose nature has not been identified so far, between supporting cells and/or sensory cells and nematocytes. This aspect is more complicated in acontia, since acontial nematocytes (Salleo et al., 1994: 148) do not seem to be activated by stimuli that are adequate for tentacular ones. In acontia of *Calliactis parasitica*, (Couch, 1842), nematocyst discharge induced by either isotonic SCN^- (Santoro & Salleo, 1991a: 701), 80 mM KCl (Salleo et al., 1993: 565), or hyposmotic shock (Salleo et al., 1994: 148) spreads sequentially along the filament, which suggests that an

unknown signal is transmitted through the entire tissue. Discharge has been shown to be calcium-dependent in acontia (Santoro & Salleo, 1991a: 701; 1991b: 173, Salleo et al., 1993: 565), and, subsequently, in tentacles of Anthozoa (Watson & Hessinger, 1994: 473) and of *Hydra vulgaris* Pallas, 1766 (Gitter et al., 1994: 115). Since discharge is prevented by Ca^{2+} channel blockers, Ca^{2+} dependence is based on Ca^{2+} influx, whose occurrence has been putatively localized in the nematocyte membrane in *Hydra* (Gitter et al., 1994: 115). Mire-Thibodeaux & Watson (1993: 335) observed that *N*-acetylated sugars induce an increase in number of rare epidermal cells, possibly sensory cells, having an unusually high Ca^{2+} concentration. The main difficulty in identifying the functional characteristics of nematocytes results mainly from the unresponsiveness of isolated nematocytes to either chemical stimuli or to depolarization. In viable nematocytes, enzymatically isolated (Anderson & McKay, 1987: 215; McKay & Anderson, 1988a: 273; 1988b: 47) from various species, nematocyst discharge could not be induced by either membrane depolarization or various chemical stimuli. It was suggested (McKay & Anderson, 1988a: 273) that either voltage insensitive channels, lack of adequate mechanical stimulus, or lack of a regulatory function of adjacent cells could prevent discharge in isolated nematocytes. Hidaka (1993: 45) suggested the possibility that transduction mechanisms could have been impaired by isolation. The unresponsiveness of isolated nematocytes discouraged further attempts to investigate the functional characteristics of nematocytes in such a preparation. Since we developed a non-enzymatic method for isolating nematocytes from acontia we compared the activation of isolated nematocytes, devoid of any influence of adjacent cells, to those of in situ ones. Our results suggest that i) the isolation method does not impair the discharging capacity of nematocysts in isolated nematocytes, and ii) the discharging effectiveness of depolarization on in situ nematocytes is exerted on other cells, such as supporting and/or sensory cells, that in turn activate the nematocyte and its nematocyst.

Materials and methods

The experiments were performed on specimens of *Aiptasia diaphana* (Rapp, 1829) collected in Lake Faro (Messina, Italy), maintained in closed circulation aquaria at 19-21°C and fed weekly with shrimp meat. Acontia were extruded by gentle mechanical stimulation of the column. The excised acontia, placed in a depression slide, were rinsed with normal artificial sea water (ASW) having the following composition (in mM): NaCl 520, KCl 9.7, $MgCl_2$ 24, $MgSO_4$ 28, $CaCl_2$ 10, Imidazole 5, pH 7.6. To lower the $[Ca^{2+}]_o$ in the tissue, such a solution was replaced by a similar one having a Ca^{2+} concentration of 0.01 mM. Then the isolating solution (isotonic 605 mM NaSCN solution containing 0.01 mM $CaCl_2$) was applied. Within 5 min. nematocytes containing microbasic mastigophores protruded from the acontial surface and were released into the medium. The SCN^- solution was then withdrawn and replaced by Ca^{2+} free ASW, which was gradually replaced by normal ASW. Since tissue contraction occurred when $[Ca^{2+}]_o$ increased, additional nematocytes were released. As also found by Anderson & McKay (1987: 215) the isolated cells, unlike isolated undischarged nematocysts, had a clearly visible centrally placed bulge, containing most cytoplasm, nucleus and organelles. Cell details were observed with a differential

interference contrast microscope (Zeiss, Axioplan). Isolated nematocytes were also observed with SEM. The cells were isolated on small coverslips, fixed with 2.5% glutaraldehyde, dehydrated in an alcohol series before critical point drying, and the gold-coated specimens were observed with a field emission scanning electron microscope (Hitachi, S-800) (fig. 1). To obtain acontia with protruding nematocytes the detachment of nematocytes from the SCN^--treated acontia was blocked by the following procedure. The excised acontia were transferred to a glass vessel covered with Sylgard and fixed at both ends with *Opuntia* spines. As soon as the protrusion of nematocytes started, the SCN^- solution was replaced by Ca^{2+} free ASW. ASW containing 10 mM l^{-1} Ca^{2+} was then gradually substituted for Ca^{2+} free ASW. Although a number of nematocytes detached from the tissue during this procedure, the acontium showed a multitude of large microbasic mastigophore containing nematocytes protruding various lengths from the surface. Such a feature was observed also by SEM (fig. 2).

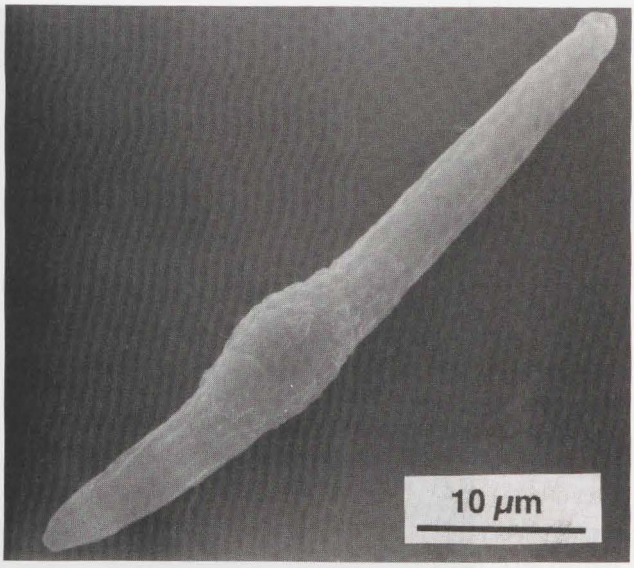

Fig. 1. Isolated nematocyte viewed by scanning electron microscope. Note the bulge containing cytoplasm and nucleus.

Two platinum wire electrodes (diameter = 70 µm) insulated except at the tip and connected to a stimulator (Syntronic model PS 1020) were placed on opposite sides of the acontium. The stimulus was a single square wave 1 ms in duration and 6-8 V in amplitude, so that, the electrodes being 280-300 µm apart, the acontial tissue was submitted to an electric field gradient ranging from 20-28.5 mV/µm. The same stimuli were applied to isolated nematocytes, untreated acontia and acontia with protruding nematocytes. In most tests the electric field gradient was either 23.3 or 25 mV/µm. Repeated stimuli of increasing intensity were not applied because subthreshold stimuli, although ineffective in inducing nematocyst discharge, cause contraction of the acontial tissue that, in turn, pulls outward the protruding nematocytes.

Most observations were performed with a video microscope (Hirox, model KH-2200-MD2) connected to a video recorder and a monitor. The recorded events were transferred to a computer and processed by a suitable image analyzer to measure on print-outs the protruding length of nematocytes preceding the electric stimulus. By examining accurately the entire sequence of images it was possible in most cases to identify the cells that had discharged their nematocysts and to measure their protrusion length, which was compared to cells with undischarged nematocysts. Student's t test was applied for statistical significance. Such a procedure was performed on 101 cells observed in 11 tests.

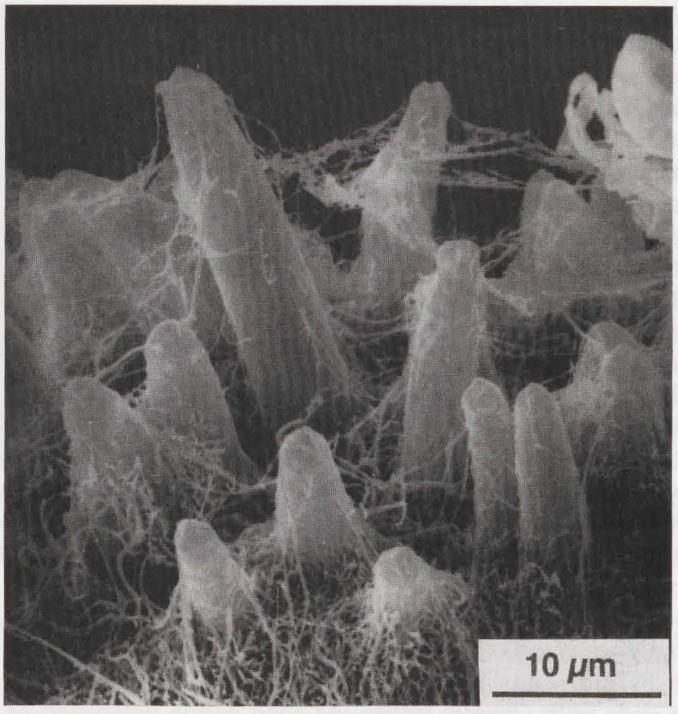

Fig. 2. SEM view of nematocytes protruding from the acontial surface following treatment with SCN^-. The SCN^- solution was removed by rinsing with Ca^{2+} free ASW before the release of nematocytes.

The effect of high $[K^+]_o$ was tested by applying through the 30 μm tip of a glass cannula 605 mM KCl solution, containing also 10 mM $CaCl_2$, to both isolated nematocytes and protruding ones.

NO-containing ASW was prepared by equilibrating ASW with chemically generated NO gas; 0.1 M of ascorbic acid, dissolved in 0.1 M HCl, and 0.1 M of sodium nitrite were reacted in a 1 l vacuum flask connected to a 0.5 l gas-tight flask containing 10 ml of ASW. Mixing of the reactants was performed only after complete removal of oxygen. ASW was left to equilibrate under the resulting atmosphere of NO (ca. 1.5 bar) for two hours with constant stirring. The final solution had a NO concentration of about 0.4 mM as assessed, after exposure to air, by colorimetric determination of nitrites with the Griess reagent (Tracey, 1992: 125).

Treatment of isolated cells with NO was performed as follows within 3 hours of isolation. ASW containing NO was collected in a gas-tight syringe that was connected through a glass cannula having a tip inner diameter of about 20 μm. The tip of the cannula, operated through a micromanipulator, was placed close to a group of cells. Less than 1 μl of test solution was ejected under microscopic observation in approximately 300 μl of suspension of isolated cells in ASW.

To exclude any pH-induced artifact, due to a lowering of pH in the medium when the NO containing ASW was perfused in the cell suspension, the effect of acidic pH on isolated nematocytes was tested. In cell suspensions placed on a glass slide

under a coverslip supported by two strips of adhesive tape, the suspending medium (ASW) was absorbed at one side of the coverslip and completely substituted with ASW, supplied at the opposite side, whose pH had been previously lowered to either 2, 3, 4, or 5 with nitric acid.

Results

Discharge of in situ nematocysts of untreated acontia occurred promptly as soon as the electric stimulus was applied. The minimum effective field gradient for inducing the discharge in untreated acontia was 23.3 mV/μm. The discharge was always observed at the cathode in agreement with previous investigations (Gitter et al., 1994: 115; Holstein & Tardent, 1984: 830; Salleo et al., 1993: 565). Also the high K^+ solution applied to intact acontia induced a prompt discharge in the acontial segment facing the cannula. Nematocysts of isolated nematocytes never discharged following electric stimulation even at higher intensity and duration, as well as under high K^+ treatment.

Nematocysts in cells protruding from acontia could be discharged by electric stimulus. Fig. 3a shows three cells protruding in the suspending medium. The nematocysts of two cells discharged simultaneously under an electric field of 23.3 mV/μm (fig. 3b). Fourty cells, whose nematocysts discharged, out of the 101 examined, had an average protruding length of 22.7 ± 2.1 mm before the electric stimulus, while 61 with an average protruding length of 35.8 ± 1.9 mm did not discharge. The difference between the mean protrusion length of discharged nematocysts and undischarged ones was highly significant at $P < 0.0001$. The measured 101 nematocysts were grouped in the 5 classes of protrusion length shown in fig. 4. The percentage of discharge in each class decreased as protrusion length increased.

In all tests the local application of 605 mM KCl in the presence on 10 mM $CaCl_2$ induced discharge of protruding nematocysts. A detailed analysis of the protrusion length of K^+-discharged nematocysts was not performed because the test solution flow and the acontial contraction do not allow a precise measurement of the protru-

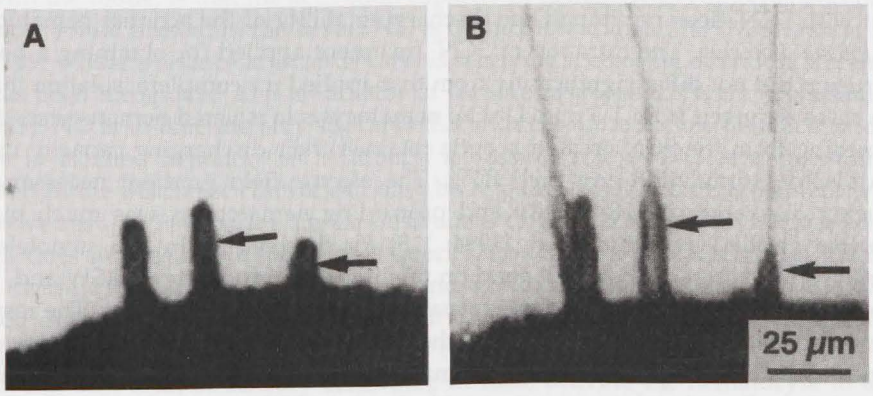

Fig. 3. A. Interference contrast microscope view of three nematocytes protruding from the acontial surface. Nematocysts of two cells (arrows) discharged under the electric stimulus (B); the tubule on the left was everted from a capsule not visible in A.

sion length immediately before the discharge.

Most nematocysts in isolated nematocytes in the proximity of the cannula discharged as soon as the ASW containing NO was perfused (fig. 5). The discharge generally occurred one sec. after the perfusion started and stopped within 10 sec. Cell membrane remnants were generally observed on discharged capsules, since the membrane is perforated by the everting tubule. In some cases a partial eversion of the tubule occurred that was completed after a few seconds. The discharging effect of NO was also seen by repeating the perfusion with the NO solution in different regions of the same cell suspension. Isolated nematocysts, a minority in cells suspension, never discharged under NO perfusion.

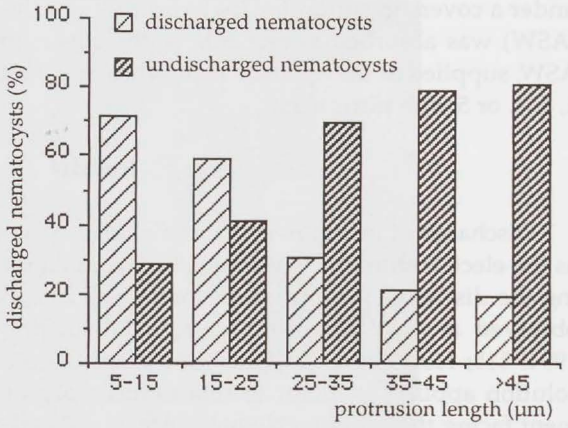

Fig. 4. Percentage of nematocysts discharged by electric stimulus in 5 classes of protruding length measured prior to the electric stimulus.

Acidic medium proved effective in discharging nematocysts in isolated nematocytes only at pH 2. Nevertheless, the medium at pH 3, although not inducing discharge, caused cell damage, so that nematocysts did not discharge when pH 2 was applied.

Discussion

Nematocysts of isolated nematocytes did not discharge at high $[K^+]_o$ that induces discharge of in situ nematocysts in a number of species (McKay, & Anderson, 1988a: 273; Salleo et al., 1993: 565; Gitter et al., 1994: 115). The unresponsiveness of SCN^--isolated nematocytes to depolarization is confirmed. Our results show that the treatment with SCN^- does not impair the discharging ability of the acontial nematocysts of *Aiptasia diaphana*. The duration of SCN^- treatment applied for obtaining a partial protrusion did not differ significantly from that applied for complete isolation that in both cases it ranged from 1-5 min. Unlike nematocysts in isolated nematocytes, those protruding from the SCN^- treated acontia retained their discharging capacity under both electric stimulation and high $[K^+]_o$. The electric field gradient necessary for inducing discharge of both in situ and protruding nematocysts was much higher than that applied by Gitter et al. (1994: 115) for discharging in situ stenoteles of *Hydra*. Such a difference could depend on the higher conductivity of ASW and, consequently, of the interstitial fluid of *Aiptasia*, with respect to fresh water. The responsiveness to electric stimuli depended on the degree of protrusion from the tissue. We conclude that the responsiveness of nematocytes to electric stimuli disappears as most of the cell surface looses contact with the tissue. We reason that any degree of protrusion interrupts the possible intercellular junctions of nematocytes with other cell components of the tissue, including synapses. Therefore, in such a condition, the

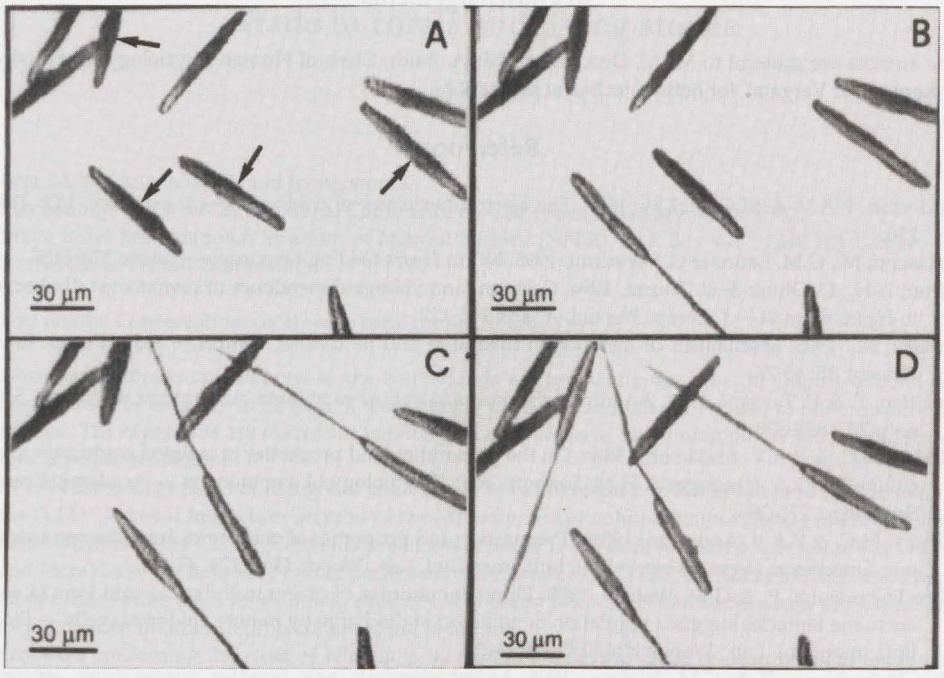

Fig. 5. Interference contrast microscope view of nematocyst discharge induced by treatment with NO. A. Resting nematocytes; arrows indicate the cells undergoing nematocyst discharge following perfusion with NO containing ASW. B. One sec. after NO perfusion. C. Three sec. after NO perfusion. D. Four sec. after NO perfusion.

activatory signals cannot be directly transmitted to nematocytes from other cells through specific and localized structures. A likely explanation of the observed dependence of sensitivity to depolarization on the amount of cell surface still in contact with the tissue, is that the activation capacity of nematocytes could depend on a diffusible solute present in the tissue. NO is a diffusible transmitter (Moncada et al., 1991: 109) whose presence in invertebrates is receiving increasing attention. Recently NO has been recognized as a modulator of feeding response in *Hydra* (Colasanti et al., 1995). NO is to date the only stimulus that induces reproducible nematocyst discharge in isolated nematocytes. Although a possible role of NO as a transmitter involved in the discharge control can only be hypothesized, it is in agreement with the above results. Finally, the unresponsiveness of isolated nematocytes to electric stimuli does not seem to depend on a specific impairment of transduction mechanisms leading to nematocyst discharge, and is expected to prevent also the NO-induced discharge. It is therefore likely that the effectiveness of electric stimuli and high $[K^+]_o$ on in situ nematocytes depends on depolarization of other cells, such as supporting cells and/or sensory cells, that, in turn, activate the nematocyte to discharge its nematocyst.

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Trade in corals and living stones

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Key words: Conservation; coral reefs; coral trade; CITES.

Abstract: Harbours and airports in the Netherlands are frequently used for the import of coral reef organisms. The majority of cargoes of these mostly protected animals is in transit to other countries in Europe. The organisms are traded for various purposes: aquaria, swimming pools, decoration, souvenirs, precious stones, etc.
In 1994 three large loads of living and dead corals were intercepted in Rotterdam and Amsterdam by the A.I.D. (General Inspection Service) of the Ministry of Agriculture, Nature Management and Fisheries. Officers of the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) have the authority to confiscate material protected by CITES, that lacks a CITES document. About these three cases an ex officio expertise was asked from the National Museum of Natural History in Leiden in order to proceed according to the law.
Problems concerning the issue of what is to be regarded as living, dead or (unprotected) fossil coral are discussed, and general questions are raised in order to support the creation of an international approach and to formulate clear criteria.

Introduction

During the last five years an expanding trade in marine organisms is perceptible. This is not only the result of increasing interest in unknown marine life, but also of the speedy and frequent airline facilities. Fragile reef corals and reef fishes can be transported easily within a day. As long as the trade is restricted to cultivated organisms it is acceptable, but it also concerns wild plant and animal species that are, or may become rare, and as a result of this exploitation, may end up as "endangered species".

Exploitation of and international trade in wild animals and plants may not only reduce populations to the point of no return, but it may also destroy whole ecosystems.

In the first half of this century this concerned mainly the large land animals like elephant, tiger, crocodile, etc., for their skins or teeth. After the second world war, with growing economy and transport possibilities, many other wildlife "objects" became targets of man's interest.

Inevitably, this growing business in wild living plants or animals had to be regulated.

The problems were discussed internationally at an assembly of the IUCN (International Union for Conservation of Nature and Natural Resources) in 1960. The IUCN General Assembly decided to create "an international convention on regulations of export, transit and import on rare or threatened wildlife species or their skins and trophies". This CITES (Convention on International Trade in Endangered

Species of Wild Fauna and Flora) was signed by 21 countries in 1973. Now over 130 countries are parties to CITES.

CITES establishes an international legal framework for the prevention of trade in endangered species and for an effective regulation of trade in others (Wijnstekers, 1992). The problems about exploitation and conservation of wildlife remain to be discussed internationally, because the concerns in these trades of producer countries and consumer countries are very different. In this respect it is worth noting the definition of conservation given in the Worlds Conservation Strategy:

Conservation is the management of human use of the biosphere so that it may yield the greatest sustainable benefit to present generations while maintaining its potential to meet the needs and aspirations of future generations.

This definition certainly is relevant in the trade of coral reef organisms. Most reef organisms are not directly endangered, but damaging of the coral reef framework by for instance harvesting coral blocks, deteriorates the whole rich ecosystem.

Trade in corals: living, dead and fossil

Regulation

The trade in hard corals (mainly Scleractinia) became regulated by CITES in 1985.

At first only some genera were included in the list of protected animals, but because it is hard for law enforcement officers to identify these animals, they had problems with the sorting of protected and non-protected animals in shipments. Fortunately the proposal of Israel to put all Scleractinia and other hard corals belonging to the Antipatharia, Milleporidae, Stylasteridae, Coenothecalia and Tubiporidae on the list of CITES appendix II, was accepted in 1989. It concerns live and dead colonies, not fossil material.

CITES Appendix II includes:

a. All species which although not necessarily now threatened with extinction may become so, unless trade in specimens of such species is subject to strict regulations in order to avoid utilization incompatible with their survival.

b. Other species which must be subject to regulation in order that trade in specimens of certain species referred to in subparagraph a of this paragraph may be brought under effective control.

There is a possibility for a quotum system for Appendix II species in the framework of CITES, which means that organisms mentioned in Appendix II can be exported from the producer country or imported into the consumer country with a CITES document. The regulation of these quota is controlled by the national legislative authorities in each country. This is administrated by the W.C.M.C. (World Conservation Monitoring Center) in Cambridge, in order to gather statistics of the amount of trade in relation to the status of each species. The quota, the administration and the regulation of the CITES certificates are of course very difficult to control. It is not the subject of the present paper to discuss this, but it is certainly a field of much concern.

In 1989 al Scleractinian corals (the most important objects for coral trade) were placed on the CITES list and regarded as protected animals. Ever since, shipments of

these corals can only pass international borders with the required CITES document. In spite of this many coral shipments without a CITES document however still passed Dutch customs.

Examples

Between 1985 and 1989 several shipments of corals were intercepted by the A.I.D. and had to be inspected by NNM specialists in order to identify the species. Those species not on the CITES list could pass the customs, and other species mentioned in the list had to be confiscated. It was an enormous task, because the trade in corals and other marine organisms like molluscs increased. The demand in consumer countries grew steadily and the supply (mainly from developing countries) became better organised, so that the trade became more and more profitable. Several trading firms such as Neptunus (Texel) and Marine Life (Spijk) flourished.

However, after 1989 the coral shipments without an export CITES document could be confiscated completely. Some examples are:

- In 1992 a shipment of 80 large cases of corals from the Philippines containing thousands of dead coral colonies without an export certificate was confiscated and deposited in the NNM after court judgement, to be used for non-commercial (e.g. educational) purposes. Expositions are, or will be set up for the public with the aim to explain the basics of the necessity to protect wildlife.

- In 1993 a large shipment of living and dead coral arrived from Indonesia. It concerned about 500 colonies and fragments of colonies. Part of the animals were dying; about 70% were Scleractinia or coral colonies with encrusting algae or other marine invertebrates. The shipment was directly confiscated and transported to the Aquarium of the Rotterdam Zoo (Blijdorp). After closer inspection part of the material was returned to the trader, because it concerned "stones" with unprotected marine organisms like Alcyonaria, Gorgonaria, Pennatularia, etc. The NNM specialist did not agree, because these "living stones" were dead coral colonies. Thus confusion remained for both the A.I.D. and the dealers. Matters became even more complicated with some cases in 1994.

- In July 1994 a container with 13296 kg coral blocks from Miami arrived by ship in Rotterdam. An accompanying letter from the trader said:

"What I shipped is not coral, but rock that is made up of calcium carbonate (limestone). It is the same rock that is used to build highways, fill for yards and cement. I have a yard full of it that is used to make my driveway. The particular rock sent to Mr. de Jong was taken from the water so that it would have more colour and the appearance would be natural for use in Aquariums. The purple on the rock is calcareous algae. I had personally gone over each rock and can assure you there is no Coral".

The judgement of the specialist was that we were dealing here with dead Scleractinian coral colonies mainly belonging to the genera *Diploria, Porites, Montastrea, Agaricia* and *Siderastrea.*

Encrusting organisms were calcareous algae, sponges, bryozoans, molluscs, etc. Extraction of such coral blocks means further destruction of the already badly affected coral reef ecosystems of the Florida Keys. The shipment was meant for a seawater

swimming pool in the Netherlands. After some hesitation the shipment was released following great pressure of the traders.

- In August 1994 a large shipment of living and dead coral from Singapore arrived at Schiphol airport. It was intercepted by the A.I.D. and transported to the Aquarium of "Artis", Amsterdam Zoo. A judgement was asked of the NNM and the following statement was given:

"The coral material inspected contains approximately 700 coral colonies, of which 20% are living Scleractinia belonging to the genera *Trachyphyllia, Goniopora, Plerogyra, Physogyra, Euphyllia, Tubastrea, Porites, Goniastrea.* The other 80% concerns dead coral colonies on which other marine organisms settled, mainly soft corals, but also sponges, algae and Actiniaria".

These encrusting organisms are not protected and the trader asked the return of these "living stones". This case reached many newspapers and magazines, so both parties decided to make it an official court case. In December 1994 the Court of Justice of Haarlem decided, after having heard the testimony of the coral expert about the meaning of the term "living stones", to confiscate all coral material and to give it to Artis Aquarium.

- In September 1994 a crate with large (20 to 60 cm) coral blocks from Mexico was intercepted by the A.I.D. After inspection by the specialist, it was concluded that most "coral" blocks were constructed by various marine organisms, such as calcareous algae, molluscs, worms and corals. All blocks were encrusted by still living organisms like *Halimeda*, soft corals, sponges, Bryozoa, boring molluscs and scleractinian corals belonging to the genera *Cladocora, Phyllangia, Isophyllia.* These living organisms proved that the coral blocks came from a living coral reef ecosystem and not from a fossil reef as contended.

Because there was no CITES certificate, the material was confiscated and given to the Zoo in Rotterdam.

In conclusion

The coral shipments mentioned, containing either living coral, dead coral or "living stones" (also called "life rock"), are only some examples of the immense worldwide trade in the main builders of our richest marine ecosystem, the coral reef ecosystem.

Because the control concerning coral shipments had been rather chaotic during 1994 the A.I.D. asked instructions about the interpretation of the biological, geological and trade terms of "coral" and "living stones". There were problems about protected and not protected coral material.

Definitions of 1. fossil coral, 2. limestone, and 3. living stone, were formulated by the A.I.D.

1. Fossil coral rock is constructed by coral colonies more than 10.000 years ago

2. Limestone is a calcium carbonate conglomeration constructed by marine organisms.

3. A living stone is a stone or lime stone on which living organisms have settled.

This coral material is not protected, unless live coral is found on any of these coral blocks.

Coral specialists do not agree with the last definition, because harvesting of dead

coral blocks with encrusting marine organisms means deteriorating the health and sustainabiliy of the coral reef ecosystem.

In the court case of December 1994, the judges accepted the declaration of the experts and overruled the definition as used by the A.I.D.

In order to support the enforcement officers, Dutch experts (in cooperation with the International Society for Reef Studies and the IUCN) formulated their criteria for the existing trade in corals.

The following declaration was formulated by M. Borel Best (NNM, Leiden) and G.J. Boekschoten (Institute of Earth Sciences, VU, Amsterdam) in order to be used by the A.I.D. officers and to be discussed on the 8th International Coral Reef Symposium in Panama (June, 1996).

Living and dead coral

About the trade and harvesting of living coral (Scleractinia and other groups) and dead coral (living stones, life rock) the following:

In tropical coastal waters, several types of coral reef may be formed such as fringing reefs, patch reefs, barrier reefs and atolls. These reefs are constructed by marine organisms, plants or animals, but mainly coral animals, that are able to absorb calcium ions from the sea water and deposit this as a skeleton of calcium carbonate. The mineralogical composition is aragonitic.

By way of budding coral colonies are formed. Through continuous growth, a reef is formed. All sorts of other marine organisms settle in, on and between coral colonies. Thus the reef is consolidated and a reef wall is formed. Reefs can be hundreds and thousands of years old. By simultaneous physical and chemical processes, parts of the reef wall break off and fall on the sandy bottom.

The currents and wave action erode these blocks further. Larvae of all sorts of marine organisms, also larvae of the corals themselves, can settle on dead coral blocks, So these dead coral blocks are then the "living stones" of the reef and of extreme importance for the survival of the reef organisms and whole ecosystem. By continuous erosion coral sand is formed, which is important for the further consolidation of the coral reef wall. Because one of the functions of the reef is protection of the coast line, harvesting of too much coral sand weakens the coast.

In short, we can state that reefs, as important protectors of coasts and supporters of the most biodiverse marine ecosystem on earth, are constructed by living corals and other marine organisms. The dead eroded parts of the reef function as substrate for the larvae of numerous reef organisms, and coral rubble and sand contribute to the stability of the reef wall. We are dealing with a cyclus of construction and destruction in which all stages of the coral (life, dead as block, rubble or sand) are important to keep this ecosystem healthy and to maintain its biodiversity. All these coral stages should therefore be protected.

During the millions of years of the earth history these calcium carbonate walls have been formed. Coral reefs are already known from the Ordovicium (450 million years ago). By the tectonic movements of the earth crust, continental drift and sea level changes these coral reefs may also be found on land. These are fossil reefs. The

fossilisation process can take thousand of years, during which the aragonitic calcium carbonate is transformed into calcite; the skeleton is crystallised. Calcite is easily recognised because it is shiny and glitters along the fractures. Fossil coral does not contain organic material, in contrast to dead and living coral.

Fossil corals do not take part in the functioning of the reef ecosystem and therefore do not have to be protected.

The contents of this statement will be discussed on International Coral Reef Symposia. Only with an international approach we may succeed to convince producing and consuming countries involved in coral trade to stop their activities by not issuing CITES documents.

The main producer countries of coral material are the Philippines, Indonesia and some Pacific nations (Wells & Barzdo, 1991). During the last two years shipments with a CITES document from the Philippines decreased, while those from Indonesia increased. Strict regulation of the coral export in the last-named country is certainly necessary in order to protect the Indonesian reefs from fast deterioration (Best et al., 1992; Best, 1994).

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Diversity of hydroidomedusan life cycles: ecological implications and evolutionary patterns

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Key words: Hydroidomedusae; life cycles; ecology; evolution.

Abstract: Hydroidomedusan life cycles are characterized by an unparalleled variety and plasticity and new types have been recently discovered, suggesting that their diversity is not completely explored. Heterochrony, in terms of paedomorphosis through progenesis or neoteny, is widely invoked for the explanation of saltational evolution, but the hydroidomedusae have been ignored in the development of such views. Reduction and suppression of the adult stage (the medusa) by progenesis or neoteny is the trademark of hydroidomedusan life cycles, especially in Antho- and Leptomedusae. The expression of the medusa is linked to the timing of gamete maturation: when gametes become ripe in the polyp, the medusa is suppressed. Such proximate (physiological) explanation is to be coupled to an ultimate (ecological, evolutionary) one. Medusae have different requirements than polyps, being more sensitive to competition for limited resources (due to their top role in food webs), so representing an obstacle to the potential adaptive radiation of the polyp stage. The concentration of studies on a few genera (e.g., *Aequorea, Polyorchis, Hydra, Tubularia, Hydractinia, Podocoryna, Laomedea*) has probably obscured the diversity of the group. Recent research is showing that even inconceivable phenomena, such as the inversion of the ontogenetic sequence, can occur in hydroidomedusae (the adult medusae of *Turritopsis nutricula* McCrady, 1857, transform into hydroids), offering new perspectives in developmental and evolutionary biology.

Hydroids and medusae

The complete cycle of hydroidomedusae usually comprises a benthic modular stage which reproduces asexually and an individual planktonic stage which reproduces sexually. The links between the two are the gametes and the planula. Besides the Actinulida and the Trachy- and Narcomedusae, the other orders, namely the Limno- Lepto- and Anthomedusae, should be considered as meroplanktonic. The sexually-reproducing, planktonic medusa is the adult by definition, whereas in most marine phyla it is the larva to be planktonic. Other modular organisms do not show such ecological, morphological and cytological differences between two long-lived stages.

Edwards (1973) interpreted the hydroid stage as a way to overcome adverse conditions for the medusa stage. The adult, in fact, disappears in the "bad" season. But

also the presence of the hydroid can be discontinuous. Hydroid formation can be delayed by egg encystment or, more often, the colonies are able to regress to hydrorhizae, in which they store the potential for future regrowth. When the conditions become favourable, new colonies originate from the encysted eggs or the resting stolons (Boero & Fresi, 1986; Calder, 1990). These organisms, thus, have a finely tuned interaction with the environmental conditions and have evolved complex life cycles to face many environmental subtleties.

Heterochrony

Most Antho- and Leptomedusae have medusoids either retained by the colony, or liberated when already mature, so functioning as simple gamete carriers. Istock (1967) argued that life cycle simplification by reduction or abolition of one stage is of general occurrence in organisms with complex life cycles. The original pattern (with both polyp and medusa) can be reduced by heterochrony, through a change in the timing of sexual maturation, leading to adult reduction and to sexually mature larvae or juveniles: paedomorphosis. In the hydroidomedusae, paedomorphosis has been considered for a long time as identical with neoteny (see Kühn, 1910), a retardation of somatic development, with sexual maturity occurring in organisms with juvenile characters. Species with large colonies might fit with the definition of neoteny, since they seldom produce free medusae (Cornelius, 1992). Such colonies could be interpreted as the result of delayed sexual maturity of larvae which continue to grow and, eventually, become sexually mature without "metamorphosing" into adults. In recent times, paedomorphosis in the hydroidomedusae has been interpreted as progenesis: precocious sexual maturation of an organism that is still at a morphologically juvenile stage (Kubota, 1983; Boero & Bouillon, 1987; Boero et al., 1993). Through progenesis, species reach sexual maturity as small colonies or individual polyps, such as *Eugymnanthea*, as suggested by Kubota (1983). Neoteny seems more widespread than progenesis, since most species with reduced or lost medusa stage have rather large colonies (Eudendriidae, Sertulariidae, Aglaopheniidae, Campanulariidae, Haleciidae, etc.).

Boero et al. (1993) tried to explain paedomorphosis by a proximate (genetic and cellular) and an ultimate (ecological) process. As suggested by Weismann (1883), and reviewed by Berrill & Liu (1948), gamete differentiation and maturation could regulate medusa expression: when occurring in the polyp, they could reduce or completely suppress medusa expression. If gametes are not mature or absent in the polyp, the medusa is expressed at various degrees of development: medusae liberated with ripe gametes are short-lived (hours-days), those without gonads at liberation can live for a long time (weeks-months) and undergo spectacular modifications. Medusa expression could thus be regulated by changes in the genes controlling the timing of gamete maturation (heterochrony), with no extensive genetic changes affecting the phenotype. This simple mechanism (see Weigel & Nilsson, 1995, for a developmental switch regulating flower initiation) could explain why medusa reduction occurred so many times in hydroidomedusan evolution (fig. 1).

However, the proposal of a mechanism regulating medusa expression does not justify why medusa reduction is selected for. The ultimate cause(s) must be ecological.

Medusae are seasonal top carnivores, competing with each other for the utiliza-

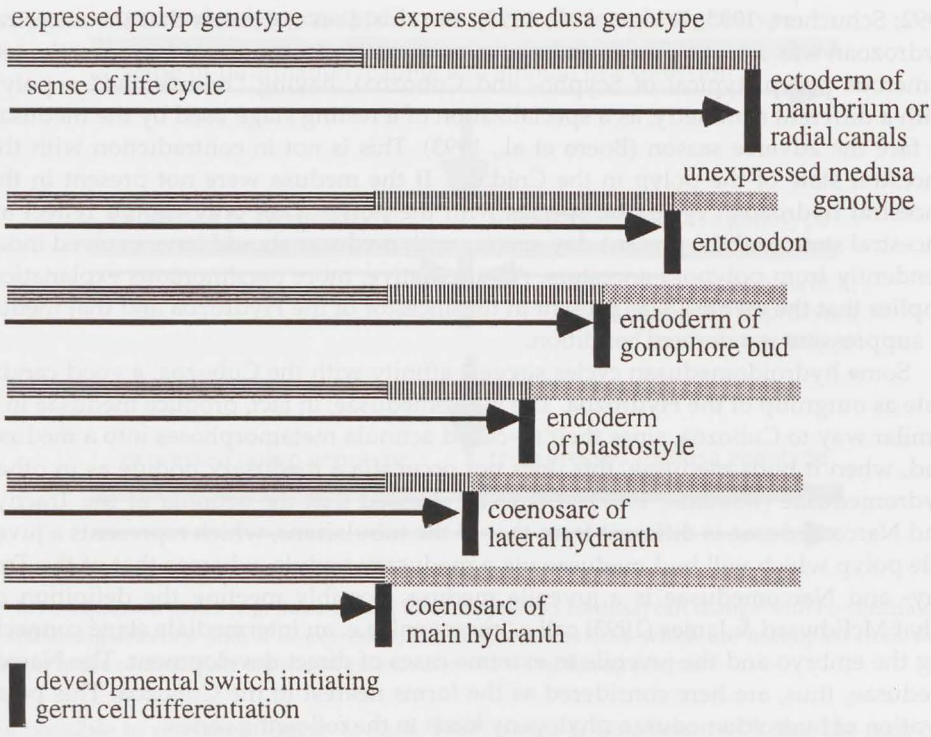

Fig. 1. Hypothetical scenario of regulation of medusa expression according to Weismann's stages of status of sexual generation linked to the site of germ cell differentiation.

tion of resources limited both in quantity and availability, whereas hydroids have multiple ways of extracting resources from the environment (see Boero et al., 1992). The species as a whole, thus, has a strong environmental constraint at the level of the medusa, and it is possible that suppression of this stage allowed a wider radiation. The most speciose genera, in fact, are to be found in the Eudendriidae, Haleciidae, Plumulariidae, Aglaopheniidae, and Sertulariidae, all without free medusae.

A further reason for medusa reduction could be the advantage of the permanent occupation of a given site. Boero & Bouillon (1993), however, showed that half of the endemic Mediterranean species do have medusae, whereas medusae are suppressed in the majority of cosmopolitan species. Dispersal, in this framework, can be considered as a by-product of the presence of the medusa, and not the "agent" selecting for such feature. Many species with particular habitat selection by the hydroid, such as those inhabiting bivalves, sponges, tunicates, bryozoans, and other sessile organisms, have long-lived medusae (Brinckmann-Voss, 1979; Kubota, 1983; Boero & Bouillon, 1989a). On the other hand, the hydroids living exclusively on sea-grass leaves are mostly without medusa (Boero, 1987). These examples suggest that the two life cycle patterns, taken as dispersal "strategies", cannot be related to particular distributions, indicating that they are just epiphenomena and not real causes for a given distribution.

Recent investigations indicate a polyp as the ancestral cnidarian (Bridge et al.,

1992; Schuchert, 1993; Bridge et al., 1995). But this does not mean that the ancestral hydrozoan was a polyp. The founder species of the Hydrozoa could have lost the tetramerous polyp (typical of Scypho- and Cubozoa), having "re-invented" a polyp with a different symmetry, as a specialization of a resting stage used by the medusae to face the adverse season (Boero et al., 1993). This is not in contradiction with the ancestral state of the polyp in the Cnidaria. If the medusa were not present in the ancestral hydrozoan cycle, the species with the polyp stage only should reflect an ancestral state, and the present-day species with medusae should have evolved independently from polypoid ancestors. An alternative, more parsimonious explanation implies that the medusa was present in the ancestor of the Hydrozoa and that medusa suppression is a derived condition.

Some hydroidomedusan cycles suggest affinity with the Cubozoa, a good candidate as outgroup of the Hydrozoa. The Narcomedusae, in fact, produce medusae in a similar way to Cubozoa, since their so-called actinula metamorphoses into a medusa and, when it buds medusae, this does not occur via a medusary nodule as in other hydromedusae (Bouillon, 1987). It is to be stressed that the actinula of the Trachy- and Narcomedusae is different from that of the tubularians, which represents a juvenile polyp which will bud medusae via a medusary nodule, whereas that of the Trachy- and Narcomedusae is a juvenile medusa, possibly meeting the definition of what McEdward & Janies (1993) call a "mesogen", i.e. an intermediate stage connecting the embryo and the juvenile in extreme cases of direct development. The Narcomedusae, thus, are here considered as the forms nearest to the Cubozoa. This polarization of hydroidomedusan phylogeny leads to the following series:

1. Narcomedusae (with "actinulae" originating from medio-lateral development of the planula and medusae with tentacular statocysts, produced by metamorphosis and never by a medusary nodule).

2. Trachymedusae (with "actinulae" originating from antero-posterior development of the planula and medusae with tentacular statocysts, produced by metamorphosis and never by a medusary nodule).

3. Actinulida (with actinulae originating from antero-posterior development of the planula and giving rise to specialized mesopsammic medusae with tentacular statocysts by metamorphosis and never by a medusary nodule).

4. the clade Limno-, Antho- and Leptomedusae, with antero-posterior development of the planula into a hydroid giving rise to medusae by a medusary nodule.

The Laingiomedusae, a morphological link between the Narcomedusae and the other orders, are still insufficiently known to be fitted into this scheme.

Medusa re-expression

The clades Limno-, Antho- and Leptomedusae probably derive from ancestors with a complete life cycle, but some medusae have features so peculiar as to allow the development of hypotheses that consider them as re-introduced in the life cycle. This implies that ancestral morphologies that have been suppressed in the phenotype are retained in the genotype and can be re-expressed (atavisms). McEdward (1995) suggested a possible re-evolution of pelagic development after larval suppression, with morphs that resemble larvae but are to be interpreted as juveniles. In this framework,

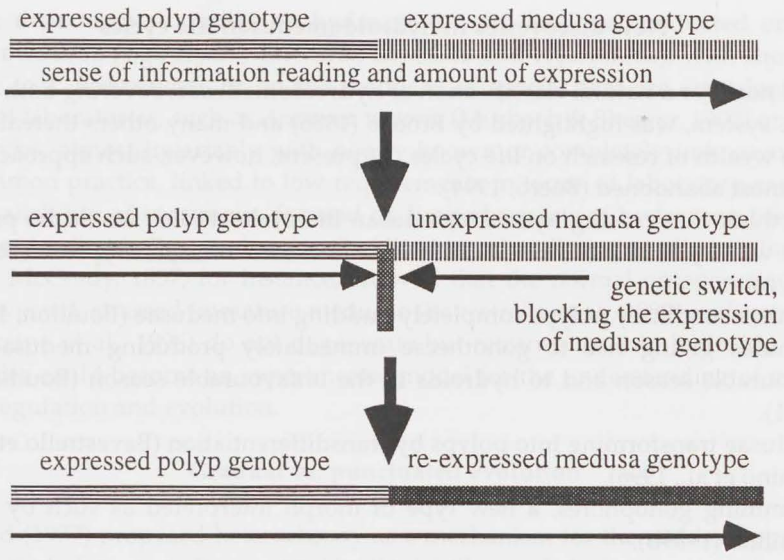

Fig. 2. Unexpressed genes (e.g. those coding for a suppressed medusa) can change with no constraint by stabilizing selection and, if re-expressed, can originate novelties which are uncoupled from the changes of the continuously expressed genes, subjected to stabilizing selection.

it is possible to envisage (Boero & Sarà, 1987; Boero & Bouillon, 1987) that the part of the genome encoding the medusa is not irreversibly lost when the medusa stage is absent and that, being present, it is subjected to molecular evolution (i. e. genomic change) uncoupled from organismal evolution (i. e. phenotypic change) (fig. 2).

If the absence of the medusa is not the result of gene deletion, the other cause for medusa reduction is that modifications occurred in regulatory genes that avoided the activation of the program leading to a medusa. A further modification might induce medusa re-expression, an event which could lead to species with similar hydroids (having been subjected to the most widespread form of selection: stabilizing selection) and different medusae (having evolved without selection in their unexpressed molecular codification). There are some cases that could be explained by such pattern. One is *Obelia*, as argued by Boero et al. (1996). The others are the "medusoids" of Leptomedusae with gonads on the manubrium, interpreted by Boero & Bouillon (1989b) as "swimming gonophores": a mosaic of fixed gonophores and medusae. These hypotheses are based on comparative analyses of recent species; molecular investigations may shed some more light on the validity of such speculations. In this context, it is noticeable that in the medusaless *Hydra* spp. the homeobox genes *cnox-1* and *cnox-2* are expressed in a graded fashion along the body column (Schummer et al., 1992; Shenk et al., 1993) but in *Podocoryna* the corresponding genes *cnox1-Pc* (Aerne et al., 1995) and *cnox2-Pc* (Aerne et al., in preparation) are present only in the medusa. Whatever the mechanism(s) regulating life cycle patterns, however, even the diversity of hydroidomedusan life cycles is still largely unknown.

New discoveries in hydroidomedusan life cycles

The need for a natural classification of hydroidomedusae, covering both stages in a single system, was highlighted by Brooks (1885) and many others thereafter, leading to a wealth of research on life cycles. At present, however, such approaches have been almost abandoned (Boero, 1994).

We think that ceasing hydroidomedusan life cycle studies would be premature and we will try to demonstrate this with a list of new life cycle types discovered in recent years:

- Planktonic solitary polyps completely budding into medusae (Bouillon, 1983).
- Planulae giving rise to gonothecae immediately producing medusae in the favourable season and to hydroids in the unfavourable season (Bouillon et al., 1991).
- Medusae transforming into polyps by transdifferentiation (Bavestrello et al. 1992; Piraino et al., 1996).
- Swimming gonophores, a new type of morph interpreted as such by Boero & Bouillon (1989b).
- Thecate polyps detaching from the colony and living freely in the plankton (Gravier-Bonnet, 1992).
- Medusae producing frustules that fall on the bottom and transform into polyps (Carré & Carré, 1990).
- Asymmetric medusae, benthic as juvenile and planktonic as adult (Boero, Bouillon, Gravili, in preparation).

This list proves that the lack of information on hydroidomedusan life cycles is not only quantitative (the majority of the species have still unknown cycles) but also qualitative (new cycles often cannot be included in the already known types).

Obelia, the classical textbook example of hydrozoan life cycles, has such an "untypical" medusa that an argument has been developed about the possibility of it being derived by polyp metamorphosis and not by a typical medusary nodule (Boero et al., 1996).

According to Bouillon (1985), 113 out of 388 genera of Antho- and Leptomedusae are based on a single stage or have poorly known life cycles. Furthermore, many species considered as having "fixed gonophores" are based on observation of preserved material: the discovery of swimming gonophores in the Lafoeidae, Aglaopheniidae, Sertulariidae, Haleciidae, for instance, suggests that several species could produce liberable morphs.

The majority of the insufficiently known genera is based on medusae and could have cycles that escape the "normal" patterns, possibly with reduction or even suppression of the polyp stage. The life cycle pattern of *Eirene hexanemalis* (Goette, 1886), with solitary and short-lived polyps, could be widespread in several medusa-based genera or, also, the polyps could be specialized to particular habitats, like those of *Bythotiara* (ascidians), *Hydrichthys* (fishes), *Larsonia* (fishes), *Octotiara* (bryozoans), *Pelagiana* (algal masses), *Teissiera* (serpulid opercula), *Pteroclava* (anthozoans), *Zanclea* (bryozoans, corals, bivalves), *Eugymnanthea* (bivalves), *Polypodium* (sturgeon eggs), etc.

Most experimental studies on hydrozoans were and are conducted on forms without medusae (e.g., *Hydra*, *Tubularia*, *Laomedea* and *Hydractinia*), with short-lived medusae (e.g. *Podocoryna*), or on the medusae of species that are abundant in the vicinity of laboratories, such as *Aequorea victoria* (Murbach & Shearer, 1902) or species of *Polyorchis*, almost invariably with poorly known or completely unknown cycles. This common practice, linked to low requirements in terms of laboratory care or on ready availability of specimens, focused on limited aspects of hydrozoan life cycles, sometimes leading to "generalizations" of limited value. Recent studies on *Turritopsis nutricula* McCrady, 1857, for instance, showed that the normal ontogenetic pattern can be reversed: stressed immature medusae (Bavestrello et al., 1992) and spent medusae (Piraino et al., 1996) do not degenerate but retransform into hydroids. Hence, this species could become an experimental model for the understanding of morphogenesis regulation and evolution.

Gradual vs. punctuated evolution

Gould (1977) proposed heterochrony as a mechanism for the sudden appearance of new morphologies, as documented by the fossil record. A classic case of neoteny is axolotl, which can be mature at an advanced larval stage with gills, or that can metamorphose into a "normal" salamander. Changes in ontogeny timing can lead to large phenotypic changes in a saltational way. Throughout his book, Gould (1977) put forward cases which are almost exceptions within regular patterns, and no attention was paid to hydrozoans, where heterochrony occurs in almost all families, and in the majority of the species.

On the one hand, medusa suppression can be a "punctuated" event leading to the expression of new phenotypes, such as *Obelia* or swimming gonophores, since there are no signs of gradual evolution of such morphs. On the other hand, the morphological comparative analysis of even recent species shows a gradual series of intraspecific variations and interspecific connections in the way the medusa stage is expressed (see Boero & Sarà, 1987).

The vast array of life cycle modifications in the hydroidomedusae led to such difficulties in classification that Hennig (1966: 123) wrote: "in the Hydrozoa the classification of the medusae is still rather independent of that of the polyps. For entire families of medusae we still do not know to which polyp families they belong as alternation of generation forms. This group presents an opportunity to test the efficacy of the methods of phylogenetic systematics by using them in all strictness first to produce independent classifications of the medusa and polyp generations. Then the "incongruences" between the two classifications would have to be tested according to the viewpoints sketched above". Naumov (1960-69) developed such an approach (but without using cladistics). Since the hydroid is a constant in most species whereas the medusa can be absent, he combined the classifications of medusae and hydroids, using just hydroid names.

Petersen (1979, 1990) argued that medusa suppression occurs so easily that monophyly of genera based on this sole feature is doubtful. It is possible that an event of medusa suppression gave rise to the ancestor of a new monophyletic clade of species with no medusa stage, but it is also possible that some forms with fixed

gonophores resulted from independent events of medusa suppression within the same clade, their grouping into separate genera leading to polyphyletic taxa.

Cunningham & Buss (1993) tested the presumed polyphyly of medusa suppression in the Hydractiniidae and showed, by molecular techniques, that the genera *Hydractinia* (with fixed gonophores), *Stylactaria* (with releasable eumedusoids) and *Podocoryna* (with free medusae) do not represent monophyletic clades.

The phylogenetic analysis of hydrozoans suggested by Hennig will be successful only if both stages change at the same rate. If in some species the medusa changes faster than the hydroid (or vice versa) the two independent classifications will reveal "incongruences" which will have to be solved case by case. Proper character choice should resolve such problems, as suggested by Petersen (1990) and by Boero et al. (1996).

Conclusion

The exploration of hydrozoan life cycle diversity is far from being completed, and has not yet led to a classification reflecting phylogeny, but the scientific community is abandoning this field (and the study of hydrozoan diversity in general). Yet, exciting things are still being discovered, and the findings deriving from hydrozoan studies may be fitted in more general frameworks. Possibly, important processes, such as the evolution by heterochrony, will be better understood by extending and generalizing the scattered observations on hydrozoan life cycles.

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Reproductive biology of the Antarctic octocoral *Thouarella variabilis* Wright & Studer, 1889

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Key words: Octocorallia; *Thouarella*; Antarctica; reproduction.

Abstract: Amongst the primnoids collected by the Brazilian Antarctic Expeditions, the genus *Thouarella* is the most abundant and *Thouarella variabilis* Wright & Studer, 1889, the dominant species. An investigation of the reproductive biology of this species was undertaken. The results of this study showed that colonies of *T. variabilis* are gonochoric brooders. The polyp fecundity is low with only one larva being released per female polyp at a time. The presence of oocytes in different stages of development within the same polyp suggests a two year cycle of oogenesis or continuous gametogenesis. The developing oocyte grows to a size of 660 µm. A non-feeding, non-pelagic lecithotrophic planula larva is retained within the polyp until full development is attained. The larva reaches a length of 860 µm and occupies on average 80% of the polyp volume. Spawning occurs along the Antarctic summer and the swimming planula most likely settles soon after release in a site not far from the parent colony, which probably explains the patchy distribution of the species. Brooding provides protection for the embryo, increasing the chance of survival and compensating for the low fecundity of the polyp, assuring the successful colonization of this species, which is abundant and widespread in Antarctic waters.

Introduction

The knowledge about Antarctic and deep-sea octocorals had been, until recently, restricted to taxonomic studies, resulting from the scientific expeditions realized mostly in the second half of the last century and the beginning of this century. Little is known about their biology. The conditions for investigating the biological aspects of Antarctic octocorals was far from ideal. The adversities and difficult access of the study site was the main limitation, adding to the lack of continuous sampling. The establishment of shore-based research facilities allied to the development of underwater activities have improved the conditions for quantitative and experimental studies on chemical ecology, growth and reproduction of octocorals have been developed.

The information about the reproductive biology of Antarctic octocorals is mostly limited to a few notes accompanying taxonomic descriptions. Based on the existing data, gonochorism associated to brooding seems to be the trend in Antarctic and deep-sea octocorals (Wright & Studer, 1889; Versluys, 1906; Thomson, 1907; Roule, 1908; Gravier, 1913; Kükenthal, 1924; Brito, 1993).

The reproductive biology of some other Antarctic invertebrates have been well investigated and some general assumptions can be established. Three major modes

of reproduction have been observed in antarctic invertebrates: pelagic planktotrophy, pelagic lecithotrophy and non-pelagic lecithotrophy, which involves the production of demersal or crawling larvae. Species which produce non-feeding, non-pelagic, lecithotrophic larvae produce a few large eggs that will be brooded until the larva is mature. The larvae have demersal habits or settle soon after being released. This will increase the chance of survival compensating for the low fecundity, but it will decrease the dispersal capability, which, in many cases, suggests patchy distribution and endemism. The study of the reproductive biology of the primnoid *Thouarella variabilis* Wright & Studer, 1989, has shown that this species follows similar reproductive patterns.

It is the objective of this paper to describe the main features of the reproductive biology of *T. variabilis*, concerning sexuality, reproductive mode, fecundity, gametogenesis, gametogenic cycles and distribution of the reproductive stages throughout the colony.

Material and methods

The material for this study was collected by recent Brazilian expeditions to the South Shetland Archipelago and Bransfield Strait, Antarctica. Deep water sampling was conducted by means of dredges and nets, beam-trawl and otter trawl. Additional sampling was conducted by SCUBA-diving in the shallow waters of Admiralty Bay, King George Island.

The colonies were divided into three regions: distal (top of the colony); middle; and proximal (base). The branches from each region were divided into two portions: peripheral (tip) and internal (middle and base of the branches). Five polyps were removed from each portion, so that, ten polyps were taken from each region and thirty from each colony.

Light microscope histochemistry.— The polyps were decalcified in Bouin's solution for at least 12 hours and then transferred to alcohol. After being decalcified in Bouin's solution, the tissue was dehydrated through ascending concentrations of ethanol: 30% (30 min.); 50% (30 min.); 70% (30 min.); 90% (30 min.); twice in 100% (30 min.); and twice in histoclear (30 and 45 min., respectively). The samples were embedded in paraffin wax, which was replaced four times at intervals of 1 hour, whilst kept in an oven at 65°C. The preparations were sectioned transversely or longitudinally at 5 µm (occasionally at 10 µm) and mounted on glass slides. The sections were stained with haematoxylin, eosin or Masson's trichrome.

Scanning electron microscopy (SEM).— The specimens preserved in 70% alcohol were dehydrated gradually to 100% alcohol and critically point dried with CO_2. Once dry, the polyps, eggs, embryos and sperm sacs were mounted on aluminium stubs by a carbon impregnated film. Some preparations were coated with gold/palladium but most were coated with 20 nm of gold in a Hummer VI sputter coater. The preparations were examined with three different scanning electron microscopes (S.E.M.): JSM - P15 (JEOL); ISI 60A and JSM 6400 (JEOL) at an acceleration voltage of 20 kv.

Transmission electron microscopy (TEM.).— The TEM processing involves the embedding of the tissues in epoxy resin and the staining of the tissues with heavy metal salts to improve contrast. The tissue was placed in 2% osmium tetroxide solution (2 hours); rinsed in distilled water; transferred to 2% uranyl acetate (30 min.); rinsed in distilled water; dehydrated with ethanol 70% (10 min.), 90% (10 min.), and absolute ethanol (3 × 10 min.); placed in histosol (30 min.); transferred to 50% histosol : 50% resin (1 hour); and soaked in spurr's resin (24 hours). The resin was polymerised by being heated in an oven at 60°C for 16 hours. The solid block of resin was posteriorly sectioned (around 70 nm) and the sections examined with a transmission electron microscope, Hitachi H - 7000.

Results

Those specimens of *Thouarella variabilis* examined showed both colony and polyps prevalently gonochoric. The colonies were, with a few exceptions, in reproductive phase. Not all the polyps in a fertile colony were breeding, but the polyps with gonads presented similar reproductive stages all along the colony.

The presence of larvae inside the polyp cavities of many examined colonies indicates that brooding is the reproductive mode. The fecundity of this species seems to be very low, with only one larva being released per female polyp at a time. The presence of oocytes in different stages of development within the same polyp suggests a two year cycle of oogenesis or continuous gametogenesis with release of larvae occurring throughout the year.

The first reproductive stage of either male or female polyps could be identified by the swollen appearance of the mesenteries in the gastric cavities (fig. 3), where the gonads will be developed. Only the four lateral mesenteries were observed to bear developing genital cells. The sperm sacs and oocytes seem to be developed preferentially on the two lateral mesenteries which are close to the asulcal mesenteries on the adaxial side of the polyp (fig. 4).

After fertilization the egg moves to the upper part of the polyp cavity where the larva develops. The larva is retained by the polyp until maturation is attained (fig. 6). If other young oocytes are being developed at the same time, these will be confined to the lowermost part of the polyp together with the mesenteries. The maximum length of larva planula observed was 860 µm. The larva was estimated to occupy 80% of the total volume of the polyp and sometimes almost 100% of its cavity.

In both male and female colonies, there are relatively more polyps in the first reproductive or immature stages in the distal region with the mature stages distributed relatively evenly between the middle and proximal regions. The peripheral polyps are mostly in the first reproductive stage or immature (fig. 2). The later stages of maturation are found in the internal polyps.

Discussion

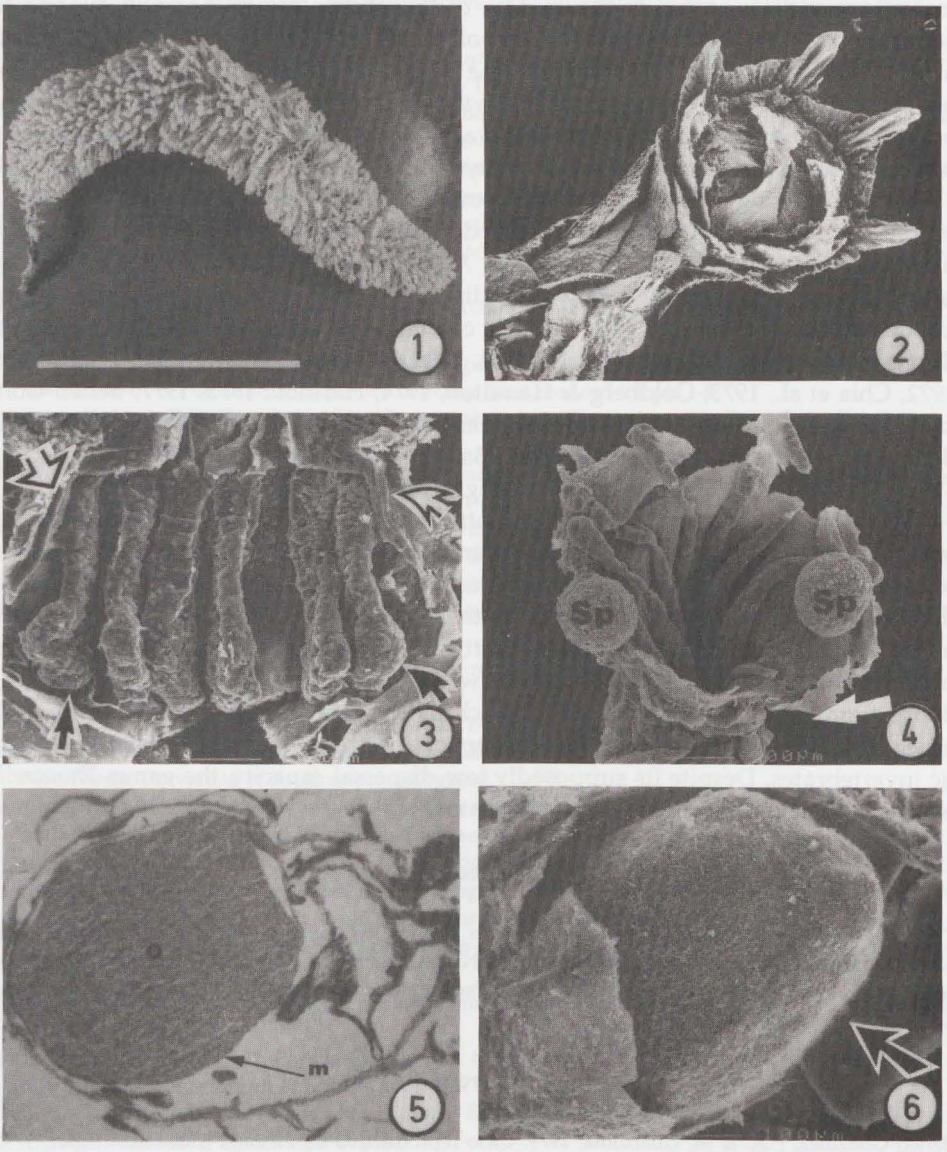
The bottlebrush colonies of *Thouarella variabilis* (fig. 1) are composed of non-retractile polyps (fig. 2), which attain an average length of 1 mm, half of which is occupied by the gastric cavity. The basal parts of the gastric cavities are interconnected by sole-

nia which are covered by a relatively thin coenenchyme (unlike the alcyonaceans which have a thick coenenchyme and are able to develop long gastric cavities). The short polyp has its volume even more limited by the calcareous armature which surrounds it, although this armature apparently has a high capacity for expansion. Benayahu & Loya (1984) suggested that fecundity depends on the length of the polyp cavity. *Thouarella variabilis*, having a short gastric cavity, shows a low reproductive output, producing only one egg to maturity at a time. The species develops large eggs (600 µm in diameter) which in some specimens can occupy 60% of the polyp volume (fig. 5). Other eggs were observed in development but only in the lower portion of the gastric cavity, sometimes compressed to the walls of the polyp as the oocyte in maturation demands additional space.

Three stages of oocyte development have been observed in a single polyp: previtellogenic oocytes; a developing oocyte; and a mature oocyte. Whether after the release of the mature oocyte, the developing oocyte can reach maturation in the same season is unknown. However, it is most probable that the polyp releases just one larva per summer and that the developing oocyte reaches maturation in the following year and that, at least, one of the youngest oocytes reaches maturation in two years time. The developing oocyte might have a slow period of growth during the long Antarctic winter and a rapid growth in the eutrophic summer months. Unfortunately, no winter samples were available for this study. Fat granules were observed in massive concentrations in polyps producing first stage oocytes. The considerable decrease of these energetic reserves, observed in the later stages of oocyte development, suggests that the production of a single egg is very costly. The replenishment of energetic reserves might be a slow process making it more difficult for the polyp to accomplish the maturation of a second oocyte within the same season. These octocorals could have continuous gametogenesis instead, and release larvae all year around as shown for other octocorals (Benayahu et al., 1989) and for other Antarctic invertebrates (Pearse et al., 1991). Despite this hypothesis, it is important to re-emphasize that to produce a large yolky egg is expensive and the polyp would have to continuously build up energetic reserves.

Similar reproductive stages were observed along the colonies of *Thouarella variabilis* but not all the polyps in a colony were bearing gonads. In contrast to Lawson's study (1990) of *Acanella arbuscula* Johnston, 1862, the polyps on the periphery of the branches were mostly immature although displaying adult size. The polyps which were reproducing were mainly located on the middle and base of the branches. Hartnoll (1975) noted that in the later stages of oocyte development in *Alcyonium digitatum* Linnaeus, 1758, the colony would enter in quiescent phase and would not feed. It is not hard to believe that if the polyp has its cavities blocked with reproductive products, it would stop capturing food. If particulate food is the main source of energy for *T. variabilis*, the polyps which have most of their volume occupied by oocytes or sperm sacs, would be disadvantaged in capturing food. This function of acquiring food could be fulfilled by the non-reproductive peripheral polyps. These "end" polyps would be in favourable feeding position and would ideally capture the particulate food and transfer the dissolved compounds to the rest of the colony via the solenia.

In *Thouarella variabilis* fertilization is internal. The ripe egg detaches from the



Figs 1-6. Fig. 1. Colony of *Thouarella variabilis;* scale bar = 100 mm. Fig. 2. Detail of the polyp showing its calcareous armature; 50 ×. Fig. 3. Exposed gastric cavity showing the markedly expanded mesenteries (black arrows) and the asulcal mesenteries (white arrows); scale bar = 100 µm. Fig. 4. Developing sperm sacs (Sp) attached to the lateral mesenteries close to the asulcal mesentery (arrow); scale bar = 100 µm. Fig. 5. Female polyp with only one oocyte (O), occupying the whole gastric cavity, showing a very stretched endoderm and a thin mesogloea (m); 70 ×. Fig. 6. Developing planula larva (arrow) in the polyp cavity; scale bar = 100 µm.

mesentery and is located in the middle of the gastric cavity that will expand to accommodate the large egg. The female polyp was found to increase its average volume by 85%, mainly in the region of the base and the neck, since the developed oocyte and larva will occupy most of cavity space. The embryo remains inside the polyp cavity until a mature planula larva is developed (fig. 6). The fertilized egg passes to the upper part of the polyp cavity but no special brooding pouch is formed.

Despite having a low reproductive output, *Thouarella variabilis* has evolved a reproductive mode that improves its reproductive efficiency by protecting the embryo until the planula larva is fully developed. As suggested by Babcock (1990), brooding is an alternative to enhance survival avoiding larval predation and compensating for low fecundity. This reproductive mode is common amongst octocorals as is the production of large eggs (Hickson, 1895; Hill, 1906; Matthews, 1916; Gohar, 1948; Vighi, 1972; Chia et al., 1973; Goldberg & Hamilton, 1974; Hartnoll, 1975, 1977; Beheti-Gonzalez & Guardiola, 1979; Dinesen, 1985; Farrant, 1985; Brazeau & Lasker, 1989, 1990; Benayahu, 1989; Benayahu et al., 1989; Babcock, 1990; Benayahu, 1991; Rice et al., 1992). The benthic habit of the planulae might add to their survival capacity.

The non-feeding, non-pelagic, lecithotrophic larvae of *Thouarella variabilis*, like other brooding octocorals and some Antarctic invertebrates, might settle soon after release, and as a consequence are likely to have low dispersal capacity. Evidence of this is the patchy distribution shown by these octocorals in underwater films taken in the Weddell Sea, Antarctica (Julian Gutt, pers. comm.). In these films an undetermined bottlebrush species of *Thouarella* was found to be very abundant, forming dense aggregates. The colonies within each patch were disposed very close to each other. Brooding associated with low dispersion is a common characteristic of Antarctic invertebrates. Despite its supposedly low dispersal capacity, the genus *Thouarella* is known to be widespread in Antarctic waters.

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Acclimation, adaptation and algal symbioses in reef-building scleractinian corals

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Key words: Acclimation; adaptation; reproduction; mutation; evolution; selection; symbiosis; zooxanthellae; corals; Scleractinia; clonal organisms.

Abstract: Zooxanthellate scleractinian corals exhibit both sexual and asexual reproduction, a reproductive biology more similar to many plants than to higher animals, and (in some taxa) a tendency to hybridize. These characteristics suggest the likelihood of somatic mutation and genetic mosaicism within colonies. This genetic diversity may not only be a component of rapid adaptation to environmental change, but may enhance and interact with acclimative mechanisms stemming from the potential formation of stable, environmentally sensitive symbioses with a variety of taxa of zooxanthellae. This conceptual model has implications of importance in current coral research.

Introduction

Rate of change in environmental parameters is an issue of major concern with regard to responses of organisms and ecosystems to global change. Foremost among cnidarians of concern are scleractinian corals; there are urgent questions about the extent to which they and coral reef communities can adapt to rapid and extensive climatic and local anthropogenic environmental changes (Buddemeier, 1995).

"Adaptation" in the general, popular sense, has two components in more rigorous scientific terminology – acclimation and adaptation in the strict sense. Acclimation is the process by which an organism adjusts its behaviour or physiology in response to altered environmental conditions; changes resulting from acclimation occur within the organism's lifetime and are not heritable, although the capability of making such changes is presumably genetically transmitted. In the strict sense, adaptation describes a process of genetic modification through selection that results in heritable changes over evolutionary time scales.

Knowledge of the ranges of conditions or rates of change to which organisms can acclimate, and of the mechanisms of acclimation, is extremely limited. Earth history suggests that corals must be adapted by evolution to cope with frequent and relatively rapid environmental changes, but laboratory and field observations indicate vulnerability to changes in many environmental parameters. Hypotheses concerning mechanisms of acclimation and adaptation can be useful tools for exploring both short-term environmental sensitivities and long-term evolutionary processes.

Recent conceptual developments in the field of coral reef science call for re-exam-

ination of ideas about both acclimation and adaptation, and about interactions and relationships between the two processes. In the area of acclimation, it has been demonstrated that a coral host can form stable symbioses with multiple taxa of zooxanthellae and that the preferred partnership may depend on environmental factors (Rowan & Knowlton, 1995). This supports the "adaptive bleaching hypothesis," which postulates that the coral "ecospecies" is determined by the nature of the particular host-alga partnership, and that exchange of symbionts may be a means of rapid acclimation to changing conditions (Buddemeier & Fautin, 1993). Model calculations show that this concept can account for acclimation responses with time scales of days to decades (Ware et al., 1996).

Adaptation and genetic diversity

Corals also may possess genetic adaptive mechanisms not normally associated with animals. Many aspects of their reproductive biology are more akin to plants than animals (Fautin, 1996; Veron, 1995). In an asexually reproducing (growing) colony, a somatic mutation occurring in one polyp can be transmitted to that polyp's asexual progeny and maybe to its sexual progeny. Although a coral colony (some of which achieve ages of centuries with average polyp budding time constants on the scale of years) is made up of seemingly identical units, it may actually represent a genetic mosaic. Fautin (1997) cited a number of examples that can be interpreted as evidence of genetic variation within a clone.

Such genetic mosaicism is well known among plants (Gill et al., 1995). One objective of this paper is to examine the potential consequences of this mechanism in corals. To do so, we present some illustrative, order-of-magnitude model calculations. Estimation of applicable mutation rates is difficult, since coral-specific data do not appear to exist. However, order-of-magnitude estimates can be made by extrapolating from other taxa. King (1993) summarized data on chromosomal mutations for a variety of taxa, expressed as numbers of individuals with mutations per generation or per population; since these are not appropriate for clonal organisms, we have converted them to rates per year per individual by estimating lifetimes. On this basis, the mutation rates cited range from $<10^{-2}$ to about 10^{-6} per year, with the lower rates representing mutations in higher animals.

Of particular interest from the coral standpoint is the likelihood that hybrid organisms are more susceptible to mutation than non-hybrids (King, 1993). Veron (1995) describes coral evolution as reticulate, characterized in some taxa by extensive and repeated hybridization. A propensity for hybridization thus provides two mechanisms for maintaining high levels of genetic diversity within a lineage: the hybridization itself, and the consequent elevated mutation rates.

Fig. 1 shows sample calculations depicting the growth of an idealized hemispherical coral colony with a constant linear extension rate and uniform polyp size. Figures 1a and 1b depict the non-linear increase in polyp number and decrease in reproduction (budding) rate per polyp that is a consequence of the hemispherical geometry. Figs 1c and 1d show the fraction and the number, respectively, of mutated polyps in the colony as a function of time and mutation rates (the polyp is treated as an individual clonal organism in this calculation, so rates are per polyp per year). Mutated polyps are assumed to transmit their mutations to descendants and to bud (repro-

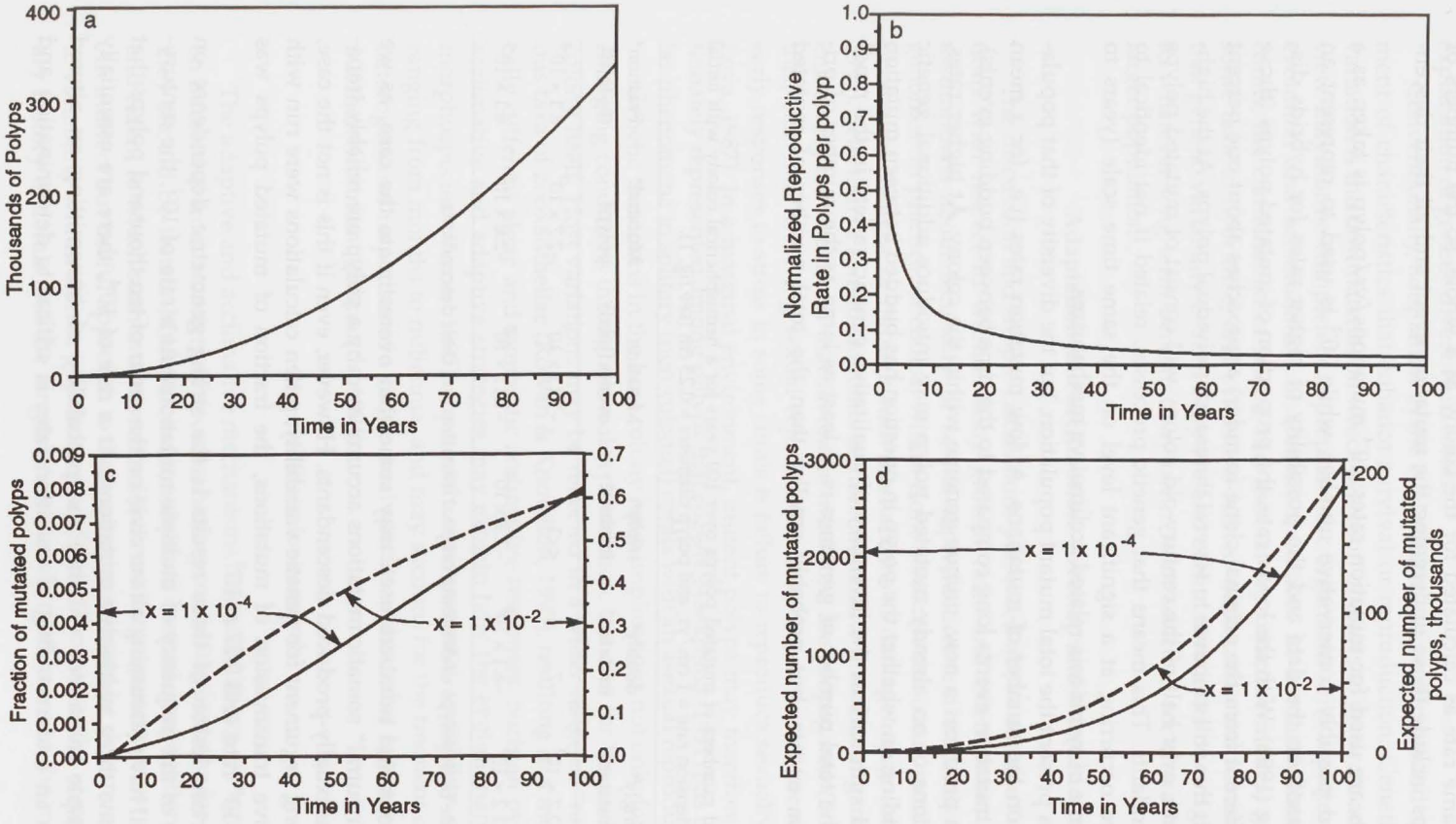

Fig. 1. Model calculations for a hemispherical coral colony with uniform polyps of 0.25 cm diameter, an initial diameter of 5 cm, and a constant linear extension rate of 1.0 cm/yr. (a) Number of polyps on the surface of the colony as a function of time (age). (b) Rate of polyp reproduction (budding) as a function of colony age (size). Note that the rate becomes relatively low and stable with increasing age. (c) Fraction and (d) Number of total polyps mutated (= different from initial clone founder) as a function of time for two different mutation rates (see text for discussion). Data on the number of different mutants are presented in table 1, and include both original mutation events and the descendants of the originally mutated polyps.

duce) at the same rate as calculated for the colony as a whole, so the numbers of mutated polyps include those undergoing the mutation event and all their descendants.

Two values are used for mutation rates: 10^{-4} mutations/yr/polyp is taken as a reasonable and possibly conservative number, while 10^{-2} is used to represent an upper limit based on the data and the possibility of higher rates for hybrids discussed by King (1993). With the lower rate, the proportion of mutated polyps (those genetically different from the original clone founder) approaches about one percent of the total, but this still amounts to several thousand individual polyps. At the higher mutation rate, over half of the century-old colony will consist of mutated polyps or their descendants. This means that genetic processes, related if not identical to adaptation, are occurring at a significant level on the same time scale (years to decades) as some of symbiosis-related acclimative mechanisms.

The figures present the total mutant population, but the diversity of that population depends on the number of mutations. At low mutation rates (i.e., for a mean time between mutation events long compared to the time between budding events), each mutation produces a new, unique genome within the colony. At higher rates, further mutations of an already mutated polyp may introduce additional genetic novelty, depending on whether the polyp in question has budded between mutation events. Table 1 summarizes the inventory of qualitative genetic variety in the modeled colony; the total number of genomes is at least as large as the number of primary mutation events, but probably smaller than the total number of mutated polyps.

Table 1. Expected numbers of mutated polyps over 100 years for a hemispherical colony with initial radius = 5 cm, extension rate = 1 cm/yr, and polyp diameter = 0.25 cm (see fig. 1).

Mutation rate/polyp	singly* mutated	doubly* mutated	triply* mutated	Mutation events (total)	Mutated* polyps	Fraction* mutated
10^{-4}/yr	$\sim 2.7 \times 10^{3}$	~7	<<1	$\sim 1.13 \times 10^{3}$	$\sim 2.7 \times 10^{3}$	8.1×10^{-3}
10^{-2}/yr	$\sim 1.2 \times 10^{5}$	$\sim 6.1 \times 10^{4}$	$\sim 1.9 \times 10^{4}$	$\sim 1.13 \times 10^{5}$	$\sim 2.0 \times 10^{5}$	6.1×10^{-1}

* Numbers include both polyps undergoing original mutation and their descendants.

The fractions and numbers used may somewhat overestimate the case, as we assume that "neutral" somatic mutations accumulated by a polyp are reliably transmitted to its asexually-produced descendants. However, even if this is not the case, there is a strong argument for genetic variability; when calculations were run with no reproductive transmission of mutations, the fraction of mutated polyps was 0.0035 for the 10^{-4} rate, and 0.27 at 10^{-2}.

An important lesson of these results is the strong geometric dependence on mutation rate of the frequency of multiple mutations. At a rate of 10^{-2}, the century-old colony will have (assuming all survive) on the order of ten thousand polyps that have undergone three successive mutations. At a rate of 10^{-4}, there are essentially none. If multiple mutations enhance the probability of transmitting an altered genome, then an understanding of mutation rates is critical to determination and

explanation of genetic diversity in corals.

Conventionally, adaptation in the evolutionary sense is regarded as the development of characteristics that enhance survival or reproduction. Genetic diversity or variation is the raw material on which selection operates to generate adaptation. This view may not be completely appropriate for organisms with a pattern of reticulate evolution and accumulation of somatic mutation. In zooxanthellate scleractinian corals, the combination of sexual and asexual reproductive modes, a propensity of some taxa to hybridize, and symbiosis-related acclimation mechanisms may mean that genetic variability is both adaptive and acclimative in its own right, in addition to whatever long-term benefits may occur from selection for characters within the larger population.

Adaptation and acclimation – overlap and interaction

The discussion and simple calculations presented above assume "neutral" mutations with respect to within-colony growth rates and interactions, and make no assumptions about fecundity or sexual reproduction and transmission of altered genomes. In practice, of course, within-clone selection can occur, manifested as morphological changes, or through such mechanisms as damage repair (Meesters & Bak, 1993). Selection of somatic mutants is more immediate than that of gametogenic ones, enhancing the likelihood that favorable mutations are propagated (Buss, 1983). Further, selection involving sexual reproduction may be enhanced because one of the early responses to stress in some corals is failure to reproduce sexually (Rinkevich & Loya, 1987). In a marginal environment, mutant polyps may therefore be disproportionately represented among polyps that spawn. Hoeksema (1991) has discussed similar phenomena in solitary (non-colonial) corals with an asexual reproduction mode.

Genetic variations in the host colony have implications not only for adaptation to changing conditions through sexual reproduction, but also for the acclimation of the colony itself. Host variations may be reflected in a wider range of "niches" for various taxa of zooxanthellae (Rowan & Knowlton, 1995), resulting in a mosaic of genetically different algae and symbiotic acclimative responses. Such an overlap between acclimative and adaptive strategies may explain how the evolutionarily stable coral morphospecies (Veron, 1995) cope with environmental variability on time scales ranging from months to millennia, and may account for the frequent observations of heterogeneous responses of a single coral species within what appears to be the same microenvironment (Edmunds, 1994).

Implications for research

The adaptive and acclimative mechanisms discussed above are hypotheses based on inferences from a variety of sources; having major implications for coral research, they should be tested. Mutation rates, extent of asexual propagation of mutations, and extent to which such mutations find their way into gametes must all be assessed. An indirect but useful approach to these questions would be determination of genetic variability within clones, populations, and taxa. Because of the complex evolutionary history of corals and the length of time since divergence of the major scleractin-

ian lineages, it is important to avoid the common simplifying assumption that mechanisms or characteristics, once determined, can be applied to a wide range of taxa.

The implications of these concepts are particularly profound in terms of relating laboratory or local field observations of stress or environmental responses to the larger issue of the resilience of organisms and communities at regional or global levels. Not only is identification of the "ecospecies" incomplete without characterization of the algal symbiont(s) (Buddemeier & Fautin, 1993), but the arguments presented in this paper suggest that experimental observations represent snapshots of a single point in a large and dynamic continuum of genetic time and space. The critical point is that such snapshots cannot be treated as would be reasonable in the case of exclusively sexually reproducing, solitary higher organisms – they are far less representative of the larger world of genetically "fuzzy" animals such as corals, characterized by reticulate evolution, both sexual and asexual reproduction, and multiple symbioses.

This concept – intellectually appreciated and experimentally implemented – could ultimately resolve with mechanistic understanding the debate over whether reefs are "fragile" or "robust" (Done, 1991). Such a resolution will require placing community-specific ecological observations and organism-specific physiological experiments into a much broader context ranging from genetic characteristics to biogeographic and evolutionary models. We predict that if this is done, the results will reveal a much wider range of adaptive and acclimative features within coral taxa than we assume from the perspective of our natural bias as terrestrial vertebrates.

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Recent advances in cnidarian venom research 1991-95; clinical, chemical and immunological aspects

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Burnett, J.W., D. Bloom, S. Imafuku, H. Houck, S. Vanucci, L. Aurelian & F. Kokelj. Recent advances in cnidarian venom research 1991-95; clinical, chemical and immunological aspects.
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Key words: Cnidaria; jellyfish; venom.

Abstract: Several new advances have been reported in cnidarian research. First, patients affected by the neurotoxic and hepatotoxic actions of cnidarian venoms have been reported. Second, *Stomolophus nomurai* (Kishinoue, 1922) is now known to be a killer and juvenile forms of *Linuche unguiculata* Schwartz, 1788, have been revealed as pathogens in Seabather's eruption. Third, tissue culture assays for the effects of cnidarian venoms on mammalian liver and kidney cells demonstrated significant cytological damage within hours. Fourth, the human delayed hypersensitivity to cnidarian venoms appears to be conducted with a specific subset of lymphocytes and the reaction can be altered by either prior or subsequent ultraviolet exposure. Finally, a case of contact dermatitis to nematocysts was documented. This rare phenomenon complicates our understanding of cnidarian envenomation.

Introduction

Important clinical, chemical, and immunological advances in cnidarian venom research have been made in recent years (Burnett & Calton, 1987). Perhaps the most dramatic progress has been in communication of research data and clinical cases between investigators in this field. Results have been processed by the International Consortium for Jellyfish Stings through their newsletter and the coming publication of the Marine Stinger Reference Book by the University of New South Wales Press.

Clinical case reporting

Four significant envenomation sequelae were newly described.

First, eight deaths from pulmonary edema or cardiac arrest followed envenomation by *Stomolophus nomurai* (Kishinoue, 1922)* in the Bohai waters of China (Zhang & Qin, 1991). These stings produce severe systemic symptoms, coma, convulsions and psychoses.

Second, a rapid fatal hepatocellular failure occurred in a young man who one year previously had no signs of liver disease (Garcia et al., 1994). He had been SCUBA-

* Some authorities suspect it to be conspecific with *S. meleagris* L. Agassiz, 1862.

Table 1. Jellyfish envenomation syndromes. Those marked with * are uncommon. Others have numbers as a postfix which indicate total of known cases.

Local Reactions

Toxin-induced to skin, mucosa, and cornea
Exaggerated local reaction (angioedema)
Recurrent reactions up to four episodes
Delayed persistent reactions up to several months (*)
Distant site reactions (1)
Local lymphadenopathy
Seabathers' dermatitis

Long-term Reactions

Keloids (*)
Pigmentation
Fat atrophy (1)
Contractions (>4)
Gangrene (>4)
Ulceration
Vascular spasm (>6)
Mononeuritis (>10)
Autonomic nerve paralysis (1)
Ataxia (*)
Increased ocular pressure (2)
Mondor's disease (thrombophlebitis) (1)
Cold urticaria (1)
Raynaud's syndrome (1)
Guillain Barre syndrome (1)
Deep vein thrombosis (1)

Post-episode Dermatitis

Herpes simplex (1)
Granuloma annulare (1)

Reactions from Jellyfish Ingestion

Gastrointestinal symptoms
Urticaria

Systemic Reactions

Toxin-induced
Irukandji reaction
Respiratory acidosis, pulmonary edema
Blurred vision (1)
Monarticular arthralgia and reactive arthritis (*)
Pronounced vomiting
Psychosis, convulsions, coma, stupor (*)
Fever
Muscle spasm

Fatal Reactions

Toxin-induced
- Immediate cardiac arrest
- Rapid respiratory arrest
- Delayed renal failure (>5)
- Liver destruction (1)

Anaphylaxis (1)

diving a mile and a half off the St. Thomas shore in 150 ft deep water when he encountered a cnidarian in a 30 ft. deep cave-like structure. The patient received a sting over a 2-3 cm diameter area on the scapula. The skin vesiculated and eventually ulcerated. Nine days later he died during liver transplantation surgery. The offending anemone-like organism had a white base and tentacles which were white proximally but had blue tips. These tentacles had knob-like structures on their end and looked like orchids. No further differentiation could be given by this patient. No hepatotoxic medications were administered. Postoperative serum was tested to the only anemone available in our laboratory, *Condylactis gigantea* Weinland, 1860. The titer was 1:450, a moderately elevated titer. Although the patient had no previous episode of being stung by an anemone he had encountered fire coral and unknown jellyfish. Nine days was a short period of time for a moribund patient to mount any significant primary antibody response. At autopsy significant liver regeneration, and massive hepatocellular destruction were observed. This case demonstrated that severe hepatic damage can follow a cnidarian sting adding significance to the previous observations that animals and humans have elevated liver function tests after envenomation (Burnett, 1992; Muhvich et al., 1991).

Third, jellyfish envenomation syndromes have been tabulated and a current list is presented in table 1 (Burnett, 1992; Burnett et al., 1994). A sixth case of mononeuritis multiplex was recorded in 1994. A 52 year old healthy Caucasian female was stung off the coast of Mandang, Papua New Guinea, in the mid morning of a calm day while backing out of $3^1/_2$ ft. deep surf. She was stung on her thighs, arms and left forehead 15 feet from shore (figs 1-2). The patient was immediately gasping for breath because she was screaming with agony. The pain, was perceived instantly, intensified for six hours then disappeared in a few days. She had seen a clear jellyfish and manually removed several two foot long gelatinous tentacles from her leg. Her past medical history had been negative for medication and atopic disease. Although she should have had a menstrual period a few days before the sting, it had not occurred. Cold water was rapidly placed on the welts which appeared and intravenous analgesia with subsequent oral prednisone, tetracycline and intramuscular sedatives were administered. The swelling on her arm increased over the next three days before desquamation occurred and distal numbness commenced. Two months after her sting she still noticed hyper and hypesthesia of the left arm. Analysis of arm muscle strength showed weakness and slightly restricted range of motion which began to diminish by two months post sting. Serologic examination of this patient done six weeks after her sting showed a titer of 1:450 against *Chironex fleckeri* Southcott, 1956, and 1:900 against a Thailand *Carybdea* species (Burnett et al., 1988). This case is the sixth patient with this post sting mononeuritis reported in the world: five of which have occurred in the waters off New Guinea, Indonesia and Malaysia.

Fourth, Seabather's eruption occurring off the east lower Atlantic coast and in the Caribbean has been found to be due to envenomations by planula larvae of *Linuche unguiculata* Schwartz, 1788 (Wong et al., 1994). This cutaneous eruption is produced by envenomation from this larva entrapped in a bathing suit. Within two days after the patient emerges from the surf a papular, pustular, pruritic eruption occurs predominantly under the bathing suit. Elevated antibody titers appear within two weeks (Burnett et al., 1995) and may persist for several months. The patients may have only one primary episode of this pruritic eruption which lasts on the aver-

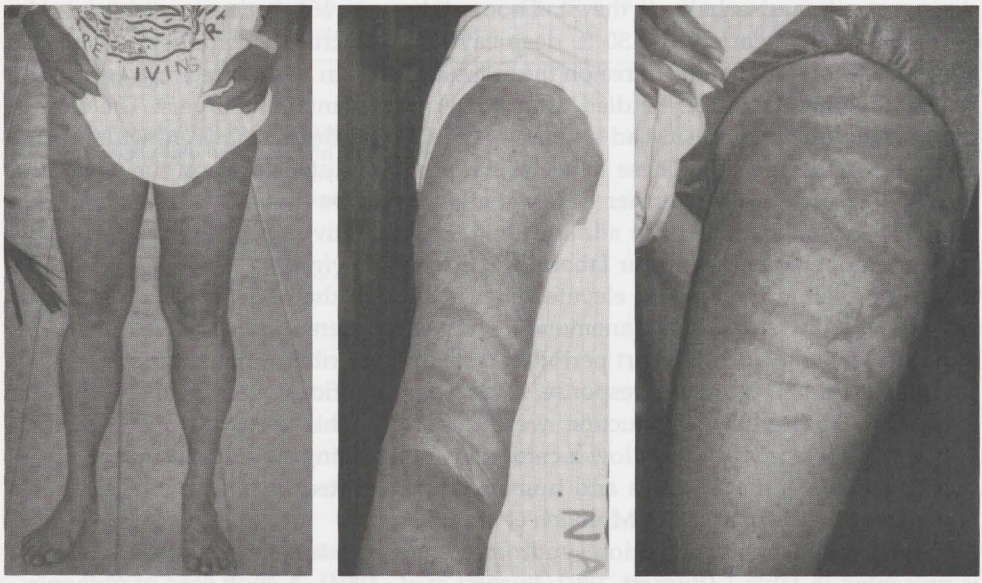

Fig. 1. Multiple erythematous linear urticarial lesions 4 hours post sting in a patient who developed mononeuritis in the left arm.

Fig. 2. Cutaneous lesions 50 days post sting.

age of 12 days or he may have multiple recurrences for several weeks. A third clinical form is a urticarial eruption which appears after a few days. Recurrent urticarial eruptions can also appear for many months after the sting. While therapy initially with ultrapotent corticosteroids looked promising, subsequent tests have shown them to be ineffective.

Chemistry

Efforts continue to purify cnidarian venoms. Techniques using capillary electrophoresis have allowed venoms to be rapidly separated into measurable fractions. Binding with hyperimmune serum occurs within seconds as demonstrated by alterations in the capillary electrophoresis chromatograms. These results corroborate the potential of early intravenous antivenom therapy in life-threatening situations.

Tissue culture experiments

Crude fishing tentacle nematocyst venom of *Chrysaora quinquecirrha* (Desor, 1848) (Burnett et al., 1992) or freeze-dried *Chironex fleckeri* tentacles obtained from Philip Alderslade of Darwin, Australia, were dialyzed against cold 3% sodium chloride for several hours. The dialyzed tentacles were screened through a fine metal mesh screen and washed with additional cold 3% saline. Nematocysts were allowed to settle overnight in a conical centrifuge tube before the supernatant was discarded. The pellets were subjected to sonic treatment (Branson sonifier, Danbury, Connecticut) in an ice bath in 3, 30 second bursts interspaced for 1 minute. Nematocyst rupturing was checked microscopically. After complete rupturing occurred the capsules were sedimented ultracentrifically at 20,000 g (Sorvall RC2-B centrifuge, Sorvall Instruments, Wilmington, Delaware). The *Chironex* nematocyst supernatant was then stored at -70° until use. These nematocyst venom preparations were then inoculated into confluent culture primary rat liver and kidney tubular epithelial cells and cytological damage was measured spectrophotometrically by release of lactic dehydrogenase (Houck et al., 1996). Contact of *Chrysaora quinquecirrha* venom with cultured rat hepatocytes requires only minutes before damage is irreparable. Cytotoxicity could be seen in one hour at a dose of $0.6LD_{50}$ mouse intravenous dose in the case of *Chrysaora* venom and cultured liver cells. The same venom required a dose of 1.5 LD_{50} for more than 2 hours contact to damage kidney cells. *Chironex* venom injured rat kidney cells within 2 hours dosage of 1 mouse IV LD_{50}. Verapamil (100 mg/ml) did not protect renal cells from injury by either venom. Heat treatment (60°C/30 min) inactivated the cytolytic factors of both venoms in both preparations. An interesting side of these experiments was the fact the young rat liver cells were more resistant to the sea nettle venom than were liver cells taken from adult rats. Calcium free media did not alter the kinetics of cytolysis induced by nettle venom. Prior inoculation of kidney cells with media mixed with *Chironex* antivenom (Commonwealth Serum Laboratory, Melbourne, Australia) at a 1:1 ratio (3 minutes prior to challenge) produced protection against that venom. Pre-incubation of *Chironex* and *Chrysaora* nematocyst venoms with high titer (ELISA> 1000) convalescent human serum from patients who had been repeatedly stung did not prevent the cytotoxic reaction. These results may be explained by a dosage basis in that convalescent human sera contains much less antibody than that obtained by inoculating sheep with crude *Chironex* venom.

Immunology

Ultraviolet light studies

It has previously been shown that exposure to light pre or post venom challenge

can result in diminished proliferation of peripheral blood leukocytes in the presence of jellyfish venoms in mice (Miura et al., 1993). Also, it is known that leukocytes of sensitized humans proliferate in response to cnidarian venoms. Ultraviolet light decreases this proliferation and its effect is widespread extending to responses against pathogens (herpes simplex), mitogens (concanavalin A and phytohemagglutinin) as well as endogenous proteins (heat shock protein). The decrease in the human blood cell proliferation occurred after small doses of ultraviolet light (less than 1 minimal erythema dose, 95% of body surface) (Miura et al., 1996). Other experiments have shown that this effect upon peripheral blood monocyte proliferation can last for several days. The importance of this study is that humans may have a different reaction to venom depending upon the dosimetry of beach sun exposure. Ultraviolet light can alter human immunity by many means including injuring circulating T-cells. Since venoms act by immunological as well as toxic pathways any abrogation of normal immunity could enhance their action on man. The clinical significance of these studies is not yet evident, but since venom itself can depress cellular proliferation that action added to that resulting from light might be combined to amplify factors important in the pathogenesis of the sting (Wachsman et al., 1995).

Contact dermatitis

Last year, contact dermatitis to *Olindias* venom or its capsule was reported in a patient (Kokelj et al., 1992). This individual, stung many times previously developed an exaggerated clinical response after his last exposure to tentacles of *Olindias sambiquenesis* F. Müller, 1861 (Kokelj et al., 1995). Patch testing, scratch testing and patch scratch testing were conducted on isolated nematocyst preparations. In contrast to 11 other normal volunteers, this individual was the only person who exhibited a positive patch test reaction at 48 hours. Contact dermatitis to jellyfish products have been reported in individuals preparing these animals as food. This delayed reaction is very rare but now complicates our understanding of the pathogenesis of future stings in this patient.

T-cell studies

Peripheral blood mononuclear cells from a patient who had latent herpes simplex labialis and who had been challenged with intradermal injections of *Chrysaora quinquecirrha* crude fishing tentacle venom were studied. Three intradermal injections were given at two day intervals. On days 1 and 5 the patient received 0.1 and 0.2 mg of heated (60°C, 30 m) fishing tentacle nematocyst venom respectively (unheated potency, 1 IV LD_{50}=0.01 ml, 2.6 mg protein/ml). On day 3, 0.03 ml of non-heated venom from the same sample was inoculated. Three days after the last injection his peripheral leukocytes were cultured with phytohemagglutinin, herpes simplex virus (HSV) antigen, mock antigen (prepared from uninfected cells) or heated *Chrysaora* venom. Proliferation of cells was measured by radiolabeled thymidine incorporation. Some of the cultures were done in the presence of antibodies to interleukin (IL) 2, 4 or 15 (50 ug/ml) and proliferation was observed for all three antigens. IL antibodies had no effect upon the proliferative response in the presence of phytohemagglutinin

or sea nettle antigen. However, anti-interleukin 2 aborted the proliferation in the cultures exposed to HSV antigen as previously shown by Imafuku et al. (1995). The T-cell repertoire affected by the presence of herpes simplex, phytohemagglutinin and sea nettle antigen were found to differ. T-cell families v beta 10, 13.1, 13.2, 14, 15 and 16 were preferentially used in response to sea nettle venom whereas v beta 1, 2, 6, 7, 12, 19 and 20 were preferentially used in response to phytohemagglutinin and v beta families 1, 2, 6, 7, 8 and 12.2 for herpes simplex antigen. These studies demonstrate that the T- cell response in man to sea nettle venom and to herpes simplex antigen is conducted by different cell populations with different proliferative mechanisms.

Final remark

The scientific investigation of the nature and action of jellyfish venoms now amalgamates several different experimental disciplines. Future discoveries will occur with increased communication and exchange of ideas between individuals versed in these various fields.

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Synopsis of hydroids from 1000 m and deeper in the western North Atlantic

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Key words: Abyss; bathyal zone; deep sea; Hydrozoa; hydroids; North Atlantic Abyssal Province; northwest Atlantic; organismal biodiversity; West Atlantic Abyssal Province.

Abstract: Based on current knowledge, hydroids of the western North Atlantic Ocean attain maximum diversity bathymetrically in the neritic zone (0-200 m). Although some 423 species are currently recognized from the entire study area, fewer than 9% (37) have been reported from depths of 1000 m or greater in the region. Records of these 37 species, comprising three anthoathecates and 34 leptothecates, are summarized herein. Of 12 family-group taxa represented, those with the most species were Sertulariidae (9), Lafoeidae (8), and Aglaopheniidae (8). Many of the species are quite eurybathic. Moreover, several predominantly neritic species from boreal and subarctic regions reportedly penetrate to considerable depths (>2000 m) at high latitudes. Zoogeographically, numbers of hydroid species were nearly equal in the western North Atlantic sectors of the North Atlantic (11) and West Atlantic (12) abyssal provinces. However, these two provinces seemed very different in the known composition of their hydroid faunas, with only one of the species listed in common, viz. *Acryptolaria conferta* (Allman, 1877). Relatively few hydroid species may be known from the sea floor of the deep ocean realm, but the fauna of this vast and frontier environment has been infrequently sampled and is still very poorly known.

Introduction

Hydroids are distributed bathymetrically in the oceans from intertidal regions to the hadal zone. The greatest diversity of species, however, has been recorded from neritic waters (0-200 m) (Fraser, 1944; Naumov, 1960; Millard, 1975; Cornelius, 1995). Few of these cnidarians occur as part of the intertidal biota (Calder, 1991), and they likewise appear to be a minor component of profundal benthos (Kramp, 1951, 1956; Vervoort, 1966; Calder, 1996).

No general synopsis exists of hydroid species reported from great depths in the western North Atlantic. Fraser (1944) included bathymetric data, together with distribution records, as part of a comprehensive taxonomic survey of hydroids from much of the region. Records in his report were overwhelmingly of species from relatively shallow bottoms (<200 m). However, a few were collected during extensive deep sea dredging operations off the east coast of the United States, the Caribbean Sea, and the Gulf of Mexico in the latter half of the last century on cruises by vessels such as the BLAKE, HASSLER, BACHE, and ALBATROSS (Allman, 1877; Clarke, 1879; Fewkes, 1881; Nutting, 1900; Fraser, 1944). Hydroids from bathyal and abyssal collections in Baffin Bay, Davis Strait, the Labrador Sea, and Denmark Strait (between Greenland and Iceland) were dealt with by Broch (1918), Kramp (1932,

1951), and Vervoort (1972). Several species from the high latitude deep sea in these reports are much better known from the neritic zone of boreal/subarctic regions, and were reported at depths considerably below their usual lower bathymetric limits. New information on hydroids from lower latitudes in the deep western North Atlantic has been contributed most recently by Vervoort (1972) and Calder (1996).

Given the current prominence assigned to issues of "global organismal biodiversity" (e.g., Hawksworth, 1995), the main objectives of this study were to assess the number of hydroid species, exclusive of stylasterids, reported from depths of 1000 m or more in the western North Atlantic Ocean, and to provide an initial inventory of the known fauna of the region.

Materials and methods

The reported bathymetric ranges of some 423 species of hydroids from the western North Atlantic and adjacent waters were compiled from literature records. The primary source of data was Fraser's book *Hydroids of the Atlantic coast of North America* (1944), containing distribution and depth records of some 426 nominal species. Other relevant works included those of Nutting (1900), Broch (1918), Kramp (1932, 1951), Vervoort (1972), and Calder (1996). Bonnevie (1899) reported several species of hydroids from depths exceeding 1000 m in the Greenland Sea west of the Mohns Ridge, but all were from waters considered more Arctic than Atlantic and are not included here. Synonymy and nomenclature of each nominal species in the preliminary list were updated, and depth data were converted from fathoms or feet to metres where necessary. Reports of hydroids from depths of 1000 m or more have been included in this synopsis, together with an indication of the overall bathymetric range of each species (e.g., neritic-abyssal). Depth data regarded as highly questionable for relatively well known species were omitted. Also excluded were records of hydroids that were dead when collected, or specimens judged allochthonous to lower bathyal and abyssal depths.

Abbreviations

NAAP = North Atlantic Abyssal Province; WAAP = West Atlantic Abyssal Province (abyssal zoogeographic provinces as defined by Vinogradova, 1979).

Species synopsis

Family HYDRACTINIIDAE L. Agassiz, 1862

Stylactaria arctica (Jäderholm, 1902). Baffin Bay (74°41'N 70°30'W), 1200 m (Kramp, 1932: 12). Bathyal-abyssal.

Stylactaria ingolfi (Kramp, 1932). Labrador Basin (60°17'N 54°05'W), 3229 m; (58°20'N 40°48'W), 3192 m (Kramp, 1932: 13). Abyssal. NAAP.

Family EUDENDRIIDAE L. Agassiz, 1862

Eudendrium planum Bonnevie, 1898. Baffin Bay (74°41'N 70°30'W), 1200 m (Kramp, 1932: 20). Bathyal.

Family LAODICEIDAE L. Agassiz, 1862

Modeeria rotunda (Quoy & Gaimard, 1827). Continental slope E of Cape Cod (41°47'N 65°37'30"W), 1238 m; continental slope E of New Jersey (40°01'N 68°54'W), 1170 m (Fraser, 1944: 179). Neritic-bathyal.

Stegopoma plicatile (M. Sars, 1863). Continental slope E of New Jersey (40°01'N 68°54'W), 1170 m; (39°53'N 69°50'30"W), 1127 m; (39°49'30"N 70°26'W), 1097 m; (39°46'30"N 70°14'45"W), 1939 m; (39°47'N 70°30'30"W), 1761 m; continental slope E of Virginia (36°42'N 74°30'W), 1330 m; continental slope E of North Carolina (35°45'43"N 74°31'25"W), 1624 m (Fraser, 1944: 180). Neritic-bathyal.

Family PHIALELLIDAE Russell, 1953

Opercularella spec. Bermuda Pedestal (32°35.0'N 64°54.9'W), 3550 m; (32°34.3'N 64°54.7'W), 3011 m (Calder, 1996). Abyssal. WAAP.

Suborder CAMPANULINIDA Bouillon, 1984 (Family Incertae Sedis)

Eucuspidella pedunculata (Allman, 1877). Windward Passage (20°30'N 73°16'W), 4798 m (Vervoort, 1972: 36). Bathyal-abyssal. WAAP.

Family HALECIIDAE Hincks, 1868

Halecium dubium Fraser, 1941. Continental slope E of New Jersey (40°29'N 66°04'W), 3235 m (Fraser, 1941: 84). Abyssal. WAAP.

Family LAFOEIDAE A. Agassiz, 1865

Acryptolaria conferta (Allman, 1877). Continental slope E of Massachusetts (41°29'45"N 65°35'30"W), 2271 m (Fewkes, 1881: 128). Denmark Strait (64°34'N 31°12'W), 2377 m; (64°44'N 32°52'W), 1785 m (Broch, 1918: 18). Continental slope E of Cape Cod (41°43'N 65°21'50"W), 2394 m (Fraser, 1944: 211). Neritic-abyssal. NAAP/WAAP.

Acryptolaria longitheca (Allman, 1877). Hatteras Abyssal Plain, Northwest Atlantic Basin E of South Carolina (32°34'N 74°21.5'W), 4681 m (Vervoort, 1972: 45). Bermuda Pedestal (32°35.0'N 64°54.9'W), 3550 m; (32°34.3'N 64°54.7'W), 3011 m (Calder, 1996). (?)Neritic-abyssal. WAAP.

Cryptolarella abyssicola (Allman, 1888). Newfoundland Basin (40°33'-40°34'N 35°24'-35°52'W), 4540-4600 m (Kramp, 1951: 121). Bathyal-abyssal. NAAP.

Grammaria abietina (M. Sars, 1851). Denmark Strait (64°44'N 32°52'W), 1785 m; (65°14'N 30°39'W), 1375 m (Broch, 1918: 19). Neritic-bathyal.

Halisiphonia arctica Kramp, 1932. Baffin Bay (74°41'N 70°30'W), 1200 m (Kramp, 1932: 37). Bathyal.

Halisiphonia megalotheca Allman, 1888. Hatteras Abyssal Plain, Northwest Atlantic Basin E of North Carolina (36°23'N 67°58'W), 4680 m; continental rise E of New Jersey (39°37'N 66°45'W), 3806 m (Calder, 1996). Bathyal-hadal. WAAP.

Lafoea fruticosa (M. Sars, 1851). Continental slope E of Labrador (55°37'N 56°08'W), 2078 m (Vervoort, 1972: 66). Neritic-abyssal. NAAP.

Lictorella pinnata (G. O. Sars, 1874). Davis Strait (63°30'N 54°25'W), 1064 m (Broch, 1918: 23). Baffin Bay (74°41'N 70°30'W), 1200 m (Kramp, 1932: 41). Neritic-bathyal.

Family CAMPANULARIIDAE Johnston, 1836

?*Campanularia abyssa* Fraser, 1940. Continental slope E of New Jersey (39°22'50"N 68°25'W), 2941 m (Fraser, 1940: 576). Abyssal. WAAP.

Clytia hemisphaerica (Linnaeus, 1767). Continental slope E of Massachusetts (42°44'N 62°43'W), 1134 m (Fraser, 1944: 140). Doubtful record. Neritic- bathyal.

Family SERTULARIIDAE Lamouroux, 1812

Diphasia fallax (Johnston, 1847). Continental slope E of Labrador (55°37'N 56°08'W), 2078 m (Vervoort, 1972: 103). Neritic-abyssal. NAAP.

Salacia laxa (Allman, 1874). Continental slope E of Labrador (55°37'N 56°08'W), 2078 m (Vervoort, 1972: 189). Neritic-abyssal. NAAP.

Sertularella polyzonias (Linnaeus, 1758). Continental slope E of Massachusetts (42°47'N 61°04'W), 2295 m (Fraser, 1944: 270). Neritic-abyssal. WAAP.

Sertularella tenella (Alder, 1856). Continental slope E of Massachusetts (42°47'N 61°04'W), 2295 m (Fraser, 1944: 274). Neritic-abyssal. WAAP.

Sertularia robusta (Clark, 1877). Continental slope E of Labrador (55°37'N 56°08'W), 2078 m (Vervoort, 1972: 183). Neritic-abyssal. NAAP.

Symplectoscyphus tricuspidatus (Alder, 1856). Denmark Strait (65°14'N 30°39'W), 1375 m (Broch, 1918: 98). Neritic-abyssal.

Tamarisca tamarisca (Linnaeus, 1758). Denmark Strait (64°34'N 31°12'W), 2377 m; (65°14'N 30°39'W), 1375 m (Broch, 1918: 96). Neritic-abyssal. NAAP.

Thuiaria hippuris Allman, 1874. Davis Strait (63°06'N 56°00'W), 2193 m (Broch, 1918: 141). Continental slope E of Labrador (55°48'N 56°00'W), 2452 m (Vervoort, 1972: 187). Bathyal-abyssal. NAAP.

Thuiaria thuja (Linnaeus, 1758). Continental slope E of Labrador (55°37'N 56°08'W), 2078 m (Vervoort, 1972: 185). Neritic-abyssal. NAAP.

Family PLUMULARIIDAE McCrady, 1859

Plumularia attenuata Allman, 1877. Off Grenada, West Indies (no coordinates given), 1053 m (Fewkes, 1881: 128). Neritic-bathyal.

Family HALOPTERIDIDAE Millard, 1962

Polyplumaria profunda (Nutting, 1900). Davis Strait (63°06'N 56°W), 2257 m (Kramp, 1932: 69). Bathyal-abyssal. NAAP.

Family AGLAOPHENIIDAE Marktanner-Turneretscher, 1890

Aglaophenopsis cornuta (Verrill, 1879). Davis Strait (63°30'N 54°25'W), 1064 m (Broch, 1918: 78). Davis Strait (63°36'N 55°15'W), 1200 m (Kramp, 1932: 56). Neritic-bathyal.

Aglaophenopsis verrilli Nutting, 1900. Continental slope E of Virginia (37°41'N 73°03'W), 2738 m; continental slope E of New York (40°34'18"N 66°04'W), 3186 m (Nutting, 1900: 120). Abyssal. WAAP.

Cladocarpus crenatus (Fewkes, 1881). Continental slope E of Massachusetts (41°24'45"N 65°35'30"W), 2271 m (Fewkes, 1881: 132). Abyssal. WAAP.

Cladocarpus flexuosus Nutting, 1900. Gulf of Mexico (28°45'N 88°16'W), 1719 m (Nutting, 1900: 114). Gulf of Mexico (28°15'N 87°02'W), 1000 m; (28°56'N 87°32.7'W), 1829 m (Calder, 1984: 408). Bathyal.

Cladocarpus formosus Allman, 1874. Davis Strait (63°36'N 55°15'W), 1200 m (Kramp, 1932: 63). Neritic-bathyal.

Cladocarpus integer (G. O. Sars, 1874). Davis Strait (63°30'N 54°25'W), 1064 m (Broch, 1918: 82). Neritic-bathyal.

Cladocarpus sigma (Allman, 1877). Continental slope E of Virginia (37°21'N 74°12'W), 1573 m (Fraser, 1943: 95). Neritic-bathyal.

Cladocarpus speciosus Verrill, 1879. Continental slope E of Virginia (37°38'40"N 73°16'30"W), 2602 m (Fraser, 1944: 411). Bathyal-abyssal. WAAP.

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Effects of SCUBA diving on coral reef invertebrates in the U.S. Virgin Islands: implications for the management of diving tourism

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Abstract: The number of visitors to coral reefs worldwide has increased greatly in recent years. The negative impacts of snorkelers, reef walkers and SCUBA divers on corals are only beginning to be understood. Especially needed is information on critical limits of visitor use, above which damage to reefs may sharply rise. To quantify the effects of SCUBA diving on coral reef invertebrates, I examined seven sites in the U.S. Virgin Islands. The frequency of SCUBA dives per site was determined from log books, and varied greatly from 20 to over 400 dives in 6 months. The amount of damage at each site was assessed by counting all damaged and undamaged individuals of each of 6 major types of invertebrates in a 100 m^2 area at 4-7 m depth. Levels of water motion also were measured, and did not correlate significantly with damage to any of the reef organisms. Massive stony corals exhibited low levels of damage (up to 1.5% of individuals), which did not correlate with diving frequency. Erect sponges, and branching stony and soft corals exhibited intermediate levels of damage (up to 21.2%). In both types of branching corals, damage correlated significantly with diving activity on the reefs. Sea fans and branching hydrocorals (*Millepora* spp.) had very high levels of damage on all reefs (up to 70.0%), which did not correlate with diving activity. The results show that sessile invertebrates vary in their vulnerability to damage, and that groups with intermediate vulnerability are the most likely to reveal impacts of SCUBA diving. The data indicate a critical level of about 500 dives per year at each reef site, above which damage to some reef taxa increased greatly. This critical level is lower than those estimated for other coral reefs, and may reflect the fragility of the reefs studied here. Such information concerning the upper critical limits of SCUBA diving and other tourist activities is necessary for the effective management of coral reefs.

Introduction

Tourism associated with coral reefs is rising exponentially world wide, as documented for reef areas in the Caribbean (Tilmant, 1987: 198; Rogers et al., 1988: 405; Dixon, 1993: 39), Australia (Tilmant, 1987: 198; Neil, 1990: 221), and Red Sea (Hawkins & Roberts, 1992a: 1007; 1993: 25; Meshi & Ortal, 1995: 30). Recently, studies have shown significant damage to coral reefs from high levels of recreational SCUBA diving (Riegl & Velimirov, 1991: 249; Hawkins & Roberts, 1992b: 175; Dixon, 1993: 38), reef walking on shallow coral reef flats (Woodland & Hooper, 1977: 2; Neil, 1990: 226; Hawkins & Roberts, 1993: 28), and boat groundings and anchor scars (Rogers et al., 1988: 407). Some studies indicate that there may be a threshold or critical level of visitation on reefs, above which damage rises sharply. Estimates of critical levels of SCUBA diving on coral reefs vary from 4,000-6,000 dives/site/year for Bonaire in the

Caribbean (Dixon, 1993: 38) to 5,000-6,000 dives/site/year for Sharm-El-Sheikh in the Red Sea (Hawkins & Roberts, in press). Determination of carrying capacity or critical visitation level is a first and necessary step for the effective management of popular coral reef areas as viable, sustainable ecosystems.

I present here data from a field study in the U.S. Virgin Islands, which show that: (1) damage to certain reef organisms rises rapidly above a critical level of diving frequency, and (2) reef invertebrates vary widely in their vulnerability to structural damage.

Methods

The present study was conducted during summer 1988 on the south side of St. John, U.S. Virgin Islands, in the area around Greater Lameshur Bay. This area is in a remote location inside Virgin Islands National Park, and contains pristine fringing coral communities (Rogers et al., 1991: 190; Witman, 1992: 643). Few divers were observed to visit the area, except those directly involved in the present study (N. E. Chadwick-Furman, pers. obs.). To measure effects of SCUBA diving, I selected 6 discrete reef sites in the Greater Lameshur Bay area: Coral Gardens and Donkey Bight (protected inner bay sites), Tektite Cove and Yawzi Point (intermediate-exposure sites, described in detail by Edmunds & Witman, 1991: 201; Rogers et al., 1991: 190), and White Point and Cabrite Point (exposed sites; Cabrite Point described in detail by Witman, 1992: 643). I also examined a site at Lesser St. James Island, a small unmarked islet between St. John and St. Thomas Islands, which appeared to receive low frequencies of SCUBA dives due to its remote location.

Data on 3 parameters were collected at each site: numbers of SCUBA dives during the 6 months immediately preceding the study, frequency of damage to coral reef invertebrates, and levels of water motion. To determine SCUBA diving levels, I used log books maintained for each site by students at the Virgin Islands Ecological Research Station (VIERS) during February to July 1988. As explained above, these dives likely represent most if not all diving activity in the Greater Lameshur Bay area during this period.

To assess levels of damage to reefs at each site, I collected data on 6 major types of sessile reef invertebrates likely to incur structural damage (species examined in each group are given in parentheses): (1) massive stony (scleractinian) corals (*Colpophyllia natans, Diploria clivosa, D. labyrinthiformes, D. strigosa, Favia fragum, Montastrea annularis, M. cavernosa, Porites astreoides, Siderastrea radians, S. siderea*), (2) branching stony (scleractinian) corals (*Porites porites*), (3) branching soft (alcyonacean) corals (*Briareum asbestinum, Eunicea* spp., *Muricea* spp., *Plexaura* spp., *Plexaurella* spp., *Pseudopterogorgia* spp.), (4) gorgonian sea fans (*Gorgonia ventalina*), (5) branching hydrocorals (*Millepora* spp.), and (6) erect sponges (*Ectoplasia ferox, Haliclona rubens, H. viridis, Ircinia campana, I. strobilina, Niphates digitalis, N. erecta, Spheciospongia vesparium, Xestospongia muta*). All damaged and undamaged individuals in each group were counted in belt transects laid parallel to shore at 4-7 m depth at each site. Each belt transect measured 50 × 2 meters, thus 100 square meters of reef area were examined at each site. A damaged individual was defined as one with broken or bent branches, abraded skeleton, or tissue loss which exposed the underlying skeleton (after Riegl &

Velimirov, 1991: 250; Hawkins & Roberts, 1992b: 173). Data on sponges were not collected at the Donkey Bight site.

I also determined water motion levels at each site, as this physical factor may cause extensive structural damage to shallow reef organisms (Edmunds & Witman, 1991: 201; Rogers et al., 1991: 189; Witman, 1992: 649). Relative levels of water motion were determined using the clod-card method of Doty (1971: 32). This method provides only relative values, but allows rapid and easy quantification of water motion levels, and was adequate for the purposes of the present study. Clod cards of plaster of Paris (calcium sulfate) were placed at each site at 4 meters depth, and retrieved after 24 hours. The mass lost due to motion-enhanced diffusion was calculated for each clod, yielding an index of water motion (see Doty, 1971: 33, for method of calculation).

Results

Levels of diving activity varied greatly among the sites, from only 20 dives/6 months at Lesser St. James to 400 dives/6 months at Coral Gardens (fig. 1). Frequently-dived areas included both protected (Coral Gardens) and intermediate-exposure

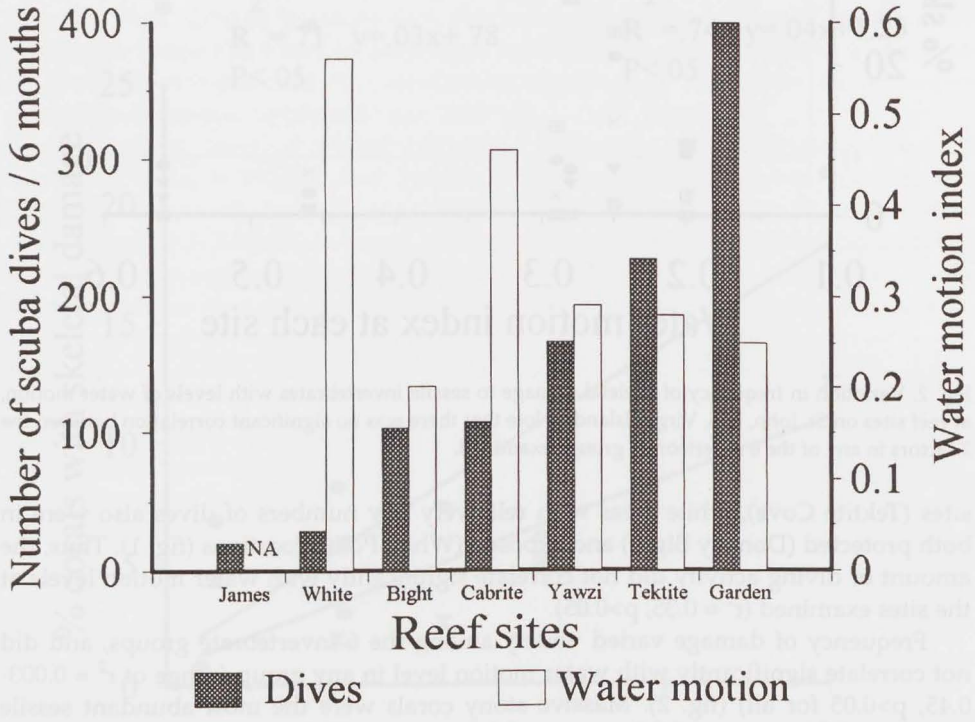

Fig. 1. Variation in levels of diving activity and water motion at seven reef sites. The site at Lesser St James (= James) was on an umarked islet between St. Thomas and St. John, U.S. Virgin Islands. The other 6 sites were on the south side of St. John, U.S.V.I., in the area of Greater Lameshur Bay. NA = not applicable, because water motion was not measured at the Lesser St. James site (= James) due to its remote location. See text for details.

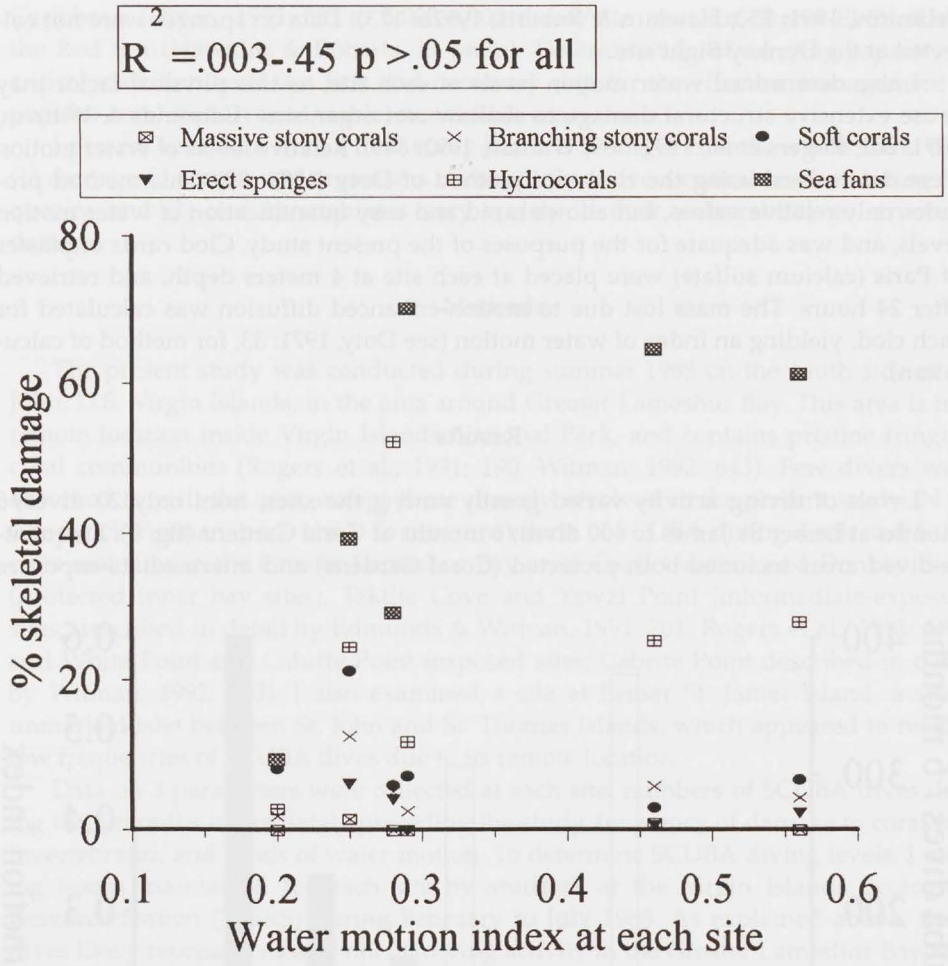

Fig. 2. Variation in frequency of skeletal damage to sessile invertebrates with levels of water motion, at reef sites on St. John, U.S. Virgin Islands. Note that there was no significant correlation between the 2 factors in any of the 6 invertebrate groups examined.

sites (Tektite Cove), while areas with relatively low numbers of dives also were in both protected (Donkey Bight) and exposed (White Point) locations (fig. 1). Thus, the amount of diving activity did not correlate significantly with water motion levels at the sites examined ($r^2 = 0.35$, $p>0.05$).

Frequency of damage varied widely among the 6 invertebrate groups, and did not correlate significantly with water motion level in any group (range of $r^2 = 0.003$-0.45, $p>0.05$ for all) (fig. 2). Massive stony corals were the most abundant sessile invertebrates at all sites examined (range = 135-625 individuals/100 m^2). Massive corals had low levels of damage (range = 0-1.5% of individuals examined), which did not correlate with levels of diving activity ($r^2 = 0.48$, $p>0.05$). Damage to massive corals was observed in the form of abrasion, crushed sclerosepta, and tissue loss.

Only one species of branching stony coral (*Porites porites*) was common on the

reefs examined here (range = 31-69 individuals/100 m^2). *P. porites* had intermediate levels of damage (0-12.5% of individuals), observed in the form of broken branches and tissue loss. Damage to these branching stony corals correlated significantly with SCUBA diving activity (fig. 3).

Branching soft corals were abundant members of the reef community (range = 45-220 individuals/100 m^2).They also exhibited intermediate levels of damage (0-21.2% of individuals), which correlated significantly with diving activity (fig. 3). The type of damage varied with species; in soft corals with thick rigid skeletons (*Briareum asbestinum*), branches were severed and lying on the surrounding substratum. In species with more flexible skeletons (*Plexaura* spp., *Pseudopterogorgia* spp.), the branches were bent or polyps were missing near the branch tips. In one case, a colony of Plexaurella spp. was observed to be almost completely detached from the substratum.

One species of sea fan (*Gorgonia ventalina*) was fairly abundant at the study sites (range = 10-49 individuals/100 m^2). Sea fans had relatively high levels of damage at all sites (9.4-70.0% of individuals), which did not correlate significantly with levels of diving activity (r^2 = 0.05, $p>0.05$). Much damage was observed on sea fans in the

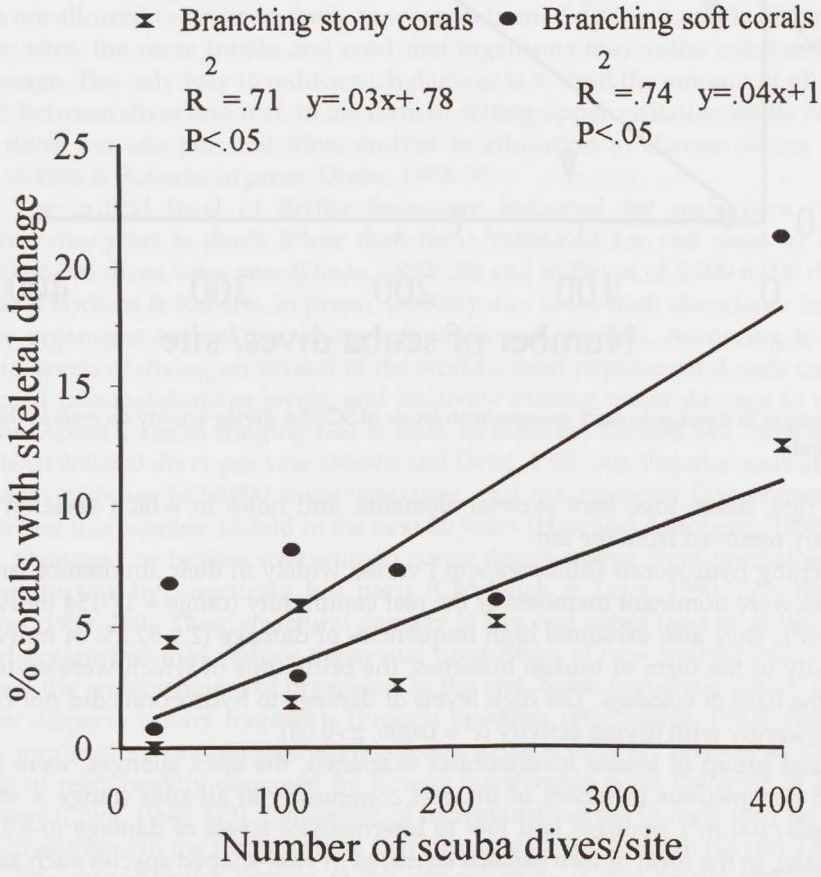

Fig. 3. Variation in skeletal damage to branching stony (Scleractinia) and soft corals (Alcyonacea) with levels of SCUBA diving activity on reefs in the U.S. Virgin Islands.

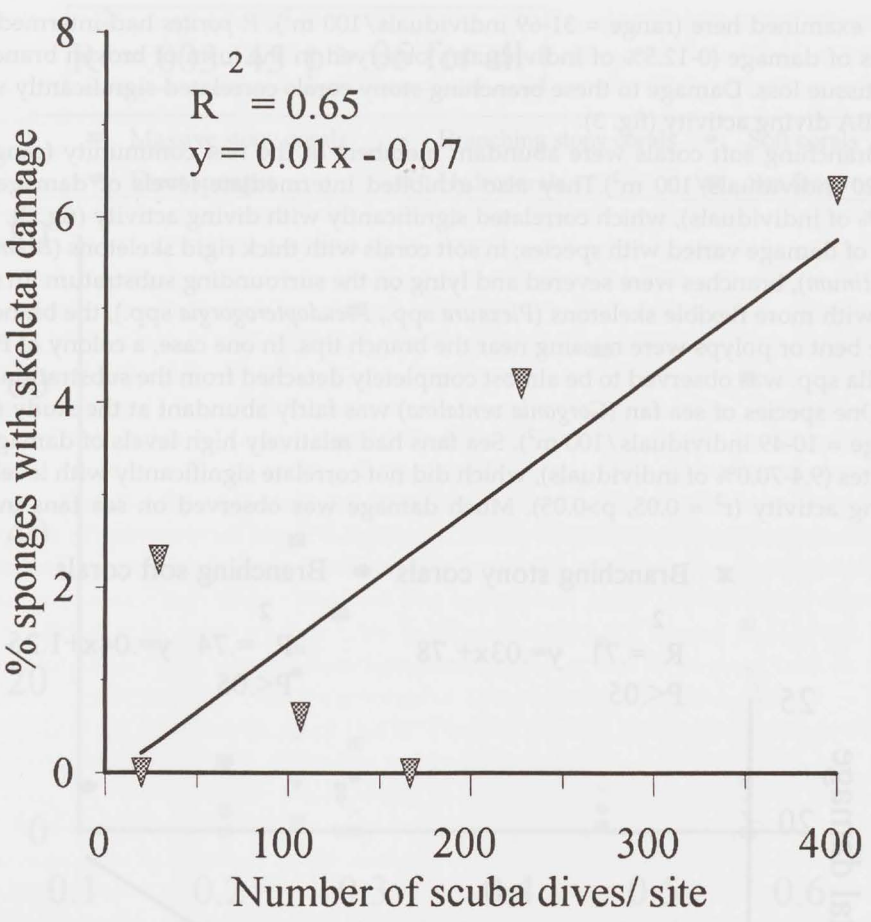

Fig. 4. Variation in damage to erect sponges with levels of SCUBA diving activity on reefs in the U.S. Virgin Islands.

form of rips, tissue loss, bent skeletal elements, and holes in which skeleton was completely removed from the fan.

Branching hydrocorals (*Millepora* spp.) varied widely in their abundance, and at some sites were dominant members of the reef community (range = 17-154 individuals/100 m^2). They also exhibited high frequencies of damage (2.9-52.2% of individuals), mostly in the form of broken branches, the brittle tips of which were scattered around the base of colonies. The high levels of damage to hydrocorals did not correlate significantly with diving activity ($r^2 = 0.006$, $p>0.05$).

The last group of sessile invertebrates examined, the erect sponges, were common and conspicuous members of the reef community at all sites (range = 46-171 individuals/100 m^2). Sponges had low to intermediate levels of damage (0-6.3% of individuals), in the form of torn skeletal elements in vase-shaped species such as *Ircinia campana*, and broken branches in branching species such as *Haliclona* spp.

Damage to sponges increased directly with frequency of SCUBA diving (fig. 4),

but the correlation was not significant ($r^2 = 0.65$, $0.05<p<0.10$), possibly due to the absence of data from one site (see Methods).

Discussion

This study shows that damage to branching soft and stony corals varies directly with levels of SCUBA diving activity on fringing reefs in the U.S. Virgin Islands. At SCUBA diving frequencies higher than about 250 dives/6 months/site (=500 dives/year/site), damage to both types of corals rises substantially (fig. 3), indicating a critical level of diving activity on these reefs. The same phenomenon is observed for sponges, in that damage rises steeply above a critical level of about 200 dives/6 months (fig. 4). Thus, data for 3 different types of reef invertebrates with branching morphologies indicate a common trend of increases in structural damage with levels of human visitation.

These findings have important implications for the management of tourism on coral reefs. They indicate that reef organisms may be able to withstand low levels of diving activity with minimal damage. If, however, high frequencies of SCUBA diving are allowed to occur on reefs, as when unlimited diving access is allowed at popular sites, the more fragile and erect reef organisms may suffer substantial skeletal damage. The only way to reduce such damage is to limit the amount of physical contact between diver and reef, in the form of setting upper visitation limits on numbers of dives per site per unit time, and/or in education to change diving behaviour (Hawkins & Roberts, in press; Dixon, 1993: 39).

The critical level of diving frequency indicated for reefs here (about 500 dives/site/year) is much lower than those estimated for reef areas in Bonaire of 4,000-6,000 dives/site/year (Dixon, 1993: 38) and in Egypt of 5,000-6,000 dives/site/year (Hawkins & Roberts, in press), possibly due to the high abundance here of delicate organisms such as branching soft corals and sponges. According to the above data, levels of diving on several of the world's most popular coral reefs currently far exceed minimal-damage levels, and are likely causing major damage to reef organisms. About 2 km of fringing reef at Eilat, in Israel on the Red Sea, currently receive at least 200,000 dives per year (Meshi and Ortal, 1995: 30). Popular reefs at Sharm-el-Sheikh have up to 50,000 dives/site/year, and the Egyptian Government plans to increase this number 10-fold in the next 20 years (Hawkins & Roberts, 1992b: 1007).

Damaged or broken scleractinian corals divert energy from normal growth and reproduction into repairing lost parts (Chadwick & Loya, 1990: 227; Rinkevich & Loya, 1989: 260). Thus, structural damage to live reef corals (and most likely to other reef organisms) may reduce the sexual production of new individuals, and greatly affect the replenishment of organisms to the reef. Some branching corals, in contrast, may disperse colony fragments through breakage (Highsmith, 1982). Nevertheless, the implications of unlimited diving access to coral reefs are serious, as the recovery rate of reefs heavily damaged by human interference is on the scale of decades or longer (Loya, 1990; 373). Limitation of recreational access to reefs may be viewed as an investment in the resource base that supports diving tourism (Dixon, 1993: 37).

The second major finding of the present study is that sessile reef invertebrates may be ranked according to their levels of vulnerability to structural damage (fig. 5).

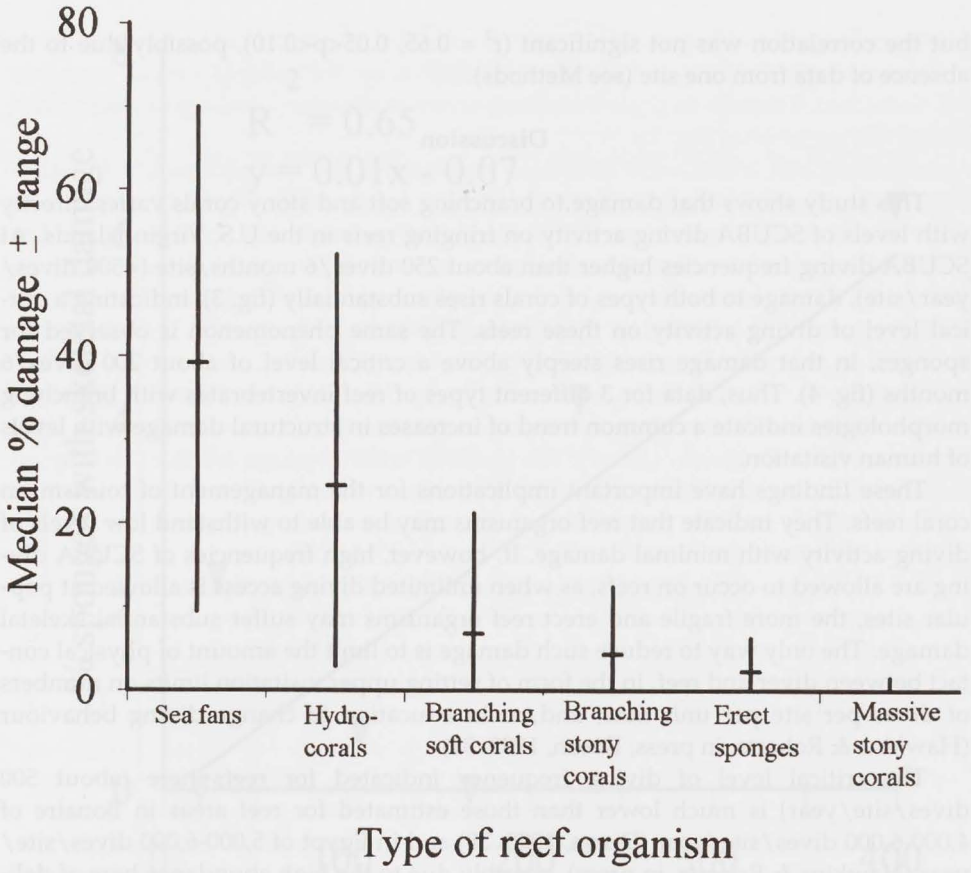

Fig. 5. Vulnerability to structural damage in 6 types of sessile invertebrates on coral reefs in the U.S. Virgin Islands. The groups are ranked according to the range of damage observed in each.

Sea fans and branching hydrocorals were the most vulnerable organisms on the reefs examined here. High damage levels to sea fans and hydrocorals on reefs with low diving activity indicate that such damage may be due to natural causes, including water motion or predation (Harvell & Suchanek, 1987: 41; Lewis, 1991: 101). The 3 invertebrate groups with intermediate levels of vulnerability (branching soft and stony corals, and erect sponges) (fig. 5) all showed a positive relationship between damage and diving activity. Thus, intermediate-vulnerability organisms are the most likely to reveal the effects of SCUBA diving and other human activities that physically impact the reef. Massive stony corals were the least vulnerable to damage of the groups examined here (fig. 5), probably due to their dense skeletons and lack of protruding structures. They are thus less likely to reveal structural effects of human activities, except under conditions of severe reef damage. Other studies have also shown variation in vulnerability to damage among sessile reef invertebrates, and in general have found that branching organisms are much more susceptible to damage than are massive organisms (Woodland & Hooper, 1977: 2; Liddle & Kay, 1987: 1; Rogers et al., 1991: 192; Hawkins & Roberts, 1992b: 176).

The present study has several weaknesses. Firstly, no attempt was made to differ-

entiate human-caused from natural damage to reef organisms. All damage observed was recorded, and if levels covaried with SCUBA diving activity, the damage was assumed to be of human origin. Thus, the data are correlative, and cause and effect may be only inferred. This is a general problem with diver-impact studies on coral reefs, due to difficulties in monitoring and manipulating the number of SCUBA dives. Small-scale field experiments, however, have directly shown negative impacts of human visitation on reef corals (Liddle & Kay, 1987: 17; Woodland & Hooper, 1977: 2). Secondly, studies conducted recently at some of the sites in Greater Lameshur Bay have revealed that, since the present survey was completed in 1988, major hurricanes have caused far greater damage to the reefs than that recorded here, and likely have obliterated most evidence of diver-related damage in the area (Edmunds & Witman, 1991: 202; Rogers et al., 1991: 192; Witman, 1992: 649). Although major marine storms obviously may cause far greater damage than SCUBA divers to reefs, the effects of divers are not benign. Reefs may be weakened by human impacts, and thus less able to withstand or recover from storms or other natural disturbances which follow (Loya, 1990: 372). A third weakness of the present study is that only a small number of sites was examined due to limitation in the SCUBA diving records available. Thus, the findings represent only a small subset of reefs, and may not be applicable to other reef areas with widely different invertebrate communities or ecological conditions. Because of ecological variation between reef communities worldwide, it is important that carrying capacity estimates of reefs for diving be viewed as elastic rather than fixed (Hawkins & Roberts, in press).

In conclusion, the present study clearly indicates that high levels of SCUBA diving activity may cause substantial damage to certain reef organisms, with implications for the structure of the reef community and the health and sustainability of the ecosystem as a whole. It is to be hoped that this type of information will be used by resource managers and environmental planners to set realistic limits of recreational use on coral reefs. Without implimentation of such limits, the most popular reef areas are likely to continue to deteriorate, leading eventually to erosion of the natural resource upon which much tourism is based.

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Biometric investigations on the cnidae of the sea anemone *Actinia equina mediterranea* form I Schmidt, 1971

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Key words: Cnidaria; Actiniaria; *Actinia equina mediterranea* form I; taxonomy; biometry; nematocysts.

Abstract: Biometric parameters of *Actinia equina mediterranea* form I Schmidt, 1971, were correlated with each other to find variables independent of size and thus useful for taxonomic purposes.
Biometric parameters of cnidae such as length, surface area and volume, are significantly correlated with individual size and weight of the anemones. The ratios length/width and surface area/volume of cnidae, were constant over a wide range of anemone sizes, however, and so these are useful characters for taxonomic purposes.

Introduction

One of the most complex and enigmatic of all eukaryotic cells is the cnidocyte with its unique secretory product, the cnida (nematocyst & spirocyst). Cnidae are found in all cnidarians and are diagnostic of the phylum. They cannot be renewed and after discharge they are extruded from the epithelium. Cnidae of different functional regions such as the tentacles, pharynx, filaments, column and basal disc show a characteristic distribution that is often taxonomically important (Carlgren, 1900, 1940; Stephenson, 1929; Weill, 1934; Schmidt, 1969, 1972; Manuel, 1981; Doumenc & Foubert, 1984; England, 1987).

Even though the biometry of cnidae can contribute significantly to the classification of Cnidaria, a number of researchers have questioned the extent to which it can be used to distinguish species. Dunn & Bakus (1977) and Dunn (1981) have reported that the size of cnidae can vary with age or size of the individual. Recently, Östman et al. (1987) found a considerable variation in cnidae biometry for Hydrozoan (Campanulariidae) populations from a variety of Mediterranean and Scandinavian shores, and Doumenc et al. (1989) concluded that the biometry of some types of cnidae (e.g., length and width) was significantly associated with age in the genus *Telmatactis*. Experiments on cnidogenesis in anemone tentacles have so far revealed little evidence for migration. In *Calliactis tricolor* (Lesueur, 1817), Sandberg et al. (1971) and Mariscal (1973) have shown a decrease in nematocyst discharge with increase in the amount of food ingested. Schmidt (1982) found that tentacles of *Anemonia viridis* (Forskål, 1775) (as *A. sulcata* Pennant, 1777) depleted of cnidae regained their normal complement after 5 or 6 days.

Although the above data seem to indicate that a number of physical and biological parameters can influence the number of cnidae present in the tissues of Cnidaria (both qualitative and quantitative, see Fautin, 1989), very few researchers have statis-

tically analysed the influence these parameters can play on the biometry of the cnidae. Additionally, the papers that have dealt with the subject, have relied on small numbers of nematocysts and individuals.

This paper deals with the biometry of cnidae using large samples of both nematocysts (over 8,000) and individuals (20) of the anemone *Actinia equina mediterranea* form I Schmidt, 1971 (see also Schmidt, 1972). The aim of the paper is to discover any correlations between cnidae biometry and size/age of anemones, in an attempt to answer the following questions :

i) Is there a relation between the size of cnidae and size/age of anemones?
ii) Which of the biometric parameters of the cnidae (length, width, surface area, volume, etc.) correlate with the size/age of the anemones, and which do not?
iii) To what extend can the biometry of the cnidae be used as reliable means of classification in sea-anemones, and in Cnidaria in general?

Material and methods

Specimens of *A. equina mediterranea* form I were collected in the spring of 1984 from the mid-shore rocky environment (Pérès & Picard, 1964) of the North Aegean Sea (Gulf of Thermaikos and Thasos Island). Twenty anemones were collected randomly with respect to size, and narcotised in 7.5% solution of $MgCl_2$ (for about 4h) and then preserved in 10% formalin. The following morphological parameters from each individual were measured after preservation: height of column (HC), diameter of oral disc (DOR), diameter of pedal disc (DPD), number of acrorhagi (NAC), number of tentacles (NT), and weight in "crude units of biomass" (WA) (Crisp, 1971). These parameters reflect with satisfactory credibility the maturity grade of the individuals, their different metabolic processes, e.g. energetic costs, or even their relative age (Bourne, 1918; Ottaway, 1979a, 1979b; Doumenc et al., 1989).

The terminology used and the method of cnidae identification are according to Carlgren (1940), Cutress (1955), and England (1987, 1991). Basitrichs are nematocysts with a relatively slender capsule and a tube armed with large proximal spines and small distal spines (England 1991). Atrichs have a thread with neither a differentiated basal shaft nor barbs (at least not discernable with optical means), and a uniform diameter (Carlgren, 1940; Cutress, 1955; see, however, Schmidt, 1969, 1972). Microbasic p-mastigophores are nematocysts with proximal shafts less than three times the capsule length and abruptly reduced to a distal thread. The armature of the shaft is distinctly longer than that of the thread (Cutress, 1955; England, 1991). Spirocysts are a major category of cnidae possessing a tube devoid of spines, which bears, when discharged, a left-hand spiral of glutinous substance (Manuel, 1981).

Squash preparations, with a drop of 7.5% formalin, were prepared from small portions of preserved tissue, from different functional regions of the anemones' bodies, in order to identify the types of nematocyst present and to measure their biometric parameters. The types of cnidae used for comparison were: basitrich and spirocyst from tentacles and pharynx; atrich, basitrich and spirocyst from acrorhagi and column; and basitrich and microbasic p-mastigophore from mesenterial filaments. Forty undischarged nematocysts of each type were measured and their length (L) and width (W), and the length of the shaft (L.sh), were recorded. This procedure was

carried out separately for each individual anemone and each examined body part. All measurements were taken using an optical microscope (magnifications with oculars 10 × and objective 100 ×) and a camera lucida. The length and width of the nematocysts, and the surface area (SA) and volume (VOL) of every type of nematocyst, were calculated using the mathematical formula of Thomason (1989).

$$SA = 2\pi (W / 2 L - W) + 4\pi (W / 2)^2$$
$$VOL = 4 / 3\pi (W / 2)^3 + \pi (W / 2)^2 (L - W)$$

where: SA = surface area; VOL = volume; L = length; W = width.

As the distribution of the data was unknown, a non-parametric test was required. Any relationships between anemone weight (WA) and the means of nematocyst parameters (length, surface area, volume, L.sh, and the ratios SA/VOL, L/W, and L/L.sh) were determined using the non-parametric Spearman's rank correlation coefficient, usually abbreviated as r_s ($p = 0.05, 0.01$), especially suitable for small data sets (Siegel 1956, Dagnelie 1986, Brown & Downhower, 1987). This was done in order to find out if the weight and other morphological parameters of the animals were associated with cnidae size.

The diameter of the pedal disc or the column of anemones, and also the number of the acrorhargi, have been used by Ayre (1984) and Ottaway (1978, 1979a, 1979b) as indicative characteristics in the definition of size class and similarity of the maturity of the individuals of *Actinia tenebrosa* Farquhar, 1898. Juveniles of *A. tenebrosa* were usually 2-11 mm long and adults were usually 17-36 mm (Ottaway, 1979a). These characteristics were also used by Quicke et al. (1983; 1985) and Donoghue et al. (1985), in their study of *A. equina*.

Results

1. Biometry of anemones (tables 1, 2).— The results of the correlation of the anemones' biometric parameters are summarised in tables 1 and 2. All the parameters show some degree of positive correlation, though values of r_s are not significant ($r_s < 0.46$, $p > 0.05$) for HC with DOR, HC with DPD, HC with NT and HC with NAC (table 2).

In general, the results of the correlations are as expected, especially the correlations with the weight of the anemones, which is positively correlated with all the other parameters estimated. This indicates that the weight of the individual is a repre-

Table 1. Morphological variables of 20 individuals of *Actinia equina mediterranea* form I. Abbreviations: HC = height of column, DOR = diameter of oral disc, DPD = diameter of pedal disc, NAC = number of acrorhagi, NT = number of tentacles, and WA = weight.

	HC (mm)	DOR (mm)	DPD (mm)	NT	NAC	WA (gm)
mean	16.4	11.16	18.19	162	52	4.14
standard deviation	5.03	6.6	7.2	40.7	28.44	2.9
standard error	1.12	1.47	1.61	9.1	6.4	0.6
range	4.15-23.2	2.3-22.6	3.25-30.1	102-241	13-122	0.1-10.3

Table 2. Spearman's rank correlation coefficients among morphological variables of 20 individuals of *Actinia equina mediterranea* form I. Abbreviations: HC = height of column, DOR = diameter of oral disc, DPD = diameter of pedal disc, NAC = number of acrorhagi, NT= number of tentacles, and WA = weight. **Bold numbers**: there is no correlation ($p > 0.05, 0.01$).

	HC	DOR	DPD	NT	NAC	WA
HC		**0.18**	**0.39**	**0.25**	**0.45**	0.73
DOR			0.77	0.52	0.82	0.74
DPD				0.47	0.78	0.82
NT					0.47	0.48
NAC						0.86
WA						

sentative parameter for anemone size. A certain body weight, though, may not be the result of a normal development, but also the result of a better nutritional state or of lack of parasitism, and other ecophysiological parameters (Shick 1992).

2. Correlations of cnidae biometry with weight of anemones (table 3).— Table 3 gives the correlations between variables of the cnidae and of weight. The lengths of the cnidae, except for basitrichs of the actinopharynx, were found to be positively correlated with weight.

Only the surface area of the microbasic p-mastigophores of the filaments and the basitrichs of the actinopharynx were not correlated with weight.

In respect to the correlation between the L/W ratio and weight, it was found that only the basitrichs of the filaments showed a correlation with weight ($p < 0.05$).

In respect to the correlation between the SA/VOL ratio and weight, it was only present in the basitrichs of the column ($p < 0.05$) and of the filaments ($p < 0.01$), and in the atrichs of the acrorhagi ($p < 0.05$).

The correlation between shaft length of microbasic p-mastigophores and weight was significantly negative ($r_s = -0.54$, $p < 0.05$), whereas no correlation was found between the length of the capsule of microbasic p-mastigophores and shaft length ($r_s = 0.15$). These results show that shaft biometry is not dependent on capsule length. The ratio of capsule length to shaft length is significantly correlated with weight ($r_s = 0.70$, $p < 0.01$).

Discussion

A number of theories concerning the relation of the biometry of the cnidae with size, age and various physiological functions exist. Actinians are equipped with different types of cnidae which function in food capture, defence and aggression. Mariscal (1973, 1974), Fautin (1989) and Robson (1989) reported that various biological factors can affect the categories of cnidae present in individual actinians. Östman et al. (1987) reported that differences in the dimensions of the nematocysts of species of the hydroid family Campanulariidae, from one geographic area to another, may be caused by differences in metabolism.

Schmidt (1982) showed experimentally that, after discharge, nematocysts are replaced

Table 3. Spearman's rank correlation coefficients between variables of the cnidom and weight of individuals of *Actinia equina mediterranea* form I. Abbreviations: L = length, SA = surface area, VOL = volume, W = width, DRB = different functional regions. **Bold numbers**: there is no correlation ($p > 0.05, 0.01$).

Type of cnida/DRB	Length	Surface area	Volume	L/W ratio	SA/VOL ratio
b-mast. of tentacles	0.76	0.60	0.56	**0.15**	**-0.45**
spirocyst of tentacles	0.58	0.48	**0.44**	**0.14**	**-0.40**
atrich of acrorhagi	0.63	0.69	0.66	**0.00**	-0.50
b-mast. of column	0.52	0.58	0.56	**-0.06**	-0.55
b-mast. of actinopharynx	**0.20**	**-0.02**	**-0.06**	**0.21**	**0.07**
p-mast. of filaments	0.48	**0.43**	**0.42**	**-0.18**	**-0.38**
b-mast. of filaments	0.64	0.70	0.72	-0.49	-0.72

in 5 to 6 days. The absence of some types of nematocyst from the tissues of some sea-anemones, may lead to classification problems. No correlations between physiological functions of the organisms (such as reproductive periods, food gathering ability, defence, etc.) and nematocyst biometry have yet been proven. Nevertheless, the majority of cnidarian taxonomists still regard the biometry of cnidae as a crucial morphological characteristic for the classification.

As can be seen, the importance of the biometric characteristics of the anemone's cnidae may be evaluated at two levels. At one the biometry is an important characteristic which can serve as a sound base for classification and taxonomy, while the other has some reservations about their value in this regard. Some authors have suggested the use of certain statistical tools in the study of cnidae morphology (such as the median, mean, standard deviation and range), in order to overcome the differences in morphology used (Schmidt, 1972; Doumenc et al., 1985, and others). Unfortunately, no standardized methods or techniques have been evaluated, resulting in the inability to use parametric data for classification and taxonomy.

Our study shows that length, surface area and volume of several of the cnidae do not constitute a significant morphological characteristic of *Actinia equina mediterranea* form I, as these parameters are related to body weight. On the other hand, because the size of the basitrichs of the pharynx is not related to weight, these nematocysts provide a significant morphological characteristic: the biometry of this type of nematocyst could be considered as a stable characteristic of *Actinia equina mediterranea* form I. The cnidae ratios L/W and SA/VOL, unlike length, surface area and volume of cnidae (table 3), are in most cases independent of weight.

It should be noted that this is the first time that a positive correlation between the length of the shaft and weight is demonstrated. Also positively correlated were the capsule length/shaft length ratio with weight. No correlation was observed between capsule length and shaft length. These results seem to imply that the length of the shaft is not dependent on the size of the capsule, which means that these two parameters are independent of each other. This study has also shown that the length of the shaft is negatively correlated with body weight, i.e., the filaments of large individuals contain large microbasic p-mastigophores with short shafts.

Conclusion

From the present study the following conclusions can be drawn:

i) Of the biometric parameters investigated (see materials and methods) in relation to body weight it was found that the most dependable parameters were the ratio L/W followed by SA/VOL. These parameters could be considered as reliable morphological characteristics of species.

ii) For this type of biometry to be used constructively in sea-anemone classification, their stability in relation to the sample size and the conditions under which the animals were found must be known. Additionally, the type of food remains present in the coelenteron, and/or the presence of symbiotic organisms, could be helpful in establishing a more precise picture of the behavioural and physiological states of the organisms.

iii) The use of parametric and non-parametric statistical methods for analysing quantitative data from cnidae for use in the classification of Cnidaria, is both desirable and attainable.

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Keys to the genera of Cubomedusae and Scyphomedusae (Cnidaria)[1]

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Key words: Scyphomedusae; Cubomedusae; identification.

Abstract: Provisional identification keys are provided to the genera of Cubomedusae and Scyphomedusae of the world. The keys have largely been prepared from the literature. Wherever possible the characters used are easy for a novice to see, and many are consequently non-systematic. It is thought that no generic key to either group world-wide exists already. Difficulties in their construction are mentioned, these mainly being availability of literature. The Natural History Museum in London is one of perhaps half a dozen places world-wide where a convenient selection of the necessary literature can easily be seen. Literature availability continues to hold up systematic research on the Scyphomedusae, but Cubomedusae literature is mostly later and easier to obtain.

Introduction

The available revisions of the Cubomedusae and Scyphomedusae of the world are those of Mayer (1910), Kramp (1961) and Franc (1995a, 1995b). Though all these works include generic diagnoses useful to systematists able to interpret anatomical details, none is helpful to a non-specialist making identifications.

Lobanov et al. (1995) described a computer-based key to all the medusa families. However, though logically conceived, it will not be useful in routine identification of individual specimens beyond family level. Family definitions can also be obtained and compared from Kramp (1961).

The present keys may be the first to include all the genera. Genera, of course, do not exist in nature. Essentially, the diagnosis of each reflects a hypothesis that the included species are closely related. But in practice the convenience of the grouping, and the idiosyncrasies of authors, also play their parts in genus definition. Hence some cubomedusan genera may alter in scope in the future. It is relevant that some, though poorly known, are monotypic or nearly so. In contrast many of the scyphomedusan genera have received much attention and refinement during and after Mayer's time and may prove more stable. In this group most of Kramp's (1961) generic definitions are still accepted though much revision has still to be done on the nominal species he listed. Still, some of the nominal scyphomedusan genera included in the keys have been considered dubiously based (e.g. *Leptobrachia*, by Kramp, 1961) or have seldom been reported and then only inadequately, but they are provisionally included in case some might prove valid when material becomes available. These are marked with an asterisk (*).

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The keys are easy to use. They are almost entirely based on characters that are readily seen in both live and preserved specimens, and mostly not on characters important systematically or on those absent from many specimens such as features of the gonads. Consequently, some genera key out in more than one place.

Accounts of the general anatomy and biology of the groups have been provided by Russell (1970, Scyphomedusae), Werner (1984, Cubomedusae and Scyphomedusae), Franc (Scyphomedusae, 1995a; Cubomedusae, 1995b) and Arai (1997, Scyphomedusae). Literature on the taxonomy is still so scattered that to list even just the latest items relevant to each genus would be a substantial bibliographic undertaking, and space does not allow it here. The most recent taxonomic accounts of the species of Scyphomedusae are those by Mayer (1910, partially illustrated) and Kramp (1961, comprising diagnoses and literature lists, not illustrated), and of the venomous species of Cubomedusae that by J. Rifkin, in Williamson et al. (1996). An internal report on the SE Asian species has recently been prepared by Heeger (1994). The abstracting journal Zoological Record also provides an index to major literature items.

Several genera of hydromedusae include species which attain diameters of 5 cm or more, thus exceeding in size many Scyphomedusae and hence superficially resembling them. Most, with the common and notable exception of *Aequorea*, are distinguished from the present groups by having just four radial canals and the tentacles inserted directly onto the bell. *Aequorea* has several dozen radial canals. Cubomedusae have four radial canals but their tentacles are inserted on pedalia, which have no counterpart in hydromedusae. Synopses of the hydromedusan genera were provided by Bouillon (1985, without illustrations; 1995, partially illustrated) and identification keys with illustrations of the species then known by Kramp (1959, 1968).

Terms used in the keys are explained in the glossary (pp. 116-121) and in figs 1-2.

The author will welcome comments on and corrections of the keys so that a better version might be produced in the future.

Key to the four Orders

1. Without marginal tentacles 2
- With marginal tentacles on or near perimeter of bell or disc [do not confuse with elongate marginal lappets of *Lobonema* (fig. 2C), for which go to key to **Rhizostomeae** (p. 113)] 3
2. Mouth-arms fused somewhere along their length; bearing mouths ..... **Rhizostomeae** (p. 113)
- Mouth-arms not fused; not bearing mouths ..... Genera *Deepstaria*, *Poralia*, *Stygiomedusa* (**Semaeostomeae**)
3. Tentacles 4, or in 4 groups of 2-15 or a few more ..... **Cubomedusae** (p. 110)
- Tentacles more than 4 and not in 4 groups 4
4. With mouth-arms (fig. 2A) ..... **Semaeostomeae** (p. 112)
- Without mouth-arms (fig. 1F) ..... **Coronatae** (p. 111)

Key to the genera of Cubomedusae

1. Tentacles 4, or in 4 groups of 3 or 2, but each tentacle inserted separately on its own pedalium (family Carybdeidae) (fig. 1G) 2

- Tentacles in 4 clusters each on a branched, roughly palmate pedalium; in all but smallest specimens several tentacles in each cluster (family Chirodropidae) (fig. 1E) 6
2. One tentacle at each corner 3
- Two or three tentacles at each corner ..... *Tripedalia*
3. Tentacles forked (dubious genus possibly based on an unusual specimen) ..... *Manokia**
- Tentacles not forked 4
4. Lacking gastric cirri ..... *Carukia*
   Having gastric cirri (usually visible on floor of stomach, through transparent top of bell) 5
5. Gastric cirri in brush-like bundles at corners of stomach or in roughly crescent-shaped areas extending *horizontally*; opening to rhopalar niche Y-shaped or a vertical slit like a keyhole (fig. 1I) ..... *Carybdea*
- Gastric cirri in interradial bands extending *vertically* along walls of stomach; opening to rhopalar niche a roughly horizontal slit (fig. 1H) ..... *Tamoya*
6. Three nominal genera: *Chironex, Chiropsalmus* and *Chirodropus*. Species referred to the first two genera are frequently responsible for human deaths. But despite intense medical and recreational interest in them, the characterization of all three genera is currently debated and simple key characters cannot be confidently included. The species are described and discussed by Rifkin (in Williamson et al., 1996).

Key to the genera of Coronatae

1. Bell as tall as wide, or taller 2
- Bell less tall than wide 4
2. Top of bell flat; sides vertical, fluted longitudinally; coronal groove (fig. 1F) near top of bell [thimble-sized, often swarming] ..... *Linuche*
- Top of bell domed to pointed; sides sloping, without longitudinal fluting; coronal groove not near top of bell 3
3. Tentacles 4 ..... *Pericolpa*
- Tentacles 12 ..... *Periphylla*
4. Tentacles more than 16 in adult 5
- Tentacles fewer than 16 in adult 7
5. Marginal lappets (fig. 1F) twice as many as tentacles ..... *Atolla*
- Marginal lappets about equal in number to tentacles 6
6. Tentacles 28, marginal lappets 32 ..... *Nauphantopsis*
- Tentacles 20, marginal lappets 24 ..... *Periphyllopsis*
7. Rhopalia (fig. 1F) 4, tentacles 12 ..... *Paraphyllina*
- Rhopalia more than 4, tentacles 6 or 8 8
8. Tentacles 6 ..... *Atorella*
- Tentacles 8 9
9. (Mature specimens only) Gonads 4 ..... *Palephyra*
- (Mature specimens only) Gonads 8 ..... *Nausithoe*

Key to the genera of Semaeostomeae.

The species of some genera key out at differing places. These genera are indicated by a +.

1. Marginal tentacles (figs 1F, 2A) longer than disc diameter (may be contracted if preserved) 2
- Marginal tentacles shorter than disc diameter, or absent 10
2. Tentacles arising from very edge of disc, albeit between lappets 3
- Tentacles arising some way in from edge of disc, in some only a short distance 7
3. Marginal tentacles in adult 16 or fewer 4
- Marginal tentacles in adult 24 or more 5
4. Marginal tentacles 8 ..... *Pelagia*
- Marginal tentacles 16 ..... *Sanderia*
5. Ring canal absent (fig. 1A) ..... *Chrysaora*+
- Ring canal present (figs 1C, D) 6
6. Marginal lappets in mature specimens 32 ..... *Floresca**
- 48 ..... *Discomedusa*
- 64 ..... *Parumbrosa**
7. Tentacles not grouped in clusters; arising across most of central region of subumbrella ..... *Drymonema*
- Tentacles in 8 clusters; situated near edge of subumbrella 8
8. Bases of tentacles in U-shaped clusters (or in young specimens in compact patches) ..... *Cyanea*
- Bases of tentacles in straight clusters 9
9. Mouth-arms much longer than disc diameter; sides of mouth-arms extended into curtain-like folds ..... *Desmonema*
- Mouth-arms much shorter than disc diameter; probably lacking curtain-like folds ..... *Sthenonia**
[Problematic taxon, not reported since 1829.]
10. Marginal tentacles 72 or fewer, none in a few species 11
- Marginal tentacles 100 or more 18
11. Marginal tentacles absent 12
- Marginal tentacles present 14
12. Jelly solid; exumbrellar surface having coronal groove (fig. 1F) ..... *Stygiomedusa*
- Jelly thin and delicate; exumbrellar surface lacking coronal groove 13
13. Gastrovascular system a network ..... *Deepstaria*
- Gastrovascular system with distinct radial canals ..... *Poralia*+
14. Marginal tentacles 16 or fewer 15
- Marginal tentacles 24 or more 16
15. Marginal tentacles 8 ..... *Ulmaris*
[Bell flat; marginal lappets 16; reported only from St Helena & Malaya; possibly young stage of *Diplulmaris*.]
- Marginal tentacles 16 ..... *Diplulmaris*
[Slightly domed, transparent, top surface warty; like *Aurelia* but marginal tentacles thicker, 16 only, spaced apart; radial canals restricted to outer $^1/_3$ or so of bell radius. May be oceanic rather than coastal; distribution believed world-wide.

16. Stomach occupying roughly half disc diameter; with anastomosing radial canal network in outer $^1/_3$ only (fig. 1D); the 4 gonads forming a single, almost continuous, ring around stomach ..... *Discomedusa*
- Stomach occupying central $^1/_3$ only; no anastomosing radial canals in outer region 17
17. Mouth-arms 4 - c. 18, shorter than bell diameter; having radial canals ... *Poralia*$^+$ [Typically deep-water, possibly also in upwellings. Minute dark reddish pigment granules in mesoglea.]
- Mouth-arms 4, longer than bell diameter; no radial canals, having instead broad extensions of stomach, the stomach pouches (tentacles abraded) ..... *Chrysaora*$^+$ [Typically colourful, may have radiating lines or triangles extending outwards from near centre of bell; mouth-arms hanging conspicuously below.]
18. Marginal tentacles arising some way in from edge of disc ..... *Phacellophora*
- Marginal tentacles arising from edge of disc 19
19. Mouth-arms forked ..... *Aurosa**
- Mouth-arms not forked ..... *Aurelia*

Key to the genera of Rhizostomeae

The species of some genera key out at differing places. These genera are indicated by a $^+$.

Some rhizostome species have mouth-arm clubs and filaments that either autotomize following mechanical disturbance (for example the terminal clubs of *Mastigias papua*) or which may be grazed by fish (for example the filaments of *Acromitus flagellatus*), leading to their total absence in a few specimens. Despite such occasional specimens, it is assumed in the key that at least some of these helpful structures remain. In practice all but a few specimens will have at least some remaining. Exceptions are, again, *Mastigias papua* (see for example Mayer, 1910: fig. 415), and *Pseudorhiza haeckeli*, both of which may lack the normally conspicuous terminal club(s).

1. Exumbrella surface essentially smooth, its contour not interrupted (fig. 2B) 2
- Exumbrella not smooth, its surface finely granular or with protuberances (fig. 2D), warts, or grooves which may branch; or with a more or less delimited central mound; or having other interruptions, perhaps fine, to its smoothness or contour 20
2. Having clubs, albeit short, or filaments, terminally on mouth-arms (figs 2B-D) 3
- Lacking such structures terminally 9
3. Bell flat, or rounded but not hemispherical 4
- Bell hemispherical or nearly so 6
4. Mouth-arms directed sideways, and having lateral branches; in life typically resting upside-down on sea bed ..... *Cassiopea*$^+$ (most species)
- Mouth-arms hanging down, not having lateral branches; not resting upside-down on sea bed 5
5. Mouth-arms having filaments (figs 2C-D) on sides, and terminal whip-like clubs on ends ..... *Acromitus*$^+$
- Mouth-arms having no filaments on sides, and terminal clubs thicker than whip-like (fig. 2B) ..... *Mastigias*$^+$

6. Mouth-arms having scapulets (fig. 2B) 7
- Mouth-arms lacking scapulets 8
7. Mouth-arms having numerous small clubs or filaments (figs 2C-D), and in most species also a terminal club (fig. 2B); intracircular canal network (fig. 1D) with fine meshes ..... *Rhopilema*+
- Mouth-arms each having a large terminal club but no other clubs (fig. 2B), and no filaments; intracircular canal network with meshes few and large ..... *Rhizostoma*
8. Mouth-arms broad laterally, branching pinnately; terminal region recurved, J-shaped, with membrane joining arms of J; in older specimens the membrane having 2-4 'windows' or fenestrae (fig. 2C) ..... *Phyllorhiza*
- Mouth-arms narrow laterally, not branching pinnately; without distal recurved portion and lacking membrane and 'windows' (fig. 2B) 42
9. Having filaments or clubs along the mouth-arms amongst the mouths (fig. 2C) ... 10
- Lacking filaments and clubs in these positions 14
10. Bell flat; in life, exumbrellar colour-pattern with a radial component..... ..... *Cassiopea*+ (some species)
- Bell domed, at least in central region; ?typically lacking a strong radial component to its colour pattern 11
11. Exumbrellar pattern dominated by roughly circular pale patches, irregular in size and distribution (except around margin where somewhat regular) [Damaged specimen, lacking mouth-arms clubs] ..... *Mastigias*+

Exumbrellar pattern not so 12

12. Bell a high, smooth dome surrounded by a wide 'gutter' (fig. 2D) ..... *Cotylorhiza*
- Exumbrella evenly rounded, lacking dome in centre (fig. 2B) 13
13. Alternate radial canals communicating with canal anastomosis just in from intermediate ring canal, and connected to circular canal alone (fig. 1D) .... *Acromitus* +

[A nominal *Acromitus* species in which the mouth-arms reportedly lack a long, whip-like terminal club.]

- All radial canals connected peripherally to ring canal alone .... *Crambione*
14. Mouth-arms longer than bell diameter, possibly only just longer in non-mature specimens; genital ostia 3-4 times as wide as pillars between ..... *Thysanostoma*+
- Mouth-arms equal to or shorter than bell diameter; genital ostia roughly same width as pillars between, or narrower 15
15. Bell roughly flat ..... *Acromitoides*
- Bell roughly hemispherical 16
16. Mouth-arms neatly conical, their overall outline even and in some species tapering gradually downward; bunched together along their length ..... *Catostylus*+
- Mouth-arms not so, their overall outline uneven 17
17. Marginal lappets (fig. 1F) in adult c. 5-10 (or, in one genus, up to 5 'doubles') in each octant 18
- Marginal lappets in adult about 16 in each octant; exumbrella completely smooth 19
18. Bell up to c. 70 mm diameter; exumbrella flatter than a hemisphere, having numerous fine nematocyst warts; scapulets absent ..... *Mastigietta*
- Bell so far recorded up to c. 400 mm diameter; exumbrella deeper than a

hemisphere, 'covered evenly with fine granules'; scapulets present .... *Eupilema*+
19. Bell-shape slightly more than a hemisphere ..... *Stomolophus*
[Known from NW Atlantic, also parts of N Pacific including Japan, perhaps in some areas by introduction.]
- Bell-shape slightly less than a hemisphere ..... *Pseudorhiza*
[A specimen lacking its single mouth-arm club; known only from Australia.]
20. Exumbrellar surface with radial grooves or a network of grooves 21
- Exumbrellar surface with minute to large papillae, warts, swellings or fine granulations; or with a central delimited mound 27
21. One or more mouth-arms rather or much longer than bell diameter 22
- Mouth-arms all about equal to or less than bell diameter in length 24
22. One mouth-arm longer than bell diameter, its length due to enormous terminal club (may be missing) ..... *Pseudorhiza*
- All mouth-arms as long as or longer than bell diameter (some may be missing from some specimens) 23
23. Mouth-arms having mouths all along length; exumbrellar surface without furrows ..... *Thysanostoma*+
- Mouth-arms with terminal region devoid of mouths, there being either a terminal club or the terminal $^1/_4$ lacking mouths; possibly having exumbrellar furrows extending up from clefts between lappets (Mayer, 1910) or other furrows on exumbrellar ..... *Leptobrachia**
[Seldom reported; identity problematic. Reported exumbrellar furrows considered by Kramp (1961) possibly caused by net during collection.]
24. Bell roughly hemispherical 25
- Bell slightly to much flatter than a hemisphere 26
25. Outline of mouth-arms somewhat ragged, with numerous small clubs projecting ..... *Versuriga*
- Outline of mouth-arms neat, tapering slightly; no clubs projecting ..... *Catostylus*+
26. Exumbrellar surface with radiating ribs ..... *Lychnorhiza*
- Exumbrellar surface with anastomosing network of grooves having elevations between ..... *Versuriga*
27. Exumbrellar surface with conspicuous papillae 28
- Exumbrellar surface finely granular or finely warty; no conspicuous papillae 30
28. Papillae present all over exumbrella ..... *Lobonema*
- Papillae present just in centre of exumbrella, or just towards edge 29
29. Exumbrellar protuberances peripheral ..... *Catostylus* +
- Exumbrellar protuberances central ..... *Cephea*
30. Exumbrellar surface having warts 31
- Exumbrellar surface finely granular 35
31. Mouth-arms having terminal club (fig. 2B) ..... *Anomalorhiza*
- Mouth-arms lacking terminal club (four poorly known nominal species, some of uncertain validity) 32
32. Mouth-arms without filaments or clubs 33
- Mouth-arms having filaments or clubs (figs 2B-D) 34
33. Mouth-arms shorter than disc radius ..... *Mastigietta*
- Mouth-arms as long as disc diameter ..... *Lychnorhiza*

34. Mouth-arms as long as disc radius; 8 (i.e. 2 × 4) marginal lappets in each octant ..... *Lychnorhiza*
- Mouth-arms as long as umbrella diameter; 4 large marginal lappets in each octant ..... *Lychnorhiza* +
35. Bell domed, to hemispherical or nearly so 36
- Bell much flatter than a hemisphere 40
36. Having filaments arising along the mouth-arms (fig. 2C) 37
- Lacking filaments along the mouth-arms 38
37. Mouth-arms having scapulets (fig. 2B) ..... *Rhopilema*+
- Mouth-arms lacking scapulets ..... *Acromitus*+
38. One or more mouth-arms having long terminal club (which may be lost) ..... *Pseudorhiza*
- Mouth-arms without terminal clubs 39
39. Mouth-arms having scapulets (fig. 2B); turned outward distally, projecting below bell margin $^1/_3$ or less so far as bell height ..... *Eupilema*+
- Mouth-arms lacking scapulets; hanging vertically downward, neatly conical, not out-turned, projecting $^2/_3$ or more so far as bell height ..... *Catostylus*+ (some species)
40. Mouth-arms narrow, outline parallel-sided, in adults much longer than diameter of umbrella ..... *Thysanostoma*+
- Mouth-arms broad, tapering distally, just slightly longer than diameter of umbrella, or shorter 41
41. Mouth-arms broad, much folded ..... *Lychnorhiza*
- Mouth-arms compact, their outline roughly conical or pyramidal, albeit in some species with long filaments projecting from the overall contour ..... *Acromitus*+
42. Marginal lappets parallel-sided, 4-8 times longer than wide, together forming a distinct skirt around edge of bell; exumbrella uniformly coloured, or with typically dark, irregular blotches of pigment ..... *Crambionella*
- Marginal lappets semi-circular in outline, not forming distinct peripheral skirt; exumbrellar pigment in most species typically in pale, roughly circular patches with smooth outline ..... *Mastigias*

Glossary
(figs 1-2)

Only terms used in the present article are included. For explanations of other terms for Scyphomedusae see Russell (1970) and Arai (1997), and for Cubomedusae Southcott (1967) and Rifkin, in Williamson et al. (1996); and for both groups Werner (1984) and Franc (1995a, 1995b). Another glossary is available in Stachowitsch (1992).

adradial - See perradial.

anastomosing network - See radial canal.

bell - The dome-shaped body of a medusa, excluding both structures hanging down from it and the marginal tentacles. There is no clear distinction between bell, umbrella and disc, the last two being typically applied to the flatter forms. The exumbrella, or exumbrellar surface, is that of the upper and outer side; the subumbrella, or subumbrellar surface, the lower or underside. The space enclosed by

a bell of convex shape is the subumbrellar cavity, which is in continuity with the surrounding sea-water.

circular canal - See radial canal.

cirri, gastric - See gastric cirri.

club - A club-shaped extension (lateral or terminal) of the mouth-arm, typically differing noticeably in structure from it (fig. 2B).

coronal groove - A groove around the exumbrellar surface of a medusa, typically over a zone of relative thinness of the underlying mesoglea (jelly). In many species it delimits one region of the bell or disc from another (fig. 1F).

disc - See bell.

epaulette - See scapulet.

exumbrella(r) - See bell.

filament - A narrow filamentous extension of the mouth-arm or other structure; typically densely armed with nematocysts and assumed to be used in defence or for the stunning of prey. Both terminal and lateral filaments occur, this varying with the species (figs 2C-D). See also gastric cirri.

gastric cirri - Short filaments, barely longer than wide, located in groups (phacellae) on the stomach linings in some Cubomedusae. Reportedly absent from *Carukia*. Gastric filaments, occurring in Scyphomedusae, are typically longer.

gastric filament - See gastric cirri.

gastric pouch - See gastrovascular.

gastrovascular - The gastrovascular system comprises the centrally placed stomach, the radial canals and in many species their anastomoses, and the circular canals (figs 1C-D). Some genera have an open gastric system, comprising gastric pouches separated by septa (figs 1A-B).

genital ostium, genital pillar - In rhizostome medusae: a genital ostium is a gap between adjacent mouth-arm bases. The basal regions of the mouth-arms are in that region often termed 'pillars' in distinction from the ostia between. The relative widths of these pillars and gaps are diagnostic of certain genera and species. Variously-shaped papillae intrude into the ostia in some species, and the shape can be diagnostic of some species.

interradial - See perradial.

inter-rhopalar - See rhopalium.

intracircular canal network - See radial canal.

lappets - Flaps on the margin of the disc, somewhat regular in arrangement; much varied in size but typically from 8 to c. 100 in number (fig. 1F).

marginal tentacle - A tentacle situated on the edge of the bell (fig. 2A). Not present in rhizostome medusae (fig. 2B).

mouth-arms - Typically thick tentacle-like structures around the mouth in Scyphomedusae, in most four or a low multiple (fig. 2A); in Rhizostomeae the four divide to make eight, and in most are fused just distal to the base (fig. 2B); but in Semaeostomeae the four are undivided and free. Coronatae and Cubomedusae lack them (figs 1F-G).

mouth-arm club - See club.

nematocyst warts - Wart-like structures richly supplied with nematocysts, or 'sting capsules'; found on various parts of the epidermis of many medusa species.

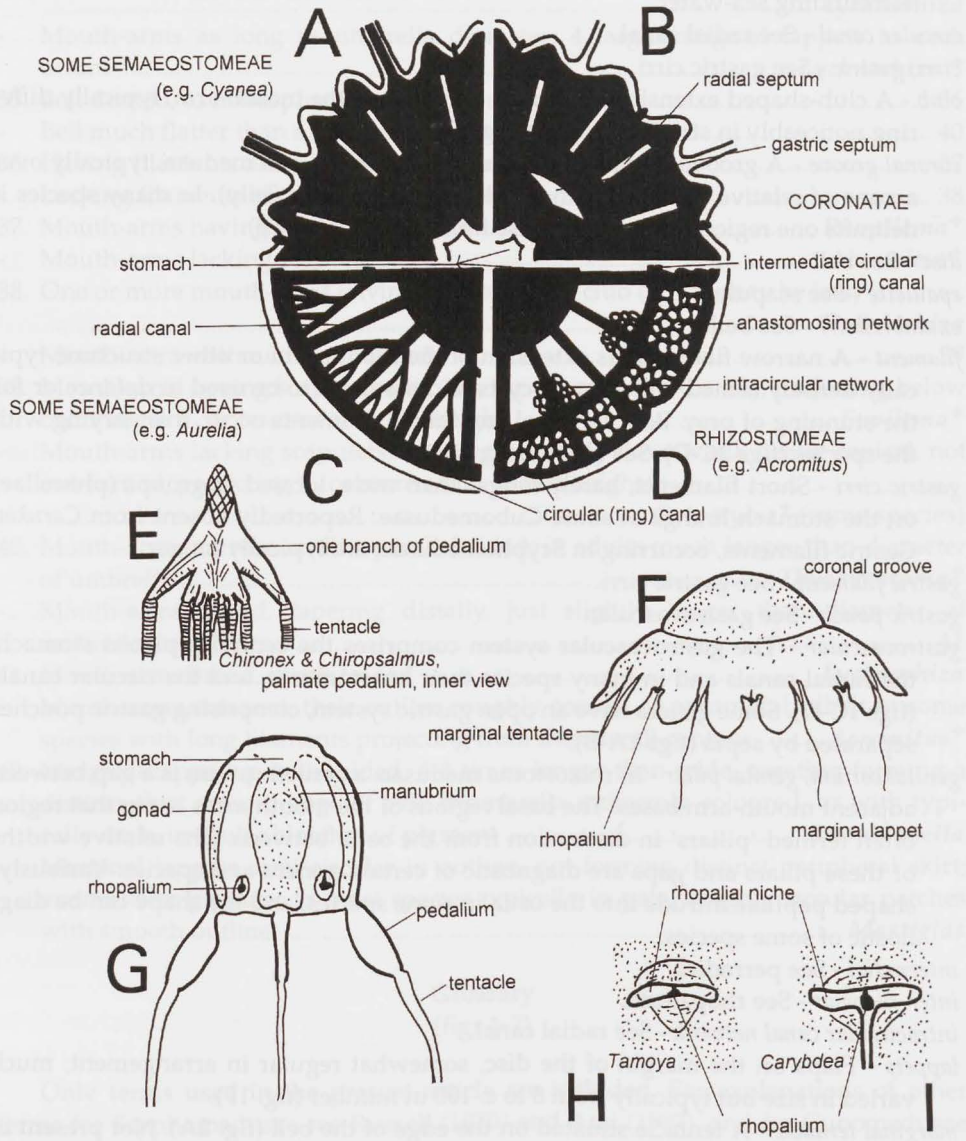

Fig. 1. Diagrams to show various medusa terms (see also fig. 2). A-D, after Russell (1970); E, after Southcott (1967); F, *Nausithoe*, after Russell (1956); G, *Carybdea*, after Conant (1898); H-I, rhopalial niches of two closely related and similar genera, after Bigelow (1938).

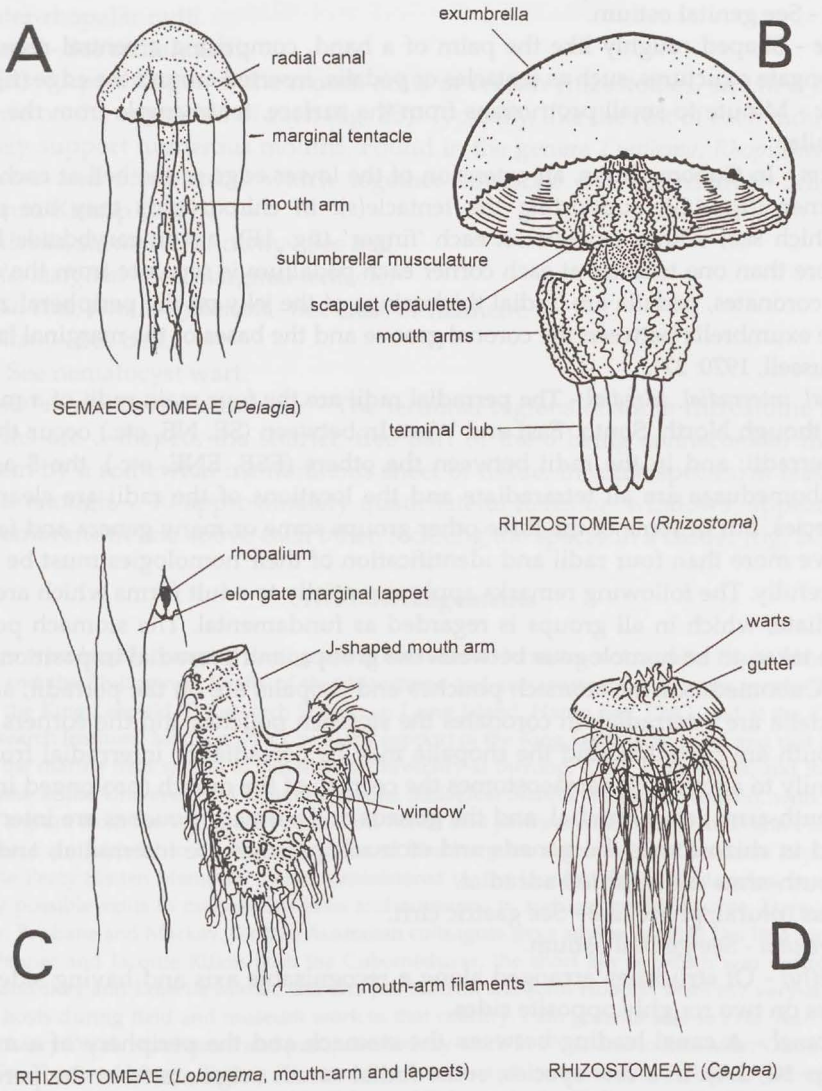

Fig. 2. Diagrams to show various medusa terms (see also fig. 1). A, after Naumov (1961); B, after Russell (1970); C, after Mayer (1910); D, after Kishinouye (1902).

octant - A one-eighth segment of the bell or disc of a medusa.

ostium - See genital ostium.

palmate - Shaped roughly like the palm of a hand, comprising a central region and elongate structures, such as tentacles or pedalia, inserted around the edge (fig. 1E).

papillae - Minute to small protrusions from the surface, for example from the exumbrella.

pedalium - In Cubomedusae, an extension of the lower edge of the bell at each lower corner (fig. 1G), supporting the tentacle(s): in chirodropids they are *palmate* (which see) with a tentacle on each 'finger' (fig. 1E); but in carybdeids having more than one tentacle at each corner each pedalium is separate from the others. In coronates, pedalia are 'radial thickenings of the jelly on the peripheral zone of the exumbrella between the coronal groove and the bases of the marginal lappets' (Russell, 1970: 27).

perradial, interradial, adradial - The perradial radii are the four main radii of a medusa, as though North, South, East and West. In between (SE, NE, etc.) occur the four interradii; and in the radii between the others (ESE, ENE, etc.), the 8 adradii. Cubomedusae are all tetraradiate and the locations of the radii are clear in all species, but in some of the three other groups some or many genera and families have more than four radii and identification of their homologies must be traced carefully. The following remarks apply essentially to adult forms which are tetraradiate, which in all groups is regarded as fundamental. The stomach pouches are taken to be homologous between the groups, and perradial in position. Thus in Cubomedusae the stomach pouches and rhopalia are on the perradii, and the pedalia are interradial; in coronates the stomach pouches and the corners of the mouth are perradial, and the rhopalia may be perradial or interradial from one family to another; in semaeostomes the corners of the mouth (prolonged into the mouth-arms) are perradial, and the gonads and stomach pouches are interradial; and in rhizostomes the gonads and stomach pouches are interradial, and the 8 mouth-arms are regarded adradial.

phacellus (plural, phacellae) - See gastric cirri.

pillar, genital - See genital ostium.

pinnate(ly) - Of structures arranged along a recognisable axis and having side branches on two roughly opposite sides.

radial canal - A canal leading between the stomach and the periphery of a medusa (figs 1C, 2A). In a few species, some radial canals originate from the periphery and end blindly without reaching the stomach. The branching patterns of the radial canals are used in many scyphozoan generic diagnoses. In many species a circular or ring canal links the outer ends of the radial canals peripherally (figs 1C-D). In a few, an intermediate circular canal occurs linking points mid-way along the radial canals (fig. 1D). In many species the radial canals are linked laterally by an anastomosing network of branches, more or less orderly in arrangement, the intracircular canal network (fig. 1D).

rhopalium, rhopalar, inter-rhopalar, rhopalar niche - A rhopalium is a small, roughly cylindrical organ, typically situated in a cleft in the umbrella margin (in Scyphomedusae, fig. 1F) or in a niche or pit on the side of the bell (in Cubomedusae, figs 1G-I). Most support light-receptors and posture- and movement-detecting

organs. The radii in which they occur are termed rhopalar radii, those in between inter-rhopalar radii.

ring canal - See radial canal.

scapulets - Appendages of the mouth-arms of certain rhizostomes, situated near the base on the outer side of each (fig. 2B). Typically, like the rest of each mouth-arm, they support numerous mouths. Found in the genera *Eupilema, Rhopilema, Rhizostoma* and *Stomolophus* which together comprise the Superfamily Scapulatae sensu Kramp (1961).

subumbrella, subumbrellar cavity - See bell.

tentacle, marginal - See marginal tentacle.

terminal club, terminal filament - See club, or filament.

umbrella(r) - See bell.

wart - See nematocyst wart.

'window' in mouth-arm membrane - The terminal regions of some rhizostome mouth-arms are J-shaped, the shorter, free part of the J being connected to the main stem by a somewhat membranous sheet of tissue. In some species, at least when old enough, 2-10 approximately quadrilateral holes or 'windows' appear in the membrane in line above each other, recalling the spaces in a ladder (fig. 2C).

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Anthozoan endosymbiosis

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Key words: Symbiosis; carbon; nitrogen; zooxanthellae; symbiosome.

Abstract: An overview is presented of some recent developments in understanding functional aspects of anthozoan symbioses. Advances in molecular taxonomy of zooxanthellae suggest a greater genetic diversity than hitherto thought. The nature of the carbon flux from symbiont to host is still equivocal, but the active component of the 'host factor' in homogenate of host cells, which stimulates the translocation of photosynthetically fixed carbon in freshly isolated zooxanthellae, has finally been identified as a suite of free amino acids. Zooxanthellae are probably nitrogen limited in symbiosis, since addition of NH_4 to seawater, increases their growth rate. The paradox of why they should translocate nitrogen compounds to the host, rather than use them for their own growth has not been resolved. Both respiration and photosynthesis are also limited, by diffusion of O_2 and CO_2 respectively, in symbiosis. However, in the light photosynthetic oxygen is made available to the host cells, whose increased respiration provides carbon dioxide to enhance the rate of algal photosynthesis. Accumulating evidence on the nature of transport mechanisms in the host cell vacuolar membrane, suggest that the host may exercise more control of zooxanthellae function than realised. This makes the concept of the alga in its vacuole as an organelle, the 'symbiosome', an attractive option in the development of hypotheses on control and regulation in anthozoan symbiosis.

Introduction

Cnidarians form symbioses with two groups of microalgae: the Chlorophyceae, of which the *Chlorella/Hydra* symbiosis is the best known, and the Dinophyceae or dinoflagellates. I shall confine my review to symbioses with dinoflagellates, or zooxanthellae, because most work has been carried out on these, and because they appear to differ in many respects from other symbioses.

The term 'zooxanthellae' has no real taxonomic value, but will probably continue, because of its current widespread use. The most important genus is *Symbiodinium*. Until recently it was commonly thought that all zooxanthellae comprised the single species *S. microadriaticum*. Following the work of Schoenberg & Trench (1980) and Blank & Trench (1985), who identified genetic differences between zooxanthellae from different hosts, Rowan & Powers (1991) and Banaszak et al. (1993) using ssRNA gene sequencing, were able to separate a further nine species. The realisation of the diversity of species involved in cnidarian symbioses will probably help the interpretation of apparent anomalies in the physiological and biochemical properties of zooxanthellae from different hosts .

Zooxanthellae are in most cases confined to the gastrodermal cell layer where they are contained in a phagocytic vacuole. It will probably prove to be helpful to adopt the concept of the algal cell in its host cell vacuole as an organelle, the 'symbiosome' (Roth et al., 1988). Rands et al. (1993) used cytochemical methods to show the

presence of ATPases associated with the symbiosome membrane. These would provide a mechanism for the inward and outward active transport of molecules such as amino acids. Control of this flux is therefore by the host cell rather than the symbiont.

Another useful concept is that of the symbiotic unit, comprising an epidermal cell and adjacent gastrodermal cell, since it helps to visualise the various fluxes between the host cell and zooxanthellae (fig. 1). Understanding the nature and regulation of these fluxes, and the regulation of the growth and division of the symbionts, are major unresolved areas, which have been the subject of active research in recent years.

Translocation of photosynthetically fixed carbon

Despite nearly 30 years of research since Muscatine's 1967 paper describing the translocation of ^{14}C labelled compounds from zooxanthellae, the nature of the products translocated is still controversial. This stems from the difficulty of determining this in symbiosis, and concern about possible artefacts arising from the use of freshly isolated zooxanthellae (f.i.z.). Research during the 1970s, using both f.i.z and zooxanthellae in symbiosis, suggested that the major product translocated was probably glycerol, with smaller quantities of glucose, alanine and a range of other compounds. (Muscatine & Cernichiari, 1969; Trench, 1971). Subsequently it was proposed that in addition to glycerol, lipids are other major translocation products (Patton et al., 1977).

Support for this came from the observation that f.i.z. from the tropical sea anemone *Condylactis gigantea* Weinland, 1860, when viewed with Normarski interference or phase contrast optics, appear to have lipid blebs attached to their surface, distending the symbiosome membranes (Kellogg & Patton, 1983). However, Muscatine et al. (1994) challenged this interpretation by showing that the blebs do not stain with Sudan Black, but do stain with Hoechst 33258, which is a DNA-binding fluorochrome, and are therefore probably host-cell nuclei of the still-intact gastrodermal cells which had not ruptured during the isolation procedure.

So whilst one of the main arguments for lipid translocation has been weakened,

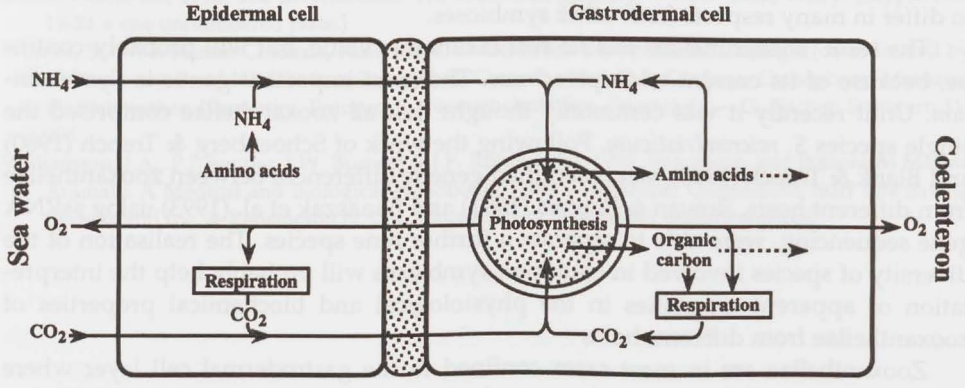

Fig. 1. Cnidarian symbiotic unit, summarising the major fluxes involving epidermal and gastrodermal cell layers during the day.

so has the argument in favour of glycerol. Streamer et al. (1993) carried out very short-term labelling of the photosynthetic products within zooxanthellae. In the first minute, 3-phosphoglycerate was rapidly labelled. Thereafter the label appeared in glucose and amino acids but not in glycerol. The possibility remains that some other molecule such as 3-phosphoglycerate is translocated and is immediately metabolised to glycerol and other compounds on the host membrane - which is often still attached to the algae in freshly isolated preparations. A summary of possible carbon metabolic pathways is given in fig 2.

Evidence is now accumulating for a reverse flux during darkness, from host to symbiont. In f.i.z from the scyphozoan *Cassiopea*, glucose is taken up by a Na^+ dependant symport-system, probably located in the host membrane, energised by a Na^+ gradient generated by a Na^+/K^+ ATPase. Glycerol may be taken up by simple or facilitated diffusion (McDermott & Blanquet, 1991). However, Ritchie et al. (1993) showed that in zooxanthellae from the coral *Plesiastrea*, the permeability to glycerol is low, and the inward flux has both transport-mediated and diffusion-mediated components.

Control and regulation of carbon efflux

The possibility of regulation of translocation by the host was first raised by the observations of Muscatine & Cernichiari (1969) and Trench (1971) that freshly isolated zooxanthellae when incubated with $H^{14}CO_3$ in sea water, released very little photosynthate. Since translocation could be stimulated by incubating the f.i.z in a homogenate of host tissues, it was suggested that the homogenate contained translocation-regulating molecules which were loosely termed the 'host-factor'. However,

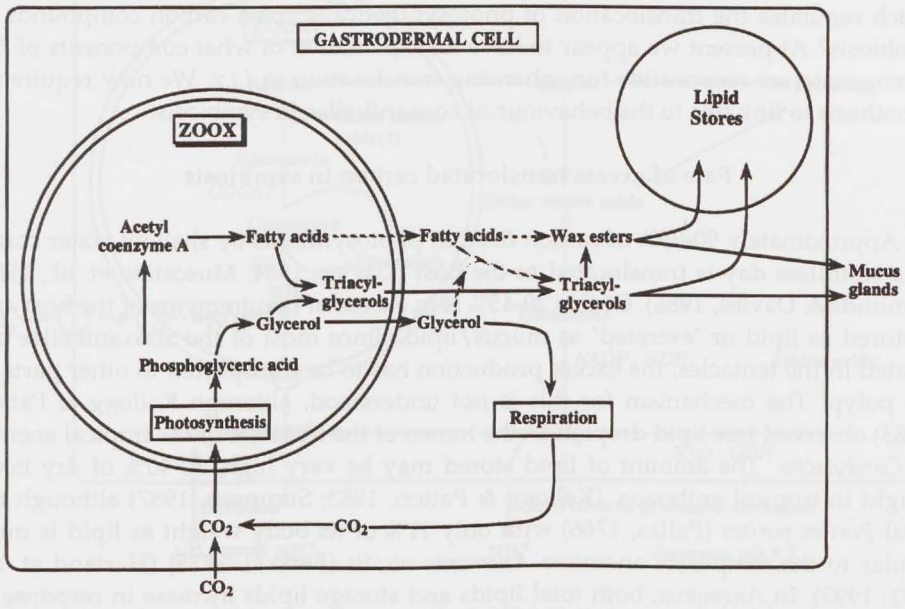

Fig. 2. Summary of the main carbon pathways in a gastrodermal cell in the light.

the hypothesis that host factors increase the permeability of the algal membrane to glycerol, received a setback from the observation that in *Plesiastrea*, host homogenate reduced the inward flux of glycerol rather than increasing it (Ritchie et. al., 1993).

Since the original observations of Muscatine & Trench, little progress has been made, although Sutton & Hoegh-Guldberg (1990) demonstrated very little effect of host-factors in three species investigated, and it was only in *Plesiastrea* that up to 42% of the net-fixed carbon was translocated. However, Gates et al. (1995) provide a basis for the identification of the active components in host homogenate.

Homogenate of tissues of the coral *Pocillopora damicornis* Linnaeus, 1758, and the anemone *Aiptasia pulchella* Carlgren, 1943, were shown to elicit release of ^{14}C labelled compounds in freshly isolated zooxanthellae. Size exclusion separation on a Sephadex column indicated that the active factor had a molecular weight of < 4 kDa and an absorption peak at 320 nm, a wavelength characteristic of mycosporine-like amino acids which are abundant in marine cnidarians (Dunlap et al., 1986). Separation of these mycosporine-like amino acids showed six separate compounds each of which was contaminated by free amino acids. Surprisingly, the host-factor activity was found to reside in all amino acids present in the host homogenate. When f.i.z. were incubated with individual amino acids at a strength of 50 mM, they were all found to increase the percentage of fixed carbon released when compared to controls in seawater. Part, but not all, of the response can be attributed to a stimulation of net photosynthesis.

Synthetic free amino acid mixtures, based upon analysis of the free amino acid pool of tissues from two different colonies of *Pocillopora damicornis* had similar effects to the whole homogenate. Furthermore the mixtures also stimulated free living *Chlamydomonas reinhardii* in stationary phase culture to release 23% of the carbon fixed.

Do the interesting data of Gates et. al. (1995) verify the existence of a host factor which regulates the translocation of photosynthetically fixed carbon compounds in symbiosis? At present we appear to have an explanation of what components of the homogenate are responsible for enhancing translocation in f.i.z. We now require an hypothesis to link this to the behaviour of zooxanthellae in symbiosis.

Fate of excess translocated carbon in symbiosis

Approximately 90-95% of carbon fixed in photosynthesis by shallow water corals on a cloudless day is translocated to the host (Davies, 1984; Muscatine et. al., 1984; Edmunds & Davies, 1986). Of this 20-45% is in excess of requirements of the host and is stored as lipid or 'excreted' as mucus/lipid. Since most of the zooxanthellae are located in the tentacles, the excess production has to be transported to other parts of the polyp. The mechanism for this is not understood, although Kellogg & Patton (1983) observed free lipid droplets in the lumen of the tentacles of the tropical anemone *Condylactis*. The amount of lipid stored may be very high, 30-40% of dry body weight in tropical anthozoa, (Kellogg & Patton, 1983; Stimpson, 1987) although the coral *Porites porites* (Pallas, 1766) with only 11% of its body weight as lipid is more similar to the temperate anemone *Anemonia viridis* (Forskål, 1775) (Harland et. al., 1991; 1993). In *Anemonia*, both total lipids and storage lipids increase in response to elevated levels of illumination (Harland et al., 1992). Of these, the storage lipids are

potentially of most interest, since they are metabolically labile. However, they are difficult to separate, and real progress will be dependent upon the development of a fast and simple separation and measurement procedure.

Nitrogen metabolism

In light, NH_4 is taken up from the surrounding seawater. Fig. 3 gives an interpretation of the possible major metabolic pathways. Rees (1987) proposed that the symbionts in the *Chlorella/Hydra* symbiosis were nitrogen-limited, since the major NH_4 anabolic pathway was that of the animal cell, so that very little of either the animal excretory NH_4 or NH_4 taken up from surrounding water was assimilated by the algae. Rees has developed the hypothesis subsequently, suggesting that the benefit of the algae to the symbiosis is to provide the carbon skeletons, via the translocation of photosynthetically fixed carbon, for the assimilation of NH_4 in the animal cytoplasm. This would permit nitrogen conservation, since less of the host cell amino acids would be used as substrates for respiration. As a corollary to this, NH_4 would not be recycled through the algae.,

There are various indications that Rees' hypothesis does not apply to zooxanthellae. For instance Rahav et al., 1989 showed that by inhibiting the NH_4 assimilatory enzyme GOGAT (glutamine 2-oxoglutarate amido transferase) with azaserine, uptake of NH_4 into the coral *Stylophora* ceased, and animal excretory NH_4 diffused out to the surrounding water. More recently Wilkerson & Kremer (1992) showed that

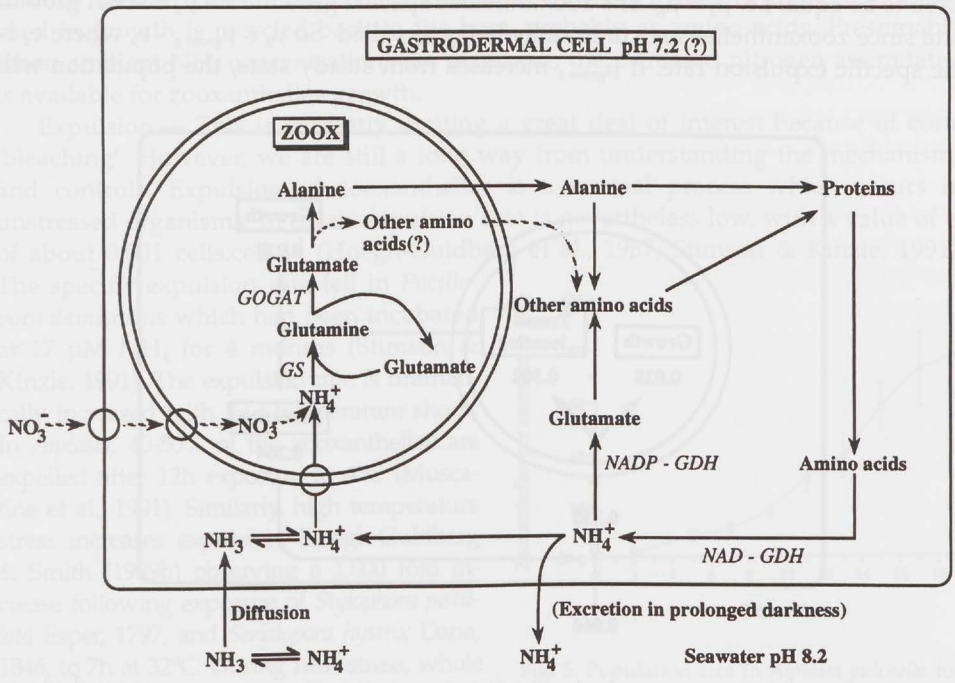

Fig. 3. An interpretation of the major nitrogen pathways in a gastrodermal cell. Uptake of nitrate does not appear to occur in a number of symbioses.

uptake of $^{15}NH_4$ took place preferentially into the zooxanthellae of the scyphozoan *Linuche*.

It is likely that zooxanthellae when in symbiosis, are nitrogen limited, since their growth rate and population size increases when the whole organism is incubated in elevated levels of NH_4 (Muscatine et al., 1989b). Preliminary nitrogen budgets (fig. 4) suggest that nitrogen, probably in the form of amino acids (as in host factor stimulated f.i.z.) is translocated to the host. It is not known whether essential amino acids are translocated, although it has recently been shown that cultured zooxanthellae exude a complex glycoconjugate containing all the essential amino acids (Markell & Trench, 1993). However, the paradox of why nitrogen-limited zooxanthellae liberate nitrogen compounds to the host instead of using them for their own growth, has yet to be resolved.

Regulation of the population of zooxanthellae

The size of the population of zooxanthellae appears to be regulated. In corals, the population size varies between species from 0.5-5 $\times 10^6$ zooxanthellae.cm^{-2} (Muscatine, 1980). In the anemone *Aiptasia pulchella* the population size is about 0.5-2.5 zooxanthellae.mg protein^{-1} (Muller-Parker, 1984; Berner et al., 1993). The mechanism of regulation of the population size is not known, but we can make some generalisations.

For a stable population, the specific growth rates of the symbionts and host cells have to be equal i.e. $\mu_z = \mu_h$. The zooxanthellae specific growth rate μ_z is a net growth rate since zooxanthellae are continually being expelled. So $\mu_z = \mu_{gross\ z} - e_z$ where e_z is the specific expulsion rate. If $\mu_{gross\ z}$ increases from steady state, the population will

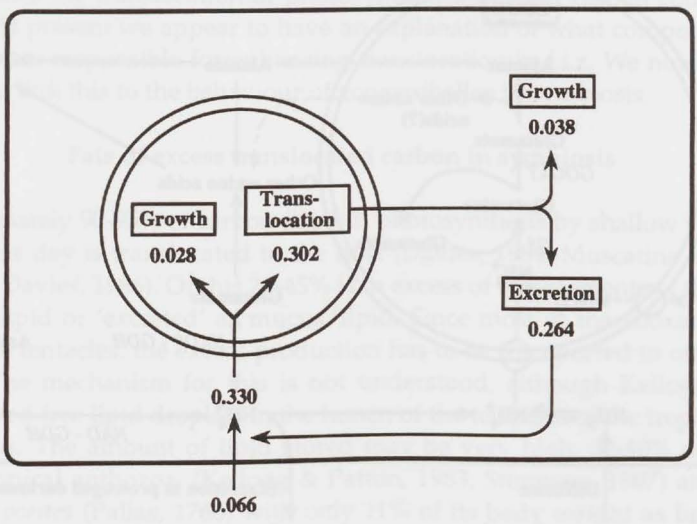

Fig. 4. Summary of 24-hour nitrogen budget for *Stylophora pistillata*. The units are μmol N.cm^{-2}.d^{-1}. The inorganic nitrogen influx was not measured but has been calculated, to balance the budget. (Derived from Rahav et al., 1989).

increase. Conversely if e_z increases, the population will decline, as happens in coral 'bleaching'. So either growth or expulsion rate could be the main regulator of population size.

Growth.— There is some evidence that growth rate is limited by the availability of inorganic nitrogen to the zooxanthellae, since coastal waters normally have very low levels, and in tropical waters, concentrations are usually below 0.1 µM (Muscatine & Weis, 1992). When shallow water corals were incubated in water enriched to up to 20 µM NH_4, the growth rate of zooxanthellae increased, and the population density rose 2-3 fold (Muscatine et al., 1989b; Hoegh-Guldberg & Smith, 1989a). Further indirect evidence comes from experiments on the reinfection of aposymbiotic *Aiptasia pulchella* with zooxanthellae (Berner et al., 1993). The algae injected into the coelenteron were initially phagocytosed in the mesenteries. After a lag period there was an exponential increase in the population size between days 9 and 15, when the specific growth rate was 0.4 cells.cell.d^{-1}, compared with the steady state of 0.005-0.025 cells.cell.d^{-1} for laboratory symbiotic *Aiptasia* (fig. 5). One explanation for this is that during the exponential growth phase, the zooxanthellae were experiencing a high NH_4 environment, since all of the animal excretory nitrogen was available to the low algal biomass .

In the steady state situation of corals living in oligotrophic waters, we nevertheless have the paradox, which is revealed by the construction of a nitrogen budget (derived from data in Rahav et al., 1989), that although the combined flux of excretory NH_4 plus that assimilated from seawater is low, there is still more available than is actually used in growth. (fig. 4). The difference between available nitrogen and that used in growth is recycled back to the host, probably as amino acids. Presumably when ambient NH_4 concentrations are increased, the increased nitrogen assimilated is available for zooxanthellae growth.

Expulsion.— This is currently exciting a great deal of interest because of coral 'bleaching'. However, we are still a long way from understanding the mechanisms and controls. Expulsion of zooxanthellae is a normal process which occurs in unstressed organisms. In corals expulsion rate is nevertheless low, with a value of e_z of about 0.001 cells.cell.d^{-1} (Hoegh-Guldberg et al., 1987; Stimson & Kinzie, 1991). The specific expulsion rate fell in *Pocillopora damicornis* which had been incubated at 17 µM NH_4 for 4 months (Stimson & Kinzie, 1991). The expulsion rate is dramatically increased with low temperature shock. In *Aiptasia*, 40-50% of the zooxanthellae are expelled after 12h exposure to 4°C (Muscatine et al., 1991). Similarly, high temperature stress increases expulsion, Hoegh-Guldberg & Smith (1989b) observing a 1,000 fold increase following exposure of *Stylophora pistillata* Esper, 1797, and *Seriatopora hystrix* Dana, 1846, to 7h at 32°C. During heat stress, whole gastroderm cells detach and are voided intact through the mouth (Gates et al., 1992). The

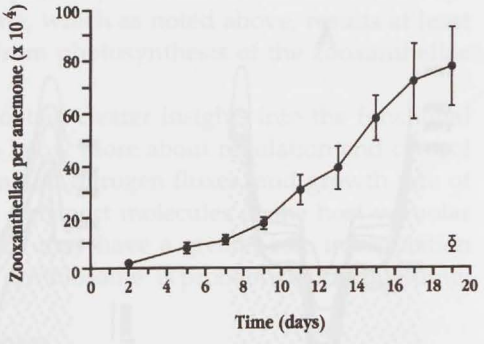

Fig. 5. Population size in *Aiptasia pulchella* following reinfection of aposymbionts, showing the exponential growth phase between days 9 and 15. (From Berner et al., 1993).

host cells rapidly disintegrate, releasing the zooxanthellae. It is not known whether all zooxanthellae are released in this way during temperature shock, nor whether this mechanism operates in expulsion caused by continuous darkness or low salinity. Nor is it known whether corals 'bleach' in this way.

In summary, it appears that population levels of zooxanthellae are normally sustained by a low growth rate resulting from a low rate of inorganic nitrogen flux. These population levels can be perturbed by increasing growth rate (by increased nitrogen flux) or increased expulsion rate (by a range of environmental stresses).

Constraints and limitations in symbiosis

In addition to the limitation of the growth of zooxanthellae by the availability of inorganic nitrogen, there is now evidence that both respiration and photosynthesis are limited by diffusion of oxygen and carbon dioxide to the tissues.

Limitation of respiration by oxygen diffusion.— For many years it was thought that in cnidarians, diffusion of oxygen to the tissues and to the symbionts would not be limiting , since the tissues are only two cells in thickness (Kanwisher & Wainwright, 1967). However, Dennison & Barnes (1987) showed that in the coral *Acropora formosa* (Dana, 1846), respiration rate was 25% lower when there was no water motion over the surface of the colony, suggesting the presence of a diffusion boundary layer (DBL). The existence of a DBL has been verified by Shashar et al. (1993) using oxygen microelectrodes which were accurately positioned relative to the surface of the colony with a micromanipulator. In unstirred water, in darkness, the DBL was about 3 mm in thickness, and the oxygen concentration fell to about 20% saturation at the surface of the coenosteum, and to anoxia over the centre of the oral disc (fig. 6). These observations, together with field observations (Patterson et al., 1991) emphasise the importance of water motion to maintain respiration of these sessile organisms which lack a ventilated respiratory surface.

Even in stirred water, oxygen delivery can be a problem. Respiration rates of a number of symbiotic cnidaria increase when they are exposed to hyperoxic seawater, indicating that diffusion is not enough to meet respiratory requirements in stirred normoxic water (Shick, 1990; Harland & Davies, 1995). A further indication of this

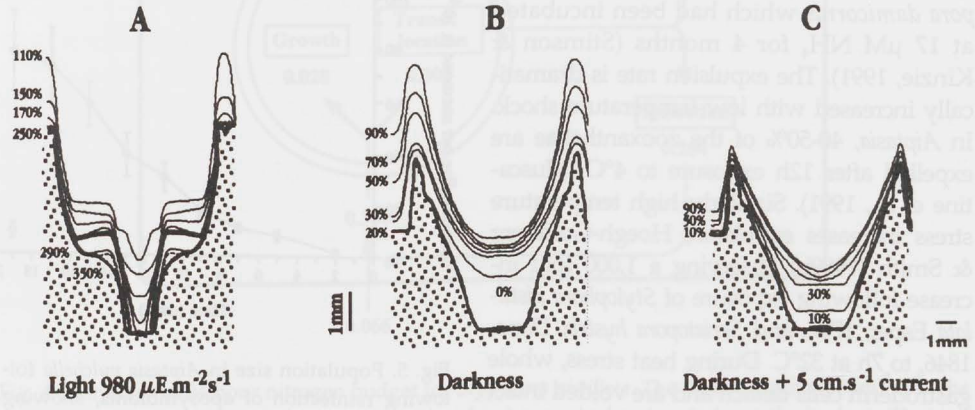

Fig. 6. Oxygen boundary layers surrounding *Favia favus* coral polyps. A. During exposure to light of 980 $E.m^{-2}.sec^{-1}$. B. In darkness. C. In darkness with a 5 $cm.s^{-1}$ current. (From Shashar et al., 1993).

problem is the low concentration of oxygen which has been measured in the coelenterons of the symbiotic anemone *Anemonia viridis* during darkness (Harland & Davies, 1995) and in other non-symbiotic anemones (e.g. Brafield & Chapman, 1965). Conversely, during photosynthesis in the light, superficial tissue oxygen concentrations may rise to 200-300% saturation (Dykens & Shick, 1982; Kuhl et al., 1995), creating a reverse DBL in unstirred water (Shashar et al., 1993; Kuhl et al., 1995). Oxygen concentrations also increase in the water in the coelenteron, but to a lesser extent (Harland & Davies, 1995).

These observations suggest that in darkness, respiration rates of the deeper tissues are reduced as a result of local hypoxia, and that in the light in symbiotic cnidaria, the respiration rate is restored, utilising the oxygen produced within the tissues by the zooxanthellae.

Limitation of photosynthesis by carbon dioxide diffusion.— Under high levels of irradiance, the ratio of gross photosynthesis to respiration in symbiotic Anthozoa is often between 3:1 and 6:1. Since values for the respiratory quotient (carbon dioxide out/ oxygen in) are usually between 0.65 and 0.9 (Gattuso & Jaubert, 1990), it is clear that only a small proportion of the carbon dioxide for photosynthesis comes from the respiration of algae and host tissue. The remainder is derived from the bicarbonate reserves of the seawater, and enters by diffusion. Diffusion boundary layers are again important, and photosynthesis is reduced in static water (Dennison & Barnes, 1987). Limitation of photosynthetic rate by CO_2 availability is indicated by the observation that photosynthetic rate increases when bicarbonate is added to the water (Burris et al., 1983). Further evidence of CO_2 limitation come from studies on ^{13}C fractionation in corals (Muscatine et al., 1989a), and from the observation that carbonic anhydrase (CA) (which catalyses the conversion of HCO_3^- to CO_2) is about 29 times higher in symbiotic than non-symbiotic cnidarians (Weis et al., 1989). The rate of photosynthesis is reduced when CA is inhibited by acetazolamide. The CA activity is considerably lower in the zooxanthellae than in the animal tissue, where it is primarily located on or near the host membrane surrounding the zooxanthellae (Weis, 1991). These observations all indicate problems with CO_2 delivery to the algae for photosynthesis. It is of interest thereore that following exposure to light in *Anemonia viridis*, the rate of photosynthesis increases, probably as result of the enhanced supply of CO_2 from the increased respiration rate, which as noted above, results at least in part, from the increased flux of oxygen from photosynthesis of the zooxanthellae (Harland & Davies, 1995).

In summary, it is clear that in order to obtain clearer insights into the functional aspects of anthozoan symbioses, we have to know more about regulation and control processes, particularly those affecting carbon and nitrogen fluxes, and growth rate of the symbionts. The demonstration of active transport molecules in the host vacuolar membrane suggests that the host cell nucleus may have a greater role in regulation than previously thought. The concept of the 'symbiosome' is probably worth pursuing.

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An unusual sea anemone, *Dactylanthus antarcticus* (Clubb, 1908) (Order Ptychodactiaria), on gorgonians in Chilean fjords

P.K. Dayton, K.W. England & E.A. Robson

Dayton, P.K., K.W. England & E.A. Robson. An unusual sea anemone, *Dactylanthus antarcticus* (Clubb 1908) (Order Ptychodactiaria), on gorgonians in Chilean fjords.
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Key words: Sea anemone; Ptychodactiaria; *Dactylanthus antarcticus*; behavioural ecology; predator of gorgonians; *Primnoella compressa*; *Thouarella* spec.; distribution; Chilean fjords; recruitment.

Abstract: During exploratory diving observations to monitor kelp abundance in fjords in Chile in 1972 and 1973, the senior author (P.K.D.) recorded a ptychodactiarian sea anemone, *Dactylanthus antarcticus*, feeding on gorgonian colonies, *Primnoella compressa* and *Thouarella* spec. These observations extend the known distribution range of *D. antarcticus*, and reveal new aspects of its biology as a mobile predator.

Introduction

The observations reported here were made in November 1972 and May 1973, during two visits to Chile to monitor the abundance of kelp in fjords (Dayton, 1985). They extend the known range of distribution of *Dactylanthus antarcticus* (Clubb, 1908) and give new insight into the biology of this anemone as a predator of gorgonians.

Anemones of the order Ptychodactiaria are of interest because they are regarded as primitive (Carlgren, 1949) and are quite rare (Dunn, 1983). The order includes three species: *Ptychodactis patula* Appellöf, 1893, recorded from the vicinity of Norway, Iceland and Bering Strait, is an arctic-boreal and possibly panarctic species (Carlgren, 1921, 1942); *Dactylanthus antarcticus* found in the Antarctic and subantarctic, is possibly circumpolar in distribution (Clubb, 1908; Carlgren, 1911, 1927; Stephenson, 1918; Dunn, 1983); and *Preactis millardae* England & Robson, 1984, is known only from the Western Cape, South Africa.

The classic work on Ptychodactiaria is based on infrequent specimens obtained by dredging. Two species of these anemones have now been observed during SCUBA dives by marine ecologists. They are found in moderate currents, and are associated with filter feeding communities living on hard substrates. They appear to be specialized predators of octocorals. *Preactis millardae* feeds on a soft coral, *Capnella thyrsoidea* Verrill, 1865 (Dr T. M. Gosliner in England & Robson, 1984; Dr G.C. Williams, personal communication). *Dactylanthus antarcticus* preys on gorgonians (as described below). For the present, nothing is known about the behaviour of *Ptychodactis patula* but it is interesting that it was first found on gorgonians in Trondheim fjord (these were referred to by Appellöf (1893) as *Muricea placomus* and *Primnoa lepadifera*, now respectively *Paramuricea placomus* (Linnaeus, 1758) and *Primnoa resedae* (Gunnerus, 1763) (Dr M. Grasshoff, personal communication)). The site, at 200 m depth, was described as "Korallengrund".

Field observations on *Dactylanthus antarcticus* (by P.K. Dayton)

Location 1.— Chile, Canal Darwin, Punta Quinlan area, 45°23.5'S 74°0.80'W, high current SCUBA dive. Moderately exposed habitat (Dayton, 1985); 16.xi.1972, am.

In this area very strong tidal currents (perhaps 15 m/s) go back and forth, with a tidal range of up to 3m. Canal Darwin is swept free of sediment and the seawater is well mixed and rich in plankton. The estimated annual temperature range is 4-10°C.

The upper 5 m was very bare coralline pavement. Below this was a suspension feeding association typical of areas with strong currents. The walls were 50-80% covered with *Megabalanus*, with the rest of the substratum covered with clumps of anemones, compound tunicates, sponges, and hydroids. In deeper (25-40 m) eddy areas behind small reefs there were forests of sea whips (gorgonians). The anemones were collected off sea whips. They have very sticky papillae which adhere to the gorgonians very quickly and efficiently (fig. 6), and help them to regain their position when dislodged. In the field they may be flattened and appear shapeless (figs 1, 8). At times they look very much like the sessile ctenophore *Lyrocteis* spec. (Robilliard & Dayton, 1972). They actually appear to be eating the gorgonians as one end of the blob is pressed against gorgonian flesh and the rest of the gorgonian is stripped behind it. When collected they look more like an empty bag with mesenteries inside, or else more like holothurians.

Four specimens were collected in jars, taken with pieces of gorgonian to avoid the remarkably strong adhesion of their papillae to neoprene diving gloves. They were treated for several hours with magnesium chloride and preserved in 4% formaldehyde. These specimens were of average or large size. The natural colour is pale yellow.

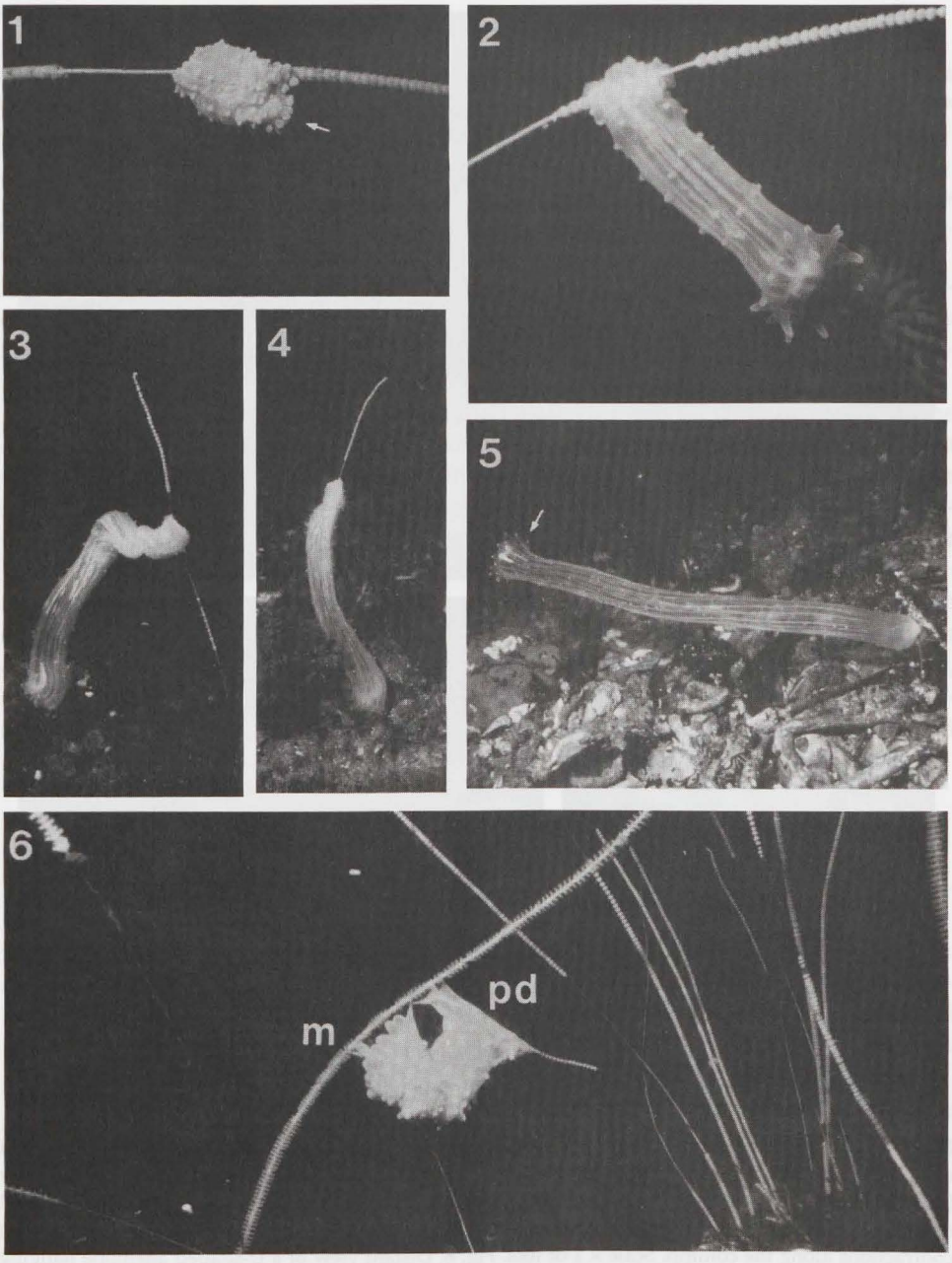
Photographs were taken of other specimens (figs 1-12) including a sweeping anemone hanging on to its sea whip perch by its basal disk and expanded to about three times its normal body length, which seemed to be dragging its tentacles over the substratum (figs 3-4). The extended length of large anemones is perhaps 15-20 cm.

Location 2.— Chile, Estrecho Collingwood, 51°52'S 73°43.6'W, SCUBA. Relatively protected from oceanic waves; current speed (perhaps 15 m/s) higher than at Punta Dashwood (Dayton, 1985); 19.v.1973, pm.

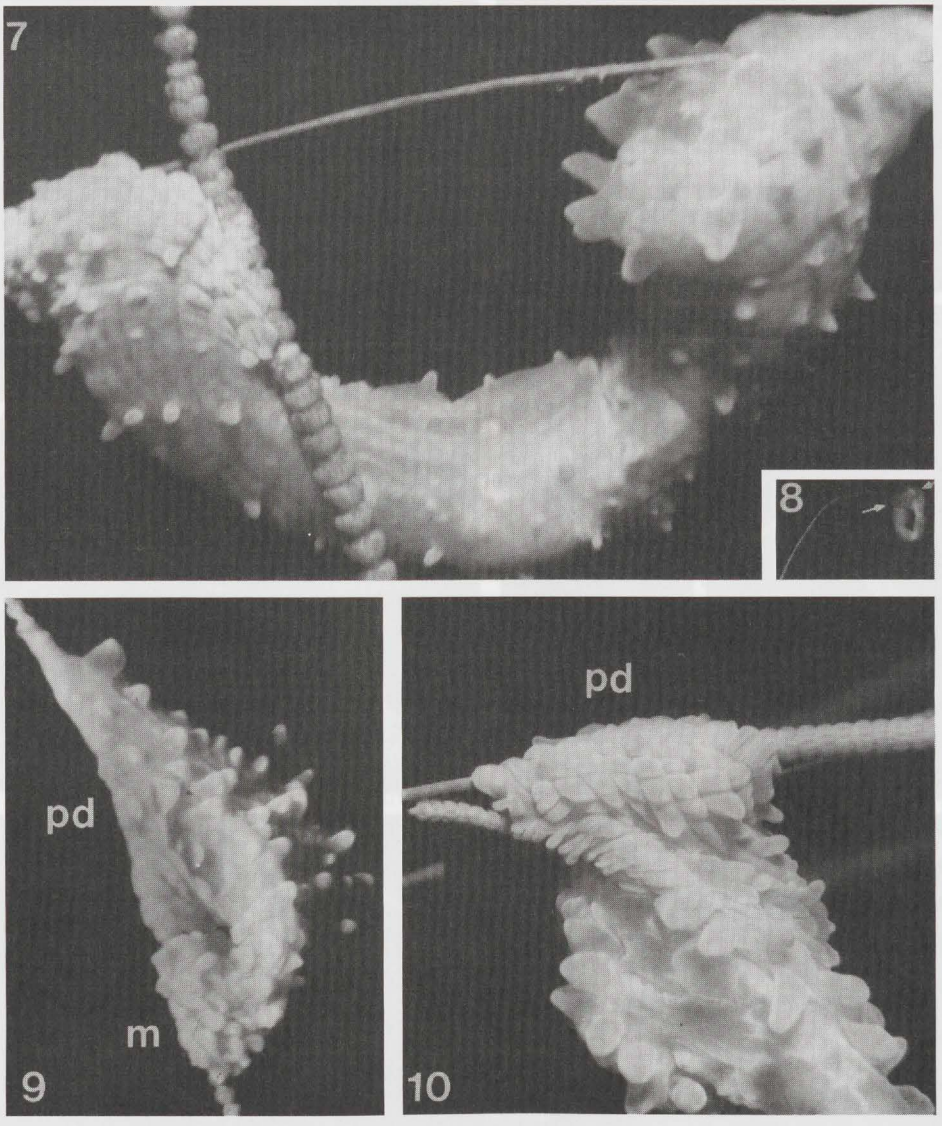
This area is current swept and free of sediment. There were many ledges at various depths. One at ± 30 m had 4 compound tunicates with hydroids on them. A deeper ledge had very high sponge diversity. A piece of *Isodactya* not unlike an antarctic sponge was collected there. Much of the area was covered with large tubes of "*Diopatra*-like" polychaetes.

There were many gorgonians and quite a few of the anemones. In a number of cases they were sweeping the bottom from their perch on gorgonians (see fig. 4). When stretched out they were practically transparent. There were three which really seemed to be eating the gorgonian as they had their tentacles around the dissolved-appearing fleshy part of the colony. The tentacles were observed as they wrapped around small pieces of the gorgonian (fig. 7). The anemones also moved their oral areas in a circular path around the edge of the colony.

Predators of sea whips include a *Tritonia*-like nudibranch. Two white-tipped aeolids were seen crawling on the gorgonian, but they did not eat it.



Figs 1-6. *Dactylanthus antarcticus* on sea whips (*Primnoella compressa*); all figures less than × 1. Fig 1. Small anemone, crown to the right (arrow); adjacent bare skeleton suggests predation. Fig. 2. Young specimen, fully extended except at the adhering pedal disk, concealed by basal folds of the column. Filaments and perhaps fusion zone of mesenteries are just discernable. Figs 3-4. A specimen "sweeping" the substratum. Fig. 5. Young anemone extended on substratum, its contracted base attached to a narrow support. White siphonoglyphs opposite arrow. Fig. 6. Anemone moving towards the only intact colony of *Primnoella* in view: note how papillae adhering to gorgonian polyps are drawn out to sharp points. The pedal disk (pd) enwraps another sea whip; tentacles and mouth (m) are to the left.



Figs 7-10. *Dactylanthus antarcticus* on sea whips (*Primnoella compressa*), continued. Fig. 7. Extended anemone with inflated crown, possibly feeding. The pedal disk (left) adheres to an intact colony (polyps closed) as well as to a stripped axial skeleton, to which the oral disk or mouth is applied (right). Note marginal tentacles, arrangement of papillae and mesenteries, and peristaltic (circular) contractions along the column. Scale × 2 or less. Fig. 8. Distant view of a U-shaped anemone hanging from a sea whip. Arrows at crown (right) and pedal disk (left). Fig. 9. Partly contracted anemone on two gorgonians. The pedal disk (pd) is firmly wrapped round one colony, while the crown and mouth region (m) are applied to another. Photograph turned clockwise by 90°. Scale less than × 1. Fig. 10. Proximal view of an anemone hanging from a sea whip, whose skeleton is partly exposed (left). Note thin-walled mobile column and base, insertions of mesenteries and inflated papillae. Scale approximately as in fig. 7.

Descriptive notes on the specimens and identification (by K.W. England)

Material.— Chile, Canal Darwin, 25 m, fjord, 45°26'S 74°05'W, 16.xi.1972, SCUBA, coll. P.K. Dayton, 4 specimens (with 2 pieces of gorgonian). The specimens have been deposited at the National Museum of Natural History, Smithsonian Institution, Washington (USNM 96507, 96508).

External appearance.— Column tall with vertical rows of tentaculate vesicles, one row per endocoel and exocoel. Continue to edge of disk. No apparent margin. Oral disk with wide central mouth, no long tentacles. Pedal disk wide, strong.

Anatomy.— Actinopharynx with two aborally prolonged siphonoglyphs, supported by directives. Mesenteries, 6 pairs perfect and 6 pairs imperfect, all with filaments and gonads. Mesenteries fused for approximately $^1/_4$ of their length at aboral end. Filaments without ciliated tracts. Double retractors, pharyngeal and main, present.

Remarks.— The specimens are identified as *Dactylanthus antarcticus*. They fit the original description of that species (Clubb, 1908) and that of Carlgren (1911) except that the V-shaped funnels on the top of the filaments of the imperfect mesenteries are somewhat different, being more diffuse and almost palmate, and attached to the column/disk. The nematocyst data are in agreement with the types and size ranges for *D. antarcticus*.

Notes added by E.A. Robson.— A sketch by the late K.W. England, referring to the distal ciliated structures described by Carlgren (1911) shows "Funnels on mesenteries of second cycle. Not as prominent as usually depicted". Nematocysts of the vesicles, throat and filaments were examined from two specimens with sketches and measurements comparable to those reported for *D. antarcticus* (BMNH 1918.5.12.4: in England & Robson, 1984).

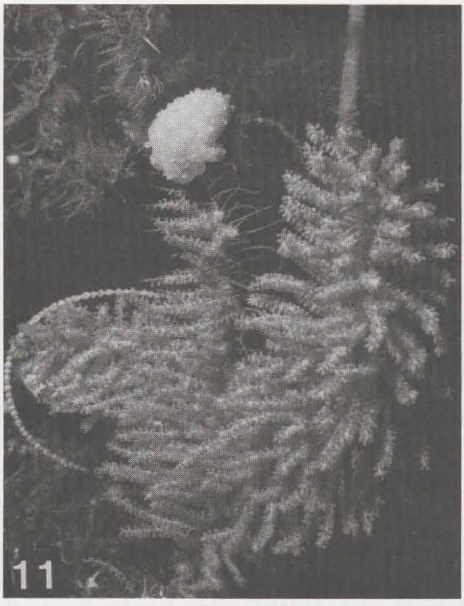
Identification of gorgonians

A specimen of the sea whip from Canal Ocasion, Chile, 54°33.4'S 71°59.7'W, collected and preserved in formaldehyde by P.K. Dayton, 17 May 1973 (USNM 96506), was examined by Dr F.M. Bayer and identified by him as *Primnoella compressa* Kükenthal, 1908 (personal communication). The two pieces of similar gorgonian collected with anemones from Canal Darwin (see above) are regarded as being also of this species (Dr T.A.S. Brito and Dr M. Grasshoff, personal communication). This sea whip appears in figs 1-4, 6-10.

The pink "bottle brush" gorgonian from Canal Darwin shown in figs 11 and 12 is regarded as *Thouarella* spec. (Dr T.A.S. Brito and Dr M. Grasshoff, personal communication) .

Comments

The behaviour of *Dactylanthus antarcticus* is reported for the first time. Field observations and photographs show a mobile, thin-walled anemone capable of extensive changes in shape. Re-expansion by filling the coelenteron would depend upon peristalsis and ciliary inflow (siphonoglyphs, e.g. fig. 5) rather than on elastic properties of relatively thin mesoglea. The extended form is cylindrical, the expanded oral disk up to 25% wider (fig. 2.). The pedal disk may contract to a small area



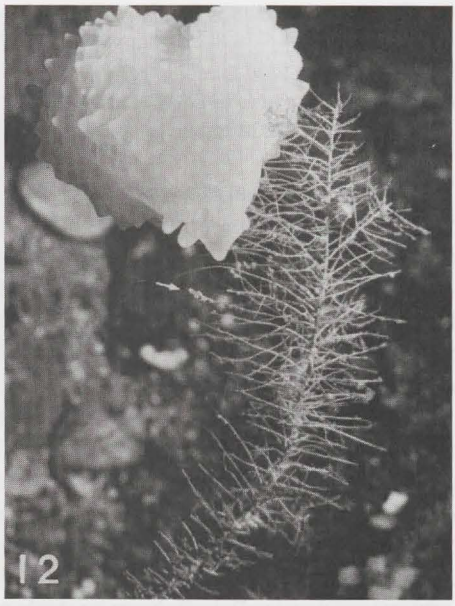


Fig. 11. "Bottle brush" gorgonian *Thouarella* spec., with *Dactylanthus antarcticus* on a branch partly cleared of polyps. There is a small sea whip (*Primnoella compressa*) to the left.
Fig. 12. *Dactylanthus antarcticus* on a skeleton of *Thouarella* spec., whose few remaining polyps (arrow, centre) suggest that the colony was destroyed recently (by predation?). The anemone's basal region is in view. Photograph turned clockwise by 90° (i.e., the anemone would hang down). Reduced perhaps $\times 2/3$.

when attached (figs 2, 5; and Dunn, 1983) but basilar muscles are absent (Carlgren, 1949). The unusual axial fusion of mesenteries proximally could perhaps be a device related to the mobility of the foot (Carlgren, 1911).

The array of regularly spaced hollow papillae on the column is formidably adhesive (fig. 6 and field observations). As the vesicles and tentacles possess spirocysts, not found in other tissues, and atrichs, either spirocysts, atrichs or both are responsible for the notable adhesion to gorgonians and other substrates. According to Carlgren (1949) the cnidom of Ptychodactiaria includes only these two elements. Another small nematocyst, however, occurs in small numbers (Carlgren, 1940; Dunn, 1983; England & Robson, 1984; K.W. England, present results).

Figures 1, and 7-9 show elongated anemones hanging leech-like from sea whips, or humped with contracted papillae. The sea whips, often more than 50 cm long, are subject to tidal currents and eddies, but the anemones seem securely attached. As predators, however, they must not only move along the gorgonians as they feed, but eventually find new colonies. It may be supposed that if released into sufficiently flowing current, *Dactylanthus antarcticus* would be buoyed along until brought into chance contact with more gorgonians. *Preactis millardae*, which is occasionally stranded on beaches, has been observed carried along by water flow (Dr G.C. Williams, personal communication; England & Robson, 1984).

The figures suggest that *Dactylanthus antarcticus* preys on *Primnoella compressa* and *Thouarella* spec. The feeding mechanism is unusual and must depend upon

external digestion in the first instance. The mouth is oval or slit-like (figs 2, 7) and the pharynx is relatively short with unusual pockets or folds (Carlgren, 1911; England & Robson, 1984) which would permit accommodation to relatively broad prey whose tissues are presumably dissolved away from the axial skeleton.

Distribution of the anemones must depend upon that of prey species. *Dactylanthus antarcticus*, like other Ptychodactiaria, is dioecious and produces large numbers of small oocytes. If planktotrophic larvae and metamorphosed young polyps were dispersed by currents until they encountered favourable gorgonians, patchy or seasonal recruitment might be expected. It should be noted that as it appears more usual for polar species to develop large oocytes, hence lecithotrophic larvae (Arntz, Bray & Gallardo, 1994), in this respect *D. antarcticus* seems exceptional. But planktotrophic larvae occur in the Antarctic among shallow-water benthic species, a life history which would favour dispersal and rapid colonisation of unstable habitats (Pearse, McClintock & Bosch, 1991).

The locations of *Dactylanthus antarcticus* conform to an antarctic and subantarctic distribution with Magellanic affinities, and will correspond to hard substrate benthic communities (see Dayton, 1990). Its presence in south Chilean fjords would follow a north flowing coastal current or eddies due to Westerlies (Lünning, 1990; Webb, Kilworth, Coward & Thompson, 1991). The sites at Canal Darwin and Estrecho Collingwood are open to tidal inflow from nearby Pacific channels (Dayton, 1985). Due to glaciation about 18,000 years ago, the present fauna of these fjords is relatively recent and arrived by means of currents or ice rafting (Dayton, 1985; Lünning, 1990). The cold-temperate conditions here are nevertheless in contrast to those at McMurdo Bay where *D. antarcticus* was first recorded (Clubb, 1908). Species of comparable distribution occur e.g., among other anemones (Dunn, 1983), gorgonians (Broch, 1961), and sponges.

The bipolar distribution of Ptychodactiaria invites comment, although only with the help of future genetic studies may it be possible to reconstruct the evolutionary history of this group. *Dactylanthus antarcticus* has specialised features which indicate that it is no newcomer to the Southern Ocean. If *Preactis millardae*, placed in a different family (England & Robson, 1984), also had a southern ancestor, its dispersal to South Africa by the Pliocene could be attributed to the establishment of the cold Benguela Current (Lünning, 1990; Williams 1992). Concerning *Ptychodactis patula*, its arctic-boreal locations suggest that it would perhaps have dispersed to the North Atlantic from the Pacific after the Bering Strait opened 3 million years ago (Lünning, 1990; Lindberg, 1991). Bipolarity has evolved many times (Lindberg, 1991; Crame, 1995); among marine molluscs, for example, bipolar dispersal of genera known since Jurassic times may be due to progressive restriction of a cosmopolitan distribution, while later examples indicate transequatorial migration (in both directions), or else radiation from southern origins (Crame, 1996). Gorgonians, of interest as a possible substrate for Ptychodactiaria, have crossed oceanic thermal boundaries by migrating into deeper water (Broch, 1961; Grasshoff, 1979; Williams, 1992) so that an early Pacific origin for the Ptychodactiaria may perhaps be suggested. When *Ptychodactis patula* is rediscovered, a study of its taxonomic relationships may prove as interesting as its biology.

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The histological basis of tissue fluorescence in the hermatypic coral *Agaricia agaricites* (Linnaeus, 1758)

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Delvoye, L. The histological basis of tissue fluorescence in the hermatypic coral *Agaricia agaricites* (Linnaeus, 1758).
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 143-150, figs 1-12.
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Key words: Scleractinia; *Agaricia* spp.; tissue fluorescence.

Abstract: Coral tissue fluorescence in *Agaricia* spp. was studied with epifluorescence microscopy. This fluorescence is concentrated in special cells, filled with fluorescent particles. For them the names fluorocyte and fluorosome are proposed. In the agariciids, fluorocytes are associated with zooxanthellae in the surface tissues of the coral. In some histological slides and most ground sections of these corals, the natural fluorescent activity is retained, despite histological treatment and years of storage. Electron microscopy of *Agaricia agaricites* (Linnaeus, 1758) from Curaçao and *Leptoseris* spec. from the Seychelles shows great similarities in ultrastructural characteristics in their zooxanthellae, host cells and associated fluorocytes. EM-images suggest a functional relationship between these cell types in the synthesis, formation, distribution and storage of fluorosomes.

Introduction

The occurrence of natural fluorescence in scleractinians is widely known among coral researchers and aquarium hobbyists. However, only a few recent papers relating to it are known to date (Schlichter et al., 1987; Delvoye, 1992).

Fluorescence of a substance is its property to absorb light of a certain energy and to reemit it as light with a lower energy. The energy difference is lost as heat (Stokes effect).

In the coral reef environment, with increasing depth the natural light is progressively scattered and absorbed (Jerlov, 1968), leaving only the blue to ultraviolet wavelenghts. Living corals display fluorescence when exposed to radiation in the spectral range from 350 nm to 420 nm. Spectroscopy on seven species of Agariciidae and *Meandrina meandrites* (Linnaeus, 1758) showed three emission bands with peak values around 430 nm, 530 nm and 570 nm (Delvoye, 1992). These values match the maxima in the absorption spectra of the photosynthetic pigments in the zooxanthellae (Jeffrey & Haxo, 1968). Corals may fluoresce to support photosynthesis in their symbionts by converting light of short wavelengths into photosynthetically active light (Schlichter et al., 1987; Delvoye, 1992).

This paper presents a histological study of tissue fluorescence in *Agaricia* spp., and samples from some other coral species.

Materials and methods

Samples of both fluorescing forms of *Agaricia agaricites* (Linnaeus, 1758): *A. agari-*

cites agaricites and *A. agaricites purpurea* Lesueur, 1820, and other agariciids were collected at Carmabi Buoy 1 on the reef near Piscadera Bay on the SW coast of Curaçao between 10 and 50 m depth (July-August, 1992). A few samples of *Montastrea cavernosa* (Linnaeus, 1758) and *Montastrea annularis* (Ellis & Solander, 1786) were collected at the same reef at depths from 6.5 m to 10 m. A juvenile colony of *Leptoseris* spec., from a depth between 12 and 15 m, collected in January 1993 on a reef in Desnoefs, Seychelles, was processed for electron microscopy.

The samples were kept up to a week in aquariums with running seawater in a sheltered place in the open air, protected from direct skylight. A Philips 8W type 08 UV-A 'Black Light' lamp was used to test the samples for fluorescence (Delvoye, 1992).

Small pieces of coral were sliced with a rock saw, using seawater as a coolant. These pieces were mounted in seawater in a small Petri dish or on a microscope slide with Aquakit (a polyester cement) and covered with a cover slip. A Leitz Ortholux microscope, equipped with a Ploemopak 1.0 epifluorescence attachment and a high pressure mercury lamp was used to examine the living coral surface. Also examined were squashes of the same material, made by scraping the surface with a scalpel and flattening the scrapings with gentle pressure between a microscope slide and a cover slip. Photographs were taken with a Zeiss photomicrographic camera on Kodak Ektachrome 200 and 400 ASA film. Two filter combinations were used. First, two 1 mm Schott BG 12 in prefiltering, position 2 (range 370-425 nm) of the attachment, Leitz K 510 or K 530 blocking filter. Second, an 1 mm UG 5 + 2mm BG 38 (Schott) in position 2. (Schott, 1991).

For electron microscopy, selected samples were fixed in a mixture of 36% formaldehyde and seawater (1 + 9, by volume), sealed and stored in plastic bags. This solution has an osmotic pressure of about 1620 milliosmol. Up to four months later, they were post-fixed at 4°C for several days in 2% glutaraldehyde, in a pH 7.2 0.1 M sodium cacodylate buffer. By adding sodium chloride, the osmolarity of the solution was adjusted to 1620 milliosmol. The same buffer was used and the same osmolarity was maintained when post-fixing in 0.5% osmium tetroxide at 4°C for 2.5 hours. The samples were decalcified for a week in 4% aquous L-ascorbic acid which was changed daily, while being kept in a refrigerator.

Infiltration and embedding in Araldite followed after dehydration in an ethanol series of 30%, 50%, 70%, 80%, and 90% in distilled water, three changes of absolute ethanol, and a mixture of Araldite and ethanol (1 + 1, by volume), each step taking 30 minutes. After two hours of infiltration, with one change, the samples were put in gelatin capsules filled with Araldite and polymerized at 60°C for 24 hours.

Thin sections were cut on LKB and Reichert ultramicrotomes with glass knives and contrasted with uranyl acetate and lead citrate. The sections were examined with a Philips EM 300 electron microscope.

Results

Fluorescence microscopy

The living surface of *A. agaricites agaricites* was dotted with fluorescing cells, against a background of zooxanthellae glowing red from the fluorescence of chlorophyll-a (fig. 1), observed through a clear highly refractive epidermis, which distorted

the images. This became noticeable when using high power objectives. Therefore, objectives with numerical apertures of 1.00 and higher can only be used with squashes. Isolated fluorescing cells were seen to be filled with clusters of small fluorescent particles. When squashed, the contents of the fluorescent cells was better visible, indicating the presence of a dense, light scattering substance between them. The fluorescent particles were so small that it was impossible to measure or photograph them individually due to Brownian movement.

The names fluorocyte and fluorosome are here proposed for the fluorescing cell and fluorescing particle, respectively.

There are differences in fluorocyte distribution between samples from 25 m and 52 m depth (figs 1, 2). The suggestion of close association between fluorocytes and zooxanthellae, noticed when studying squashes, becomes stronger when figs 2 and 3 are compared: Both are from the same depth, but the sample in fig. 3 is an aberrant non-fluorescent form of *A. agaricites* (Delvoye, 1992). Zooxanthellae cluster around fluorocytes in the fluorescent form, in the other they spread evenly over the surface.

When observing *A. agaricites purpurea*, *A. lamarcki* (Milne Edwards & Haime, 1851), *A. undata* Ellis & Solander, 1786, and *A. humilis* Verrill, 1901, also a close association of fluorocytes and zooxanthellae is suggested. These results prompted the author to look for fluorocytes in routine histological slides and ground sections of the Agariciids and other corals produced in the course of earlier investigations (Delvoye 1989, 1992).

Indeed, in ground sections of the agariciids made between 1987 and 1989 by Donath's method (Donath, 1987) identification of fluorocytes was easy because much of the original activity is preserved, despite histological treatment, for example, *Agaricia grahamae* (Wells, 1973) (fig. 4). Recognition in old, routine histological slides followed suit (fig. 5). Because eosin and erythrosin (both fluorochromes) were originally used as stains for these sections, fluorescence microscopy alone is not decisive.

Fluorocytes are always found in the light exposed surface of coral colonies, mostly in the endodermis, occasionally in the epidermis (*A. humilis*, *Helioseris cucculata* (Ellis & Solander, 1786)). They may occur concentrated (polyp mouths and tentacles of *A. lamarcki*; polyp mouths of *A. agaricites purpurea*), or evenly distributed over the surface (*A. agaricites agaricites*, *A. undata*, *A. humilis*). Fluorocytes are always closely associated with zooxanthellae and have therefore an irregular shape.

M. cavernosa and *M. annularis* show poor fluorescence activity when irradiated with a type 08 UV-A lamp. However, under the microscope, squashes of them show a brilliant fluorescence. This apparent contradiction was solved by irradiating these corals with a quartz halogen lamp, using a 2 mm thick Schott BG 12 filter. When looking through a yellow filter a vivid emission can be seen. A Sylvania blue fluorescent tube, similar to Philips type 03, gives the same results. Hence, the required radiation for these corals to show emission must be somewhere in the range of 400 nm to 450 nm.

The ovoid shaped fluorosomes have a diameter of one to two micrometers and show internal structure in *M. cavernosa* (fig. 6) or a smooth surface as in *M. annularis*. They are part of fluorescing tissue structures, extending into the polyp's interior. In routine histological slides made by Van Veghel (1993) they can easily be identified by observing the same tissue alternately in normal light and by its fluorescence under the microscope.

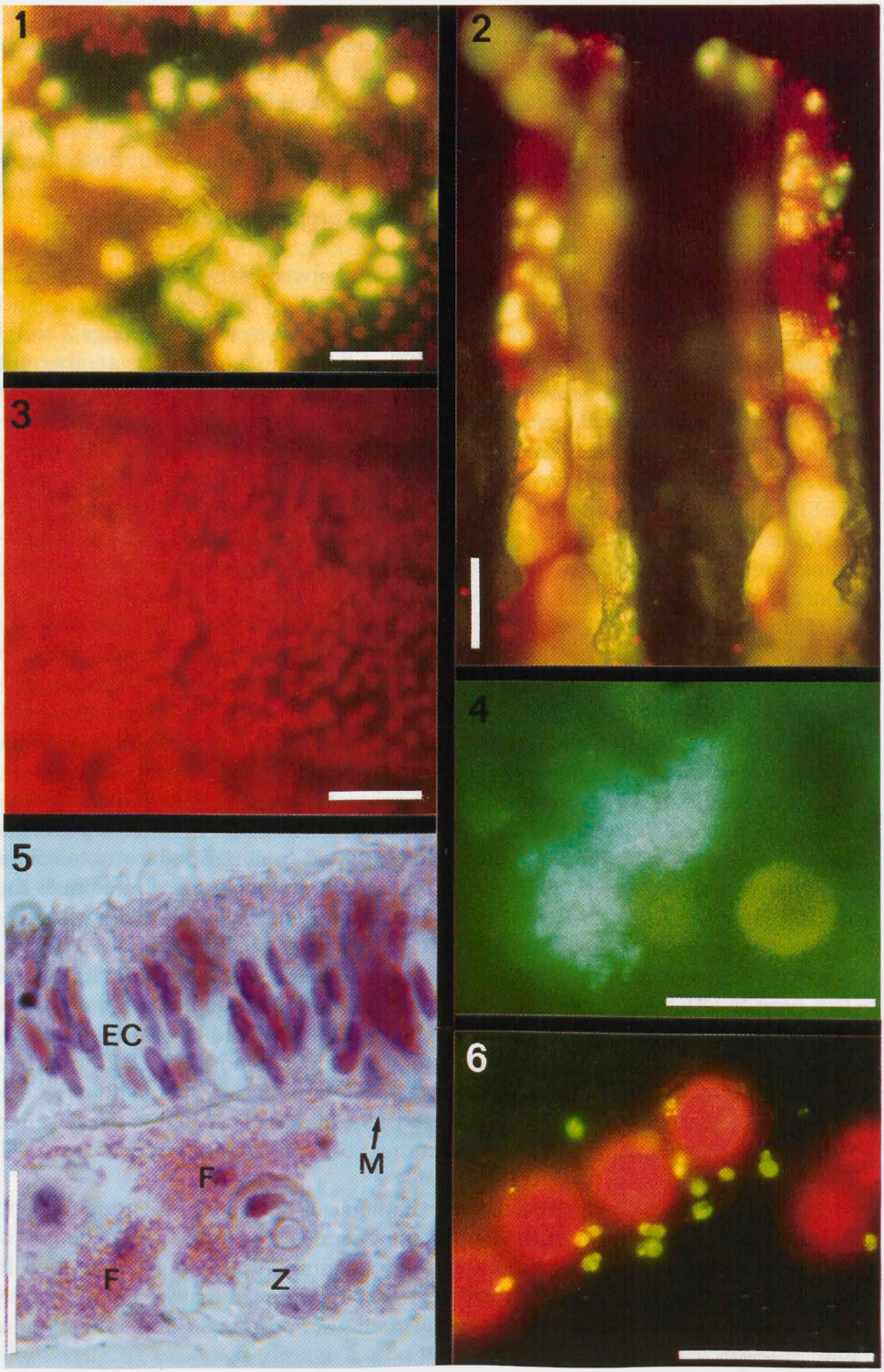
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Fig. 1. *Agaricia agaricites* from a depth of 25 m. Fluorocytes (yellow) occur scattered in the endodermis of the surface layer of the coral; between them zooxanthellae (brownish). Scale bar = 25 µm.
Fig. 2. *Agaricia agaricites* from a depth of 52 m. Fluorocytes (yellow) and zooxanthellae (red) are confined to narrow strips on both sides of a calcareous sept (the dark median band). The surface tissue contains no zooxanthellae. Scale bar = 50 µm.
Fig. 3. *Agaricia agaricites*, non-fluorescent form from a depth of 52 m. The zooxanthellae are spread over the entire surface of the coral. Scale bar = 25 µm.
Fig. 4. *Agaricia grahamae* (Wells, 1973) from a depth of 32 m. Epifluorescence of ground section made by the Donath method in 1989; photograph taken five years after preparation of slide. Superficial staining with toluidine blue; the fluorocytes are not stained and show fluorosomes; zooxanthellae light green. Scale bar = 10 µm.
Fig. 5. *Agaricia agaricites* from a depth of 25 m. Routine histological transversal section, showing ectodermis (EC), and below it endodermis with zooxanthellae (Z) surrounded by fluorosomes (F); M = mesoglea. Scale bar = 10 µm.
Fig. 6. *Montastrea annularis*. Squash of surface tissue showing zooxanthellae (red) and compound fluorosomes of type II (yellow to green). Scale bar = 10 µm.

Electron microscopy

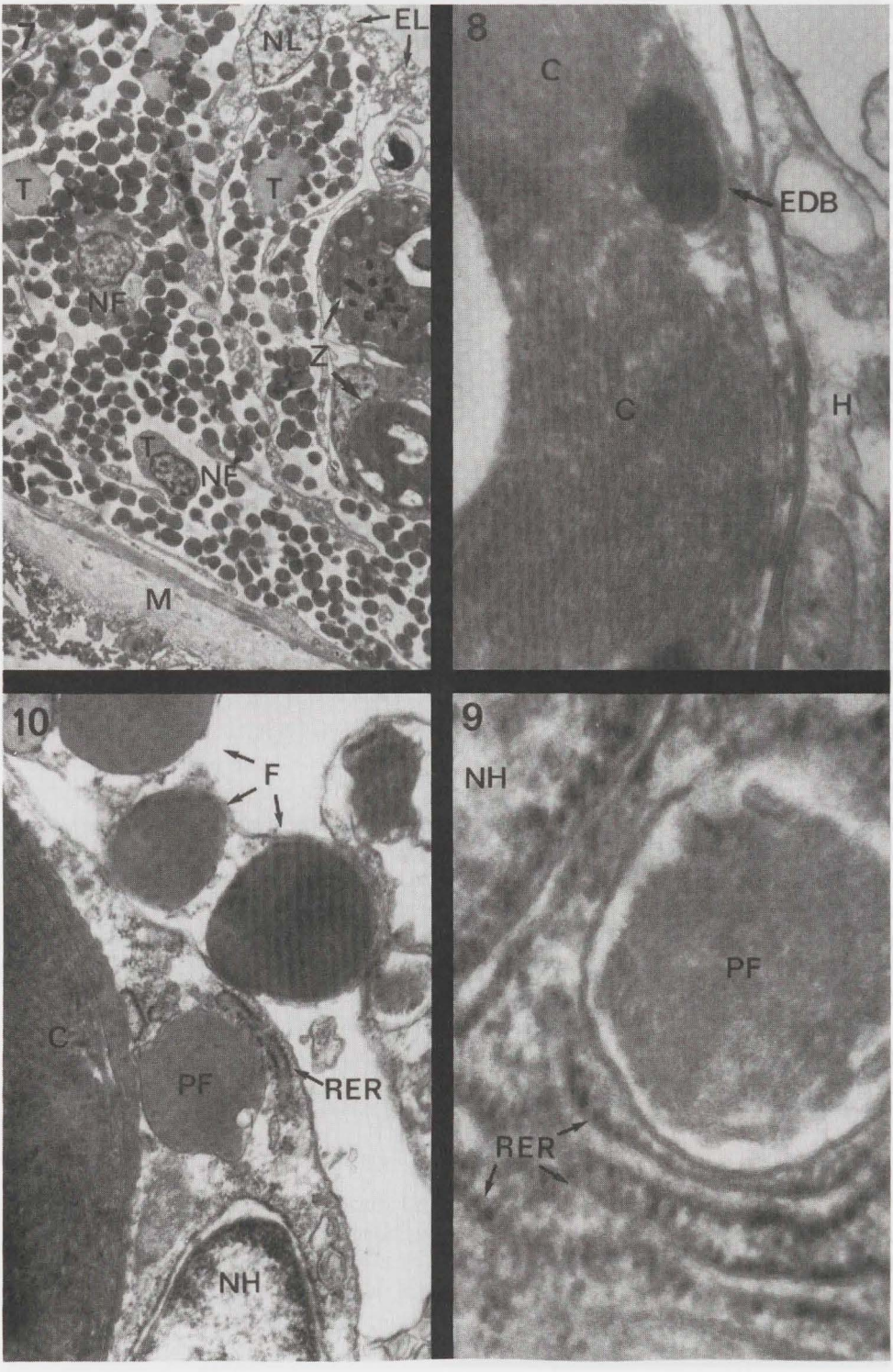
Samples of the two *A. agaricites* forms and a juvenile colony of *Leptoseris* spec. were examined. Both species, while originating from different regions in the world, have a similar ultrastructure of their zooxanthellae, host cells, fluorocytes and fluorosomes. Identification of fluorocytes under the electron microscope was made easy by comparing semi-thin and thin sections of the same blocks, the first stained with toluidine blue for light microscopy. They appear as irregularly shaped cells with small amounts of cytoplasm around the nucleus and the cell membrane. Oval tubes lined with ribosomes connect the cytoplasmic compartments. Occasionally protein-containing vesicles are also present. Most of the cellular volume is filled with a membranous network, containing fluorosomes (fig. 7). A dictyosome, indicating synthetic activity is observed near the nucleus. Strings of fluorosomes are associated with it (fig. 12). However, no precursor stages of fluorosomes were found here.

Fluorosomes are globular or ovoid shaped bodies with diameters of about 725 nm. They contain a substance of uniform density and are enveloped by a trilaminar unit membrane. In the host cells synthetic activity is found around fluorosome-like bodies (figs 9, 10). Ribosomal activity is apparent. After processing, extrusion into the fluorocytes follows (fig. 11).

Only in zooxanthellae, associated with fluorocytes electron dense bodies between the chloroplasts were found. These electron dense bodies lack a trilaminar unit membrane (fig. 8) and are smaller than fluorosomes.

Discussion

The close association of zooxanthellae and fluorocytes, seen in both epifluorescence microscopy and electron microscopy, supports the idea that tissue fluorescence in stony corals enhances the photosynthesis of their zooxanthellae (Schlichter et al., 1987; Delvoye, 1992). By converting the short wavelengths in the incident flux of daylight into photosynthetically active radiation, they extend the vertical range of these corals on the reef.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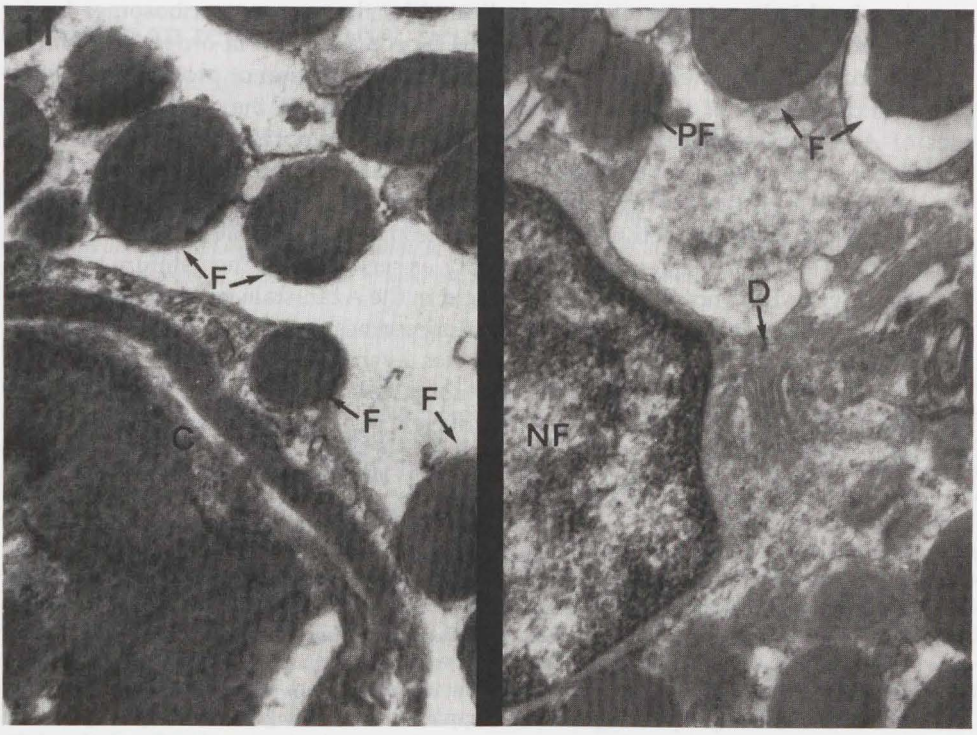

Fig. 7. *Agaricia agaricites*. Electron micrograph of transverse section. To the right two zooxanthellae (Z) in their host cells. To the left a fluorocyte with two nuclei (NF), filled with fluorosomes; the apparent empty spaces between the fluorosomes contain a highly refractive substance, probably a polysaccharid; tubes (T) of cytoplasm can be seen in sagittal and cross sections. M = mesoglea, EL = endodermal lining cells, showing one nucleus (NL). Magnification 3600 ×.

Fig. 8. Electron micrograph. Detail of zooxanthella showing electron dense substance (EDB) between two chloroplasts (C), probably the precursor stage of a fluorosome. H = host cell. Magnification 37600 ×.

Fig. 9. Electron micrograph. Detail of early stage of protein synthesis in fluorosome formation in the host cell; note ribosomal activity as indicated by presence of rough endoplasmatic reticulum (RER). NH = nucleus of host cell, PF = pre-fluorosome. Magnification 143500 ×.

Fig. 10. Electron micrograph. Detail of late stage of protein synthesis in fluorosome formation in the host cell; the rough endoplasmatic reticulum is still present. Magnification 43300 ×.

Fig. 11. Electron micrograph. Extrusion of fluorosome by host cell into the fluorocyte. Magnification 37600 ×.

Fig. 12. Electron micrograph. Detail of nucleus of fluorocyte with dictyosome (D). Magnification 37600 ×.

Electron microscopy of both *A. agaricites* forms and *Leptoseris* spec. suggests a three step sequence in the synthesis of fluorosomes:

The first step is the production of a substance by the zooxanthellae that is probably a precursor of the fluorochrome that fills the fluorosomes, which are extruded into the host cells (fig. 8). Production of the pre-fluorochrome(s) (derivatives of photosynthetic pigments?) by the zooxanthellae can explain why the spectral composition of the fluorescence emissions are about the same, regardless of the coral species (Delvoye, 1992). The second step is the processing of the pre-fluorochrome(s) into fluorosomes by the host cell and their extrusion into the fluorocytes (figs 9-11). This

may involve binding to a protein, as indicated by the numerous ribosomes in the rough endoplasmatic reticulum. This may be a necessary step in order to become a fluorochrome. If so, a mutation that results in the inactivation of protein production or in the production of a dysfunctional protein may explain the occurrence of colonies of *A. agaricites* that are partly "dark" and partly fluorescent (Delvoye, 1992). Another indication for protein binding is the stability of the fluorochrome(s) in the fluorosomes: treated with formaldehyde and/or glutaraldehyde, both protein fixing substances, much of the activity is retained despite histological processing. Treatment with heavy metals quenches this activity, as seen when using osmium tetroxide in postfixing for EM and phosphotungstic acid in the Azan-stain combination.

The last step involves the storage of the fluorosomes in the membranous network in the fluorocyte. By distributing fluorosomes evenly in the cellular space, their natural light absorption and hence their emission will be optimized. This packaging and distribution activity is found near the nuclei (fig. 12). However, the flow direction of this activity (towards the nucleus or away from it) could not be determined.

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Cnidarian reproduction: assumptions and their implications

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Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 151-162, fig. 1.
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Key words: Corals; sea anemones; somatic mutation; vegetative propagation.

Abstract: At least some individuals of the actinian *Bathyphellia australis* at 4100 m off California spawn annually, contrary to the expectation that reproduction in such a constantly cold, dark environment would be continuous. This hypothesis was based on what seemed, in the absence of empirical data, to be reasonable premises, just as had been the inferences that the abyss would be devoid of life and that deep-sea animals would be genetically less variable than shallow-water ones. These discredited paradigms were based on flawed premises.

Understanding reproduction of other cnidarians in other habitats may similarly require reassessing long-held assumptions and considering additional factors. Evolutionary forces affecting release, fertilization, and distribution of propagules may be similar for sessile organisms, be they plants or animals, living in a fluid medium sufficiently dense to transport the propagules. Beyond morphological and functional parallels is the cytological one of absence of a germ-cell lineage, which, in plants permits propagation of genetic novelty vegetatively and into the next generation. The accumulation of somatic mutation is consistent with some data on cnidarian reproduction.

The nearest analogs to some aspects of cnidarian reproductive biology are in plants; other aspects are clearly not plant-like. The implications of these similarities and differences relate to deeply-held and seldom-discussed assumptions about the nature of animals, as contrasted with that of plants, that should be addressed explicitly in future studies of cnidarian reproductive biology.

Introduction

Despite empirical evidence to the contrary, prior to the mid-19th century, most scientists reasoned that the deep sea should be lifeless because of its high pressure, low temperature, and darkness (Gage & Tyler, 1991). Subsequently, the constancy in many parameters of the physical environment led to the hypothesis that genetic variability, including taxonomic diversity, should be much less in deep than shallow seas. Such reasoning was also belied by empirical evidence (summarized by Gage & Tyler, 1991).

Because changes in day length and ambient temperature, the Zeitgebers for reproductive periodicity in terrestrial and shallow-water organisms, are absent at depth, sexual reproduction in deep-sea animals was predicted to be continuous (Orton, 1920). As sufficiently large samples have been examined, this expectation, too, has not been borne out entirely. Reproductive studies on seven species of deep-sea hexacorals — all from the North Atlantic — have been published. Four sea anemones — *Paracalliactis stephensoni* (see Van-Praet, 1990), *Phelliactis hertwigi* and *P. robusta* (see Van Praët et al., 1990), and *Amphianthus inornata* (see Bronsdon et al., 1993) — spawn annually, whereas in the zoanthids *Epizoanthus paguriphilus* and *E. abyssorum*, and the actinian *Kadosactis commensalis* (see Bronsdon et al., 1993), sexual reproduction is continuous (Muirhead et al., 1986).

Bathyphellia australis

I have studied reproduction in the acontiate sea anemone *Bathyphellia australis* Dunn, 1983, from collections made three times a year for five years from a site at 4100 m in the Northeast Pacific 220 km off the coast of south-central California (34°50'N 123°00'W). The animals came from a single population, in contrast to those of previous studies that were from multiple sites; the North Pacific differs in biological oceanography from the North Atlantic (Smith et al., 1992); and the depth from which animals were collected is greater than those of previously studied anemones. The animals are gonochoric, with some of both sexes spawning in the interval between collections of October and February (Fig. 1), at the time particulate organic carbon and sediment-community oxygen consumption are at their minimum annual values (Smith et al., 1992). As for the four sea anemones mentioned above, reproductive cyclicity appears to be entrained by periodicity in the rain of organic material from the surface waters, which, in turn, is controlled by annual cycles in day-length and temperature (Smith et al., 1994). I shall publish a complete study of reproduction in *B. australis* elsewhere.

Expectations and hypothesis testing

The history of deep-sea biology teaches us that expectations based on what seem to be reasonable assumptions may not be borne out. When assumptions are not recognized, empirical results based on them may be misinterpreted. In the rest of this paper, I discuss some preconceptions about reproductive biology of cnidarians (primarily anthozoans, with which I am most familiar), and offer some alternative interpretations.

An example of how hypotheses have been challenged in face of empirical evidence concerns mating systems. Cnidarians exhibit a wide range of sexual/reproductive patterns. This may be related to the simplicity of gamete production in them (Fautin, 1991). Various forms of hermaphroditism exist, and among some simultaneous hermaphrodites self-fertilization, which is generally considered deleterious, is possible (e.g. Bucklin et al., 1984; Heyward & Babcock, 1986). An expectation of even greater detriment is associated with hybridization. Upon learning of the mass spawnings on the Great Barrier Reef (references in Harrison & Wallace, 1990), many biologists wondered how hybridization is avoided. The assumption persists (e.g. Palumbi, 1994) that pre-fertilization mechanisms must exist to avoid gamete wastage; after years of searching, a sperm attractant has been identified in one species of mass-spawning coral (Coll et al., 1994). Failing pre-fertilization mechanisms, hybrid inviability was postulated (e.g. Willis, 1990). However, not only does hybridization readily occur between some taxa in the laboratory (Wallace & Willis, 1994), even intergeneric hybrids can form during mass spawnings (Kenyon, 1995). Diversity in the major reef-forming genus *Acropora*, and the lack of congruence between species defined on genetic, morphological, and reproductive criteria may be due in part to hybridization (Wallace & Willis, 1994).

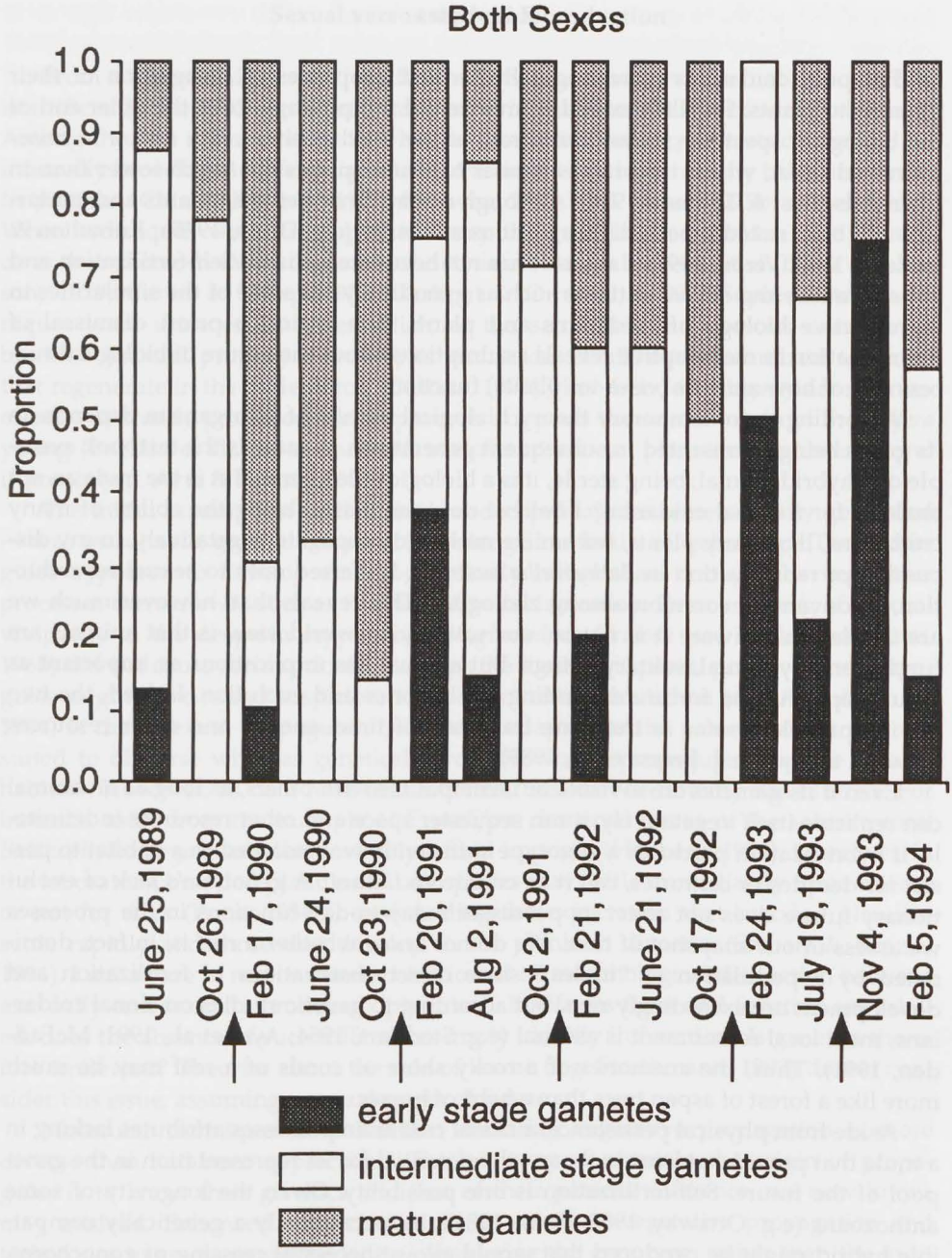

Fig. 1. Gametogenic cycle of the abyssal sea anemone *Bathyphellia australis* during 1989-1994. The proportion of animals of both sexes possessing immature, maturing, and mature gametes is plotted; arrows indicate probable times of spawning. Ten animals were sampled per time; sterile ones are not represented in this plot.

Zoophytes

Polypoid cnidarians were originally termed zoophytes in recognition of their likeness to plants. Parallels extend from external morphology to, at the other end of the biological spectrum, substitution rate at the third position of a codon in mitochondrial DNA, which in corals is similar to that in plants but much lower than in mammals (Best & Thomas, 1994). Although resemblance between plants and cnidarians has been noted repeatedly for various attributes (e.g. Dunn, 1975a; Knowlton & Jackson, 1993; Veron, 1995), I assert it has not been internalized. Self-fertilization and hybridization, and sexual patterns such as gynodioecy are some of the similarities in reproductive biology of cnidarians and plants. The typical a priori dismissal of hybridization as maladaptive reveals assumptions about the nature of biological success and of how animals (vis-à-vis plants) function.

According to contemporary theory, biological success of an organism depends on its genes being represented in subsequent generations. A mule is the textbook example of a hybrid animal; being sterile, it is a biological dead end. But is the mule an apt analogy for a clonal cnidarian? I believe not, one reason being the ability of many cnidarians, like many plants, but unlike mules, to propagate vegetatively. In my discussion of reproduction in *Bathyphellia australis*, I referred only to sexual reproduction, a convention common among zoologists. This reveals that, however much we are intellectually aware it is not so, our underlying world view is that animals are fundamentally sexual, solitary beings. But asexual has implications as important as sexual reproduction for understanding life histories and evolution. Indeed, the two are inseparable insofar as there are trade-offs of time, energy, and other resources between the two (e.g. Pearse et al., 1989).

Even if its gametes are inviable or incompatible with others, as long as an animal can replicate itself vegetatively, it can sequester space and other resources indefinitely. If hybridization produces a genotype sufficiently well adapted to a habitat to persist for decades or centuries, can it be counted a failure? A genotype's lack of evolutionary future does not affect its possessor's day-to-day functions in the processes we assess in our snapshot of time. We do not know whether a reef is, in fact, dominated by a population of "mules," since direct observations of fertilization and development are exceedingly rare. But according to genetic studies on clonal cnidarians, most local recruitment is asexual (e.g. Stoddart, 1984; Ayre et al., 1991; McFadden, 1991). Thus, the anemones of a rocky shore or corals of a reef may be much more like a forest of aspen trees than a herd of horses.

Aside from physical persistence, a clonal cnidarian possesses attributes lacking in a mule that provide, at least in theory, the potential for its representation in the genepool of the future. Self-fertilization is one possibility. Given the longevity of some anthozoans (e.g. Ottaway, 1980; Potts, 1984a, b), occasionally a genetically compatible hybrid might be produced that would allow successful crossing of gonochores, or a mutation might overcome self-incompatibility in a hermaphrodite. As Yoshioka (1994) recognized, critical events in the lives of such longevous organisms are likely to be infrequent.

Sexual versus asexual reproduction

From a theoretical perspective, the line between sexual and asexual reproduction is artificial (Buss, 1983). As a practical matter, in cnidarians it is increasingly blurred. Among the first sea anemones in which reproductive ecology was studied were the internally brooding *Actinia equina* and *A. tenebrosa*, and the externally brooding *Epiactis prolifera* (Chia & Rostron, 1970; Ottaway, 1979; and Dunn, 1975b, respectively). When techniques were developed to assay the genetics of individuals, it became clear that young in the two species of *Actinia* are asexually generated (Black & Johnson, 1979; Carter & Thorp, 1979; Gashout & Ormond, 1979). (Sophisticated techniques were unnecessary to establish, at the same time, an obvious example of brooded asexual progeny: *Bunodeopsis medusoides* swallows autotomized tentacles that regenerate in the coelenteron [Cutress, 1979].) But brooded progeny are not necessarily asexually produced: electrophoresis supports the sexual origin of *E. prolifera* young (Bucklin et al., 1984), and both sexual and asexual planulae have been found in corals (e.g. Ayre & Resing, 1986).

The initial assumption — that the brooded propagules were sexually generated — was reasonable, given the state of knowledge and limits of technology. The propagules appeared to develop embryogenetically rather than through the vegetative processes then known, such as fission and pedal laceration. Even knowing of asexual viviparity in the deep-sea scyphozoan *Stygiomedusa fabulosa* (see Russell and Rees, 1960) did not cause serious doubt. Perhaps the limited dispersal power of the propagules might have raised suspicion, for the conventional wisdom (e.g. Ayre & Dufty, 1994) is that asexual propagules, being well adapted to the parent habitat, are not suited to disperse whereas genetically variable sexual propagules disperse to new habitats. However, as data have accumulated, this neat dichotomy has blurred. For instance, aside from the example of *E. prolifera*, demersal larvae of the solitary coral *Balanophyllia elegans* are demonstrably sexual in origin (Hellberg, 1994). Moreover, in the laboratory, brooded planulae of one species may settle immediately upon release or not for days (Potts, 1984b), confounding the expectation that brooded planulae settle rapidly and those that develop in the plankton disperse (Stobart & Benzie, 1994). I am aware of no consistent behavioural or morphological distinction between asexually and sexually produced "planulae". Insofar as form and function are related, does the absence of such differences imply identity in function? Is genetics therefore irrelevant? Theories about the role of larvae (e.g. Strathmann, 1993) do not consider this issue, assuming a sexual origin of larvae.

Molecular techniques revealed that cnidarian vegetative propagation is more diverse than had been appreciated. The best way to ascertain the origin of propagules is to know their genetics and that of their parents; inference, we now know, may mislead. Enhanced appreciation of the extent of vegetative propagation and techniques to assess genotype led to considering the genotype, rather than the individual (such as a polyp), the relevant unit of selection in clonal organisms (e.g. Hoffmann, 1976). (I use the term "clone" to mean a collection of individuals descended vegetatively from a single sexually-produced progenitor; they may or may not be physically linked.)

Clonemates are assumed (sometimes explicitly, often implicitly) to be genetically

identical. As such, Hughes & Cancino (1985: 176) proposed that clonal organisms provide replicates "for testing life-historical theories: they can be separated into genetically identical units." Doing such testing, McManus et al. (1994: 103A) found "Growth rate ... surprisingly variable within clones" of *Haliplanella lineata* in the laboratory. Timing and number of spawnings vary among clonemates of another anemone, *Nematostella vectensis*, held under identical conditions (Hand & Uhlinger, 1992, 1995). Tissue grafting has been used to assess genetic identity among colonial anthozoans, but results from this assay do not always agree with those using other techniques (e.g. Willis & Ayre, 1985). Neigel & Avise (1983: 443) attributed the one "anomalous rejection response" in 24 experimental grafts of *Acropora cervicornis* to a mistaken identification of the experimental subjects as clonemates, rejecting the possibility it was due to "divergence in histocompatibility identity among clonemates," an unpublished idea they attributed to Tunnicliffe.

Genetic mosaicism

I know of no empirical evidence that clonemates are genetically identical, an assumption that seemed reasonable but increasingly does not fit the evidence (above). The possibility that cnidarian clonemates may differ genetically has been mentioned (e.g. Neigel & Avise, 1983; Sebens & Thorne, 1985; Hughes et al., 1992), but in no study of which I am aware have the full implications of this possibility been seriously considered; we continue to function as if clonemates were identical.

In discussing the importance of knowing whether a clone is genetically homogeneous, Avise (1994) considered only fusion of genetically unlike larvae or colonies as a possible cause of genetic mosaism. A more common source is likely to be somatic mutation. Given other resemblances between plants and cnidarians, I expect genetic variability to accumulate in cnidarian clones as it does in long-lived plants. For long-lived clonal cnidarians, such as corals, "in a temporally and spatially heterogeneous environment that has the potential to exert variable selective pressures" (Potts, 1984b: 1072), it is difficult to comprehend how a single genotype could persist through space and time. Buss (1985) discussed mechanisms of creating variability and their consequences, one of which is fine-tuning to microenvironmental differences (Buss, 1983). Genetic heterogeneity is manifest as ecotypic variation, which is as typical of clonal cnidarians (some studies on corals summarized by Willis, 1990) as it is of plants (Whitham & Slobodchikoff, 1981). Even modules such as branches of a single tree may differ genetically due to accumulated mutations, and compete with one another. In consequence, the genotype of a plant can change through time as better-adapted modules prosper and those more poorly adapted wither (reviewed by Gill et al., 1995); the original genotype may even disappear (Whitham & Slobodchikoff, 1981). If parts of cnidarian clones accumulated differences, they could compete directly with one another or indirectly, through competition for resources, just as do conspecific clones originating from different founders (e.g. anemones: Francis, 1973; corals: Willis & Ayre, 1985).

Chromosomal mutation rate in hybrids of many taxa is higher than in non-hybrids (King, 1993), which adds to the probability of some corals, at least, accumulating genetic variability. Selection of somatic mutants is more immediate than that of gametic ones (Buss, 1983), enhancing the likelihood that favorable mutations are

propagated. Hughes (1989: 29) provided no evidence for the assertion that "somatic division or modularity itself will tend to limit the spread of mutant cells." Genetic mosaicism allows potentially long-lived organisms the flexibility to survive in face not only of variations in the physical environment, but against predatory and disease-causing organisms that could otherwise evolve to overcome the larger organism's defenses (Whitham & Slobodchikoff, 1981). Details of the mechanism by which mutants accumulate would be expected to differ between plants and cnidarians; but meristem is unnecessary for the same pattern to be manifest — mutations that occurred in the more distant past or that resulted in a genotype that propagates more readily would tend to be more widespread and common than more recent or less prolific ones.

Somatic mutations are potentially heritable in plants and cnidarians (as well as in animals of 18 other phyla) due to absence of a germinal cell line (Whitham & Slobodchikoff, 1981; Buss, 1983, 1985). Indeed, "Mutations arising somatically in plants have a greater probability of being incorporated into the gene pool than mutations that arise in the gametes of either plants or animals" (Whitham & Slobodchikoff, 1981: 288). This possibility further blurs the line between sexual and asexual reproduction in cnidarians, and therefore a distinction between growth (propagation of a particular genome) and reproduction (propagation of novel genomes) (e.g. Pearse et al., 1989) may be unrealistic. At least some apomictic plant populations are neither genotypically nor phenotypically less diverse than strictly sexual ones (Whitham & Slobodchikoff, 1981), and so are not, as is often asserted (e.g. Pearse et al., 1989), less evolutionarily flexible (Silander, 1985). This is also true in the hermatypic corals studied by Budd (1990). By analogy, although "continued presence of a sexually reproducing portion of the population" (Sebens & Thorne, 1985: 373) may reduce the chances of a lineage's extinction (Budd, 1990), it may be far from true in cnidarians that "recruitment of sexually derived propagules is probably the most common mechanism generating clonal diversity" (Sebens & Thorne, 1985: 373). To the attributes of the clonal soma listed by Hughes & Cancino (1985) — morphological flexibility, potential to accumulate large biomass, and often potentially rapid growth — can be added potential genetic flexibility. This potential could be the engine driving creation of genotypes that, when incorporated into gametes, also contribute to success in sexual reproduction, particularly, perhaps, in face of environmental change.

Expectations and hypothesis testing revisited

Genetic data are used increasingly "to infer the mode of reproduction and the extent and directionality of dispersal" (Ayre, 1990: 403) in cnidarians. Explanations invoked to account for the poor fit between data and expectations in many studies are *ad hoc*, and include founder and Wahlund effects (e.g. Black & Johnson, 1979; Ayre & Dufty, 1994, respectively), and balancing, disruptive, and random selection (e.g. Hoffmann, 1976; Potts, 1984b; Burnett et al., 1994, respectively). Although these factors may be relevant in some cases, the discrepancy between expectations and data warrants examination of underlying assumptions. Models from which such inferences are made assume particular attributes of populations (Avise, 1994); if the assumptions are wrong, the inferences are likely to be, too.

Populations in which sexual reproduction predominates are expected to be in

Hardy-Weinberg equilibria and have high genotypic diversity, whereas excesses and deficits of heterozygotes and low genotypic diversity are expected in populations dominated by vegetative propagation. Brazeau & Harvell (1994: 57) found "genotypic diversities equal to those expected for a population dependent solely on sexual reproduction" in an octocoral population they knew propagates vegetatively. Had they lacked independent evidence, they might have drawn an erroneous conclusion about reproductive mode.

The poor fit between expectations and data may be because the expectations were developed for organisms unlike cnidarians. Gene flow differs among taxa (Avise, 1994), so metrics for intrataxon variability cannot be generalized: populations of the anthozoans studied by Solé-Cava & Thorpe (1991) have high levels of heterozygosity relative to other taxa. If somatic mutation were important, high genotypic diversity could characterize a population of clones that had successfully adapted to a variety of microenvironments through a long time. Low genetic diversity in asexual populations can be expected only if, in addition to somatic mutations not accumulating, lifespan is short relative to the time constant of relevant selective factors (above). In addition to lifespan and generation time, which Potts (1984a) recognized can affect the predictions of genetic models, frequency of sexual recruitment, ploidy, and deviations from panmixis, among other parameters, will affect interpretation of gene frequencies, so must be assessed in creating models that will more successfully explain empirical data.

The population genetics of cnidarians are described by many authors as unusual. Presumably the peculiarity is relative to expectations based on other taxa, but is normal for cnidarians. The challenge is to understand cnidarians on their own terms.

Implications for systematics

The paradigm of modern genetics is that sexual reproduction maintains gene flow and thereby cohesiveness among members of a species; without gene flow, genetic divergence accumulates, ultimately leading to fragmentation of the species (e.g. Harrison & Wallace, 1990; Palumbi, 1994). But reproductive isolation seems neither necessary nor sufficient for demarcating species of clonal cnidarians. Genetic "cohesiveness shows little consistent relationship with degree of outcrossing" in reef corals studied by Budd (1990). The lack of correspondence among reproductive, morphological, and molecular characters discussed by Wallace & Willis (1994) is consistent with the conclusion of a symposium on species and evolution in clonal organisms (Budd & Mishler, 1990: 168) that "Speciation is something more than the acquisition of reproductive isolation." Such within-species coherence is also true of non-clonal cnidarians: the anemone *Urticina eques*, with a larva poorly adapted to disperse, is genetically uniform over great distance (Solé-Cava et al., 1994), and life history traits of the external brooder *Epiactis prolifera* are similar at two distant sites (Fautin & Chia, 1986).

The degree of variability considered to characterize a species need not be the same across taxa (above). Modern techniques have revealed the existence of many previously unrecognized species (e.g. Knowlton, 1993; Stobart & Benzie, 1994), but molecular criteria led Burnett et al. (1994: 158) to conclude "there has been a general

exaggeration in the numbers of extant zoanthids" and to anticipate "a substantial reduction in the number of species." Evolution of corals has been proposed by Veron (1995) to be reticulate within metaspecies, the largest unit in which hybridization does not occur. This does more than shift the level of analysis: it potentially separates morphologically recognizable units from genetically connected units, and it means that all members of a species do not necessarily share a single history.

Conclusion

At this early stage of genetic analysis of cnidarian reproduction, we must take care not to reason from unfounded assumptions, no matter how rational they seem or how applicable they are to other organisms. Until we have established patterns empirically, based on numerous instances, we risk circularity in reasoning inductively.

Most plants and polypoid cnidarians (clonal or not) are sessile, and may rely on a fluid medium for dispersal of their gametes/propagules (the greater density of water than air probably accounts for an apparent absence of "pollinators" among aquatic invertebrates). Similarities in these attributes presumably result in similar evolutionary pressures on the two and therefore analogous adaptations, such as aversive chemicals or structures for protection. Because of these similarities, a priori assumptions about reproduction (including genetics and population biology) should not necessarily be made by analogy with other animals. There are, to be sure, fundamental differences between plants and cnidarians (e.g. Willis, 1990; Knowlton & Jackson, 1993). I aver the focus has been on these differences because of a deeply-held dichotomous perception about the nature of plants and animals. Removing the blinders of kingdom chauvinism may bring theory into closer correspondence with empirical observations.

Discussion has centered on clonal cnidarians not only because they represent the majority of cnidarians and most reproductive research has been on them. They are more plant-like than non-clonal cnidarians in possessing vegetative propagation and the associated possibility of somatic mutation, which profoundly affect life history parameters. Although unlikely to reconcile all incoherent observations on population genetics, systematics, and reproductive biology of cnidarians, incorporation of these biologically reasonable possibilities into models may improve understanding of how these animals function, and thereby the fit between data and expectations.

Reproduction of cnidarians, as has been pointed out repeatedly (e.g. Fautin, 1991; Gage & Tyler, 1991), is highly variable, making patterns difficult to abstract and predictions difficult to make. Just as we should not generalize to clonal cnidarians from models and metrics constructed for other sorts of animals, we should remain open to the possibility that different rules may govern reproduction and evolution in non-clonal (if they truly exist!) and clonal cnidarians, and even in long-lived and short-lived clonal taxa (e.g. anthozoans versus hydrozoans and scyphozoans).

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The karyotype of the Japanese sea anemone *Haliplanella lineata* (Verrill, 1869) (Cnidaria: Actiniaria)

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Fukui, Y. The karyotype of the Japanese sea anemone *Haliplanella lineata* (Verrill, 1869) (Cnidaria: Actiniaria).
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Key words: Actiniaria; *Haliplanella lineata*; karyotype.

Abstract: The karyotype of the acontiate sea anemone *Haliplanella lineata* (Verrill, 1869) (= *H. luciae* (Verrill, 1898)) from Japan was studied in embryos by a Giemsa staining and banding method. *Haliplanella lineata* has 16 pairs of homologous chromosomes that form two groups by size and shape. Of the first group comprising 6 pairs of larger chromosomes (pairs 1 through 6), one pair is metacentric and the other pairs are subtelocentric. The numerical dominance of subtelocentric pairs in the first group and of metacentric or submetacentric pairs in the second group (pairs 7 through 16) characterizes the karyotype of *H. lineata*.

Introduction

Karyotypes of many animals have been determined by banding techniques. Previously reported chromosome numbers of Cnidaria, for instance those of *Hydra* (Niiyama, 1944; Datta,1970), were usually obtained by sectioning or squash methods. In Anthozoa, the squash method with orcein staining for preparation of mitotic chromosomes from embryos yielded the first karyotype of the coral *Goniopora lobata* Milne-Edwards & Haime, 1860 (2n = 28), as described by Heyward (1985). A similar method was used by Van-Praet & Colombera (1985) to demonstrate haploid chromosome numbers of 17 and 15 in spermatocytes of the sea anemones *Phelliactis robusta* Carlgren, 1928, and *Amphianthus radiatus* Carlgren, 1928. Recently, Fukui (1993), using an air drying-Giemsa staining method with early embryos, found that the diploid chromosome number of the anemone *Haliplanella lineata* from Japan was 32. However, no details of karyotype analysis have yet been provided. The purpose of the present work was to examine the karyotype of early embryos of *H. lineata* and to develop a method which allows application for banded karyotypes.

Material and methods

Specimens of *Haliplanella lineata* were collected in the Miura district, Kanagawa Prefecture, near Tokyo. The chromosome preparations were made from embryos at the 2- to 32-cell stages. Methods for preparation of chromosomes have been described previously (Fukui, 1993) but were used here with some modifications. Prior to the stage chosen for analysis, embryos were incubated for 30 min in a solution of 0.05% w/v colchicine or 2.5 µg/ml colcemid in sea-water. To prepare chromosome spreads for air drying, the method developed for Hydra, as modified by Rahat et al.

(1985), was used. The preparations were stained with 4% Giemsa in phosphate buffer (pH 6.8) for 30 min. The banding patterns were obtained by the ASG technique (acetic/saline/Giemsa) described by Sumner et al. (1971) with slight modifications.

Results and discussion

The morphology of the chromosomes of *Haliplanella lineata* was studied in early embryos using conventional Giemsa staining. In addition karyological analysis using the ASG method was carried out.

A typical metaphase plate after Giemsa staining is shown in figure 1. The diploid number of chromosomes was 32, confirming previous results (Fukui, 1993). Unlike in previous preparations, the positions of the centromeres were easily visible in most cases, probably because of the higher concentration of the colchicine solution. The banding patterns facilitated the identification of all the chromosomal pairs. The chromosomes were serially arranged by size and centromere position (fig. 2). The karyotype consisted of 16 pairs of chromosomes that could be divided into two groups according to their sizes and shapes. The first group (pairs 1-6) comprised 1 metacen-

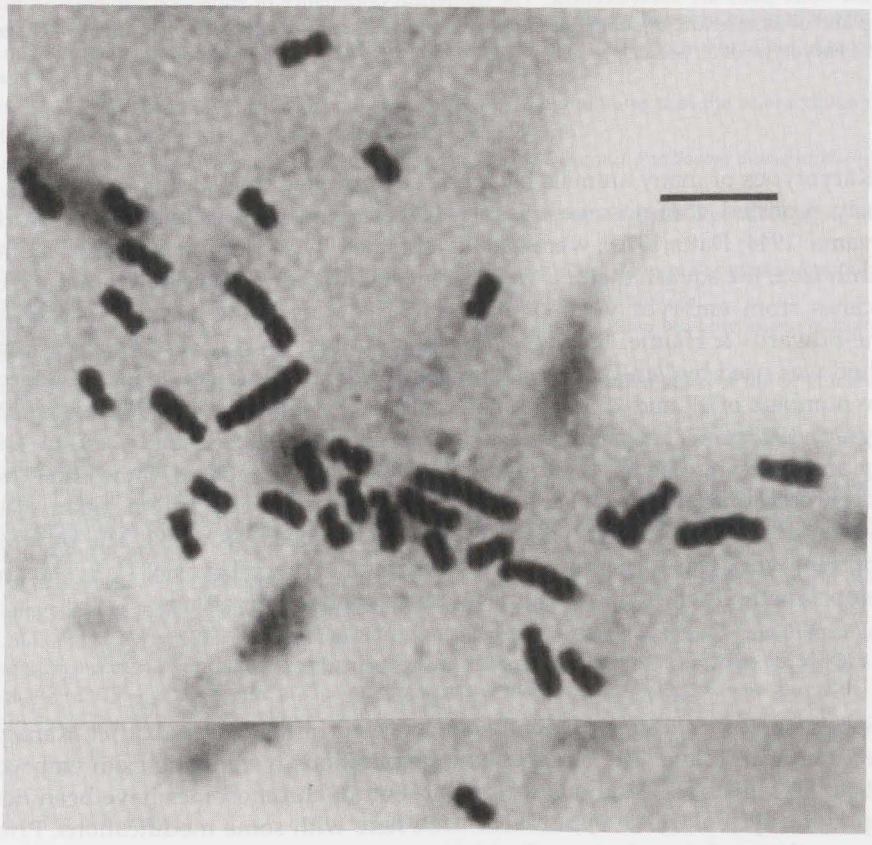

Fig. 1. Typical diploid components of conventional Giemsa-stained metaphase of *Haliplanellla lineata*, 2n = 32. Bar represents 5 µm.

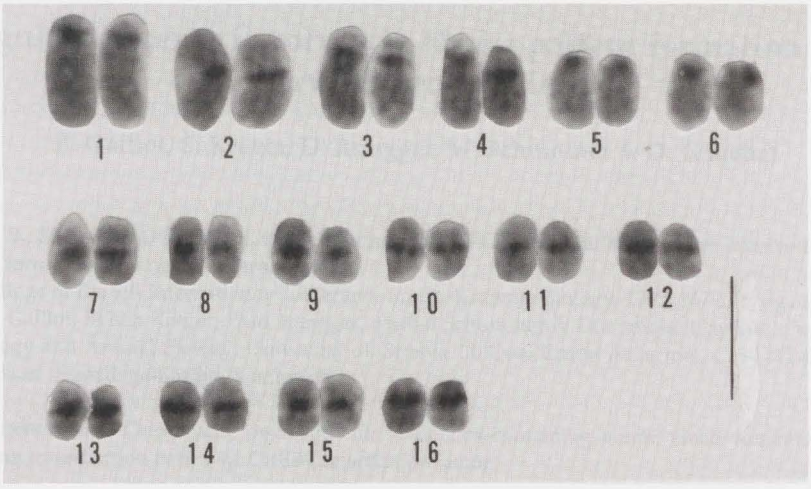

Fig. 2. Karyotype of *Haliplanella lineata*. Colcemid was applied at 16- to 32-cell stages and the preparation was made by the banding method. Bar represents 5 μm.

tric and 5 subtelocentric pairs of larger chromosomes. Each of these six pairs could be identified by their lengths, shapes and banding patterns. The remainder (pairs 7-16) comprised a second group. There was no marked difference in size beween pairs 6 and 7, which therefore did not separate distinct size groups, although their shapes were different. Most pairs belonging to the second group were close to metacentric and submetacentric shapes with sizes from medium to small; but one pair (16) appeared to be subtelocentric. The preparations obtained using the present air-drying technique were also suitable for studies on banded chromosomes.

Haliplanella lineata is widespread along Japanese coasts. Moreover, this species is characterized by a high degree of morphological and ecological diversity (Uchida, 1932). The presence of karyotypic variation in this species therefore seems likely. Hence, it is necessary to perform karyological analyses of further specimens from additional localities.

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Regulatory genes involved in *Hydra* pattern formation and nerve differentiation

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Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 167-175, figs 1-5, tab. 1.
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Key words: *Hydra*; *Chlorohydra*; development; homeobox-containing genes; Head Activator; cAMP signalling transduction pathway; CREB transcription factor.

Abstract: *Hydra* is one of the most primitive organisms displaying an apical to basal differentiation process and understanding the molecular mechanisms underlying pattern formation in such a simple model system might highlight some basic processes involved in development. Therefore in a first approach we have isolated regulatory genes, namely homeobox-containing genes, which in more complex animals have been proved to specify positional information along antero-posterior axis of developing embryos. By screening cDNA libraries from two *Hydra* species, we isolated ten different homeobox-containing genes: Among them, two are related to the HOM/HOX genes, *cnox1* (*labial* type) and *cnox2* (*Deformed* type) and two encode a paired-type homeodomain highly related to that of *aristaless*. At the level of gene expression, our previous results showed that *cnox1*, and *cnox2* display a significant differential temporal expression during apical regeneration (Schummer et al., 1992: 1815). In this report, we show that *prd-a* expression is restricted to the apical region, in few cells of the central part of the mouth ectoderm and is not detectable within first hours of apical regeneration suggesting a role for *prd-a* in late differentiation of ectodermic mouth cells. Following another approach we investigated the signal transduction cascade followed by the neuropeptide Head Activator (HA) which is involved in apical-specific differentiation. In conditions where HA leads to nerve cell differentiation, after HA treatment or upon cutting, we measured an activation of the cAMP signalling pathway, both at the cytoplasmic and at the nuclear level suggesting that the *Hydra* cAMP Response Element Binding protein (CREB) might play a key role, as a transcription factor in the induction of final nerve cell differentiation observed within first hours of regeneration. Thus the CREB nuclear regulatory protein might be an useful marker of early stages of regeneration.

Introduction

Morphology of *Hydra* adult polyps results from a continuous process of differentiation from the undifferentiated gastric region towards the extremities of the animal. In addition, *Hydra* is capable of epimorphic regeneration, of reaggregation, and of asexual reproduction through budding, whereas few species readily display sexual development. All these processes represent different developmental contexts but might all be the result of a similar cascade of events leading to pattern formation. In order to understand the molecular mechanisms underlying these processes, we started to characterize genes encoding regulatory proteins, namely transcription factors, and to analyse their specific regulation during regeneration. By analogy with pattern formation mechanisms delineated in more complex animals, we first focused on homeobox-containing genes (for an explanation of technical terms, abbreviations and

acronyms, see the end of this paper). So far we have isolated ten different *Hydra* homeobox-containing genes from cDNA libraries of adult polyps of *Hydra vulgaris* Pallas, 1766, and the "multiheaded" mutant of *Chlorohydra viridissima* (Pallas, 1766). The deduced homeodomain sequences show in most cases a high degree of similarity with arthropod or vertebrate homeoproteins, signing thus their relatedness to specific classes of homeodomains. The question is whether this structural conservation correlates with some functional pressure along evolution and whether we can trace back the ancestral function of homeogenes in development and cell differentiation in Cnidaria. As a preliminary answer, we present the expression pattern we obtained with two *paired-like* genes in adult polyps and during regeneration which suggests some functional conservation for one of them. The second approach we are currently following involves the signal transduction pathway targetted by the endogenous neuropeptide HA. HA, which has been characterised several years ago by Schaller, is released upon injury and involved in apical-specific differentiation processes (reviewed in Schaller et al., 1989: 99). At concentrations where HA exposure induces determination or differentiation of nerve cells, cytoplasmic cAMP levels are elevated and, at the nuclear level, the binding of nuclear proteins onto the cAMP response element is dramatically modified during regeneration and after HA treatment (Galliot et al., 1995: 1205). The gene encoding the cAMP Response Element Binding protein (CREB) has been isolated from *Hydra* cDNA libraries, and the sequence of its product exhibits a surprisingly high level of conservation in the domains involved in DNA-binding and cAMP-dependent phosphorylation. Some aspects of the *Hydra* CREB transcription factor activity will be discussed.

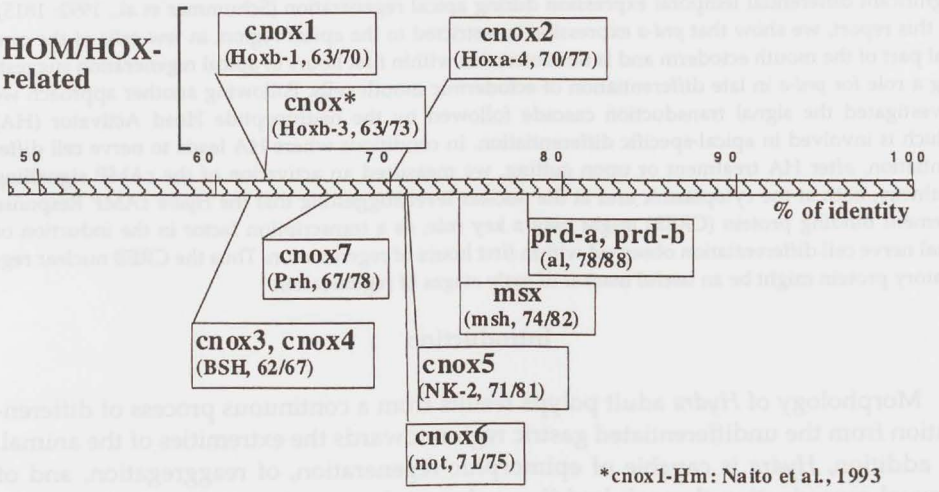

Fig. 1. Scheme depicting the relation between *Hydra* homeodomains and their relative arthropod or vertebrate cognates. Homeodomains related to the Antp-type (HOM/HOX) have been listed in the upper part of the figure. The first number corresponds to the percentage of identity between homeodomains (out of 60 residues), the second to that of similarity when conservative substitutions are included. References of the sequences are in (Duboule, 1994) except that of *al* (Schneitz et al., 1993: 114), *NK-2.5* (Lints et al., 1993: 419), *not* (von Dassow et al., 1993: 355), *Prh* (Crompton et al., 1992: 5661), *bsh* (Jones & McGinnis, 1993: 793) and *cnox** Hm (Naito et al., 1993: 271).

Results and discussion

Hydra homeobox-containing genes

In arthropods, nematodes and vertebrates, HOM/HOX genes display a conserved clustered genomic organisation and specify positional information during development according to the colinearity principle. This means that their domain of expression along the antero-posterior axis of the embryo follows their order along the chromosome (Gaunt et al., 1988: 169; Duboule & Dolle, 1989: 1497; Graham et al., 1989: 367; Wang et al., 1993: 29). Because of these highly conserved features it has been proposed that HOM/HOX genes plus some others, define the zootype (Slack et al., 1993: 490; for more details see the report of D. Miller in these proceedings). Following this hypothesis, *Hydra* would represent one of the most primitive organism displaying the zootype characteristics. Therefore in a first approach we wanted to know which homeobox-containing genes are expressed in such a simple organism and what might be their expression pattern.

By screening cDNA libraries representing mRNA of adult polyps of *Hydra vulgaris* and *Chlorohydra viridissima*, we isolated ten different homeogenes (Schummer et al.,

Table 1: Homeodomain (homeod.) sequences of 10 different *Hydra* homeogenes isolated from cDNA libraries of *Hydra vulgaris* (hv) and *Chlorohydra viridissima* (cv) adult polyps have been compared to that encoded by *Antennapedia* (*Antp*) from *Drosophila melanogaster* (dm). Dashes correspond to identical amino acids. Stars underneath the consensus sequence correspond to invariant residues, inverted triangles to highly conserved residues. PG1, PG4: homeodomains related to paralog groups (PG) 1 and 4 of the HOX genes.

```
Antp dm     ERKRGRQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN

cnox1 cv          CSFGHRKII---R--KYK-----D--L-F-RN-D-S-S---V--------Q---Q   labial
cnox1 hv    DAF-K-CSFGHRKII------KY-K--S-D--V-F-RN-E-S-S------------Q---Q   (lab, PG1)

cnox2 cv    KS--I-TA--SI-L-------QN----S-L---Q--AI-D---K-V--------V----DK   Deformed
cnox2 hv    KS--I-TA--SL-L-------QN----S-L---Q--VI-D---K-V--------V----DK   (Dfd, PG4)

prd-a hv    NER-Y-T-F-QF-LD---RA-DKTH-PDVFM-E---VRVH---ARVQV------A--R-RE   aristaless
prd-b hv    KKR-Y-T-F-TH-LD---RV-NRTH-PDIFL-E-M-VK-G---AR-QV------A--R-A     (al)

msx1 cv     ANRKP-TPFSVN-L-T--QK-KRKQ--SISE-A-LSEL-R---T----------A-Q-RSK   muscle-segment
msx1 hv     ANRKP-TPFSVN-L-A--QK-KRKQ--SISE-S-LSVM-R---T----------A-Q-RRT   homeod. (msh)

cnox5 hv    S--KP-ILFSQSQVM----K-KDQK--SASE-DQ--NK-N--PT-V------K-Y-C-NKQ   NK-2

cnox6 hv    CN--R-TIF-I--LER--C--QQQQ--VGHE-RY--KD-G-NEV-C-V------I--R-SS   not

cnox7 hv    SK-CVQVRFSHS-ST---RV-LVQK-ISPYE-KQ-SRS-D-S-----T------A--RXLK   Prh

cnox3 cv    KCRKP-TVFSDL-LMV--R--NNRK--STPQ-TNL-DR-G-NQT-V-T-Y----------T   brain-specific
cnox4 cv                                          -M--DN-V-T-Y------L-RHI   homeod. (bsh)
            1.......10.........20.........30.........40.........50.........60
consensus   ..KR.R..Y...Q...LE..F....YLT...R......L.L...QIK.WFQNRR.K.KK..
                  Δ       ΔΔ Δ    Δ                Δ    Δ  ** * * Δ Δ
```

1992: 1815; S.K. and B.G., unpublished data). Their deduced homeodomain sequences (fig. 1, see references in the legend) show that (a) two are related to the HOM/HOX genes, *cnox1* and *cnox2*, 60% and 70% identical to the lab- and the Dfd-type homeodomains respectively; a third different *Hydra* HOM/HOX-related gene has more recently been isolated (Naito et al., 1993: 271) proving that at least three HOM/HOX-related genes representing paralog groups (PG) 1, 4 and 3 are expressed in adult *Hydra*; (b) *prd-a* and *prd-b* encode a paired-type homeodomain highly related to that of *aristaless* (*al*; 78% identical); (c) msx is highly similar to the msh-type homeodomain (74% identical), (d) *cnox5*, *cnox6* and *cnox7* encode homeodomains related to that of *NK-2* (71% identical), *not* (71% identical) and *Prh* (67% identical) respectively; (e) finally *cnox3* and *cnox4*, show homeodomain sequences more distantly related to bsh (brain-specific homeodomain, 62% identical with that of cnox3).

When conservative substitutions are taken into account, these rates of homology are even higher reaching 88% in case of prd-a and prd-b homeodomains (fig. 2) although the conservation at the nucleotide level in the homeobox does not reach 50%. Outside the homeodomain, some *Hydra* homeoproteins (cnox2) contain short conserved domains (Schummer et al., 1992: 1815) but this does not seem to be the rule (S.K. and B.G., unpublished). It was previously shown that some of these *Hydra* homeobox genes, *cnox1*, *cnox2* and *cnox3* display a significant differential expression during apical regeneration in *Chlorohydra viridissima* (Schummer et al., 1992: 1815). We have investigated by Northern blot analysis and in situ hybridization the pattern of expression of the *prd-a* and *prd-b* genes which encode the mostly conserved homeodomains. This analysis shows that *prd-a* transcripts are barely detectable in gastric and basal regions but rather apically expressed. During regeneration, *prd-a* transcripts will reappear late after cutting, shortly before the time where the apical

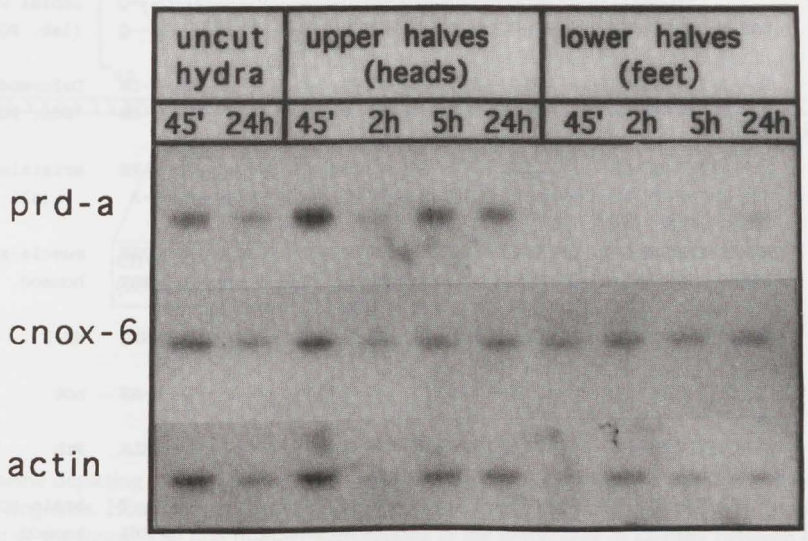

Fig. 2. Northern blot analysis performed with *Hydra vulgaris* mRNAs prepared at indicated times (hours) after cutting showing the apical-specific expression of the *prd-a* gene.

morphology becomes visible (fig. 2). In the same experiment, *cnox6* (*not*-related) does not display significant variation of its transcript levels during apical or basal regeneration. In situ hybridization performed on adult polyps show that *prd-a* is expressed in few cells of a restricted central part of the mouth ectoderm. This expression pattern might correspond to nerve cells of the inner hypostome region which are enclosed among ectodermal epithelial cells (Dübel, 1989: 99-109). In contrast, *prd-b* is expressed in patches of ectodermal cells along the gastric column and do not show any modulation of its expression during regeneration (not shown here). This expression analysis suggests first that despite highly similar homeodomain sequences *prd-a* and *prd-b* play very different roles in *Hydra* differentiation processes and second, that *prd-a* might participate to the terminal differentiation of the most apical part of the animal which is reminiscent of the role played by the *aristaless* gene in the differentiation of distal appendages of *Drosophila* (Schneitz et al., 1993: 114).

Head Activator (HA) and the cAMP pathway

HA is a conserved neuropeptide which exerts in *Hydra* three main effects: it is a mitogenic factor for all cell types, it induces nerve determination from interstitial stem cells and it leads to apical-specific differentiation of nerve and epithelial cells. These effects require HA at different concentrations. Our previous data show that, at concentrations where the determination/differentiation effect is observed, HA behaves as an agonist of the cAMP pathway. At the nuclear level the binding of nuclear proteins onto the cAMP response element is dramatically modified during apical or basal regeneration and after HA treatment at relatively high concentrations (Galliot et al., 1995: 1205). A *Hydra* cAMP Response Binding protein (CREB) related gene has been isolated which exhibits a surprisingly high level of conservation in the basic domain, the leucine zipper motif and the protein kinase A phosphorylation site (fig. 3). Polyclonal antibodies raised against the product of this gene specifically recognise the hydra CREB protein in the CRE-binding complex whose pattern on band-shift assays is drastically changed during regeneration (Galliot et al., 1995: 1205).

In order to test the transactivation activity of this transcription factor and its puta-

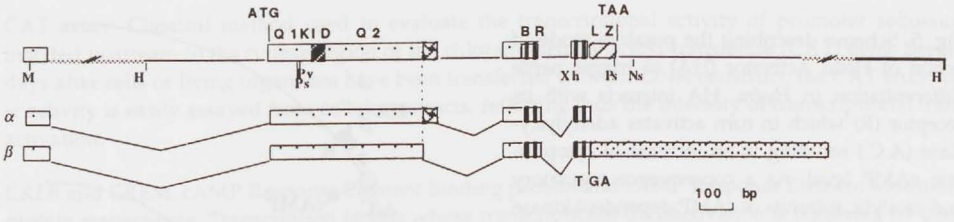

Fig. 3. Scheme depicting the highly conserved structure of the *Hydra CREB* gene when compared to the vertebrate *CREB* and *CREM* genes. Mostly conserved domains are the kinase inducible domain (KID), the basic region involved in DNA-binding (BR) and the leucin zipper domain required for dimerisation (LZ). Q1 and Q2 correspond to Glutamine rich regions. Boxes correspond to cDNA sequences, lines to genomic ones The XhoI site (Xh) and the splice site located within the basic region have been conserved from *Hydra* to vertebrate genes. As in case of the vertebrate related genes, the *Hydra CREB* gene encodes several isoforms: α and β differ by a short stretch of 17 residues.

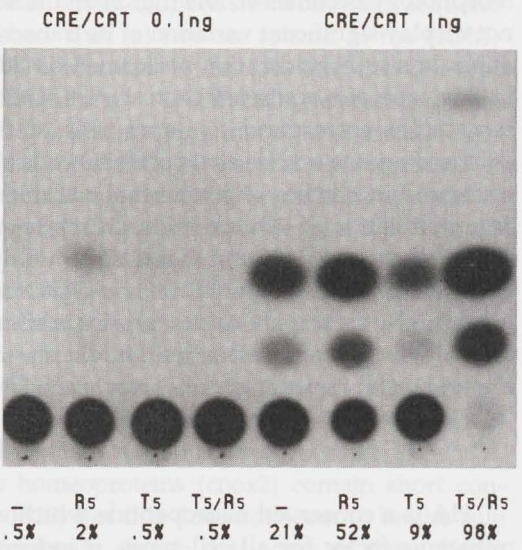

Fig. 4. Transcriptional transactivation potential of nuclear extracts from regenerating (R) or uncut (T) *Hydra* injected in *Xenopus* oocytes is deduced from CAT activity: a construct containing a CRE promoter fused to the CAT-reporter gene was coinjected and the CAT activity measured after 24 hours. This activity is expressed and shown as percentage of conversion of Chloramphenicol from the non-acetylated to the acetylated form.

tive regulation upon HA exposure, we electroporated intact specimens of *Hydra* with a CRE-containing promoter reporter construct (Montminy et al., 1986: 6682), we could detect a very clear activity suggesting that *Hydra* CREB activates transcription from this specific element (not shown here). In addition we tested the transactivation potential of extracts obtained from regenerating versus non regenerating animals by injecting them into the cytoplasm of *Xenopus* oocytes and measuring the CAT activity of a CRE-reporter construct after 20 hours. Due to the presence of endogenous *Xenopus* CREB the background activity of the reporter construct was relatively high but lowered (about twice) when nuclear extracts from intact *Hydra* were injected and increased (2.5 times) in presence of nuclear extracts from regenerating *Hydra* (fig. 4). Such variations of CAT activity were not detected when a promoter lacking the CRE was used as a control. These preliminary results would suggest that, due to the highly conserved leucin zipper domain, heterodimerisation between *Hydra* and *Xenopus* CREB proteins

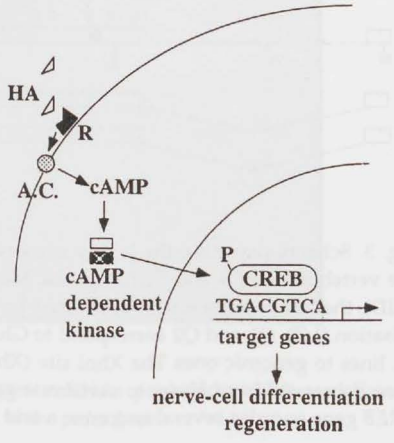

Fig. 5. Scheme describing the putative mode of action of Head Activator (HA) to trigger nerve differentiation in *Hydra*. HA interacts with its receptor (R) which in turn activates adenylylcyclase (A.C.) resulting in an increase of cytoplasmic cAMP level. As a consequence regulatory and catalytic subunits of cAMP dependent kinase (PKA) dissociate and the diffusion of the PKA catalytic subunit to the nucleus allows the phosphorylation of the transcription factor CREB modifying then its binding on cis-elements located in promoters of target genes. The modification of CREB binding might change the transcriptional activity of these target genes.

occurs and leads to repression of the endogeneous transcriptional transactivation when the *Hydra* CREB is in a predominantly inactive form as in intact *Hydra*. Conversely during regeneration, most of the *Hydra* CREB protein is in an active form responsible for the higher transactivation activity observed in the *Xenopus* oocyte.

We also confirmed that the *Hydra* CREB protein is indeed a substrate for the protein kinase A by observing an efficient phosphorylation of the *Hydra* CREB in presence of the protein kinase A (PKA) in a "in gel kinase assay" (not shown here). We are currently searching for modifications in the PKA activity of *Hydra* extracts prepared within minutes following cutting as well as modulations in the CREB transcriptional activity in the same period of time. At concentration where HA behaves as a growth factor, no modifications of cAMP levels or CRE-binding activity were noted. In addition, no data so far are available concerning the role of the cAMP pathway upon the determination and the differentiation of other cell types, namely epithelial cells. Nerve-cell differentiation is the major cellular event observed within the first 4-8 hours of regeneration and HA which is released upon cutting, is supposed to induce this effect on precursor nerve cells (Holstein et al., 1986: 9). As in these conditions HA behaves as an agonist of the cAMP pathway and as cAMP can mimick HA effect (Holstein, et al., 1986: 9; Fenger et al., 1994: 115), we propose that this HA-induced nerve-cell differentiation requires the activation of the cAMP pathway which in turn modulates the activity of the CREB transcription factor (fig. 5). This HA signal transduction pathway would thus regulate the transcriptional activity of genes responsible for early regeneration processes.

Abbreviations, technical terms and acronyms

Antennapedia (Antp): *Drosophila* homeotic gene, originally identified as a dominant mutation which transforms antennae on the head into legs. Homologous chordate HOX genes belong to paralog group 6 (for homeogenes mentionned above without references, see in Duboule, 1994).

aristaless (*al*): *Drosophila* homeogene encoding a homeoprotein containing paired-type homeodomain but no paired domain, involved in the specification of distal appendages (Schneitz et al., 1993)

bsh: brain-specific homeobox gene from *Drosophila* (Jones and McGinnis, 1993: 793)

CAT assay: Classical method used to evaluate the transcriptional activity of promoter sequences inserted upstream to the coding region of the chloramphenicol acetyl-transferase (CAT) gene. Several days after cells or living organisms have been transfected with the DNA construct, the CAT enzymatic activity is easily assayed from cellular extracts, reflecting thus the intensity of transcriptional transactivation.

CREB and *CREM*: cAMP Response Element Binding protein and cAMP Response Element Modulator protein respectively. Transcription factors whose transcriptional transactivation is regulated by phosphorylation upon cAMP levels (Borelli et al., 1993: 321).

Deformed (Dfd): *Drosophila* homeotic gene, originally identified as a dominant mutation affecting head development. Homologous chordate HOX genes belong to paralog group 4.

HOM/HOX or HOX genes: design genes which encode *Antennapedia*-type homeodomains and are clustered on the chromosome. In arthropods, homeogenes providing homeotic mutations are physically linked on a single cluster (actually broken into two complexes in *Drosophila*) and are expressed

according to the colinearity principle: Their domain of expression along the embryonic antero-posterior axis follows their order along the chromosome. In other words, products of the most 3' genes are found in anterior segments of the developing embryo and more 5' genes are expressed more posteriorly. Homologous genes with similar chromosomal organisation have been found in nematode, amphioxus and vertebrates where duplication of complexes occured (fish, rodents and man have four complexes). Expression of protochordate and chordate HOX genes follows the spatial and temporal colinearity rule.

labial (lab): *Drosophila* homeotic gene, originally identified as a recessive mutation affecting posterior head development. It is the most proximal gene of the *Antennapedia*-complex and homologous chordate HOX genes define paralog group 1.

muscle-segment homeobox (msh, msx): Highly conserved homeodomains which define their own family. In vertebrates, this homeogene is expressed in regions of interactions between ectoderm and mesoderm that will result in formations of progress zone during development (references in Duboule, 1994).

NK-2.5 : homeogene implicated in commitment and differentiation of the mesodermal myocardial lineage in vertebrates (Lints et al., 1993: 419)

Northern blot analysis: purified mRNAs representing all kind of genes expressed in the animal are run in a denaturing agarose gel, transferred to a membrane which is then hybridized to specific ^{32}P-labelled DNA probes and exposed to autoradiographic films. This method allows quantification of transcripts abundancy for a given gene.

not: homeogene expressed primarily in the gastrula organizing region of *Xenopus* (von Dassow et al., 1993: 355), tail-organizer marker in chordates (de Robertis et al., 1994: 117)

paralog groups (PG): 13 different groups of HOX genes have been characterized along the murine complexe(s) with homologs in most triploblastic species investigated so far. HOX genes belonging to paralog groups 1 (PG1) and 4 (PG4) are homologous to the *Drosophila labial* (*lab*) and *Deformed* (*Dfd*) *Drosophila* homeotic genes. Within one paralog group, homeodomain sequences are highly conserved and thus define sub-families.

Prh: vertebrate homeogene expressed in haematopoeitic cells, lung and liver (Crompton et al., 1992: 5661)

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Ecology and distribution of the limnomedusa *Limnocnida indica* Annandale, 1912, in Lake Chandubi, Assam, India

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Key words: Hydroida; *Limnocnida indica*; ecology; distribution; India; Assam; Lake Chandubi.

Abstract: The fresh water medusa *Limnocnida indica*, Annandale, 1912, was recorded in 1978 for the first time from Chandubi (Goswami, 1985), a tectonic lake in Assam, India. The occurrence of its medusoid swarms from the end of January till early in March is a regular phenomenon observed in the lake every year. The seasonality of the species is related to temperature. Since there has been no report so far on the occurrence of this species from other lentic water bodies, comprising more than 1300 lakes in Assam, its presence in Lake Chandubi seems to exhibit a highly restricted emergence in the region.
The present paper provides some preliminary information on the ecology of the species and encompasses the relevant water quality characteristics of the lake including temperature, pH, dissolved oxygen (DO), free carbon dioxide (FCO_2), total alkalinity (TA), total hardness (TH), salinity and chlorinity (Cl^-), specific conductivity, Ca^{+2}, Mg^{+2}, Fe, N, PO_4^{-3}, and SiO_2.

Introduction

A spectacular outburst of the fresh water medusa *Limnocnida indica* Annandale, 1912, was discovered in Lake Chandubi, Assam, during a limnological reconnaissance in January, 1978 (Goswami, 1985: 97), probably constituting the first record of this species from northern and north-eastern India. Earlier, *L. indica* was known to be a resident species of the Western Ghats of India (Gravely & Agharkar, 1912: 312; Annandale, 1912: 253) and there have been a number of reports on the species from south and south-western India (Rao, 1931: 971; Ramakrishna et al., 1950: 318; Jones 1951: 800; and Dumont, 1994: 3) (fig. 1, insert). Little, however, is known about its ecology. The present paper pursues to fill this gap to some extent with regard to the population in Lake Chandubi. Since its discovery in 1978, the medusoid occurs every year in a definite area of the lake (fig. 1; A), but its occurrence in two more areas (fig. 1; B and C) has been noticed since 1985 and 1990, respectively.

Study area

Lake Chandubi is of tectonic origin, having been formed during the great earthquake of 1897; it is situated at the Assam-Maghalaya border in the southern Brahamaputra Valley (25°52'15" 25°53'45"N and 91°24'15" 91°27'15"E). Some special physico-geographical features typify the lake and distinguish it from other lakes in Assam. The lake basin is dendritic, with numerous loops of depressions (mostly covered by floating swamps), a 2.5 km long perennial inlet/outlet canal, a narrow streamlet

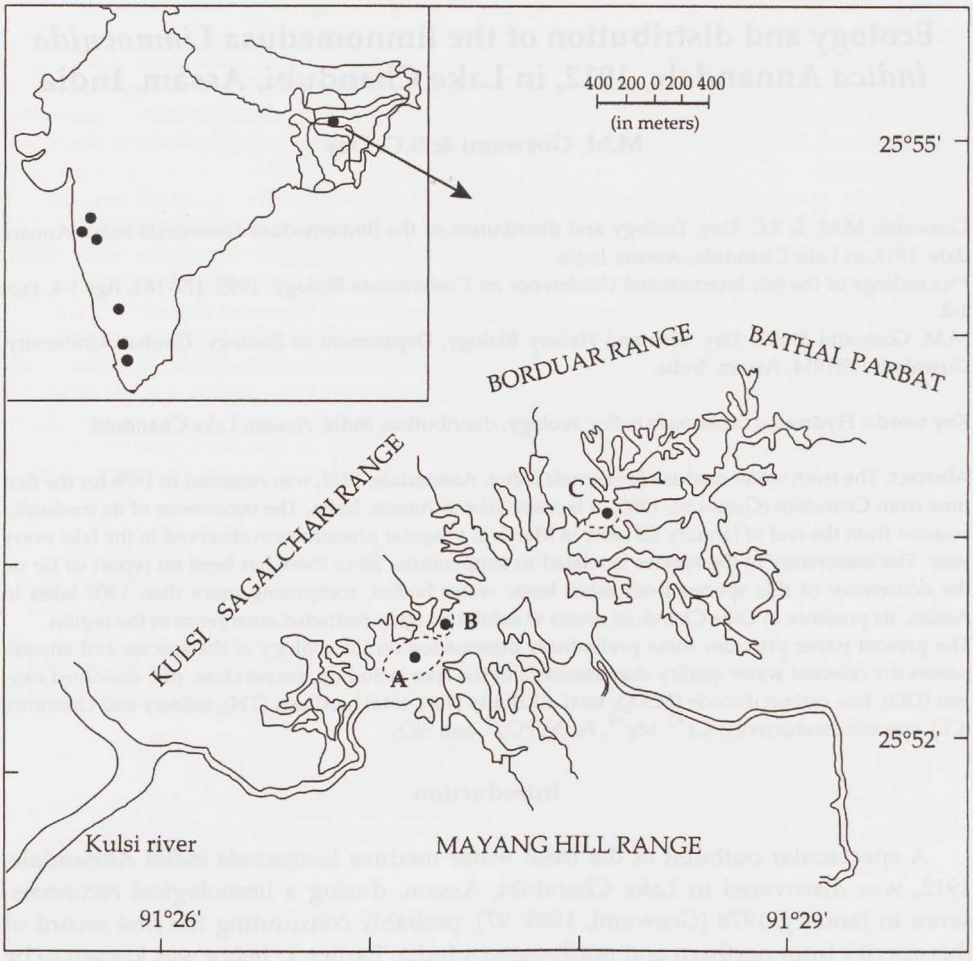

Fig. 1. Distribution of *Limnocnida indica* in India and in Lake Chandubi.

(perennial inlet) canal and six small streams coming down from the hill ranges. Throughout its length, the northern and the southern irregular shore-lines are bordered by the Borduar-Sagalchari-Kulsi Foothill Range of the Kamrup district of Assam and the Mayang Foothill Range of Meghalaya, respectively. The eastern side of the lake is blocked by hills and the western side is open to the cool down-stream water of the Kulsi tributary (fig. 1).

Two distinct seasons are mainly operating in the region; the warm and rainy summer (April-September) and the cold and fog dominated winter (December-February).

Material and methods

From 1980 to 1994 the lake was sampled each year during the period January-March with wide-mouthed nylon hand nets operated from boats. A 0.5 m^3 quadrate net of nylobolt silk cloth was used to collect data on densities. Living medusae were

studied both on the site and in the laboratory. Samples were preserved in a 3% formaldehyde solution after narcotization by warm water drops and 1% chloral hydrate. Relevant ecological parameters were analysed following the standard methodology of APHA (1981), Welch (1948) and NEERI (1988).

Biological aspects of the habitat

The habitat of *Limnocnida indica* is dominated by two important biological components, the macrophytes (table 1) and the plankton.

Table 1. Macrophyte cover (% range) in the three areas of the lake where medusae are found.

	Area A	Area B	Area C
Surface			
Trapa bispinosa	20.0-41.0	65.0-90.0	10.0-20.0
Hygrorhiza aristata	5.0-15.0	10.0-25.0	absent
Nymphaea nochali	absent	2.0-10.0	absent
Submerged column			
Ceratophyllum demersum	20.0-40.0	20.0-50.0	5.0-10.0
Hydrilla verticillata	10.0-25.0	15.0-30.0	absent
Naias indica	10.0-15.0	15.0-25.0	absent
Bottom carpet			
Vallisneria spiralis	70.0-100.0	60.0-90.0	40.0-80.0

A list of dominant planktonic species common to the three sites where medusoid swarming was recorded is presented below. All these species are common to the three areas and occur throughout the year. Species marked with an asterisk were found in the gastric cavity of medusae.

Phytoplankton: *Oscillatoria tenuis* (Cyanophyta), **Closterium acerosum*, **Cosmarium monomazum*, **Eudorina elegans*, **Staurastrum* spp, *Sphaerozoma, Spondylosium moniliformes* (Chlorophyceae) **Frustulia rhomboides*, **Fragillaria* spec., **Melosira* spec. **Navicula cuspidata*, **Pinnularia viridis* (Bacillariophyceae), **Peridinium* spec. (Dinophyceae).

Zooplankton: **Arcella discoides*, **A. megastoma*, **Difflugia urceolata*, **Lesquerensia epistomium*, *Nebela caudata, Quadrulella* spec., *Stelexomonas dichotoma* (Protozoa), **Asplanchna priodonta*, *Keratella cochlearis*, **Polyarthra vulgaris*, **Brachionus angularis*, **B. caudatus*, *B. falcatus* (Rotifera), *Alona affinis*, **Bosmina longirostris*, **Diaphanosoma* spec. (Cladocera), *Mesocyclops leuckarti*, **Neodiaptomus* spp. (Copepoda), *Nauplius larvae.

Seasonality, density, and daily vertical migration of medusae

Every year, the medusae begin to appear either in the last week of January or in the first week of February (fig. 2). After swarming in February they disappear within the first days of March. Low densities of medusae were noted in 1980, 1986 and 1990, whereas very high densities occurred in 1992 and 1993.

The medusae show the following daily cycle: In the morning, after disappearance of night fogs, they swim upward to the surface, generally showing active swim-

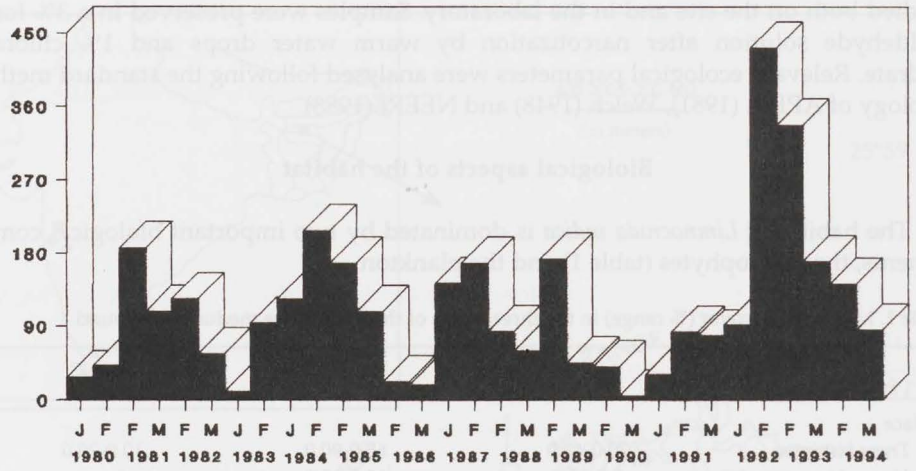

Fig. 2. Numerical density (n.m^{-3}) of *Limnocnida indica* in Lake Chandubi during the survey period, 1980-1994.

Table 2. Correlation coefficient (r) between various physico-chemical factors of the water of Lake Chandubi and the density of occurrence of medusae of *Limnocnida indica*. S = significant, HS = highly significant, NS = not significant.

Parameters	Ranges	medusa density (n.m^{-3})	r	t	Correlation
T °C	18.0≤19.0	5-186	0.647	2.4944	S
	19.0≤20.0	10-248	0.970	16.4518	HS
	20.0≤21.0	10-184	0.033	0.1095	NS
	21.0-23.0	17-432	0.112	0.3561	NS
DO mg.l^{-1}	7.0≤9.0	5-108	0.714	2.8870	S
	8.0≤8.6	10-170	0.110	0.2930	NS
	8.6-9.2	92-432	0.191	0.6162	NS
TA mg.l^{-1}	19.0≤26.0	5-84	0.994	4.0196	HS
	26.0≤29.0	42-432	0.129	0.4506	NS
	29.0-32.0	28-168	0.012	-0.0407	NS
FCO_2 mg.l^{-1}	1.0≤1.6	28-208	0.303	1.0534	NS
	1.6≤2.6	42-432	-0.206	-0.4711	NS
	2.6-4.5	5-170	-0.605	-2.1460	S
pH	6.6-6.8	5-84	0.677	2.4337	S
	6.9-7.0	28-432	0.682	2.6370	S
	7.1-7.2	78-337	-0.074	0.0536	NS
Cl^- mg.l^{-1}	2.0≤3.0	5-45	0.720	2.7439	S
	3.1≤4.0	56-170	0.659	2.1463	NS
	4.1-5.1	60-432	-0.315	-1.1497	NS
NH_4^+-N mg.l^{-1}	0.04-0.09	45-432	1.002	2.6457	S
	0.1-0.5	18-208	-0.463	-2.6667	S
	0.6-1.0	5-28	-0.278	-0.4004	NS
Mg^{+2} mg.l^{-1}	0.8-2.0	22-432	-0.408	-1.0538	NS
	2.1-4.0	5-60	-0.430	-2.2438	S
Fe^{+2} mg.l^{-1}	0.3-0.39	5-186	0.844	6.3096	HS
	0.4-0.5	10-432	0.599	2.4810	S

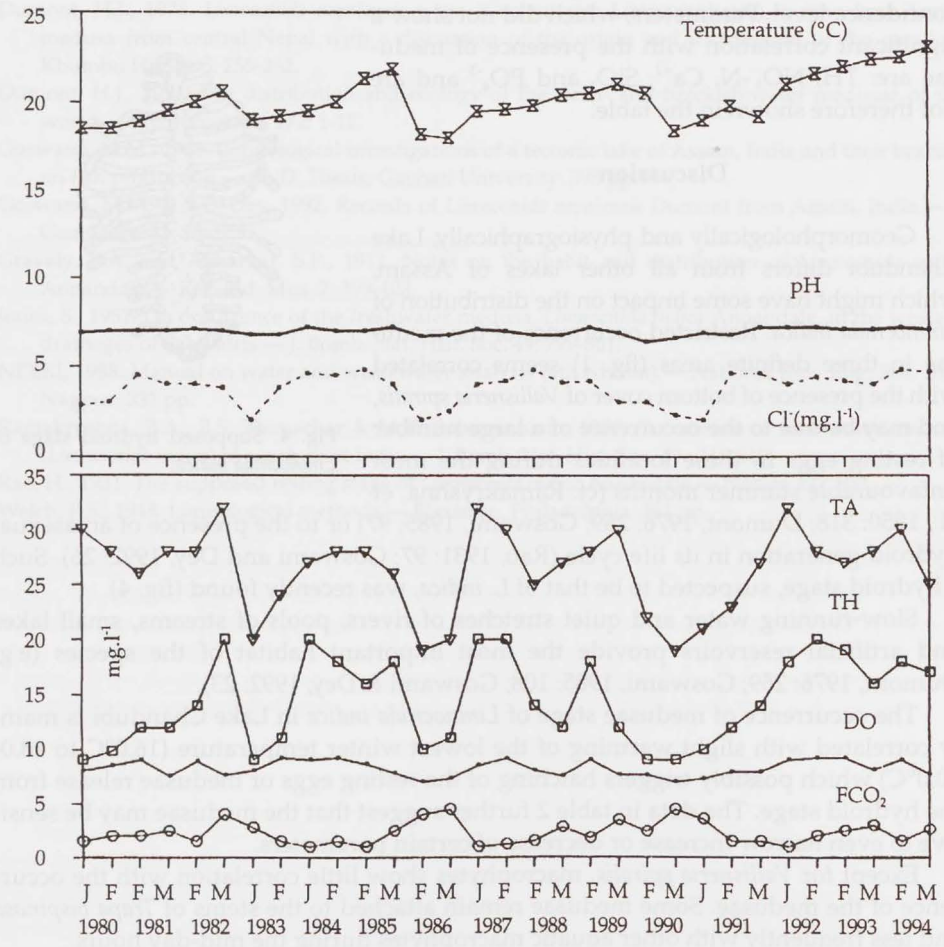

Fig. 3. Some physico-chemical parameters observed during the incidence of occurrence of *Limnocnida indica* in Lake Chandubi.

ming movements within 10-15 cm from the surface. The aggregation continues for 3-4 hours up to 10.00-11.00 hrs in the morning. Thereafter, the medusae move downward. Just before sunset some individuals again move upward to reach the surface, but most of them reach the bottom and remain there during the night.

Physico-chemical aspects of the habitat

Physico-chemical parameters of the lake monitored during the period of occurrence of the medusae are represented in table 2 and fig. 3. Low salinity (0.034-0.039‰) and specific conductivity (29.0-50.0 $\times 10^{-6}$ mhos.cm^{-1}) was recorded throughout the period of investigation. Temperature, DO, TA, pH, Cl^-, NH_4^+-N and Fe^{+2} showed a positive correlation at the 95% confidence level, whereas a negative correlation was noticed with the slightly higher ranges of FCO_2, NH_4^+-N, and Mg^{+2} at 90%

confidence level. Parameters, which did not show a significant correlation with the presence of medusae are: TH, NO_3^--N, Ca^{+2}, SiO_2 and PO_4^{-3} and are not therefore shown in the table.

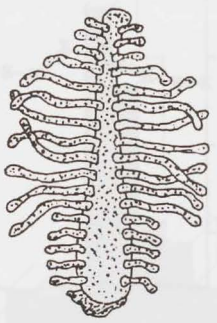

Fig. 4. Supposed hydroid stage of *Limnocnida indica*.

Discussion

Geomorphologically and physiographically, Lake Chandubi differs from all other lakes of Assam, which might have some impact on the distribution of *Limnocnida indica*. Restricted occurrence of the medusae in three definite areas (fig. 1) seems correlated with the presence of bottom cover of *Vallisneria spiralis*, and may be due to the occurrence of a large number of resting eggs in these localities during the most unfavourable summer months (cf. Ramakryshna, et al., 1950: 318; Dumont, 1976: 259; Goswami, 1985: 97) or to the presence of an asexual hydroid generation in its life cycle (Rao, 1931: 97; Goswami and Dey, 1992: 26). Such a hydroid stage, suspected to be that of *L. indica*, was recently found (fig. 4).

Slow-running water and quiet stretches of rivers, pools of streams, small lakes and artificial reservoirs provide the most important habitat of the species (e.g. Dumont, 1976: 259; Goswami, 1985: 108; Goswami & Dey, 1992: 23).

The occurrence of medusae stage of *Limnocnida indica* in Lake Chandubi is mainly correlated with slight warming of the lowest winter temperature (16.0°C to 18.0-20.0°C) which possibly triggers hatching of the resting eggs or medusae release from the hydroid stage. The data in table 2 further suggest that the medusae may be sensitive to even narrow increase or decrease of certain parameters.

Except for *Vallisneria spiralis*, macrophytes show little correlation with the occurrence of the medusae. Some medusae remain attached to the stems of *Trapa bispinosa* and less frequently with other aquatic macrophytes during the mid-day hours.

Desmids and pinnate diatoms among phytoplankton, and protozoans, rotifers and crustacean zooplankton (see "Biological aspects of the habitat") were frequently found in the gastric cavity of living medusae and, at least as far as the zooplankton is concerned, presumably constitute the major bulk of their food.

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Demography of black coral populations in Doubtful Sound, New Zealand: results from a 7-year experiment

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Key words: *Antipathes*; growth rates; mortality; recruitment; tags; ANOVA; fiords; New Zealand.

Abstract: Growth and mortality in four populations of the black coral *Antipathes fiordensis* Grange, 1990, were followed for seven years in Doubtful Sound, New Zealand. Growth rates did not differ significantly among sites or size classes, but there was significantly higher mortality of colonies less than 40 years old. One of the populations, delimited within an enclosed area, had all colonies mapped and tagged. Recruitment rates over the experimental period for this population were significantly less than mortality rates. Recruitment was highly variable and appeared to have a minimal effect on the temporal dynamics of *A. fiordensis*, unlike most marine populations. Rather, very low mortality of very old colonies allows the population to survive through prolonged periods of poor recruitment, more typical of long-lived organisms with delayed reproduction and indeterminate growth, such as forest trees.

Introduction

Following the discovery of an accessible and very large population of the antipatharian black coral *Antipathes fiordensis* Grange, 1990, along the steep rock walls of the fiords of south-western New Zealand (Grange et al., 1981), attempts have been made to quantify the resource. Initial research concentrated on the distribution and abundance and showed that this population was possibly the largest in the world within SCUBA depths (Grange, 1985), and it appeared to occupy different habitats from those considered normal for antipatharians. For instance, most of the population occurred in 10-25 m depth, in contrast to below 30 m reported elsewhere, e.g. Poor Knights Is (Kelly, 1983), Hawaii (Grigg, 1965; Grigg & Opresko, 1977), Caribbean (Opresko, 1972), Trinidad (Warner, 1981) and Panama (Opresko, 1976). The cool-temperate, enclosed, estuarine environment of the fiords was also different to the more usual offshore island, tropical habitats.

The estimated total population of over 7×10^6 colonies in depths less than 30 m throughout the fiords (Grange, 1985) has provided unique opportunities for research into this relatively little-known yet commercially important group of coelenterates. Studies have been conducted on growth-rate estimates (Grange, 1985), population structure (Grange & Singleton, 1988), cell ultrastructure (Goldberg & Taylor, 1989a; 1989b), chemical composition (Goldberg, 1991) and mechanical properties of the skeleton (Kim et al., 1992). Others have lead to the discovery of defensive sweeper tentacles (Goldberg et al., 1990), and a mutualistic relationship with the euryalanid ophiuroid *Astrobrachion constrictum* (Farquhar, 1900) (Grange, 1991).

Antipatharians are known to grow slowly and to be long-lived. Methods used to

estimate growth rates have included X-radiography of growth bands (Grigg, 1965), tagging (Grigg, 1965), and sizes of colonies on structures of known age (Oakley, 1988). Growth-rate studies in *Antipathes fiordensis* have involved correlation of colony height with X-radiographic analysis of growth rings (Grange, 1985), cohort analysis using density traces (Grange & Singleton, 1988), and the incorporation of ^{14}C-labelled proteins into the skeleton of growing colonies and subsequent autoradiographic analysis (Grange & Goldberg, 1993). More recent analysis of autoradiographic sections has suggested that the periodic bands seen in the skeleton may not be annual (Grange & Goldberg, in prep.).

This paper reports on the results of a 7-year experiment following the growth rates of four populations of *Antipathes fiordensis* in Doubtful Sound, New Zealand, and on natural mortality rates for the first time in an antipatharian population.

Methods

Four sites in Doubtful Sound (fig. 1) were selected, covering a range of habitats. Site 1 (Deep Cove) was the most sheltered, and the most influenced by fresh water runoff from the head of the fiord. The subtidal rock wall had a mean slope of 65°. There was a low diversity of other encrusting fauna at this site. Site 2 (Tricky Cove) was 15 km up-fiord from the Deep Cove site. At this site the rock wall was very steep (70°) and generally covered with an abundance of ascidians, alcyonaceans, sponges, hydroids, brachiopods, and crinoids, typical of the fiord habitat (Grange et al., 1981). Sites 3 and 4 (Hall Arm) were less influenced by fresh water runoff than the Deep Cove site, had a more gentle slope (35°), and were relatively bare of encrusting fauna, possibly as a consequence of treeslides from the mountains above dating from less than 30 years ago. The influence of these periodic tree slides on the black coral population has been outlined by Grange & Singleton (1988).

At sites 1, 2 and 3, colonies were randomly chosen between 10 m and 35 m depth, and tagged. Tags were made from

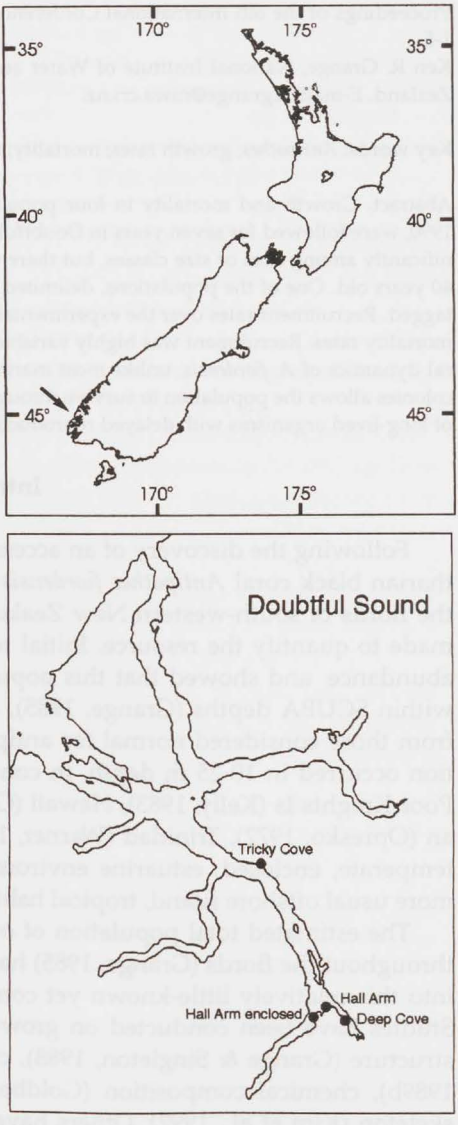

Fig. 1. Study sites in Doubtful Sound, Fiordland, New Zealand.

nylon cable ties, sleeved with numbered yellow plastic cable markers (manufactured by Critchley, Australia). A single tag was placed around the base of each colony. These tags proved resilient and encrusting coralline algae and ascidians were easily wiped off. Each tag was replaced only once during the experiment.

At site 4, an area of 8 m (vertical) and 7 m (horizontal), at a mean depth of 18 m, was delimited by anchoring masonary nails into the granite substrate at four corners and laying polypropylene string around the nails. Within this area, all colonies were tagged and mapped. At each sampling period all new recruits within the area were also tagged and their positions mapped.

A total of 80 colonies was tagged at the start of the experiment in March 1985; 15 at Deep Cove, 30 at Tricky Cove, 18 at Hall Arm, and 17 within the enclosed quadrate at Hall Arm. Each colony was sampled 13 times at approximately 6-monthly intervals between March 1985 and February 1992. Sampling consisted of measuring the length of the main branch, following curves as close as possible, using a flexible tape. The same researcher measured the branch lengths at all sampling periods to reduce inter-diver error.

Mean growth rates were calculated from the linear regression slopes of each tagged colony over time, and mortality rates were calculated from the percentage of tagged colonies remaining at each site and within each size class at each sampling period. Growth and mortality rates were compared among sites and size classes using ANOVA (SigmaStat; Jandel Scientific, USA). Where significant differences were found, Bonferroni multiple comparison tests determined which pairs of means differed significantly. Size classes were determined after the results of mean growth rates of each colony were obtained, so that each size class could be related to age estimates.

Results

Growth rates

Tags were lost from a small proportion of colonies at each site, and mortality of tagged colonies occurred during the experiment. Tagged colonies were included in the growth rate analyses only if measurements were available for more than eight of the 13 sampling periods. Growth rates were therefore possible on 11 colonies at Deep cove, 16 at Tricky Cove, 13 at Hall Arm, and 17 within the enclosed area at Hall Arm.

The growth of clonal organisms is not necessarily asymptotic, and previous estimates of growth rates and longevity of *Antipathes fiordensis* suggested that this species grew at 20-30 $mm.y^{-1}$ and could survive for centuries (Grange & Singleton, 1988; Grange & Goldberg, 1993). It was therefore assumed that growth rates over the 7-year experimental period would be linear, and least squares linear regressions were fitted to the tagged colonies height data from each site. All regression lines gave r^2 values > 0.825 (unpublished data). The slopes gave an estimate of the mean growth increment for each colony at each site (fig. 2a). ANOVA on these data showed no significant differences among sites ($F = 0.514$; $p = 0.675$) (table 1). The mean growth rate for all colonies was 24.4 $mm.y^{-1}$.

Table 1. *Antipathes fiordensis*. Results of one-way ANOVA of mean growth rates at each site, Doubtful Sound, New Zealand. Normality test passed (p = 0.175); equal variance test passed (p = 0.831).

Group	N	Mean	SD	SEM	
Deep Cove	11	20.62	11.31	3.41	
Tricky Cove	16	24.90	10.09	2.52	
Hall Arm	13	25.44	14.72	4.08	
Hall Arm Enclosed	10	26.74	12.71	4.02	
Source of variation	**DF**	**SS**	**MS**	**F**	**P**
Between treatments	3	229.88	76.63	0.514	0.675
Residual	46	6857.67	149.08		
Total	49	7087.55			

Since there were no significant differences in growth rates among sites, data from all sites were pooled to examine whether there were differences among size classes. Size classes were constructed, based on the mean growth rate of 24.4 $mm.y^{-1}$, representing colonies aged <10 y (0-249 mm), 10-20 y (250-499 mm), 20-40 y (500-975 mm), 40-60 y (976-1450 mm), and >60 y (> 1450 mm). It was recognised that colonies would move from one size class to the next over the experimental period, so all colonies were assigned to a size class at sampling period 2, September 1985. Only eight colonies grew from one size class to the next during the experiment. Mean growth rate of each size class is shown in fig. 2b. ANOVA on these data showed no significant differences in growth rates among size classes (F = 1.748; p = 0.156) (table 2).

Table 2. *Antipathes fiordensis*. Results of one-way ANOVA of mean growth rates for each size class, Doubtful Sound, New Zealand. Normality test passed (p = 0.080); equal variance test passed (p = 0.966).

Group	N	Mean	SD	SEM	
0-249 mm	20	19.65	10.88	2.432	
50-499 mm	8	31.46	12.42	4.39	
500-975 mm	9	27.51	12.05	4.02	
976-1450 mm	7	25.61	9.78	3.70	
> 1450 mm	6	25.30	14.62	5.97	
Source of variation	**DF**	**SS**	**MS**	**F**	**P**
Between treatments	4	953.31	238.33	1.748	0.156
Residual	45	6134.25	136.32		
Total	49	7087.55			

Mortality

Mortality rates were calculated from the percentage of tagged colonies that remained alive at each sampling period, for each site (fig. 3a) and each size class (fig.

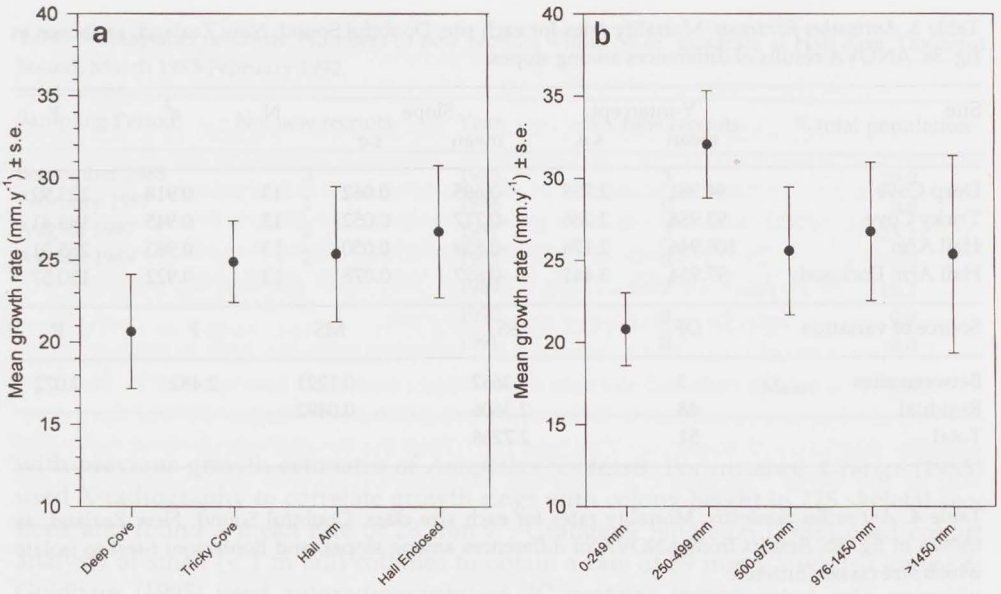

Fig. 2. *Antipathes fiordensis*; a, mean growth of tagged colonies at each site; b, mean growth of colonies of each size class; Doubtful Sound, March 1985 - February 1992.

3b), as defined above. There were no significant differences among slopes for site data ($F = 2.482$; $p = 0.072$) (ANOVA, table 3), but significant differences were found among size classes ($F = 31.804$; $p < 0.001$) (ANOVA, table 4). Pairwise multiple comparisons Bonferroni t-tests were performed to show which size classes differed (table 4). Mortality rates in the smallest two size classes were greater than in colonies greater than 40 years old, and colonies less than 10 years old had a greater mortality rate than 10-20 year-olds. Mortality rates decreased, therefore, in the order 0-10 y > 10-20 y > 20+ y. There were no differences in mortality rates among colonies older than 20 y. Mean annual mortality rates, calculated from these slopes were 12.3% (< 10 y), 8.2% (10-20 y), and 3.8% (> 20 y).

Recruitment

Within the 56 m^2 enclosed quadrate at Hall Arm, a total of 8 recruits was recorded over the 83 month experimental period (table 5). This represents 0.096 recruits per month, or 1.15 recruits per year. The mean total population in the enclosed quadrate over the experimental period was 12.9 colonies. The mean annual recruitment rate averaged over the 7 years was, therefore, 8.8%, but no recruitment was observed in 4 of the 7 years (table 5). Only 50% of the new recruits survived their first year, with a further 30% mortality in the second year.

Discussion

The growth rate of 24.4 $mm.y^{-1}$ obtained from this experiment may be compared

Table 3. *Antipathes fiordensis*. Mortality rates for each site, Doubtful Sound, New Zealand, as shown in fig. 3a. ANOVA results of differences among slopes.

Site	Y-intercept		Slope		N	r^2	F
	mean	s.e.	mean	s.e			
Deep Cove	94.981	2.738	-0.695	0.062	13	0.918	123.92
Tricky Cove	93.988	2.286	-0.717	0.052	13	0.945	189.41
Hall Arm	106.946	2.176	-0.839	0.050	13	0.963	285.51
Hall Arm Enclosed	97.954	3.441	-0.897	0.078	13	0.922	130.57

Source of variation	DF	SS	MS	F	P
Between sites	3	0.3662	0.1221	2.482	0.072
Residual	48	2.3606	0.0492		
Total	51	2.7268			

Table 4. *Antipathes fiordensis*. Mortality rates for each size class, Doubtful Sound, New Zealand, as shown in fig. 3b. Results from ANOVA of differences among slopes, and Bonferroni t-test to isolate which size classes differed.

Site	Y-intercept		Slope		N	r^2	F
	mean	s.e.	mean	s.e			
0-249 mm (<10 y)	95.405	2.460	-1.028	0.056	13	0.968	335.897
255-499 mm (10-20 y)	88.008	3.672	-0.686	0.084	13	0.859	67.235
500-975 mm (20-40 y)	103.495	2.212	-0.308	0.050	13	0.773	37.376
976-1450 mm (40-60 y)	102.727	1.992	-0.419	0.045	13	0.886	85.263
>1450 mm (>60 y)	103.569	2.040	-0.219	0.047	13	0.669	22.267

Source of variation	DF	SS	MS	F	P
Between sites	4	5.5984	1.3996	31.804	<0.001
Residual	60	2.6405	0.0440		
Total	64	8.2389			

Pairwise multiple comparisons (Bonferroni)	Diff of mean	t	p<0.05
0-249 mm vs >1450 mm	0.809	9.832	yes
0-249 mm vs 500-975 mm	0.720	8.750	yes
0-249 mm vs 976-1450 mm	0.609	7.401	yes
0-249 mm vs 255-499 mm	0.342	4.156	yes
255-499 mm vs >1450 mm	0.467	5.676	yes
255-499 mm 500-975 mm	0.378	4.594	yes
255-499 mm vs 976-1450 mm	0.267	3.245	yes
976-1450 mm vs >1450 mm	0.200	2.431	no
976-1450 mm vs 500-975 mm	0.111	1.657	no
500-975 mm vs >1450 mm	0.089	1.328	no

Table 5. *Antipathes fiordensis*. Numbers of new recruits within 56 m^2 quadrate at Hall Arm, Doubtful Sound, March 1985-February 1992.

Sampling Period	No. new recruits	Year	No. new recruits	% total population
September 1985	3	1985	3	20.0
February 1987	1	1986	0	0.0
August 1987	2	1987	3	23.1
August 1989	2	1988	0	0.0
		1989	2	18.2
		1990	0	0.0
		1991	0	0.0
			Mean	8.8

with previous growth estimates of *Antipathes fiordensis*. For instance, Grange (1985) used X-radiography to correlate growth rings with colony height in 225 skeletal sections and found a mean rate of 28 $mm.y^{-1}$; Grange & Singleton (1988) used cohort analysis of small (< 1 m tall) colonies to obtain a rate of 39 $mm.y^{-1}$, while Grange & Goldberg (1993) used autoradiography of ^{14}C proteins incorporated into growing skeleton to show rates of 16.2 $mm.y^{-1}$ over the 3-year experimental period. These methods have shown that *A. fiordensis* grows significantly slower than warmer water species, such as *Antipathes dichotoma* Pallas, 1766 (45-93 $mm.y^{-1}$) (Grigg, 1975); *A. grandis* Verrill, 1928 (64 $mm.y^{-1}$) (Grigg, 1976) and *A. pennacea* Pallas, 1766 (57 $mm.y^{-1}$) (Oakley, 1988).

Mortality in young colonies of *Antipathes fiordensis* is principally due to dislodgement during the indiscriminate grazing action of urchins, but once colonies have grown taller than 100 mm, the basal holdfast is sufficiently strong to withstand

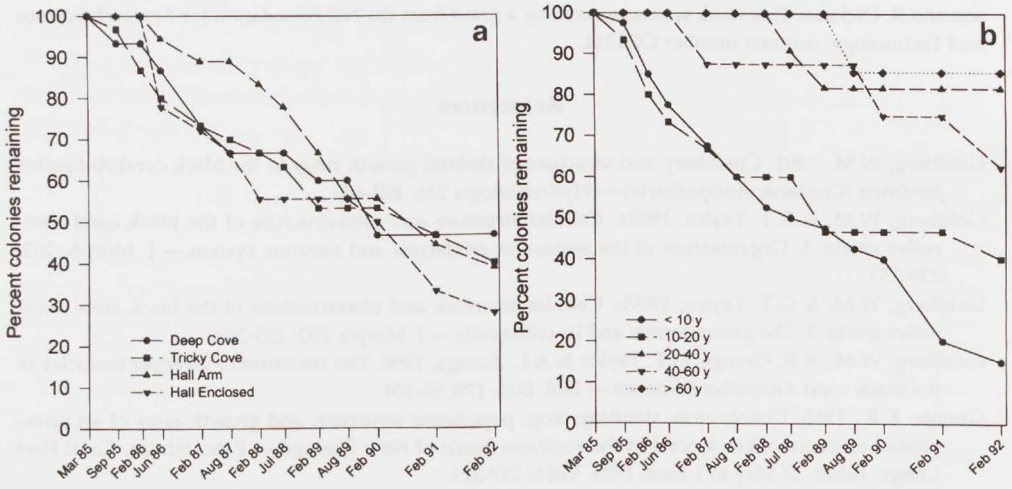

Fig. 3. *Antipathes fiordensis*; a, percent tagged colonies remaining alive at each sampling period, for each site; b, percent tagged colonies of each age class remaining alive at each sampling period; March 1985 - February 1992.

urchin grazing. Mortality of older colonies in the New Zealand fiords is due to catastrophic events which may not be biologically induced. Treeslides from the surrounding steep mountains are common (Grange & Singleton, 1988), and during periods of decreased rainfall, increased light penetration underwater can cause phytoplankton blooms that smother colonies (Grange, 1991). Increased rainfall in the surrounding catchments can increase the depth of the surface low salinity layer (Witman & Grange, submitted) and kill colonies in shallow water (< 15 m). Predation has not been observed on post-recruits of *Antipathes fiordensis*. Sweeper tentacles (Goldberg et al., 1990) and symbiotic ophiuroids (Grange, 1991) also prevent the settlement of epibionts and reduce the mortality of larger colonies.

The low annual recruitment rate of 8.8%, coupled with the high mortality in the first 20 years, means that each recruit, on average, must survive for over 30 years for the population to remain stable. Previous studies have shown that 90% of the population is less than 20 years old (Grange, 1985), but the species can grow to over 5 m tall, or 200 years. Colonies this large still reproduce (personal observations), so are contributing relatively more to the population growth than smaller colonies. It appears as though the population in the fiords survives despite very low and variable recruitment over at least a 7 year period. Very low mortality rates of very old colonies, and the ability to spawn for 200 years, allow the population to survive through prolonged periods of poor recruitment, more typical of long-lived organisms with delayed reproduction and indeterminate growth, such as forest trees.

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Outlines of Coelenterate Evolution based on principles of constructional morphology

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Key-words: Evolution; Metazoa; compartmentalisation theory; Coelenterata; Anthozoa; Tetrazoa.

Abstract: A reconstruction of the evolution of the ancestral Metazoa and of the Coelenterata is presented. The evolution of Metazoa is explained as a process of internal differentiation of an unicellular, multinuclear organism (cell compartmentalisation theory).
The evolution to coelenterates is characterised by development of a central gastric cavity with radial pouches, resulting in the completely hydraulic soft body system of sessile polyps, as represented by Anthozoa.
By fission of the upper polyp part as a normal mode of asexual multiplication the alternation of fertile medusae and sterile polyps evolved, characterising the "Tetrazoa" or "Medusozoa". Semaeostomeae and Rhizostomeae persisted at this level, with specialised medusoid stages. Stauromedusae are combined polyp-medusa individuals. In the *Stephanoscyphus*-polyp of the Coronatae a ring canal serves as a hydrostatic organ for holding the upper polyp part at the margin of the long periderm tube. The fossil Conulata, if they were coelenterates, can be explained as similar polyp structures, growing to giant size.
Among the ancestral polyp colonies mechanically supported by long periderm tubes, differentiation into feeding polyps and medusae-producing polyps took place, giving rise to the highly evolved Hydrozoa. The Cubozoa evolved by a development towards rapid medusae shedding and extreme reduction of polyp size.

Introduction

Coelenterates represent one of the main evolutionary lineages of the animal kingdom. They originated from early metazoans, and they developed remarkably complicated structures.

Questions

The answer to the question, "how coelenterate evolution began" depends upon how the first metazoans were structured. The first metazoans originated from unicellular eukaryotic organisms, and the question to be asked is: "How did the Metazoa evolve from this level, in contrast to other eukaryotic organisms such as plants and fungi?"

Subsequent questions are: "What was the structure of the "first" coelenterate, and how are the different structural types of coelenterates related to each other?" The answer to the latter question depends on the structure of the "first" coelenterate, as in evolution a certain body structure always sets the limits for subsequent transformations.

Methodology

Phylogenetic considerations are always reconstructions of evolutionary path-

ways. "Constructional morphology" (Gutmann 1989, Vogel 1991) formulates the principles of organism body construction and evolutionary transformations. The organism has to be conceived as an energy transforming system. As such, the organism has to be mechanically closed, and its inner (sub)systems have to work coherently to enable the "energy-cataract"; these subsystems have to be arranged in such a way that they do not allow arbitrary or fortuitous deformations and unnecessary or detrimental energy flow ("restriction"); last but not least, and most importantly, organisms are soft body hydraulic systems, made up of flexible membranes and fluid fillings (explained in fig. 1). Moreover, internal differentiation is considered as the main force of evolutionary change, whereas the traditional view of additionism (and hence of its implicated non-gradualism) is abandoned.

In consequence, organisms are active "subjects" of evolution, not the passive "objects" of external forces (Weingarten 1993).

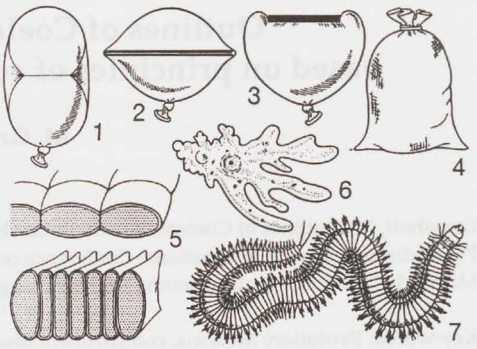

Fig. 1. A flexible membrane is set under tension and is stretched by filling with gas, fluid or granular material, in technical and organic systems. The terms "pneu" and "soft body hydraulic system" were especially coined for them (Otto, 1973; Gutmann, 1989). All organisms are basically soft body hydraulic systems, as organic chemical reactions can be active only in aqueous solutions, and as they must be held together by an envelope of flexible membrane. Rigid sclerites are always produced and preformed by the soft body system; they immobilize parts of the body.
1-3. Balloons, deformed by tethering and stiff structures: 1, with tethering rope inside, causing two funnel-shaped impressions; 2-3, with rigid sticks inside and on the surface. 4. Granular material as a filling substance: sugar sac. 5. Pneu-combinations: pillows adhering to each other, forming cushions.
Organisms: 6. A cell: Amoeba. 7. Polychaete worm.

The arguments on evolutionary change use terms as they are used in engineering, like tensile-strength, hydraulic pressure, flexibility, stiffness. The arguments are not based on phenomena alone, animal or fossil, or on so-called evidence. Phenomena exist, but they have to be explained using established principles, and incorporated into theories.

This should be kept in mind, even if my argumentation about evolutionary pathways is written in a more narrative way, thus avoiding complicate and circumstantial styling. The descriptions of the various steps of evolutionary change are always based on the principles as mentioned above, hence, the text is not a "narrative explanation" as defined by philosophy of science.

Evolution of Metazoa
The cell-compartmentalisation theory

Cell-compartmentalisation (fig. 2)

The ancestral eukaryotes were organized as unicellular organisms. In multinucle-

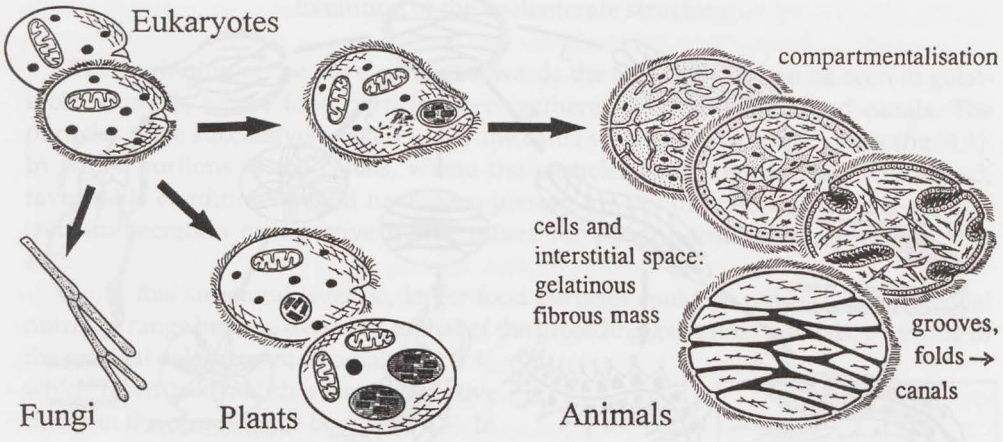

Fig. 2. Evolution of multicellular animals; explanation in text.

ar heterotrophic eukaryotes, moving with the aid of cilia, the prerequisites for several evolutionary lineages were present. The incorporation of cyanobacteria led to plants; eukaryotes secreting decomposing substances, taking up very small particles and molecules by pinocytosis, became fungi. Many of these organisms remained at the unicellular level, others developed multicellular body constructions.

The present theory explains the evolution of multicellular animals as a process of internal differentiation. The evolution of metazoans began in those eukaryotes that deposited polysaccharides and polypeptides as reserve substances into their endoplasmic reticulum.

These deposits have gelatinous and fibrous properties. Because of their viscosity, they offer support and stabilize the soft body. This mass of jelly and fibres was able to expand more and more. It gradually separated the cytoplasm into many regions, in each of which at least one nucleus was necessarily maintained, surrounded by cytoplasm. Since the endoplasmic reticulum is membranous, (it is "the" membrane of organisms), these masses of cytoplasm are surrounded by membrane and hence are, per definition, cells. The body was finally compartmented into cells and interstitial spaces containing jelly and fibres (connective tissue). In this way the body construction typical for all metazoa, and only for them, was attained.

During this process, the body, stabilized by connective tissue, has the potential to increase in size, extending the range of possible nutrition of these ancestral animals. This stabilisation, in conjunction with contractile fibre systems (the developing muscles), permitted many different body forms. Specifically it allowed the formation of various grooves and folds on the surface, which eventually closed to make canals, where food capture and uptake (and finally digestion) was performed.

The first multicellular animals

In German, we call these jelly-supported constructions "Gallertoide" (Gallerte = gelatine, jelly), and in English "gelatinoid animals". They have the structural ele-

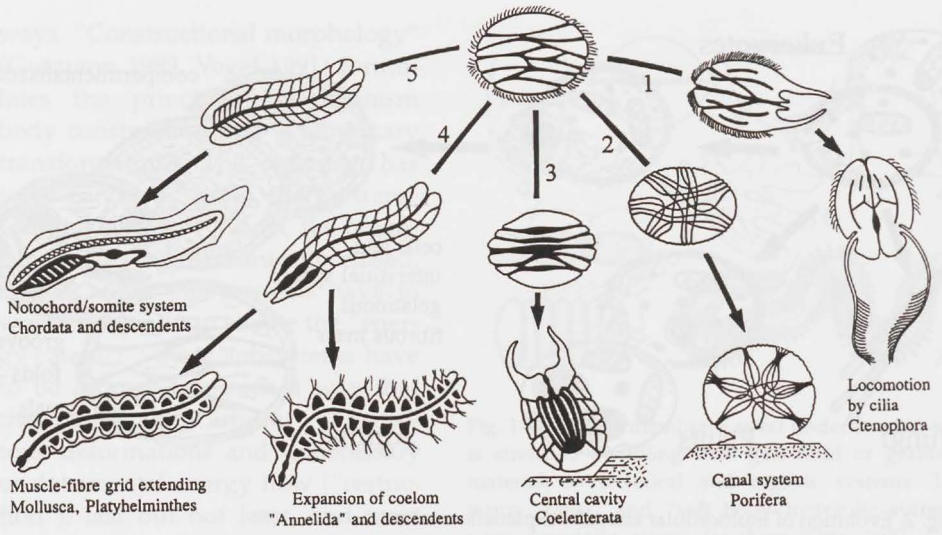

Fig. 3. Coelenterata among the main lineages of animal evolution: 1. Food capture by thin lappets, the future tentacles, swimming by ciliar movement, body construction very close to the ancestral gelatinoid connective fibre construction: Ctenophora. 2. Increase of the number of canals, canals narrow, water-currents driven by cilia, development of a fine filter system (choanocytes) for minute particles like bacteria: Sponges. 3. Coelenterata, explained in text. 4. Elongate forms, undular swimming movements, development of gut and of lateral fluid spaces (coelom sacs and transversally tethering tissue membranes in metameric arrangement); flexibility of mouth region for seizing particles: one branch of Coelomata; so-called Protostomia. 5. Similar constructions; but mouth region kept immobile, collecting food particles during swimming by filtering, drainage canals for the superfluous water (the later gill slits in the fore-gut), notochord, muscle-somites: the other branch of coelomates: Chordata. (From Grasshoff, 1993).

ments typical of animals, viz., eukaryotic cells (these are hydraulic systems) such as epithelial cells with cilia, and interstitial spaces filled with connective tissue. Various forms of cellular differentiation may occur, chiefly to muscle fibres and nerve cells. Muscle cells remain embedded in the connective tissue fibre grid; they are anchored in the fibres, so that transmission of force can be effected. Fluid filled spaces may evolve as larger hydraulic systems, starting from canals with an epithelial lining. These fluid fillings are surrounded by more complicated membranes; the form of these units is controlled by the tensile strength of the collagenous fibres and the contractility of muscle fibres. For reproduction the whole system is provided with all the prerequisites to produce eggs and sperm and to vegetatively multiply by fragmentation and tying off body parts.

From the beginning, all the structural parts which are typical for all animals were present; these elements and their properties are the invariants, persisting throughout the whole of animal evolution. These ancestral "gelatinoid animals" appear to be the only reasonable entrance into the animal kingdom, and from their body structure all main lineages of animal evolution can be derived, representing constructional options by internal differentiation (fig. 3).

Evolution of the coelenterate structure

The beginning of the development towards the Coelenterata can be seen in gelatinoid animals, where food particles were gathered in slightly enlarged canals. The particles were successively taken up by the canal wall cells by phagocytosis (fig. 4.1). In wider portions of the canals, where the particles could be collected and stored, favourable conditions would have been present for cell differentiation: cells specialized for secretion of digestive fluids, others for phagocytosis and pinocytosis (fig. 4.2).

From this structural level on, larger food particles could be utilized. The potential nutrition range became extended to that of the preceding gelatinoid animals, by virtue of the gradual enlargement of canals (fig. 4.3), which fused to establish a single digestive cavity in the centre of the body (fig. 4.4). In conjunction, a single canal entrance developed as a mouth surrounded by a crown of tentacles for seizing particles. (An alternative, viz. a free swimming animal capturing large particles using several mouths, is structurally almost unthinkable and indeed not verified to exist in nature).

One of the specific features of coelenterates is the presence of nematocysts. They evidently evolved in connection with the development of tentacles. As a beginning to nematocyst evolution, tentacular cells may have secreted a glue to aid in prey capture, as still true for several nematocyst types, notably spirocysts. From this point the differentiation to nematocysts could possibly be explained. (A continuous and comprehensive model for the evolution of these cells has not been presented so far).

Enlargement of the body cavity and increase of the ability of the ingestion canal to utilize large particles would have further extended the potential nutrition range. But, a constructional problem emerged: the more the central cavity widens, the thinner the body wall becomes, and the body then looses the support provided by the stiffness of the walls.

For further evolution there was but one option. The central cavity extended into the thick body wall forming radial pouches (fig. 4.5). This had several consequences:

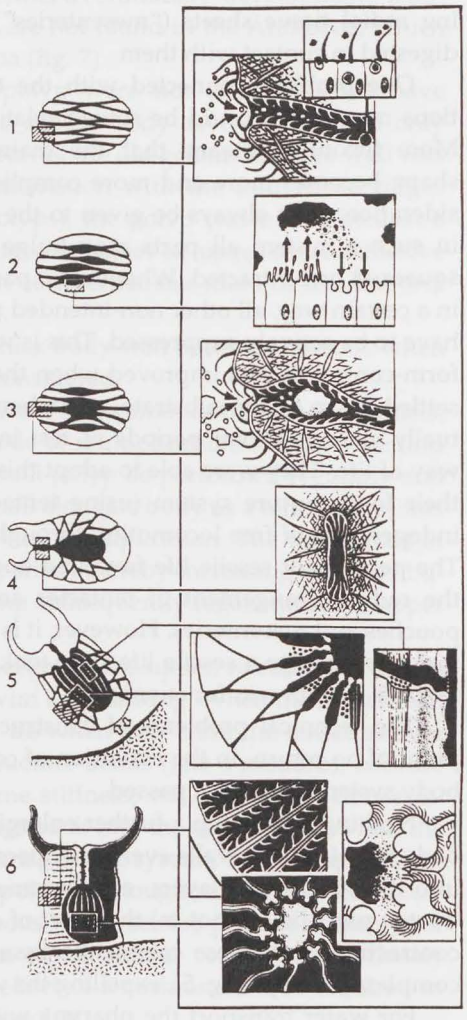

Fig. 4. Evolution of the coelenterate polyp construction; explanation in text.

(a) the muscles capable of withdrawing the mouth and tentacular crown were retained centrally, and consequently gained greater freedom of movement, as more and more fluid filling surrounded them; (b) the over-all flexibility of the body increased, as fluid (water) is easier to deform and to displace than the viscous gelatinous fibre mass; (c) as the stiff gelatinous fibre mass was reduced, the body cavity (plus the extensive canals) became filled with water, resulting in a stabilized soft-body hydraulic system; (d) food particles were kept in the center of the cavity by the edges of the remaining radial tissue sheets ("mesenteries") and were digested in contact with them.

One problem connected with the transformations mentioned, could be solved relatively easily. More flexibility means that the maintenance of shape becomes more and more complicated. Consideration must always be given to the fact of that in such a system all parts may bulge out, or be squeezed or contracted. Whenever a part is moved in a certain way, all other non-intended movements have to be actively suppressed. This is what we call form-control; it was improved when these animals settled down on the substrate, and when they eventually extended their periods of rest into a sessile way of life. They were able to adopt this lifestyle as their food capture system (using tentacles) works independent of free locomotion through the water. The permanent sessile life favoured and stabilized the radial arrangement of tentacles and of their inner counterparts, viz., gastric pouches and mesenteries. However, it is quite possible, and should not be precluded, that transition to a sessile life style took place even earlier in the evolution once the tentacle system was functioning.

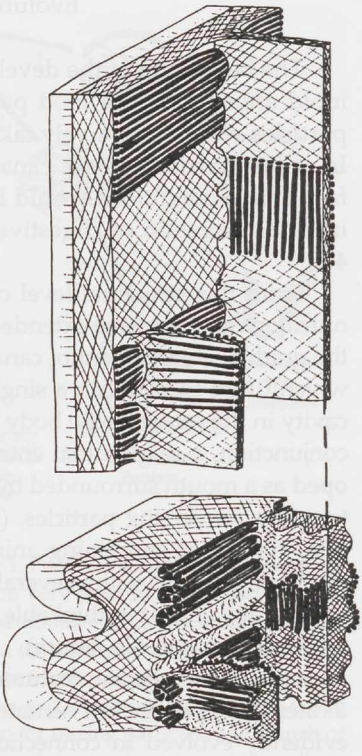

Fig 5. Arrangement of fibres and muscles in the body wall of *Metridium senile* (Linnaeus, 1761). Buckling of wall during contraction. (From Gutmann, 1966).

The technical problems of construction had been solved; the critical step, the point of no return, in the evolution of coelenterates to sessile, largely hydraulic soft-body systems had been passed.

Nothing could stop a further enlarging of the central cavity, "hollowing out" the body (fig. 4.6). The walls eventually became so thin that the muscle cells were placed into the surface cell layers, and became epitheliomuscular cells. Because their contractile parts lie on (not in) the sheet of collagenous fibres, where they are anchored, contractions fold these connective tissue sheets, and hence the body wall, in very complicated ways (fig. 5), expelling the water from the body cavities.

For water transport the pharynx with its cilia was preserved. It moreover functioned as a (laterally compressed) valve against the loss of fluid pressure, as well as a swallowing tube for large food particles. The radial tissue sheets, the mesenteries,

which remained between the pouches, kept the body form more or less cylindrical, while flattened in both the tentacular and the foot region. They hold the tentacle bases down, and the pharynx in its position against the inner fluid pressure.

Thus the hydraulic soft-body system was complete as a multi-mesenterial polyp, as we find it now in the Anthozoa (fig. 4.6).

The Tetra-polyp

Large polyps require more mesenteries than small ones to control their body form. The smallest polyp arising from the level of the "developing coelenterate" was evidently a polyp with four gastric cavity pouches, which we may call the tetra-polyp (fig. 6,3). This was the key event for further evolutionary development, leading into the other constructional designs that are not found in the Anthozoa, which we may collectively call Tetrazoa or Medusozoa (fig. 7).

The mechanical properties of the tetra-polyp were not exactly as we have described them in the anthozoans. When the central body cavity developed only four pouches, it enlargened only so far that a part of the old gelatinous fibre wall was retained. By the stiffness of the wall, in combination with the four tissue ridges (which are not called mesenteries in these polyps), the polyp was maintained in a cylindrical shape. Attachment to the substrate was achieved using some adhesive substance, leading eventually to the evolution of a permanent more or less flat and cuplike periderm base.

The advantage of preserving a relatively thick body wall becomes obvious when some special features of this construction are considered.

A small solitary polyp is unable to produce a large amount of sexual products, i.e. sperm and eggs. However, a vegetative mode of multiplication, of which we find various types in coelenterates, could in this small polyp be performed regularly and frequently, viz. transverse fission. In such a small elongate body as a tetra-polyp, this appears as the appropriate method of non-sexual multiplication. The upper part of the polyp is severed and swims for a certain period, thereby increasing the distributional range of the species, and the polyp stump subsequently regenerates the upper part in a short time.

A notable fact is that the mechanical properties of the upper, released portion of the polyp would have enabled it to actively swim by pulsating. When the muscles on the "mouth disk side" are contracted, as they do when they close the tentacles over the mouth, the whole cup-like or dish-like structure bends (like a medusa), because on the opposite side there is a cushion of some stiffness, viz., gelatinous fibre substance and cells. The cushion bends and springs back into the old position when the muscle contraction ceases. It is a very simple antagonistic system, the prerequisites of which are established in the upper part of the polyp. Although in the relatively thick-walled tetra-polyp a certain amount of gelatinous wall was also present in the upper portion, it should not be overlooked that in small animal constructions, closely packed cells may have the same mechanical effect of a cushion, as was required in the swimming upper part of the ancestral tetra-polyp.

These temporary swimming polyp sections settled down and grew into new polyps. However, it was but a question of time for those pelagic polyp sections

which extended their swimming period, to form gonads. As this process gradually developed, another critical point in coelenterate evolution was passed. Now the polyp form and the swimming stage, the "medusa", were able to evolve independently. The polyps improved the process of medusae production (strobilation), and the medusae, now unlimited in size, improved pulsatory swimming, prey capture, and gonad production.

In sexual reproduction, from fertilized eggs, the polyp stage developed again; what else could have happened? The basically metagenetic life cycle of Coelenterata-Tetrazoa had evolved.

The radial symmetry of the medusa, a free swimming organism, was inherited from a sessile organism, in which radial symmetry developed, (in several other evolutionary lineages of the animal kingdom, radial symmetry also developed in sessile animals). In the special case of the coelenterate medusa, the tetra-polyp permanently determined the basically tetra-radial construction.

An outline of phylogenetic connections

Anthozoa (fig. 6)

The coelenterates first to settle down to a sessile life style were the octocorals (fig. 6,1). In the basal part of the polyps a not insignificant amount of gelatinous wall (permeated by canals) was preserved. This mass ("coenenchyme") spread out over the substrate, and new polyps arose from it, thus producing siamese multiples called colonies or corals. Only the upper part of polyps had sufficient space within to constitute complete hydraulic systems. In the wall, calcareous sclerites could be developed; the octocorals are the only coelenterates with true internal skeletons. The few species with definitely solitary polyps, such as *Taiaroa touhou* Bayer & Muzik, 1976, can be explained as reduced stages of previously colonial forms, whereas the seemingly solitary polyps of *Umbellula monocephalus* Pasternak, 1964, are highly developed polyp colonies, marking the end of an evolutionary line.

In the various anthozoan constructional forms other than the octocorals, the whole polyp developed sufficient hollowness to become a completely hydraulic system (fig. 6.2). Amongst them the modes of growth of the cylindrical bodies, and of the insertion of the mesenteries during growth, are so different that these types cannot have transformed one into another. They arose independently from the "developing polyp" stage.

In the Actiniaria-types (plus Corallimorpharia and Scleractinia) and in the fossil Rugosa the growth zones encompass almost the whole body cylinder. Calcareous substance, if present ("corals"), is deposited in the form of septa under the radial zones between the mesenteria. In the Actiniaria-types, viz. Scleractinia, where the mesenteries are arranged in twin-pairs, each new septum begins between a mesenterial pair and hence begins as a free standing structure; this way of inserting new septa allows also non-cylindrical growth forms. A reconstruction of the mesenterial arrangement in the fossil Rugosa, based on their skeletal structures, shows that obviously single mesenteries were present. During cylindrical growth, only under one side of a new mesenterium a space was developing for a new septum, so that this

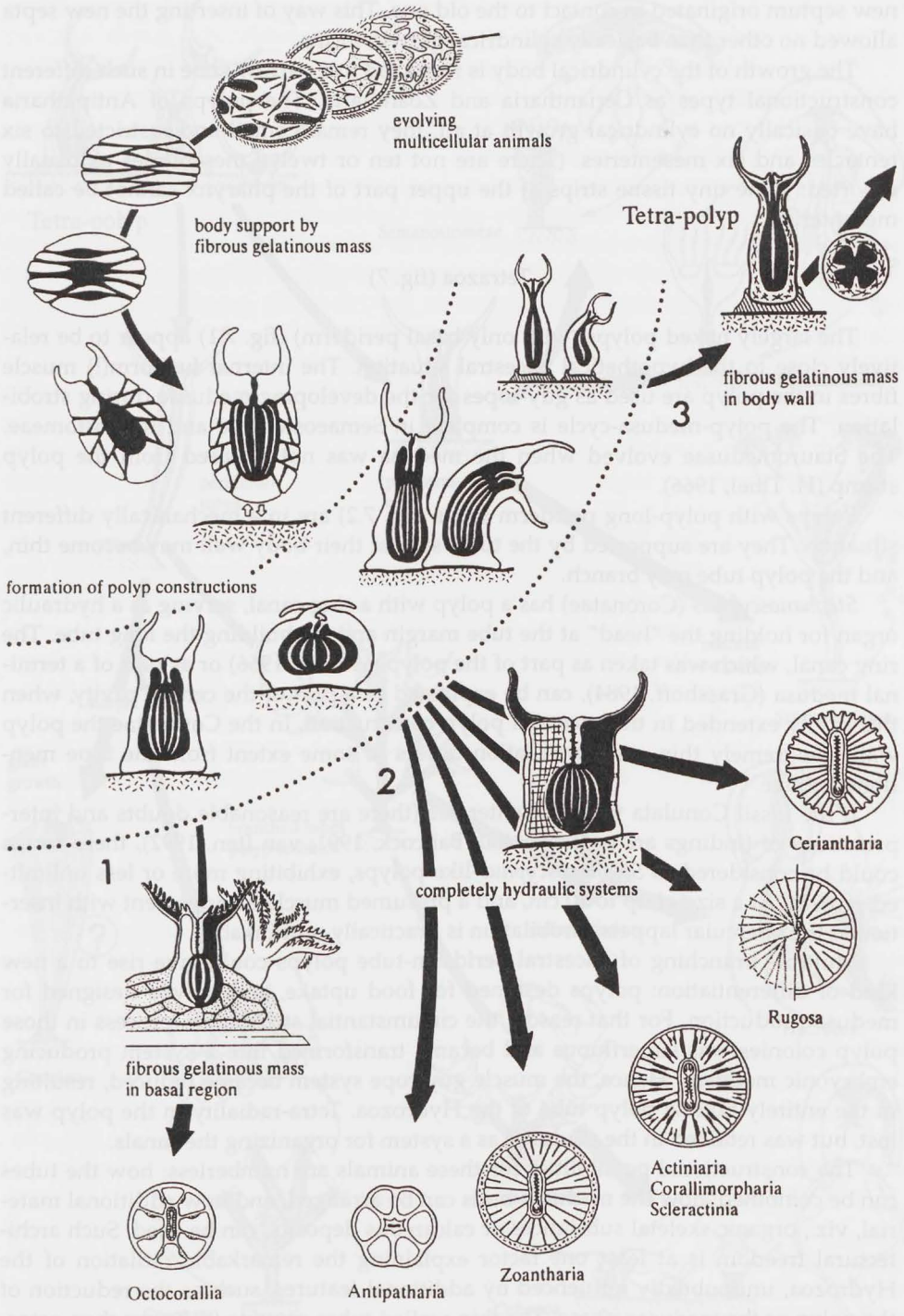

Fig. 6. Evolution of the coelenterate polyp constructions and radiation of the Anthozoa; explanation in text.

new septum originated in contact to the old one. This way of inserting the new septa allowed no other than basically cylindrical growth forms.

The growth of the cylindrical body is restricted to a narrow zone in such different constructional types as Ceriantharia and Zoantharia. The polyps of Antipatharia have basically no cylindrical growth at all, they remain small and restricted to six tentacles and six mesenteries. (There are not ten or twelve mesenteries as usually reported: some tiny tissue strips at the upper part of the pharynx cannot be called mesenteries).

Tetrazoa (fig. 7)

The largely naked polyps (with only basal periderm) (fig. 7.1) appear to be relatively close to the hypothetical ancestral situation. The internal fusiform(!) muscle fibres in the polyp are used as guy-ropes for the developing medusae during strobilation. The polyp-medusa-cycle is complete in Semaeostomeae and Rhizostomeae. The Stauromedusae evolved when the medusa was not released from the polyp stump (H. Thiel, 1966).

Polyps with polyp-long periderm tubes (fig. 7.2) are in a mechanically different situation. They are supported by the tube, so that their body wall may become thin, and the polyp tube may branch.

Stephanoscyphus (Coronatae) has a polyp with a ring canal, serving as a hydraulic organ for holding the "head" at the tube margin and for building the long tube. The ring canal, which was taken as part of the polyp (Werner, 1966) or as part of a terminal medusa (Grasshoff, 1984), can be explained as a part of the central cavity, when this cavity extended in the ancestral polyp construction. In the Coronatae the polyp wall is extremely thin, and strobilation differs to some extent from the type mentioned above.

If the fossil Conulata were coelenterates (there are reasonable doubts and interpretations of findings are controversial; Babcock, 1991; van Iten, 1992), these forms could be considered as *Stephanoscyphus*-like polyps, exhibiting more or less unlimited growth. At a size of up to 10 cm, and a presumed muscle-arrangement with insertion at the opercular lappets, strobilation is practically unthinkable.

Multiple branching of ancestral periderm-tube polyps could give rise to a new kind of differentiation: polyps designed for food uptake, and polyps designed for medusa production. For that reason, the circumstantial strobilation process in those polyp colonies was superfluous and became transformed into a system producing embryonic medusae. Hence, the muscle guy-rope system became reduced, resulting in the entirely hollow polyp tube of the Hydrozoa. Tetra-radiality in the polyp was lost, but was retained in the medusae as a system for organizing the canals.

The constructional possibilities for these animals are numberless: how the tubes can be combined; how the medusae buds can be arranged; and how additional material, viz., organic skeletal substances or calcareous deposits, can be used. Such architectural freedom is at least one factor explaining the remarkable radiation of the Hydrozoa, undoubtedly influenced by additional features, such as the reduction of the polyp or the medusae phase. The thin-walled tubes provide (different than octocorals) no internal space for skeletons: hydrozoans produce exo-skeletons, which may

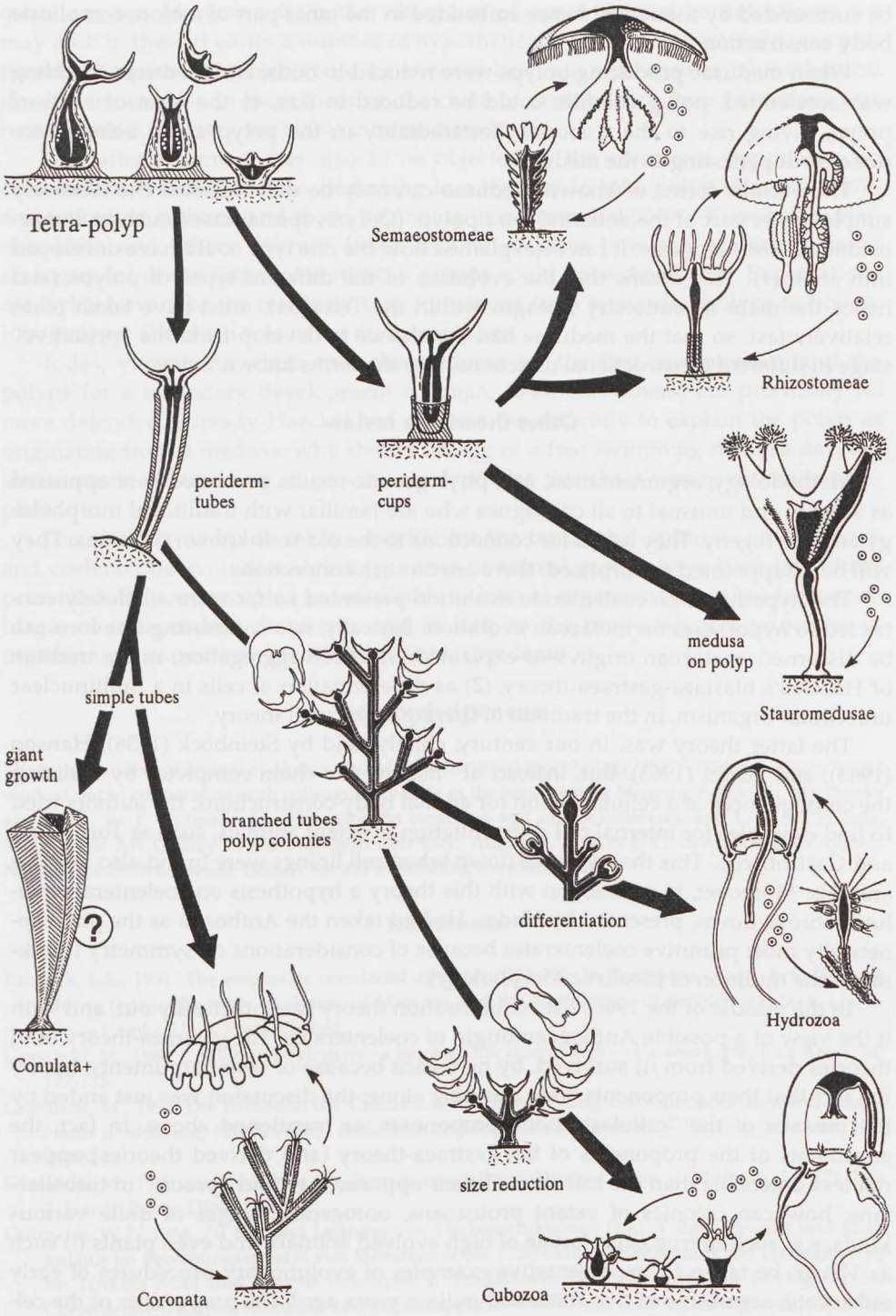

Fig. 7. Radiation of the Tetrazoa; explanation in text. (Graphic design figs. 1-4, 6-7: A. Siebel).

be surrounded by tissue and hence embedded in the inner part of a more complicate body construction.

When medusae producing polyps were reduced to buds, and medusae shedding was accelerated, polyp colonies could be reduced in size, to the form of a dwarf polyp, giving rise to the Cubozoa. Tetraradiality in the polyp again being abandoned, but persisting in the medusae.

The various forms of known medusae can only be derived from the relatively simple upper part of the ancestral tetra-polyp. (Only in special cases, such as Semaeostomeae-Rhizostomeae, it can be explained how the one type could have developed into another). This means that the evolution of the different types of polyps (and hence the main evolutionary lineages within the Tetrazoa), must have taken place relatively fast, so that the medusae had the chance to develop from the "primitive" stage in different constructional directions, into the forms known today.

Other theories, a review

Methodology, argumentation, and phylogenetic results presented here appeared as strange and unusual to all colleagues who are familiar with traditional morphology and phylogeny. They asked for connections to the old well-known positions. They will be disappointed or surprised: there are no such connections.

The hypotheses on coelenterate evolution presented so far were all closely connected to hypotheses on metazoan evolution. Basically, two contrasting opinions can be discerned: metazoan origin was explained (1) as cell aggregation, in the traditon of Haeckel's blastaea-gastraea-theory, (2) as differentiation of cells in a multinuclear unicellular organism, in the tradition of Ihering's syncytial theory.

The latter theory was, in our century, chiefly held by Steinböck (1936), Hanson (1963), and Hadzi (1963). But, instead of "making the chain complete" by realizing the consequences of a cellularisation for animal body constructions, the authors tried to find examples for internal cell differentiation in extant animals, such as Turbellaria and Gastrotricha. This theory broke down when cell linings were found also in these animals. Moreover, in connection with this theory a hypothesis on coelenterate evolution broke down, presented by Hadzi. He had taken the Anthozoa as the phylogenetically most primitive coelenterates because of considerations on symmetry (thinking in the tradition of Idealistic Morphology).

In the middle of the 1960s the cellularisation theory was practically out, and with it the view of a possible Anthozoan origin of coelenterates. The gastraea-theory (and theories derived from it) survived, by no means because of better arguments, but by the fact that their proponents were standing alone; the discussion was just ended by the mistake of the "cellularisation" proponents, as mentioned above. In fact, the arguments of the proponents of the gastraea-theory (and derived theories) appear not less untenable than the intention of their opponents to find "proofs" in turbellarians: how can colonies of extant protozoans, ontogenetic stages of quite various kinds, e.g. such of frog eggs, larvae of high evolved animals, and even plants (!) such as *Volvox*, be taken for representative examples of evolutionary procedures of early eukaryotic organisms of two-thousand million years ago? The proponents of the cellularisation theory had already asked such questions and never had gotten an ade-

quate answer. Moreover, from the viewpoint of constructional considerations we may add: in these theories a number of hypothetical transitional organisms was proposed or implicated, which could have never been alive because of biomechanical insufficiencies. Also in these theories, the chain of evolutionary transformations was never made complete over viable intermediate stages.

The latter statement has also to be objected to the connected hypotheses on coelenterate evolution. Those, beginning in a hydrozoan tube polyp (Haeckel, 1874, and following authors) and those, beginning in a tetraradial polyp (Korschelt & Heider, 1890, and others; Werner 1966), did not explain how the multimesenterial arrangement of the different Anthozan groups could have developed. Explanations are lacking even for the relatively easy transitions from tetraradial polyps to hollow tube polyps. The chain was never made complete.

Today, viewpoints which took the medusae for the first coelenterates, and the polyps for a secondary development (Hyman, 1940, and others) are practically no more defended. Already Haeckel had realized the difficulty to explain the polyp as originating from a medusa: why should an egg of a free swimming medusa develop into a sessile intermediate stage, viz., a polyp. Also this chain was never made complete.

The situation is by no means specific for the traditional opinions on metazoan and coelenterate evolution. Arbitrary series of animal groups have been plentifully presented; they are accepted as reasonable as long as organisms are taken for assemblages of independent characters, and as long as the properties and principles of organismic constructions are not taken into consideration.

Acknowledgements

The modern view of animal evolution and of constructional morphology, the "Frankfurt theory", was worked out in cooperation with colleagues, mainly in the Senckenberg Museum Frankfurt. My thanks are due to W. F. Gutmann for discussion on evolution and on coelenterates, and to P. Alderslade, Museum & Art Gallery of the Northern Territory, Australia, and to J. C. den Hartog, Nationaal Natuurhistorisch Museum, Leiden, for aid in finishing the manuscript.

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How calcium is held in nematocysts: molecular modeling of poly γ-glutamic acids and detection of a specific calcium binding protein in nematocysts of the actinian *Metridium senile* (Linnaeus, 1761)

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Greenwood, P.G. & M.J. Yunes. How calcium is held in nematocysts: molecular modeling of poly γ-glutamic acids and detection of a specific calcium binding protein in nematocysts of the actinian *Metridium senile* (Linnaeus, 1761).
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 209-214, figs 1-3, tab. 1.
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Key words: *Metridium senile*; nematocyst; calcium; calcium binding protein.

Abstract: Because little is known about the binding of cations with poly γ-glutamic acids a molecular modeling study was done to assess the possible specificity of these molecules for monovalent or divalent cations. The results show that poly γ-glutamic acids reside at a lower energy state when bound to calcium than when bound to potassium. This lower energy state could result in a slightly increased specificity for calcium ions over potassium ions. This study also investigated whether specific calcium binding proteins are present within nematocysts from several species of marine cnidarians. A calcium binding protein was found associated with the capsules or tubules of nematocysts from one species, the sea anemone *Metridium senile* Linnaeus, 1761. These results suggest that divalent cations, and particularly calcium, might be selectively retained within some nematocyst types by more than one mechanism.

Introduction

High concentrations of cations occur in the capsular matrix of all nematocyst types investigated to date. The predominant cations are typically either potassium, magnesium, or calcium depending on cnidarian species and nematocyst type (Watson & Mariscal, 1984; Gerke et al., 1991; Tardent et al., 1990; Tardent, 1995). The cations are thought to have an important role in discharge primarily by contributing to a high osmotic pressure within the capsule (Weber, 1989; Hidaka & Afuso, 1993).

Considerable attention has been paid to calcium ions because early studies indicated that divalent cations (especially calcium) stabilized nematocysts and prevented their discharge (Blanquet, 1970; Lubbock & Amos, 1981; Salleo et al., 1983, 1984). In addition, numerous studies have identified large amounts of calcium in undischarged nematocysts (Lubbock et al., 1981; Gupta & Hall, 1984; Watson & Mariscal, 1984, 1985; Mariscal, 1988; Tardent et al., 1990). How high concentrations of calcium and other cations are held within nematocysts has not been completely resolved.

It has been known for some time that large quantities of acidic amino acids are found in hydrolyzed matrix protein samples from nematocysts, and it was speculated that proteins or polypeptides containing high amounts of acidic amino acids balanced the charges of the cations present in nematocyst capsules. It is now known that the cations are probably bound to poly γ-glutamic acids, which subsequently have been found in all nematocyst types investigated to date (Weber, 1990, 1991; Tar-

dent, 1995). Most research has shown that cations can be exchanged easily across the nematocyst capsule wall (Weber, 1989, 1991; Gerke et al., 1991) suggesting that poly γ-glutamic acids do not have great cation binding specificity. In contrast, Hidaka and Afuso (1993) found that calcium ordinarily found in the nematocysts of the sea anemone *Calliactis polypus* (Forskål, 1775) is not freely exchanged with other cations.

Because little is known about the binding of cations with poly γ-glutamic acids, a molecular modeling study was done to assess the possible specificity of poly γ-glutamic acids for monovalent or divalent cations. We also investigated whether specific calcium binding proteins are present within nematocysts from several species of marine cnidarians.

Materials and Methods

Molecules of poly γ-glutamic acid were modelled using Silicon Graphics Indigo computers and the software package Quanta/CHARMm 4.0 (Biosym/Molecular Simulations). Molecules were modelled by linking 10 glutamic acid residues together with the γ-carboxyl of one amino acid residue linked to the α-amino group of the adjacent monomer. Modeling was simulated in H_2O, pH 7.0, and the structure was minimized using CHARMm. Energy calculations were done on the modelled poly γ-glutamic acids in the presence of 20 K^+ or 10 Ca^{2+} (to maintain equal charges) using CHARMm.

Undischarged nematocysts were isolated from acontia of *Metridium senile* (Linnaeus, 1761), *Calliactis tricolor* (Lesueur, 1817), and *Aiptasia pallida* (Verrill, 1864), and from tentacles of *Physalia physalis* (Linnaeus, 1758) (kindly supplied by Dr D. Hessinger) and *Chrysaora quinquecirrha* (Péron & Lesueur, 1809) (purchased from Sigma Chemical Co.). Acontia were isolated into 1 M sodium citrate and frozen overnight. The acontia were then thawed, triturated in fresh 1 M sodium citrate until the tissue was uniformly dispersed, and filtered through fine nylon mesh. The resulting slurry was further purified by differential centrifugation following the techniques of Weber et al. (1987). Nematocysts from *P. physalis* and *C. quinquecirrha* were provided as lyophilized tentacle extracts and were rehydrated in 1 M sodium citrate and further purified as above. Capsule and tubule proteins were separated from venom components by resuspending the undischarged nematocysts in ultrapure water, which caused the majority of nematocysts to discharge. The venom and capsule/tubule components were separated by centrifugation, and the capsule/tubule components were washed in ultrapure water several times. The proteins of both fractions were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), electroblotted onto nitrocellulose, and incubated with ^{45}Ca following the techniques of Maruyama et al. (1984). This ^{45}Ca overlay procedure is done in the presence of competing magnesium and potassium ions, and is an excellent means to screen mixed protein samples for specific calcium binding proteins (Maruyama et al., 1984; Macer & Koch, 1988).

Results

Poly γ-glutamic acids were modelled in the presence of potassium ions or calcium ions (figs 1, 2). The open helices of both stuctures were composed of five amino acids for each complete turn of the molecule. The ions are oriented between the oxy-

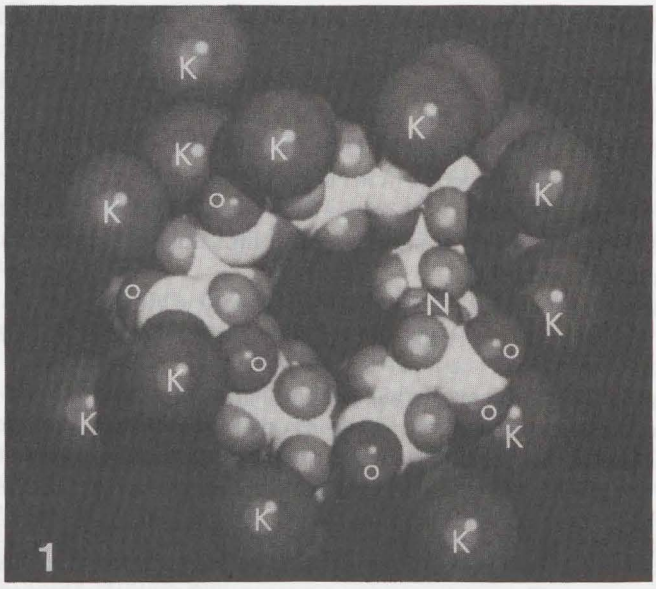

Fig. 1. Space filling model of poly γ-glutamic acid with 20 potassium ions (K). Carbon atoms are white with bound hydrogens gray (unlabelled). Oxygens are indicated (o). In this orientation looking down onto the top of the helix, the helix turns clockwise from the amino terminus (N).

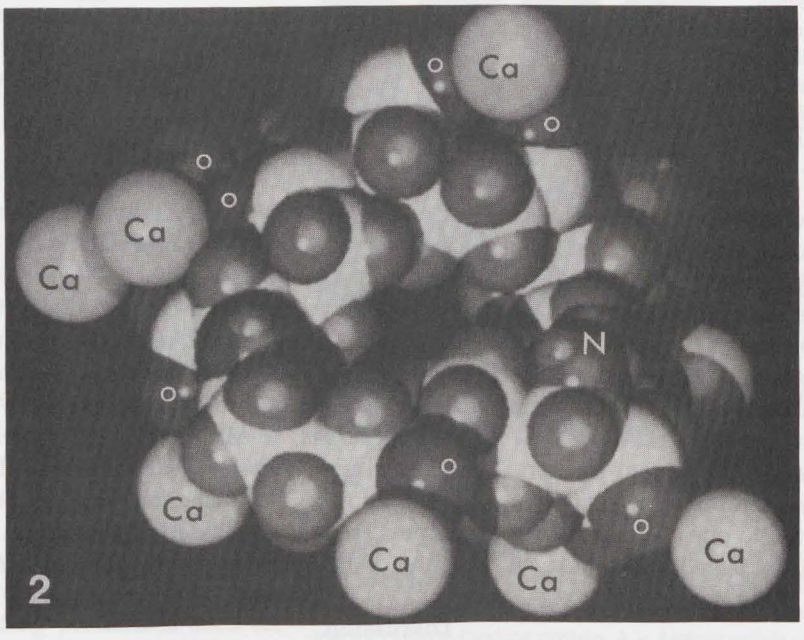

Fig. 2. Space filling model of poly γ-glutamic acid with 10 calcium ions (Ca). The orientation and labels are the same as in fig. 1.

gens of the carboxyl groups and the oxygen of the γ-carbons. The calcium ions are bound more closely to the oxygens than the potassium ions are (average distance from calcium to the closest oxygen = 2.75 Å; average distance from potassium to the closest oxygen = 3.26 Å). Hydrogen bonding with water (not shown) is extensive and appears to be important in stabilizing the helical structure. Two intrahelix hydrogen bonds were present in the structure with calcium, but only one was present in the structure with potassium (not visible in the figures). Intrahelix hydrogen bonds may become increasingly important in stablizing the molecule as polymer size increases above 10 amino acid residues.

The calculated energies for the two modelled poly γ-glutamic acids differed significantly, due almost exclusively to differences in electrostatic forces (table 1).

Table 1. The calculated energies of 10-residue poly γ-glutamic acids modelled with 10 calcium ions or with 20 potassium ions (to maintain equal charges), in kcal/mol. Lower numbers indicate lower energy states. Total CHARMm energy = sum of all steric energy contributions. Bond energy = energy required to compress or stretch covalent bonds. Angle energy = energy required to bend a covalent bond from its equilibrium angle. Dihedral energy = energy required to rotate a covalent bond around the bond axis. Improper energy = a term that maintains planarity about certain planar atoms such as carbonyl carbons. Lennard-Jones energy = a measure of the energy of Van der Waals, non-bonded interactions between atoms. Electrostatic energy = a measure of the partial interaction of electrostatic charges residing in molecules with polar bonds.

Energies	10 Calcium ions	20 Potassium ions
Total CHARMm Energy	-23300	-19568
Bond Energy	397	360
Angle Energy	409	368
Dihedral Energy	52	38
Improper Energy	8	6
Lennard-Jones Energy	244	214
Electrostatic Energy	-24411	-20554

The model with calcium ions has more favorable electrostatic interactions than the model with potassium ions, indicating that the poly γ-glutamic acid is more stable when bound to calcium than when bound to potassium.

Nematocysts isolated from the acontia of *Metridium senile* contained a specific calcium binding protein as indicated by the ^{45}Ca overlay technique (fig. 3). The molecule is a protein, as indicated by its sensitivity to Pronase E (data not shown), of apparent molecular weight 75,000 Daltons. The calcium binding protein is restricted to the capsule and/or tubule components of the nematocysts; no calcium binding proteins were detected in the venom fraction. The acontial nematocysts of *M. senile* are microbasic amastigophores and basitrichs; it is not known if the calcium binding protein is restricted to one of these nematocyst types. There were no definitive calcium binding proteins detected in nematocysts from any of the other cnidarian species investigated.

Discussion

It has been thought that cations bound to intracapsular molecules within isolated nematocysts can be exchanged easily with cations in the external environment

(Weber, 1989, Gerke et al., 1991; Tardent, 1995), but the nematocysts were typically incubated in 1 M salt solutions to load alternative cations. However, in the case of hydra, high concentrations of K^+ normally found in nematocysts in situ are readily replaced by Ca^{2+} and Mg^{2+} found only in relatively low concentrations in the tissue homogenate (Gerke et al., 1991), indicating some specificity for the divalent cations. Our results from molecular modeling suggest that poly γ-glutamic acids reside at a lower energy state when bound to calcium than when bound to potassium; it is possible that this lower energy state results in a slightly increased specificity for calcium ions over potassium ions.

Nematocysts loaded with divalent cations have lower intracapsular pressures and discharge more slowly than nematocysts loaded with monovalent cations (Weber, 1989, Tardent, 1995). The decreased discharge rate for nematocysts loaded with divalent cations may in part be due to a higher specificity of the poly γ-glutamic acids for divalent cations. Discharge might be slower because the cations are less likely to be released or exchanged. Clearly, binding studies to investigate the affinity of poly γ-glutamic acids for various cations are warranted.

In *Calliactis polypus*, the calcium found within the nematocysts is not surrendered, even when challenged with 1 M salt solutions of alternative cations (Hidaka & Afuso, 1993). Based on other studies that show considerable exchange of cations (Weber, 1989; Gerke et al., 1991; Tardent, 1995) and on our molecular modeling, it seems unlikely that the poly γ-glutamic acids alone could account for this degree of specificity for calcium. Although individuals of *C. polypus* were not available for this study, we have found a specific calcium binding protein in acontial nematocysts of *Metridium senile*. The protein appears to remain within the capsule and tubule of the discharged nematocysts, and is not found in the released venom or in the other acontial tissue. Whether or not this calcium binding protein of *M. senile* is performing a similar function of poly γ-glutamic acids in other nematocyst types is not presently known. Another alternative is that the calcium in certain nematocysts bound by specific calcium binding proteins is used for some other function than the ions bound by poly γ-glutamic acids. It is too early to tell if and where the

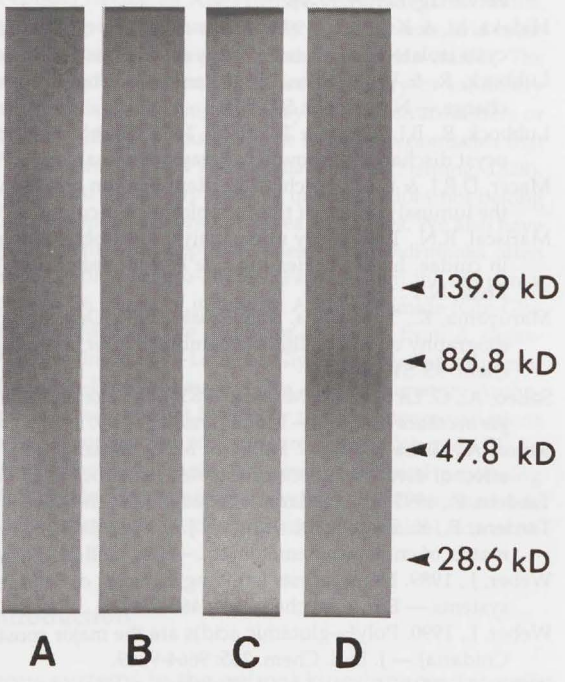

Fig. 3. ^{45}Calcium overlay of nematocyst proteins separated by SDS-polyacrylamide gel electrophoresis (4-12%). Notice that only the nematocysts from *Metridium senile* contain a specific calcium-binding protein. A = *Aiptasia pallida* capsule and tubule proteins. B = *Aiptasia pallida* venom proteins. C = *Physalia physalis* capsule proteins. D = *Metridium senile* capsule and tubule proteins.

calcium binding protein is localized within the structure of the nematocyst, although the calcium binding subunits of the nematocyst tubule reported by Watson & Mariscal (1985) might be a likely candidate. Further purification and characterization of the calcium binding protein is underway at this time.

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Function and biosynthesis of cnidarian neuropeptides

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Key words: Neuropeptide; neurohormone; neurotransmitter; preprohormone; processing enzyme; neurone; nervous system; development; metamorphosis; behaviour.

Abstract: Neuropeptides are an important group of hormones mediating or modulating neuronal communication. Neuropeptides are especially abundant in evolutionary "old" nervous systems, such as those of cnidarians, the lowest animal group having a nervous system. From a single sea anemone species, *Anthopleura elegantissima* (Brandt, 1835), we have isolated 16 different neuropeptides. All peptides are structurally related and have the C-terminal sequence Arg-X-NH_2 or Lys-X-NH_2 in common, where X is Ala, Asn, Ile, Phe, Pro and Trp. Peptides having an Arg-Phe-NH_2 (RFamide) C terminus, such as Antho-RFamide (<Glu-Gly-Arg-Phe-NH_2) appear to occur generally in anthozoans, and related RFamide peptides have also been isolated from species belonging to other cnidarian classes. The peptides are located in neuronal dense-cored secretory vesicles, and have excitatory or inhibitory actions on muscle preparations or isolated muscle cells, suggesting that they are neurotransmitters or neuromodulators. Recently, Leitz et al. (1994) have isolated a neuropeptide from *A. elegantissima* that induces metamorphosis in planula larvae of the marine hydroid *Hydractinia echinata* (Fleming, 1828). This peptide, <Glu-Gln-Pro-Gly-Leu-Trp-NH_2 (named Metamorphosin A, or MMA) does not belong to the large family of Arg-X-NH_2 or Lys-X-NH_2 neuropeptides present in sea anemones. We also have cloned the preprohormones for most of the cnidarian neuropeptides. These preprohormones often contain a high number of immature neuropeptide copies. Prepro-Antho-RFamide from the sea pansy *Renilla koellikeri* Pfeffer, 1886, for example, contains 36 copies of immature Antho-RFamide (Gln-Gly-Arg-Phe-Gly) and 2 other, putative neuropeptide sequences. The precursor for MMA from *A. elegantissima* contains 10 copies of immature MMA (Gln-Gln-Pro-Gly-Leu-Trp-Gly), 14 copies of an immature, closely related neuropeptide sequence (Gln-Asn-Pro-Gly-Leu-Trp-Gly, named immature Antho-LWamide I) and 13 other immature neuropeptide sequences that have only small sequence variations (the most frequent one: Gln-Pro-Gly-Leu-Trp-Gly, 8 copies, named immature Antho-LWamide III). In addition to classical preprohormone processing enzymes, a variety of new, or unusual processing enzymes must occur in cnidarian neurones. These include a proteinase cleaving at the C-terminal side of acidic (Asp or Glu) residues and a dipeptidyl aminopeptidase cleaving at the C-terminal side of N-terminally located X-Pro or X-Ala sequences.

Introduction

Cnidarians have the simplest nervous systems in the animal kingdom, and it was probably within these group of animals, or a closely related ancestor phylum, that nervous systems first evolved (Mackie, 1990). The primitive nervous systems of cnidarians are strongly peptidergic. Using various radioimmunoassays, we have isolated 16 different neuropeptides from a single sea anemone species *Anthopleura elegantissima* (Brandt, 1835). Thirteen peptides are listed in table 1. These peptides are all structurally related and contain the C-terminal sequence Arg-X-NH_2 or Lys-X-NH_2, where X is Ala, Asn, Ile, Phe, Pro, or Trp (Grimmelikhuijzen & Graff, 1986; Graff & Grimme-

Table 1. Neuropeptide families in cnidarians.

Species	Structure	Name
Anthopleura elegantissima	L-3-phenyllactyl-Phe-Lys-Ala-NH_2	Antho-KAamide
Anthopleura elegantissima	L-3-phenyllactyl-Tyr-Arg-Ile-NH_2	Antho-RIamide I
Anthopleura elegantissima	Tyr-Arg-Ile-NH_2	Antho-RIamide II
Anthopleura elegantissima	L-3-phenyllactyl-Leu-Arg-Asn-NH_2	Antho-RNamide I
Anthopleura elegantissima	Leu-Arg-Asn-NH_2	Antho-RNamide II
Anthopleura elegantissima	<Glu-Ser-Leu-Arg-Trp-NH_2	Antho-RWamide I
Anthopleura elegantissima	<Glu-Gly-Leu-Arg-Trp-NH_2	Antho-RWamide II
Anthopleura elegantissima	<Glu-Asn-Phe-His-Leu-Arg-Pro-NH_2	Antho-RPamide II
Anthopleura elegantissima	Leu-Pro-Pro-Gly-Pro-Leu-Pro-Arg-Pro-NH_2	Antho-RPamide I
Anthopleura elegantissima	Gly-Pro-Hyp-Ser-Leu-Phe-Arg-Pro-NH_2	Antho-RPamide IV
Anthopleura elegantissima	<Glu-Val-Lys-Leu-Tyr-Arg-Pro-NH_2	Antho-RPamide III
Anthopleura elegantissima	Tyr-Arg-Pro-NH_2	Antho-RPamide V
Anthopleura elegantissima	<Glu-Gly-Arg-Phe-NH_2	Antho-RFamide
Renilla köllikeri	<Glu-Gly-Arg-Phe-NH_2	Antho-RFamide
Polyorchis penicillatus	<Glu-Leu-Leu-Gly-Gly-Arg-Phe-NH_2	Pol-RFamide I
Polyorchis penicillatus	<Glu-Trp-Leu-Lys-Gly-Arg-Phe-NH_2	Pol-RFamide II

likhuijzen, 1988a, b; Grimmelikhuijzen et al., 1990; Nothacker et al., 1991a, b; Carstensen et al., 1992, 1993). One sea anemone peptide, <Glu-Gly-Arg-Phe-NH_2 (Antho-RFamide), appears to occur generally in anthozoans (Grimmelikhuijzen & Groeger, 1987), and related Arg-Phe-NH_2 (RFamide) peptides have been isolated from species belonging to the other cnidarian classes, such as *Polyorchis penicillatus* (Eschscholtz, 1829) (Grimmelikhuijzen et al., 1988, 1992a, table 1), *Hydra* and *Cyanea lamarckii* Péron & Le Sueur, 1809 (Moosler & Grimmelikhuijzen, unpublished). This indicates that RFamide peptides are ubiquitous in cnidarians.

All peptides (table 1) are localized in neurones (Grimmelikhuijzen and Spencer, 1984; Grimmelikhuijzen et al., 1988, 1992b; Nothacker, 1991b; Carstensen, 1992). Using immuno-electronmicroscopy, we have found that Antho-RFamide and the Antho-RWamides are localized within dense-cored neurosecretory granules (Westfall & Grimmelikhuijzen, 1993; Westfall et al., 1995). A similar vesicular localisation was found for the *Hydra* RFamide peptides (Koizumi et al., 1989).

Table 2. Excitatory (+) or inhibitory (-) actions of neuropeptides on different muscle groups of sea anemones.

Peptide	Tentacle	Body column	
	longitudinal	longitudinal	circular
Antho-RFamide	+	+	+
Antho-RWamide I, II	-	+	+
Antho-RNamide	+	+	-
Antho-RIamide I	-	-	-
Antho-KAamide	-	-	-
Antho-RPamide I	+		+
Antho-RPamide II, III	-		+

The neuropeptides are biologically active and have excitatory or inhibitory actions on intact animals, muscle preparations, or isolated muscle cells, suggesting that they are neurotransmitters or neuromodulators (McFarlane et al., 1987, 1991, 1992, 1993; Anctil & Grimmelikhuijzen, 1989; McFarlane & Grimmelikhuijzen, 1991; Carstensen et al., 1992, 1993). A summary of the actions of the various neuropeptides on sea anemones muscle preparations is given in table 2. The strongest evidence for neuropeptides being a neurotransmitter comes from work done with the Antho-RWamides: (i) there is a massive and direct innervation of the oral sphincter muscle by neurones containing the Antho-RWamides (Grimmelikhuijzen et al., 1992b), (ii) the Antho-RWamides are localized in dense-cored vesicles of synapses connected to the sphincter muscle cells (Westfall et al., 1995), (iii) addition of low concentrations (10^{-9} M) of the Antho-RWamides to isolated sphincter muscle cells causes contraction, showing that the action of the peptide is direct (McFarlane et al., 1991).

Recently, Leitz and co-workers have isolated a neuropeptide from *A. elegantissima* that induces metamorphosis in planula larvae of the marine hydroid *Hydractinia echinata* (Fleming, 1828) (Leitz et al., 1994; Leitz & May, 1995). This peptide, <Glu-Gln-Pro-Gly-Leu-Trp-NH_2 ("Metamorphosin A" or "MMA"), has an interesting structure, because it does not belong to the large family of Arg-X-NH_2 or Lys X-NH_2 neuropeptides present in sea anemones. The work of Leitz is also interesting, because it shows that neuropeptides in cnidarians, in addition to being transmitters, also can be hormones that control developmental processes such as metamorphosis.

We have also cloned the preprohormones for most of the isolated cnidarian neuropeptides. Because of space limitations, we would like to give only two examples, viz. the biosynthesis of Antho-RFamide and the biosynthesis of MMA. For more detailed reports, we refer to the original literature and to some recent reviews (Darmer et al., 1991; Schmutzler et al., 1992, 1994; Reinscheid & Grimmelikhuijzen, 1994; Grimmelikhuijzen et al., 1992b, 1994, 1995; Leviev & Grimmelikhuijzen, 1995).

Biosynthesis of Antho-RFamide

Figure 1 gives a summary of what is known for the biosynthesis of neuropeptides in higher animals. Neuropeptides are made as large prohormones that contain one or several copies of the immature neuropeptide sequence, flanked by basic amino acid

residues (marked by XX in the top of fig. 1). If the mature, biologically active neuropeptide has an N-terminal <Glu group (which protects against N-terminal degradation and which originates from cyclization of an N-terminal Gln group), the immature neuropeptide sequence has a Gln in this position. It is also known that a C-terminal amide group (which protects against C-terminal degradation) originates from a C-terminal Gly residue in the immature neuropeptide sequence. Neuropeptides, then, are generated from their prohormones in the following way. First, there is an initial endoproteolytic cleavage at the C-terminal side of paired or single basic (Arg or Lys) residues. This liberates the immature neuropeptide sequence from its precursor protein (step 1 in fig. 1). Several prohormone processing enzymes responsible for endoproteolytic cleavage at paired basic residues have been cloned, among them the prohormone convertases PC1 (also named PC3) and PC2 from mammals (Seidah et al., 1990, 1991; Smeekens & Steiner, 1990; Smeekens et al., 1991). The endoproteinase cleaving at single basic residues has not been cloned and characterized yet. It is clear, however, that cleavage is not allowed at every single basic amino acid residue, because some of the neuropeptide sequences themselves contain internal, single basic residues. Therefore, recognition sequences must exist that direct cleavage at monobasic sites. The exact nature of these recognition sequences, however, has still not been determined (Schwartz, 1986; Devi, 1991; Leviev & Grimmelikhuijzen, 1995). Second (step 2, fig. 1), the remaining basic residues at the C terminus of the immature neuropeptide are removed by a carboxypeptidase B, specific for basic amino acid residues (Fricker et al., 1989). Third, if the mature peptide carries a C-terminal amide group, a peptidyl-glycine is converted into a peptidyl-amide by the concerted action of two enzymes: peptidyl glycine hydroxyclase and peptidyl-hydroxyglycine N-C lyase (Bradbury & Smyth, 1991). Both enzymatic activities are located on a common proenzyme, the bifunctional peptidyl-glycine α-amidating monooxygenase (PAM), but this proenzyme can be cleaved by endoproteolysis into the separated enzymes (Kato et al., 1990; Katopodis et al., 1990, 1991; Eiper et al., 1992). Fourth, if the mature neuropeptide carries an N-terminal <Glu group, an N-terminal Gln is converted into a <Glu residue by the enzyme glutaminyl cyclase (Fischer & Spiess, 1987; Pohl et al., 1991). Thus, in higher animals, about five processing enzymes catalyze the generation of mature, biologically active neuropeptides from their precursor proteins.

The first cnidarian neuropeptide of which we studied the biosynthesis, was the anthozoan neuropeptide Antho-RFamide (<Glu-Gly-Arg-Phe-NH_2). We used two strategies to clone the Antho-RFamide precursor

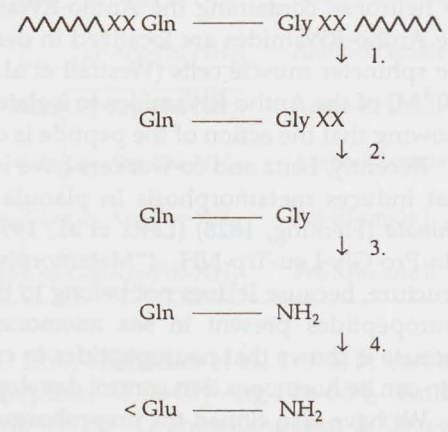

Fig. 1. Neuropeptide precursor processing in higher invertebrates and vertebrates. Top: the precursor protein contains one or more copies of an immature neuropeptide sequence flanked by basic residues (marked by XX). The cleavage or processing steps are catalyzed by the following enzymes: 1. An endoproteinase cleaving at the C-terminal sides of pairs or single basic residues. 2. A carboxypeptidase B-like enzyme. 3. Peptidyl-glycine hydroxylase and peptidyl-hydroxyglycine N-C lyase. 4. Glutaminyl cyclase.

from the sea anemone *Calliactis parasitica* (Couch, 1842) (this was the sea anemone species used for the experiments of table 2). First, we raised antibodies against the presumed immature Antho-RFamide sequence that was coupled via two flanking Lys residues (Lys-Gln-Gly-Arg-Phe-Gly-Lys) to a carrier protein, thyroglobulin. We then affinity-purified the antibodies and used them to screen an expression cDNA (λgt11) library from *C. parasitica*. The second strategy was to use a radioactive labeled pool of oligonucleotides coding for the immature sequence Gln-Gly-Arg-Phe-Gly, to screen the same cDNA library. Both strategies were successful. The Antho-RFamide precursor from *C. parasitica* contains 19 copies of immature Antho-RFamide and 7 other, putative neuropeptide sequences (Darmer et al., 1991). As we expected, each immature Antho-RFamide sequence had the structure Gln-Gly-Arg-Phe-Gly and was followed, at its C-terminus, by one or two basic amino acid residues. Thus, all processing enzymes mentioned in fig. 1 are probably present in sea anemone neurones. At the other hand, it was a surprise to see that at the N terminus of each immature Antho-RFamide sequence, basic residues were lacking and that, instead, acidic amino acidic (Asp and Glu) residues occurred. This meant that these acidic residues must represent a processing site, and that a novel processing enzyme must be present in cnidarian neurones. Such acidic processing sites were not known at that time for neuropeptide precursors from higher animals (Darmer et al., 1991).

We used the *Calliactis* cDNA coding for the Antho-RFamide precursor to screen a cDNA library from the sea anemone *A. elegantissima* (belonging to the subclass Hexacorallia) and the sea pansy *Renilla koellikeri* Pfeffer, 1886 (Octocorallia). Authentic Antho-RFamide had been isolated from both species (Grimmelikhuijzen and Graff, 1986; Grimmelikhuijzen and Groeger, 1987). The Antho-RFamide precursors from *A. elegantissima* and *R. koellikeri* showed the same features as the one from *C. parasitica*. In the precursor of *A. elegantissima*, are 13 copies of immature Antho-RFamide and 20 copies of other, putative neuropeptide sequences (Schmutzler et al., 1992). The Antho-RFamide precursor from *R. koellikeri* contains 36 copies of immature Antho-RFamide and two other, putative neuropeptide sequences (Reinscheid and Grimmelikhuijzen, 1994). The Antho-RFamide precursor from *R. koellikeri* is given in fig. 2. We have especially choosen the Antho-RFamide precursor from *R. koellikeri* for a full page representation in this review, because it shows in an impressive way how regular the immature Antho-RFamide copies are distributed over the whole precursor protein. Of the 36 copies of immature Antho-RFamide, 31 are preceded by an acidic (Glu) residue. This, again, clearly points to the presence of a novel processing enzyme specific for acidic residues.

Biosynthesis of MMA

We have cloned the preprohormone for the metamorphosis inducing neuropeptide MMA by screening a λgt11 cDNA library from *A. elegantissima* with a mixed pool of oligonucleotides corresponding to the presumed immature sequence of MMA (Gln-Gln-Pro-Gly-Leu-Trp-Gly). Fig. 3 shows the MMA precursor (Leviev & Grimmelikhuijzen, 1995). Nine out of 10 immature MMA sequences have a (Lys-Arg) dibasic processing site at their C termini, and one immature MMA sequence is followed by a single basic residue (table 3). It was an exciting surprise, however, to see

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                                                                TTTAGTATTGCGTCGTGTGTGTGTGTGTGTGTG    35
TTTGGCTCATTTTCTACGATCAACGTTGCTCTACACATTTGTATGGAGAAAACCTGTCAGTCTATAACGTATATACTTC   114
AAAATAATGGACTTAGTAATATAAGTATCAACTGCATTATTGTTGTCAATGTCCA ATG GTT AGT CTG GGT TTT   187
                                                        Met Val Ser Leu Gly Phe     6

TTC GTT CGT GAT GTT ACT CCA ACA TTC ATT GTA GAT CAT ATG TTC TAC ATG CTA TTC CCG   247
Phe Val Arg Asp Val Thr Pro Thr Phe Ile Val Asp His Met Phe Tyr Met Leu Phe Pro    26

ATG GAT TTT ACG TGT TAC GTT GCC GGG CTG TTG TTG ATA TTA AAT ACT TAC AGT TTG GCC   307
Met Asp Phe Thr Cys Tyr Val Ala Gly Leu Leu Leu Ile Leu Asn Thr Tyr Ser Leu Ala    46

GGG CCC TCA ACT AGC GAA GGA CTA AAC GAA CGG AAC TTG CTG GAT AAA ACA GAG TTG TCG   367
Gly Pro Ser Thr Ser Glu Gly Leu Asn Glu Arg Asn Leu Leu Asp Lys Thr Asp Leu Ser    66

ATA AAT GAC GAG ATA TTT AGC GAA GAT GAT GAT ATG CTG GCA AGG GAC GCT GAA GAC AAA   427
Ile Asn Asp Glu Ile Phe Ser Glu Asp Asp Asp Met Leu Ala Arg Asp Ala Glu Asp Lys    86

CAA GGA CGA TTT AAT CGT AAA TTA AAT AAC AAG TTG AAT GAA GCG GTA CAA GGA CGC TTC   487
Gln Gly Arg Phe Asn Arg Lys Leu Asn Asn Lys Leu Asn Glu Ala Val Gln Gly Arg Phe   106

GGG AGA AAT GAG AGA AAA GAA TCG GAA GAA GAA CAA GGA AGG TTC GGG CGA GAA AAT GAA   547
Gly Arg Asn Glu Arg Lys Glu Ser Glu Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   126

AAA CAA GGA AGA TTT GGA AGG GAA AGC GAG GAG CAA GGG AGA TTT GGA CGA GAA AAC AAA   607
Lys Gln Gly Arg Phe Gly Arg Glu Ser Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Lys   146

GAA CAA GGA AGA TTT GGA AGG GAA AAC AAA GAA CAA GGA AGG TTT GGA CGA GAA AAC GAG   667
Glu Gln Gly Arg Phe Gly Arg Glu Asn Lys Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   166

GAA CAA GGA AGA TTT GGA AGG GAA AGC GAG GAG CAA GGG AGA TTT GGA AGA GAA AAC GAA   727
Glu Gln Gly Arg Phe Gly Arg Glu Ser Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   186

GAA CAA GGA AGA TTT GGA CGA GAA AAC GAA GAA CAA GGA AGA TTT GGA CGA GAA AAC GAA   787
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   206

GAA CAA GGA AGG TTT GGA CGA GAA AAC GAG GAG CAA GGG AGA TTT GGA AGG GAG AAC GAA   847
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   226

GTT CAA GGA AGA TTT GGA CGA GAG AAC GAG GAG CAA GGA AGA TTT GGA CGA GAA AAC GAA   907
Val Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   246

GAA CAA GGA AGG TTT GGA AGA GAA AAC GAA GAA CAA GGA CGA TTT GGA CGA GAA AAC GAG   967
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   266

GAG CAA GGA AGA TTT GGA CGA GAA AAT GAA GAA CAA GGA AGA TTT GGA CGA GAA AAC GAG  1027
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   286

GAG CAA GGA AGA TTT GGA CGA GAA AAC GAA AAA CAA GGA CGA TTT GGA CGA GAA AAC GAA  1087
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Lys Gln Gly Arg Phe Gly Arg Glu Asn Glu   306

GAA CAA GGA AGA TTT GGA CGA GGA AAC GAG GAG CAA GGG AGA TTT GGA AGG GAA AAC GAA  1147
Glu Gln Gly Arg Phe Gly Arg Gly Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   326

GAA CAA GGA AGA TTT GGA CGA GAA AAC GAA GAA CAA GGA AGA TTT GGA CGA GAA AAC GAA  1207
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   346

GAA CAA GGA CGA TTT GGG CGA GAA AAC GAA GAA CAA GGA AGG TTT GGG CGA GAA AAC GAA  1267
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu   366

GAA CAA GGA AGG TTT GGA AGG GAG AAC GAG AAG CAA GGG AGA TTT GGA AGA GGG GAC GAA  1327
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Lys Gln Gly Arg Phe Gly Arg Gly Asp Glu   386

GAA CAA GGA AGG TTT GGA AGG GAG AAC GAG GAG CAA GGG AGA TTT GGA AGA GGG GAC GAA  1387
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Gly Asp Glu   406

GAA CAA GGA AGA TTT GGA CGA GAA AAC GAA GAG CAA GGG AGA TTT GGA AGA GAA AAC AAA  1447
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Glu Asn Lys   426

GAA CAA GGA AGA TTT GGA AGA GAA AAC GAA GAA CAA GGA AGA TTT GGA AGG GGG AAC AAA  1507
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Glu Gln Gly Arg Phe Gly Arg Gly Asn Lys   446

GAA CAA GGA AGA TTT GGA CGA GAA AAC GAA CAA GGA AGA TTT GGA AGA GAA AAC GAA GTT  1567
Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Gln Gly Arg Phe Gly Arg Glu Asn Glu Val   466

CAA GGA AGG TTT GGA AGA TTC AGT CGG GAG TTG GCG AAA GGT TTA AAG ATT GAC GAT GTT  1627
Gln Gly Arg Phe Gly Arg Phe Ser Arg Glu Leu Ala Lys Gly Leu Lys Ile Asp Asp Val   486

CTC TGA  CAATGAACTAATTACGTGAATTACTAGAAACAAACTTAGAGAAAGTTGTTTATGAATCTATCAATAGTAT  1703
Leu  *                                                                           487

TTAAAAAGCGTTTCCAAATATTTAGTGTGAAATGATATTTTAAAAAAATATACACCGAAAAAAAAAAAAAGGCCGATCG  1782
GGCCG(A)32                                                                       1819
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Fig. 2. cDNA and deduced amino acid sequence of the Antho-RFamide precursor from *R. koellikeri*. Nucleotide residues are numbered from the 5′ to 3′ end and amino acid residues are numbered starting with the first ATG codon in the open reading frame. All Antho-RFamide and Antho-RFamide-related sequences are underlined. Polyadenylylation signals in the untranslated 3′ region are marked by dotted lines. (Modified from Reinscheid & Grimmelikhuijzen, 1994).

that nine immature MMA copies were preceded by the sequence Ser-Ala-Asp-Pro and one copy by the sequence Ser-Ala-Ala-Pro (fig. 3, table 3). As authentic <Glu-Gln-Pro-Gly-Leu-Trp-NH_2 has been isolated from extracts of *A. elegantissima*, this clearly proves that there must be processing at the C-terminal sides of Pro residues. The most likely enzyme catalyzing this cleavage is dipeptidyl aminopeptidase (DPAP), which cleaves at the C-terminal sides of N-terminal X-Pro and X-Ala sequences. This stepwise removal of X-Pro and X-Ala dipeptides has already been known for the final processing of immature, N-terminally extended yeast α-mating factor, honey bee melittin and frog skin peptides (Kreil, 1990). So far, however, DPAP has not been found to be involved in the processing of neuropeptide precursor proteins.

In addition to the 10 copies of immature MMA, there is a large number of other, putative neuropeptide sequences that are closely related to authentic MMA (table 3). For reasons of simplicity, we have named the most frequent, putative peptide Antho-LWamide I (14 copies), the authentic metamorphosis inducing peptide (MMA) isolated by Leitz et al. (1994) Antho-LWamide II (10 copies), a third peptide occurring in high frequency Antho-LWamide III (6 copies) and other, closely related peptides Antho-LWamides IV-IX (table 3). The 14 immature Antho-LWamide I sequences are followed by single basic amino acid residues (Arg) and preceded by the processing sequence Lys-Arg (table 3). Thus, although Antho-LWamide I is still a putative peptide that has not been isolated yet, it is quite likely that it will exist and be released in a very high copy number from its precursor. The Antho-LWamides IV, VII and VIII have exactly the same processing sites as Antho-LWamide I, and it is likely that also these peptides will be released from the Antho-LWamide precursor. All mature Antho-LWamides I, II, IV, VII and VIII will have an N-terminal <Glu group and the C-terminal structure Gly-Leu-Trp-NH_2 (table 3).

There are other putative peptides (the Antho-LWamides III, V, VI and IX) that are very similar, or nearly identical to the authentic peptide MMA. Their immature sequences are followed by Lys-Arg residues, and they are preceded by X-Ala or X-Pro sequences (table 3), so they will probably be released and processed. Their final structures, however, are still uncertain. They might start with a <Glu group, or be N-terminally elongated by a single Gly, Leu or Arg residue, or by the sequence Gly-Ser-Gly (see Leviev & Grimmelikhuijzen, 1995, for details). Finally, there are other, putative peptide sequences flanked by basic residues, but it is not certain whether these will yield intact peptides (underlined by a dotted line in fig. 3 and table 3).

In summary, the Antho-LWamide precursor contains 37 immature neuropeptide copies that are likely to be released from the precursor. These 37 immature neuropeptide copies probably yield nine different, mature neuropeptides that are closely related, the Antho-LWamides I-IX. Table 4 shows the putative structures of these mature neuropeptides and the extent to which these peptides are structurally related. All neuropeptides have the C-terminal sequence Gly-Leu-Trp-NH_2 in common, and also in the N-terminal parts are many sequence similarities. Leitz and co-workers (1994) have found that the metamorphosis inducing potency of MMA (Antho-LWamide II) resides in the C-terminal part of the molecule, as N-terminal variants are still able to induce metamorphosis in *Hydractinia* planula larvae. Thus, it is possible that many, or perhaps all of the peptides of table 4 have a metamorphosis inducing capacity.

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CAATGAACTGAGTGGAACACAAGTAATACATATTCTTCACTTCGGTTGATA ATG GCC CTC AAG TGT CAT CTA GTT CTA CTG    81
                                                     Met Ala Leu Lys Cys His Leu Val Leu Leu    10

GCC ATT ACT TTA CTA TTA GCA CAG TGT TCA GGG TCA GTA GAC AAG AAG GAT AGT ACG ACG AAT CAC TTA   150
Ala Ile Thr Leu Leu Leu Ala Gln Cys Ser Gly Ser Val Asp Lys Lys Asp Ser Thr Thr Asn His Leu    33

GAT GAG AAG AAA ACA GAT TCC ACA GAA GCA CAT ATT GTA CAA GAA ACA GAC GCG TTA AAA GAA AAT TCT   219
Asp Glu Lys Lys Thr Asp Ser Thr Glu Ala His Ile Val Gln Glu Thr Asp Ala Leu Lys Glu Asn Ser    56

TAT CTT GGC GCC GAG GAG GAA TCT AAA GAA GAA GAC AAG AAG AGA TCC GCC GCT CCT CAG CAG CCT GGC   288
Tyr Leu Gly Ala Glu Glu Glu Ser Lys Glu Glu Asp Lys Lys Arg Ser Ala Ala Pro Gln Gln Pro Gly    79

CTC TGG GGG AAA CGC CAG AAA ATA GGA CTA TGG GGA AGA TCC GCT GAC GCA GGA CAG CCA GGC CTC TGG   357
Leu Trp Gly Lys Arg Gln Lys Ile Gly Leu Trp Gly Arg Ser Ala Asp Ala Gly Gln Pro Gly Leu Trp   102

GGC AAA CGA CAA AGT CCC GGA TTA TGG GGA AGA TCC GCT GAC GCA GGA CAG CCA GGC CTC TGG GGC AAA   426
Gly Lys Arg Gln Ser Pro Gly Leu Trp Gly Arg Ser Ala Asp Ala Gly Gln Pro Gly Leu Trp Gly Lys   125

CGT CAA AAT CCC GGA TTA TGG GGA AGA TCC GCT GAC GCA GGA CAG CCA GGC CTC TGG GGC AAA CGT CAA   495
Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser Ala Asp Ala Gly Gln Pro Gly Leu Trp Gly Lys Arg Gln   148

AAT CCC GGA TTA TGG GGA AGA TCG GCT GAC GCA GGA CAG CCA GGC CTC TGG GGC AAA CGT CAA AAT CCC   564
Asn Pro Gly Leu Trp Gly Arg Ser Ala Asp Ala Gly Gln Pro Gly Leu Trp Gly Lys Arg Gln Asn Pro   171

GGA TTA TGG GGA AGG TCC GCT GAC GCA AGA CAA CCC GGA CTC TGG GGC AAA CGT GAA ATC TAC GCA TTA   633
Gly Leu Trp Gly Arg Ser Ala Asp Ala Arg Gln Pro Gly Leu Trp Gly Lys Arg Glu Ile Tyr Ala Leu   194

TGG GGA GGA AAA CGT CAA AAT CCC GGA CTT TGG GGA AGA TCC GCT GAT CCA GGA CAG CCC GGC CTC TGG   702
Trp Gly Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser Ala Asp Pro Gly Gln Pro Gly Leu Trp   217

GGC AAA CGT GAA CTC GTC GGA TTA TGG GGG GGA AAA CGT CAA AAC CCC GGA TTG TGG GGA AGA TCG GCT   771
Gly Lys Arg Glu Leu Val Gly Leu Trp Gly Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser Ala   240

GAA GCA GGA CAG CCA GGA CTT TGG GGA AAA CGC CAA AAA ATA GGA TTG TGG GGA CGT TCG GCT GAC CCA   840
Glu Ala Gly Gln Pro Gly Leu Trp Gly Lys Arg Gln Lys Ile Gly Leu Trp Gly Arg Ser Ala Asp Pro   263

CTT CAG CCT GGC CTC TGG GGC AAA CGT CAA AAT CCC GGA TTA TGG GGA AGA TCT GCT GAC CCG CAG CAG   909
Leu Gln Pro Gly Leu Trp Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser Ala Asp Pro Gln Gln   286

CCT GGC CTC TGG GGC AAA CGT CAA AAT CCC GGA TTA TGG GGA AGA TCT GCT GAC CCG CAG CAG CCT GGC   978
Pro Gly Leu Trp Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser Ala Asp Pro Gln Gln Pro Gly   309

CTC TGG GGC AAA CGT CAA AAT CCC GGA TTA TGG GGA AGA TCT GCT GAC CCG CAG CAG CCT GGC CTC TGG  1047
Leu Trp Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser Ala Asp Pro Gln Gln Pro Gly Leu Trp   332

GGC AAA CGT CAA AAT CCC GGA TTA TGG GGA AGA TCT GCT GAC CCG CAG CAA CCT GGC CTC TGG GGC AAA  1116
Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser Ala Asp Pro Gln Gln Pro Gly Leu Trp Gly Lys   355

AGC CCC GGT TTA TGG GGA CGA TCC GCT GAC CCA CAA CAG CCT GGA CTT TGG GGG AAA CGC CAA AAT CCC  1185
Ser Pro Gly Leu Trp Gly Arg Ser Ala Asp Pro Gln Gln Pro Gly Leu Trp Gly Lys Arg Gln Asn Pro   378

GGA TTT TGG GGA AGA TCT GCT GAC CCG CAG CAG CCT GGC CTC TGG GGC AAA CGT CAA AAT CCC GGA TTA  1254
Gly Phe Trp Gly Arg Ser Ala Asp Pro Gln Gln Pro Gly Leu Trp Gly Lys Arg Gln Asn Pro Gly Leu   401

TGG GGA AGA TCT GCT GAC CCG CAG CAA CCT GGC CTC TGG GGC AAA CGT CAA AAT CCC GGA TTA TGG GGA  1323
Trp Gly Arg Ser Ala Asp Pro Gln Gln Pro Gly Leu Trp Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly   424

AGA TCT GCT GAC CCG CAG CAA CCT GGC CTC TGG GGC AAA CGT CAA AAC CCC GGT TTA TGG GGA CGA TCC  1392
Arg Ser Ala Asp Pro Gln Gln Pro Gly Leu Trp Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser   447

GCT GAC CCA CAA CAG CCT GGA CTT TGG GGG AAA CGC CAA AAT CCA GGA CTA TGG GGA AGA AGT GCT GGC  1461
Ala Asp Pro Gln Gln Pro Gly Leu Trp Gly Lys Arg Gln Asn Pro Gly Leu Trp Gly Arg Ser Ala Gly   470

TCC GGT CAA CTC GGA CTT TGG GGT AAA AGG CAA TCA CGC ATT GGA TTA TGG GGA AGA TCT GCC GAG CCT  1530
Ser Gly Gln Leu Gly Leu Trp Gly Lys Arg Gln Ser Arg Ile Gly Leu Trp Gly Arg Ser Ala Glu Pro   493

CCA CAA TTT GAA GAT TTA GAA GAT TTA AAG AAA AAA TCA GCA ATT CCC CAA CCA AAA GGA CAA TGA TAA  1599
Pro Gln Phe Glu Asp Leu Glu Asp Leu Lys Lys Lys Ser Ala Ile Pro Gln Pro Lys Gly Gln Stop Stop   514

TATCCTAGGATCTTCAAAAGTTATCCCGATCATCAATCCCCGGACAAGAGATATTTTAATTTCTGCCGCACGATTGACAGTTCCATTCCAT  1690
TACGAAGAACAAAAAGCTACGTTTCTTTAAGATAATAAATCAAATTCAATATTGTTTGAAGCAATGCACTTCAGGTTTTCACACAAAACTA  1781
ATACAAAAGTTATAAACATAAATAATAAACAAAAAAGGGGTAAGAAACCTGGTTTTTCGTTTTAGACTTTTCAAATTGGTCCTGCATGTCA  1872
TTGTAAGAGGTCGGCAGTAGAAGCTCTTTGAAACACTGTAAGTAGTCATTGAATGCACATTTATGGCAATAAAGATCACGCAGTGGTCTGC  1963
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Fig. 3. cDNA and deduced amino acid sequence of the MMA precursor from *A. elegantissima*. All authentic, immature MMA sequences are underlined and printed boldfaced type. Highly likely, but putative neuropeptide sequences are underlined only. Uncertain, putative neuropeptide sequences, or residues that might not be contained within the mature peptides, are underlined by a dotted line. Possible polyadenylylation signals are underlined twice. (Modified from Leviev & Grimmelikhuijzen, 1995).

Table 3. N- and C-terminal extensions of MMA and related, putative neuropeptide sequences.

N- and C-terminal extensions and neuropeptide sequence	Copy number	Name
Arg↓Ser-Ala-Asp-Pro-**Gln-Gln-Pro-Gly-Leu-Trp-Gly**-Lys-Arg↓ [a]	8	MMA (Antho-LWamide II)
Arg↓Ser-Ala-Asp-Pro-**Gln-Gln-Pro-Gly-Leu-Trp-Gly**-Lys↓ [b]	1	MMA (Antho-LWamide II)
Arg↓Ser-Ala-Ala-Pro-**Gln-Gln-Pro-Gly-Leu-Trp-Gly**-Lys-Arg↓ [c]	1	MMA (Antho-LWamide II)
Lys-Arg↓Gln-Asn-Pro-Gly-Leu-Trp-Gly-Arg↓ [d]	14	Antho-LWamide I
Lys-Arg↓Gln-Ser-Pro-Gly-Leu-Trp-Gly-Arg↓ [e]	1	Antho-LWamide VII
Lys-Arg↓Gln-Lys-Ile-Gly-Leu-Trp-Gly-Arg↓ [f]	2	Antho-LWamide IV
Lys-Arg↓Gln-Ser-Arg-Ile-Gly-Leu-Trp-Gly-Arg↓ [g]	1	Antho-LWamide VIII
Arg↓Ser-Ala-Gly-Ser-Gly-Gln-Leu-Gly-Leu-Trp-Gly-Lys-Arg↓ [h]	1	Antho-LWamide IX
Arg↓Ser-Ala-Asp-Ala-Gly-Gln-Pro-Gly-Leu-Trp-Gly-Lys-Arg↓ [i]	4	Antho-LWamide III
Arg↓Ser-Ala-Glu-Ala-Gly-Gln-Pro-Gly-Leu-Trp-Gly-Lys-Arg↓ [j]	1	Antho-LWamide III
Arg↓Ser-Ala-Asp-Pro-Gly-Gln-Pro-Gly-Leu-Trp-Gly-Lys-Arg↓ [k]	1	Antho-LWamide III
Arg↓Ser-Ala-Asp-Pro-Leu-Gln-Pro-Gly-Leu-Trp-Gly-Lys-Arg↓ [l]	1	Antho-LWamide V
Arg↓Ser-Ala-Asp-Ala-Arg-Gln-Pro-Gly-Leu-Trp-Gly-Lys-Arg↓ [m]	1	Antho-LWamide VI
Lys↓Ser-Pro-Gly-Leu-Trp-Gly-Arg↓ [n]	1	
Lys-Arg↓Glu-Leu-Val-Gly-Leu-Trp-Gly-Gly-Lys-Arg↓ [o]	1	
Lys-Arg↓Glu-Ile-Tyr-Ala-Leu-Trp-Gly-Gly-Lys-Arg↓ [p]	1	
Arg↓Ser-Ala-Glu-Pro-Pro-Gln-Phe-Glu-Asp-Leu-Glu-Asp-Leu-Lys-Lys-Lys↓ [q]	1	

The sites of initial cleavage at tribasic, dibasic or monobasic residues are indicated by arrows. MMA copies are underlined and printed boldfaced type. Highly likely, but putative peptide sequences are underlined only. Uncertain mature sequences or residues are underlined by a dashed line. The neuropeptide sequences given in this table can be found back in fig. 3 at the following amino acid positions: a, 285, 306, 327, 367, 388, 409, 430, 451; b, 348; c, 76; d, 127, 148, 169, 200, 231, 273, 294, 315, 336, 376, 397, 418, 439, 460; e, 106; f, 85, 252; g, 481; h, 470; i, 97, 118, 139, 160; j, 243; k, 212; l, 264; m, 181; n, 358; o, 221; p, 190; q, 492.

Table 4. Established and putative, mature neuropeptides that could be released from the MMA precursor protein.

Copies	Structure	Name
14	<Glu-Asn-Pro-Gly-Leu-Trp-NH_2	Antho-LWamide I
10	<Glu-Gln-Pro-Gly-Leu-Trp-NH_2	MMA (Antho-LWamide II)
6	Gly-Gln-Pro-Gly-Leu-Trp-NH_2	Antho-LWamide III
1	Leu-Gln-Pro-Gly-Leu-Trp-NH_2	Antho-LWamide V
1	Arg-Gln-Pro-Gly-Leu-Trp-NH_2	Antho-LWamide VI
1	<Glu-Ser-Pro-Gly-Leu-Trp-NH_2	Antho-LWamide VII
2	<Glu-Lys-Ile-Gly-Leu-Trp-NH_2	Antho-LWamide IV
1	<Glu-Ser-Arg-Ile-Gly-Leu-Trp-NH_2	Antho-LWamide VIII
1	Gly-Ser-Gly-Gln-Leu-Gly-Leu-Trp-NH_2	Antho-LWamide IX
37		

Amino acid residues that are identical with those of MMA are highlighted by boxes. Residues that might possibly not be contained in the mature peptides are underlined with a broken line.

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Hydroids from the vicinity of a large industrial area in Vitória, Espírito Santo, Brazil

P.A. Grohmann, M.M. de Souza & C.C. Nogueira

Grohmann, P.A., M.M. de Souza & C.C. Nogueira. Hydroids from the vicinity of a large industrial area in Vitória, Espírito Santo, Brazil.
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Key words: Cnidaria; Hydroida; faunal list; Brazil.

Abstract: Hydroids were collected as part of a long-term monitoring project in the vicinity of a large industrial site at Vitória, Espírito Santo, SE Brazil. The study area has been impacted by effluents from SUPEL/CVRD (Superintendência de Pelotização da Companhia Vale do Rio Doce), an iron ore pelletizing plant, and also by oil spills from the Tubarão Harbour terminal complex. In 1986, two new decanters were installed by the plant to mitigate pollution previously discharged directly into the sea, and the company has funded a monitoring program to assess the rate of recovery of biota near their installations. Considering the environmental stresses caused by the large scale industrial activity in the area nowadays, the diversity of hydroids is still high. To date, 52 species of hydroids, referrable to 35 genera and 21 families, have been reported. Of these, 5 genera (*Parawrightia, Rhysia, Sphaerocoryne, Hydranthea, Calamphora*) and 7 species (*Parawrightia robusta* Warren, 1907; *Eudendrium fragile* Motz-Kossowska, 1905; *Rhysia* spec.; *Amphinema dinema* (Perón & Lesueur, 1809); *Sphaerocoryne* spec.; *Hydranthea margarica* Huvé, 1954; and *Calamphora campanulata* (Warren, 1908)) are recorded from Brazil for the first time. Distribution records along the coast of Brazil of all 52 species are provided.

Introduction

Substantial inventories of hard and soft bottom benthos have been undertaken in Brazil since 1980, in the coastal zone of the southeastern region of the country, primarily in relation to disturbances of various kinds. The first large inventory was carried out by staff of the Universidade Federal do Rio de Janeiro at Angra-dos-Reis, Rio de Janeiro, during 1980/81 (Nogueira et al., 1997).

This report presents faunistic records of hydroids from a nine-year (1986-95) investigation on the biota in the vicinity of the Companhia Vale do Rio Doce (CVRD), at Vitória, capital of Espírito Santo state.

The land area surrouding Baía do Espírito Santo is nowadays one of the most densely populated metropolitan areas in Vitória. The region has a long history of urbanization and intensive industrial activity. In addition, Praia de Camburi is also the main source of domestic and industrial waste water of the city, discharged into Rio da Passagem, and of the newly created districts in its vicinity.

After the implantation of CVRD, in 1962, and the operation of the Tubarão maritime terminal, started in 1967, multiple pollution has incessantly been increased in the area.

In order to mitigate environmental pollution in Baía do Espírito Santo, partially caused by the effluent discharges of the company, a large monitoring program was

established by CVRD soon after the implantation of two decanters by Superintendência de Pelotização (SUPEL), its subordinate pelletizing plant, in 1986. Studies on the biota were carried out on the benthos of bottom sediments in Baía do Espírito Santo, as well as on the rocky coast at two locations: Praia de Camburi, in the vicinity of SUPEL and Praia Mole, an open sea beach, a site also subordinated to CVRD.

A surprisingly high diversity was observed in the hydroid fauna, compared to other similar areas in the coast of Brazil.

Material and methods

Since 1986, qualitative samples have been collected seasonally at two locations on the rocky shore at Vitória, state of Espírito Santo, southeastern Brazil (fig. 1). One site was located at Praia de Camburi, Baía do Espírito Santo (1) and the second at Praia Mole (2), a beach facing the open sea. Benthic biota were sampled by manual collecting on the littoral and sublittoral fringe. The samples were sorted, anaesthetized with menthol crystals, and fixed in 10% seawater-formalin. Hydroids were mounted on permanent slides following the methods of Mayal (1973), modified. Specimens were identified and classified according to Millard (1975) and Bouillon (1985).

Study area

Baía do Espírito Santo is a prime area of the city of Vitória, and important to tourism in the state of Espírito Santo. Until about three decades ago, the region also supported an important commercial fishery. Camburi, a district located adjacent to the shore, is one of the finest parts of the city. Originally comprising sandbank and mangrove habitats, and with an existing forest reserve, Camburi is becoming increasingly urbanized (Costa, 1986) due to real estate speculation.

Over the past three decades, the Camburi region has been impacted by industrial development. Operation of a pelletizing plant by Companhia Vale do Rio Doce (SUPEL/CVRD) since 1966, together with the opening of a port terminal at Tubarão in 1967, contributed environmental stresses to the area. Wastes from the washing of iron ore pellets as well as other effluents, flow into Baía do Espírito Santo. Moreover, occasional petroleum spills have taken place in the vicinity of docks within Tubarão Harbour, site of an ore quay, a coal quay and an oil terminal (CVRD Report, 1966). Two new decanters were installed by CVRD in 1986 to mitigate pollution previously discharged directly into the sea, and the company has funded a monitoring program to assess the rate of recovery of biota near their installations.

Results

Pooling data from the two sampling sites in the region, 52 species, representing 35 genera and 21 families have been identified from the study area to date (table 1). Five genera (*Parawrightia*, *Rhysia*, *Sphaerocoryne*, *Hydranthea*, *Calamphora*) and seven species (*Parawrightia robusta* Warren, 1907, *Eudendrium fragile* Motz-Kossowska, 1905, *Rhysia* spec., *Amphinema dinema* (Perón & Lesueur, 1809), *Sphaerocoryne* spec., *Hydran-*

thea margarica Huvé, 1954, *Calamphora campanulata* (Warren, 1908)) are reported from the coast of Brazil for the first time.

Discussion

In a biogeographic study of the southern coast of Brazil, Palacio (1982) recognized a Paulista Province occurring between the states of Espírito Santo and Rio Grande do Sul. The region was characterized as a transition zone, with the gradual disappearence of tropical species ranging down from the north and the appearance of

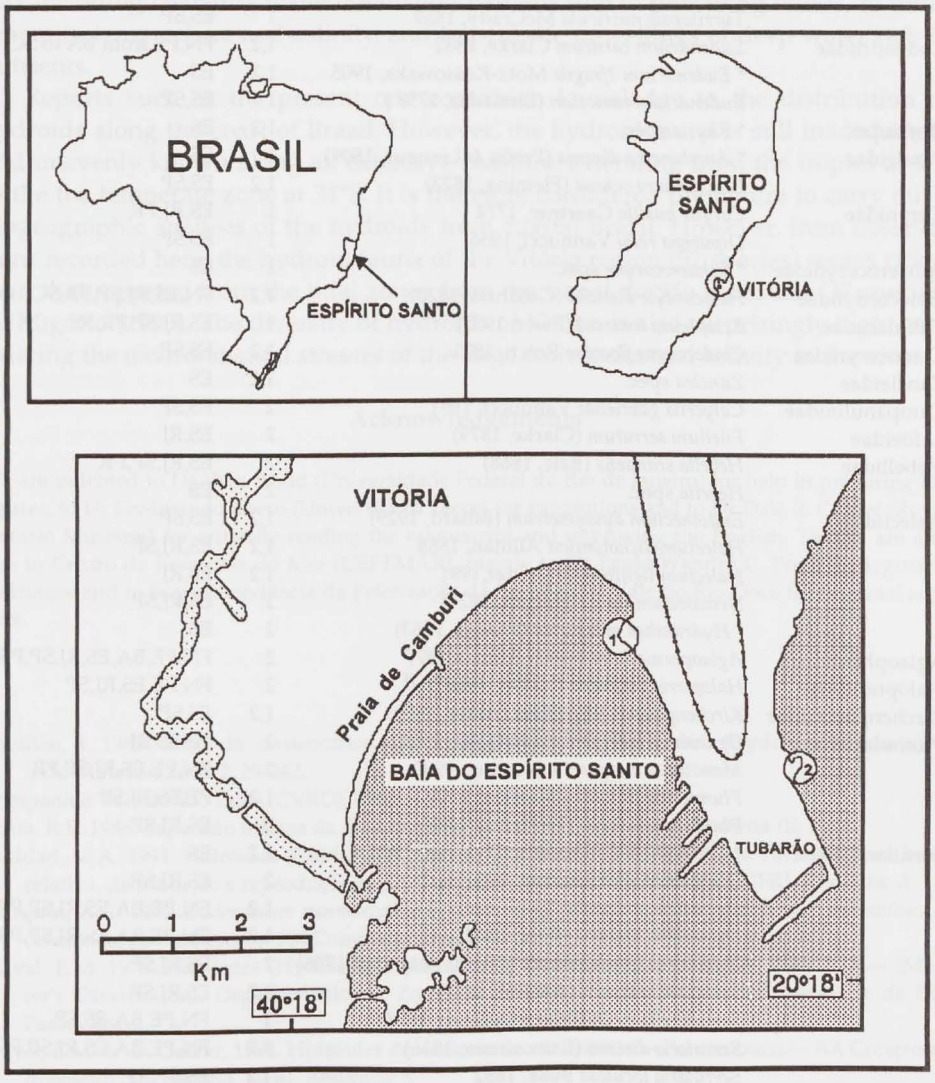

Fig. 1. Map of the Baía do Espírito Santo region showing the location of the two collecting sites at Vitória, Espírito Santo, Brazil. 1= Praia de Camburi; 2 = Praia Mole.

Table 1. Hydroid species collected at Camburi, Vitória, Espírito Santo, Brazil and the localities along the Brazilian coast from where they have been reported (after Migotto, 1993; Haddad, 1994; Marques, 1994; and Mayal & Calder, 1994); * = genus/species recorded from Brazil for the first time.
Abbreviations: FN = Fernando de Noronha; PE = Pernambuco; BA = Bahia; ES = Espírito Santo; RJ = Rio de Janeiro; SP = São Paulo; PR = Paraná; SC =Santa Catarina; SV = Sites of occurrence at Vitória (1 = Praia Mole; 2 = Praia de Camburi).

Family	Species	SV	Localities
Bougainvilliidae	*Bimeria vestita* Wright, 1859	1,2	ES,SP
	* *Parawrightia robusta* Warren, 1907	1,2	ES
Clavidae	unidentified species	1,2	ES
	Turritopsis nutricula McCrady, 1859	1	ES,SP
Eudendriidae	*Eudendrium carneum* Clarke, 1882	1,2	FN,PE,from BA to SC
	* *Eudendrium ?fragile* Motz-Kossowska, 1905	1,2	ES
	Eudendrium ramosum (Linnaeus, 1758)	1	ES,SP
Rhysiidae	* *Rhysia* spec.	1,2	ES
Pandeidae	* *Amphinema dinema* (Perón & Lesueur, 1809)	1	ES
	Leuckartiara octona (Fleming, 1823)	1,2	ES,SP
Corynidae	*Coryne pusilla* Gaertner, 1774	1	ES,RJ,PR
	Dipurena reesi Vannucci, 1956	1	ES,SP
Sphaerocorynidae	* *Sphaerocoryne* spec.	1	ES
Halocordylidae	*Halocordyle disticha* (Goldfuss, 1820)	1,2	FN,ES,RJ,SP,PR,SC
Tubulariidae	*Ectopleura warreni* (Ewer, 1953)	1	ES,RJ,SP,PR,RS
Cladocorynidae	*Cladocoryne floccosa* Rotch, 1871	1,2	ES,SP
Zancleidae	*Zanclea* spec.	1,2	ES
Campanulinidae	*Calycella gabriellae* Vannucci, 1951	2	ES,SP
Lafoeidae	*Filellum serratum* (Clarke, 1879)	2	ES,RJ
Hebellidae	*Hebella scandens* (Bale, 1888)	2	ES,RJ,SP,PR
	Hebella spec.	2	ES
Haleciidae	*Endothecium dyssymetrum* (Billard, 1929)	1,2	ES,SP
	Halecium dichotomum Allman, 1888	1,2	ES,RJ,SP
	Halecium lightbourni Calder,1991	1,2	ES,RJ
	Nemalecium lighti (Hargitt, 1924)	2	ES,RJ,SP
	* *Hydranthea margarica* (Hincks, 1863)	2	ES
Aglaopheniidae	*Aglaophenia latecarinata* Allman,1877	2	FN,PE,BA,ES,RJ,SP,PR
Halopteriddidae	*Halopteris diaphana* (Heller, 1868)	2	FN,PE,ES,RJ,SP
Kirchenpaueriidae	*Kirchenpaueria halecioides* (Alder, 1859)	1,2	ES,SP
Plumulariidae	*Dentitheca bidentata* (Jäderholm, 1920)	2	PE,ES,RJ
	Monotheca margaretta Nutting, 1900	2	FN,PE,ES,RJ,SP,PR
	Plumularia floridana Nutting, 1900	1,2	PE,ES,RJ,SP
	Plumularia setacea (Linnaeus, 1758)	2	ES,RJ,SP
Sertulariidae	* *Calamphora campanulata* (Warren, 1908)	1,2	ES
	Diphasia tropica Nutting, 1904	2	ES,RJ,SP
	Dynamena crisioides Lamouroux, 1824	1,2	FN,PE,BA,ES,RJ,SP,PR
	Dynamena disticha (Bosc, 1802)	1,2	FN,PE,BA,ES,RJ,SP,PR
	Dynamena quadridentata (Ellis & Solander, 1786)	2	ES,RJ,SP
	Sertularella conica (Allman, 1877)	1,2	ES,RJ,SP
	Sertularella cylindritheca (Allman, 1878)	1	FN,PE,BA,ES,SP
	Sertularia distans (Lamouroux, 1816)	1,2	FN,PE,BA,ES,RJ,SP,PR
	Sertularia loculosa Busk, 1852	1,2	ES,SP,RJ
	Sertularia marginata Kirchenpauer, 1864	1,2	FN,PE,ES,RJ,SP,PR
	Sertularia rugosissima Thornely, 1904	1,2	ES,RJ,SP,PR

	Sertularia turbinata (Lamouroux, 1816)	1,2	PE,ES,RJ,SP,PR
Campanulariidae	unidentified species	2	
	Clytia hemisphaerica (Linnaeus, 1767)	1,2	ES,RJ,SP,PR
	Clytia linearis (Thornely, 1899)	2	ES,RJ,SP
	Clytia paulensis (Vanhöffen, 1910)	2	PE,ES
	Obelia dichotoma (Linnaeus, 1758)	1,2	ES,RJ,SP,PR
	Obelia geniculata (Linnaeus, 1758)	2	ES,RJ,SP,PR
	Orthopyxis sargassicola (Nutting, 1915)	1,2	ES,RJ,SP

Patagonic species ranging further south. Distribution patterns were attributed to factors including changing water circulation patterns due to prevailing winds, to differences in temperature and salinity, and to periodic upwellings of deep water rich in nutrients.

Reports such as the present one contribute knowledge to the distribution of hydroids along the coast of Brazil. However, the hydroid fauna is still inadequately and unevenly known along an extensive coastline extending from the tropics at 6°S to the the temperate zone at 34°S. It is therefore considered premature to carry out a biogeographic analysis of the hydroids from coastal Brazil. However, from observations recorded here, the hydroid fauna of the Vitória region (52 species) seems richer than that reported from the tidal zones from the Canal de São Sebastião (32 species) by Migotto (1993). The diversity of hydroids of Vitória is also surprisingly high, considering the environmental stresses of the large scale industrial activity in the area.

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Stimulation of respiration and photosynthesis by light in the symbiotic anemone *Anemonia viridis* (Forskål, 1775) (Anthozoa: Actiniaria)

A.D. Harland & P.S. Davies

Harland, A.D. & P.S. Davies. Stimulation of respiration and photosynthesis by light in the symbiotic anemone *Anemonia viridis* (Forskål, 1775) (Anthozoa, Actiniaria).
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Key words: Anthozoa; Actiniaria; *Anemonia viridis*; symbiosis; respiration; photosynthesis; light.

Abstract: The dark respiration rate of *Anemonia viridis* (Forskål, 1775) increases by about 30% when exposed to hyperoxia of 150% oxygen saturation in darkness. This suggests that during the nighttime, respiration rate is reduced, probably as a result of problems of oxygen diffusion to the deep tissues, such as the mesenteries. This interpretation is supported by the fact that at night the oxygen concentration in the water of the coelenteron falls to about 72% saturation. Conversely, when exposed to saturating irradiance in the day, the coelenteric water becomes hyperoxic, with a mean of 129% oxygen saturation. The respiration rate increases with increasing exposure to saturating irradiance, and after 6 hours is about double that of the pre-irradiation rate, probably as a result of the increased energy costs of metabolising translocated carbon compounds or of the energy cost of growth. Exposure to 6 hours of saturating irradiance results in a significant 12% increase in gross photosynthesis, probably due to utilisation by the zooxanthellae of the CO_2 resulting from the enhanced respiration of the host. It is proposed that in the day time there is a reciprocal benefit between symbiont and host. The oxygen from photosynthesis of the zooxanthellae is available to maintain the respiration of the otherwise oxygen limited host tissue, whilst the carbon dioxide from increased host respiration enhances the CO_2-limited photosynthesis of the algae.

Introduction

Until recently, it was considered that respiration rate of Cnidaria is not oxygen limited, since oxygen diffusion distances would be low because of the laminate body plan, based upon tissues only two cell layers in thickness (e.g. Kanwisher & Wainwright, 1967). However, Shick (1990) cast doubt on this by demonstrating that respiration rate of symbiotic cnidarians increases when exposed to hyperoxic sea water. This suggests that the oxygen produced in vivo by the photosynthesis of zooxanthellae, which can raise tissue oxygen concentrations to 200% saturation or more (Dykens & Shick, 1982; Kuhl et al., 1995), would overcome this oxygen limitation in the daytime. It has also been proposed that the photosynthesis of zooxanthellae is CO_2-limited (Muscatine et al., 1989; Weis et al., 1989).

The present preliminary investigation was undertaken firstly to find out if the rate of respiration in the symbiotic anemone *Anemonia viridis* (Forskål, 1775) is oxygen-limited at night, and secondly, if respiration increases during the day, would zooxanthellae photosynthesis then increase as respiratory carbon dioxide is utilised?

Materials and methods

Anemones of similar size were maintained in re-circulating sea water at 15°C (± 0.4°C), salinity 33% and a saturating irradiance of about 300 μE m^{-2} s^{-1} with a 12 h light : 12 h dark photoperiod. A stream of compressed air was bubbled into the sea water to remove the oxygen produced in photosynthesis so that normoxic levels of oxygen saturation were maintained. Anemones were fed weekly on chopped mussel (*Mytilus edulis*) three days before any experiments were conducted (Tytler & Davies, 1984). All dark respiration and photosynthesis measurements were made in closed chamber respirometers at 15°C with microcathode oxygen electrodes connected to oxygen meters (Strathkelvin Instruments). The respirometer chambers were positioned underneath a light-hood fitted with fluorescent lights to enable photosynthesis to be measured at a saturating irradiance of 300 μE m^{-2} s^{-1}. Respiration was measured in total darkness (for further details see Harland and Davies (1994)).

In order to investigate whether the respiration rate of *Anemonia* is oxygen-limited at night, the dark respiration rate of 10 anemones was measured towards the end of the night-time photoperiod in normoxic (100% saturated) sea water. During the following night, oxygen was bubbled into the aquarium to maintain hyperoxic sea water (150% saturated). After 12 h exposure anemones were transferred to the respirometer which contained 150% saturated sea water, and the dark respiration rate measured.

To investigate the oxygen concentrations experienced by the deeper tissues, such as the mesenteries, in the coelenteron, a needle oxygen electrode with a diameter of 0.89 mm (Diamond General, 768) connected to a Strathkelvin Instruments 781 oxygen meter, was used. Anemones (n = 12) were maintained with a 12 h light : 12 h dark photoperiod at 15°C under saturating light for 3 weeks. At various times during the light and dark periods, the electrode was inserted through the body wall into the coelenteron and the maximum and minimum O_2 level was noted over an interval of about 5 s.

The effect of exposure to light on the respiration rate (R) was determined on five anemones over a 3 day period. On day 1, respiration was measured at the end of the night-time dark period. On each of the following 3 days, a measurement was made after cumulative exposure to either 2, 4, or 6 hours to saturating irradiance.

In an experiment conducted to see if zooxanthellae photosynthesis is CO_2 limited, both *R* and net photosynthesis (P^{net}) were measured at the beginning of the lightperiod and then after 6 h, when increased levels of respiratory CO_2 might be expected to be available for photosynthesis (n = 5).

After each experiment, anemones were buoyant weighed (Davies, 1990) and dry weights calculated from a regression of buoyant weight on dry weight (Harland and Davies 1994). Data appeared to be normally distributed and variances were homogeneous (F_{max} test). Statistical analyses were carried out using Anova, Student's t-test and t-test for matched pairs where appropriate. Multiple comparisons were done with a Tukey-Kramer test (Sokal and Rohlf, 1981).

Results

When anemones were incubated in the dark in hyperoxic sea water containing 150%

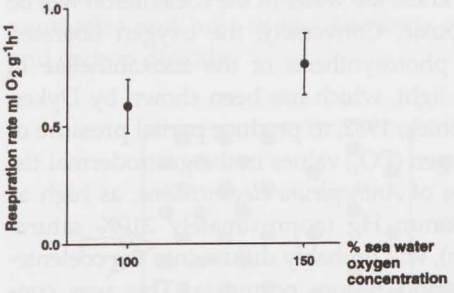

Fig 1. *Anemonia viridis*. Respiration rates of anemones measured after exposure to normoxia (100% sea-water oxygen saturation) and hyperoxia (150% sea-water oxygen saturation) in darkness. Data are means ± SD (n = 10).

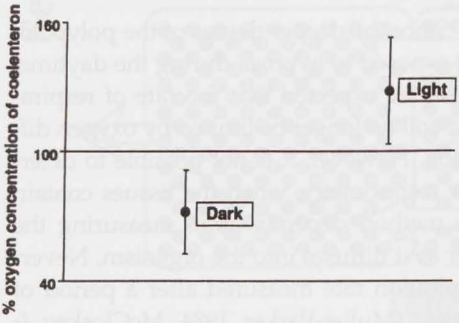

Fig 2. *Anemonia viridis*. Oxygen concentration of coelenteric fluid during the night and during the day. Measurements were made at intervals during a 12 h light: 12 h dark photoperiod. Data are means ± SD (for dark, n = 7, for light, n = 17).

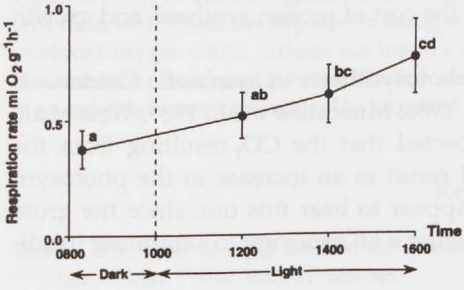

Fig 3. *Anemonia viridis*. Respiration rates of anemones measured in darkness and then at 2 h intervals in the light for 6 h. Where letters differ, means are significantly different ($P < 0.05$). Data are means ± SD (n = 5).

of normal oxygen saturation, *R* increased to 0.761 (± 0.130) when compared to a value of 0.578 (± 0.115) ml O_2 $g^{-1}h^{-1}$ under normoxia (t-test for matched pairs $P < 0.05$) (fig. 1)

The average oxygen concentration of the coelenteron in darkness was 72 (± 19.4)% saturation, compared with 128.5 (± 24.7)% during the day under saturating light conditions (Student's t-test $P < 0.05$) (fig. 2).

There was a gradual increase in R over time with 0.378 (± 0.082) ml O_2 $g^{-1}h^{-1}$ recorded at the end of the night-time dark period and a maximum of 0.767 (± 0.16) ml O_2 $g^{-1}h^{-1}$ after 6 h exposure to saturating light (Anova $P < 0.05$) (fig. 3).

Net photosynthesis increased from 1.251 (± 0.239) to 1.322 (± 0.259) ml O_2 $g^{-1}h^{-1}$ (+ 6%) after 6 h exposure to light. However, this increase was not significant (t-test for matched pairs $P > 0.05$). Gross photosynthesis (P^{gross}) (calculated from $R + P^{net}$) increased from 1.946 (± 0.279) to 2.176 (± 0.35) ml O_2 $g^{-1}h^{-1}$ (+ 12%) and was significant (t-test for matched pairs $P < 0.05$), since R had also increased during the 6 h light period, from 0.695 (± 0.08) to 0.855 (± 0.146) ml O_2 $g^{-1}h^{-1}$ (+ 23%) (fig. 4).

Discussion

When exposed to hyperoxic sea water (150% saturation), the rate of respiration increased from 0.578 to 0.761 ml O_2 $g^{-1}h^{-1}$, an increase of 32%. This is in agreement with the observations of Shick (1990) who demonstrated similar increases in a range of symbiotic Anthozoa when exposed to water of about 200% saturation. This observation suggests that diffusion alone is inadequate to meet the requirements for respiration of the deeper tissues in the polyp. It also calls into question the assertion of earlier workers (e.g. Kanwisher & Wainright, 1967) that oxygen diffusion should present no problems to organisms which are essentially only two cell layers in thickness.

The observation also suggests that in

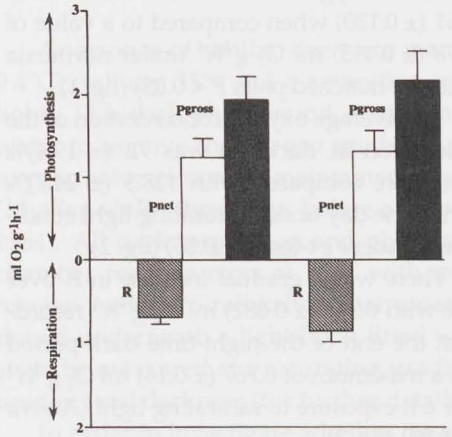

Fig 4. *Anemonia viridis*. Respiration (R), net photosynthesis (P^{net}) and gross photosynthesis (P^{gross}) [$P^{gross} = R + P^{net}$] at the start of the light period (A) and after 6 h exposure to saturating light (B). Data are means ± SD (n = 5).

darkness the water in the coelenteron will be hypoxic. Conversely, the oxygen liberated by photosynthesis of the zooxanthellae in the light, which has been shown by Dyken & Shick, 1982, to produce partial pressure of oxygen (PO_2) values in the gastrodermal tissues of *Anthopleura elegantissima*, as high as 328 mm Hg (approximately 210% saturation), will probably diffuse into the coelenteron and restore normoxia. This was confirmed by the in vivo measurements in *Anemonia viridis* showing a mean night-time oxygen concentration of 72%. In the light, the coelenteric water became hyperoxic with a mean concentration of about 129% saturation.

Since the deeper tissues of the polyp are not exposed to hypoxia during the daytime, it is to be expected that the rate of respiration will no longer be limited by oxygen diffusion. However, it is not possible to determine the respiration rate, using closed chamber respirometry, when the tissues contain photosynthetically produced oxygen, since the method depends upon measuring the depletion of oxygen from the surrounding water as it diffuses into the organism. Nevertheless, several workers have reported that respiration rate measured after a period of exposure to the light is higher than after darkness (Muller-Parker, 1984; McCloskey & Muscatine, 1984; Edmunds & Davies, 1986). In *Anemonia viridis*, the rate of respiration after 6 h of light exposure at 0.761 ml O_2 $g^{-1}h^{-1}$ was about double the pre-exposure rate. Since oxygen is diffusing from the surrounding normoxic sea water, it seems likely that the increased oxygen demand must come from the superficial tissues of the body wall and tentacles. The cause of the post-illumination increase in oxygen demand has been attributed to the cost of intermediary metabolism of carbon products translocated from the zooxanthellae (e.g. Porter et al., 1984) or to the cost of protein synthesis and growth (Edmunds & Davies, 1988).

There is some evidence that the rate of photosynthesis in symbiotic Cnidaria is limited by availability of CO_2 (Streamer et al., 1986; Muscatine et al., 1989; Weis et al., 1989; Weis, 1990). It might therefore be expected that the CO_2 resulting from the enhanced respiration rate in the light would result in an increase in the photosynthetic rate. The results of this study would appear to bear this out, since the gross rate of photosynthesis increased by 12% following a 6h exposure to saturating irradiance.

A simple model is proposed (fig. 5) in which respiration in the deep tissues is oxygen-limited during darkness. In the light, oxygen from zooxanthellae photosynthesis will create a hyperoxic environment and the translocation of carbon compounds will result in an increase in respiration. Any CO_2-limitation of photosynthesis will gradually diminish in the light as CO_2 becomes available from the increase in

respiration. It would appear therefore that there is reciprocal benefit between zooxanthellae and host in the *Anemonia viridis* symbiosis with the recycling of oxygen and carbon dioxide.

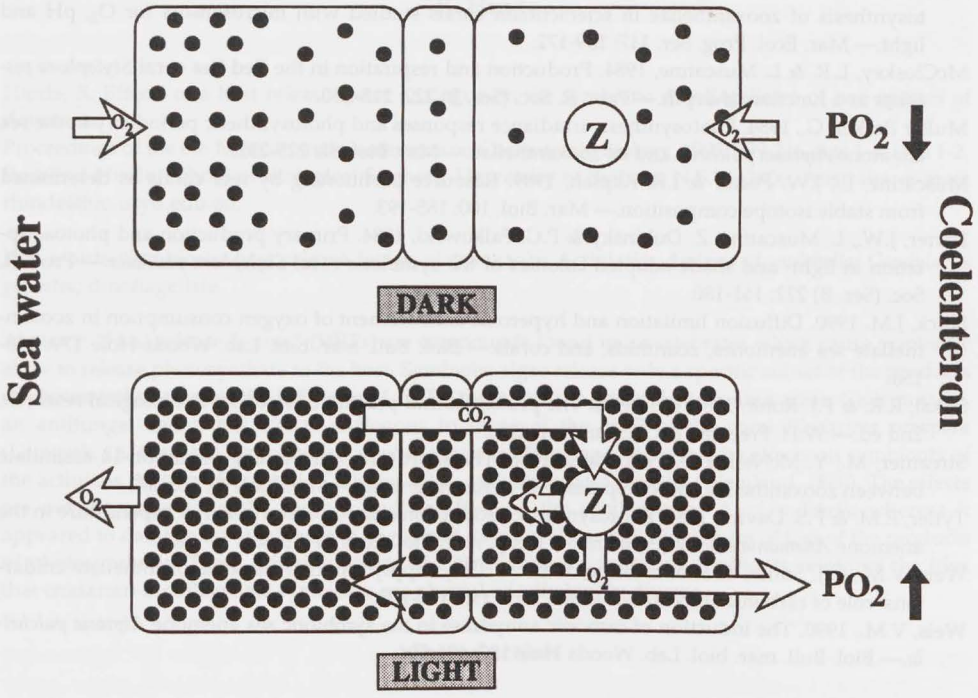

Fig 5. *Anemonia viridis*. Model to illustrate the proposed reciprocal exchange of oxygen and carbon dioxide between animal host tissue and symbiont zooxanthellae in the dark and in the light. The model shows a gastrodermal cell confluent with the coelenteron and an ectodermal cell surrounded by sea water with the mesoglea in between. Zooxanthellae (Z) are located in the gastrodermal cells. Arrows denote pathways of either oxygen, carbon dioxide or carbon movement. The density of shading shows oxygen diffusion gradients: In the dark, oxygen diffuses from the sea water and the coelenteric fluid, which becomes hypoxic. In the light, all tissues become hyperoxic from photosynthetically produced oxygen which diffuses out into the coelenteron and surrounding sea water. The water in the coelenteron becomes hyperoxic. Carbon from photosynthesis is translocated to the host resulting in an increase in respiration rate. The increased respiration rate provides CO_2 for photosynthesis.

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Effects of a host release factor analogue on the symbiotic dinoflagellates of two species of Actiniaria

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Key words: Symbiosis; host release factor; clotrimazole; Actiniaria; *Aiptasia* cf. *pulchella*; *Condylactis gigantea*; dinoflagellate.

Abstract: "Host release factors" (HRFs) are compounds found in invertebrates which cause symbiotic algae to release photosynthate to the host. Symbiotic algae release only a specific subset of the products of photosynthesis under the influence of HRFs; these include glycerol and amino acids. Clotrimazole, an antifungal agent, has effects analogous to those of the HRF of the coral *Plesiastrea versipora* (Lamarck, 1816) on the symbiotic algae of *P. versipora*. This paper describes its effects on symbionts of the actinians *Aiptasia* cf. *pulchella* Carlgren, 1943, and *Condylactis gigantea* (Weinland, 1860). The effects are not the same as in the coral: it had no effect on the symbionts of *A.* cf. *pulchella* whereas it appeared to damage the symbionts of *C. gigantea*, causing abnormally high rates of loss of the products of photosynthesis. These differing effects of clotrimazole on anemone symbionts reinforce the idea that cnidarian-dinoflagellate symbioses are physiologically highly diverse.

Introduction

Host release factors (HRFs) are as yet unidentified compounds found in many animals which have symbiotic dinoflagellates. They cause these dinoflagellates to release a large proportion of the products of their photosynthesis to the host's cells. In the presence of $^{14}CO_2$ and HRF only a subset of the compounds labelled during photosynthesis is available for release; glycerol is the main compound released, with smaller amounts of amino acids (particularly neutral amino acids), organic acids and glucose. Most studies of this phenomenon have used homogenized host tissue ("host homogenate"), since it is only recently that HRFs from various hosts have been partially purified (Gates et al., 1995; Montano, 1990; Grant & Hinde, unpublished data). In the case of the scleractinian coral *Pocillopora damicornis* (Linnaeus, 1758) a mixture of amino acids was shown to have HRF activity, and the possibility that mycosporine-like amino acids also have HRF activity was raised (Gates et al., 1995). However, the actual mechanism by which HRF induces release of glycerol is not yet known. We have shown that in a temperate population of the widespread Indo-West Pacific scleractinian *Plesiastrea versipora* (Lamarck, 1816) HRF does not significantly change the permeability of the algae to glycerol; instead HRF may stimulate release of glycerol from symbiotic dinoflagellates by causing an increase in its production or a decrease in either its rate of incorporation into other compounds or its catabolism (Ritchie et al., 1993). Crude host homogenates have been reported to increase, decrease or have no effect on the rate of photosynthesis of isolated symbiotic algae. This variability may be due mainly to the use of crude tissue homogenates, which

may vary in the concentration of HRF. However, species-specific differences, intercolonial variation and differences in experimental conditions may also contribute to variability. Host homogenate sometimes decreases the rate of photosynthesis by isolated symbionts from *P. versipora* (Hinde, 1988).

Naturally occurring mycosporines are difficult to purify and unstable (Hoogerheide & Wyka, 1982). We therefore tested the commercially available, stable mycosporine clotrimazole (1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole) and found that it stimulates the release of photosynthate by dinoflagellates isolated from *P. versipora* (Ritchie et al., submitted). Both the amount of organic carbon released and the mixture of compounds found in the released material are similar to those released in the presence of a crude host homogenate of *P. versipora.* Clotrimazole does not affect the initial gross rate of photosynthesis of the isolated symbionts of *P. versipora*, although over longer experiments (more than 30 minutes) inhibition is often observed (Ritchie et al., submitted). Thus clotrimazole appears to mimic the activity of host homogenate on the release of photosynthate and on photosynthesis. These results mean that it could be used as an internal control in experiments, for instance in the screening of host homogenate derivatives during attempts to purify and characterize the HRF of the coral.

The results reported here show that clotrimazole had different effects on symbionts from different hosts. It had no marked effect on either the rate of photosynthesis or the release of photosynthate by symbionts from the actinian sea anemone *Aiptasia* cf. *pulchella* Carlgren, 1943. It caused severe abnormalities in the symbiotic algae from another actinian, *Condylactis gigantea* (Weinland, 1860).

Materials and methods

Aiptasia cf. *pulchella* was collected from Kanehoe Bay, Oahu, Hawaii and *Condylactis gigantea* was obtained from the Long Key Marine Station, Florida. Both species of sea anemone were maintained in aquaria, in seawater (salinity 33‰, at 26°C) under fluorescent lamps (40 µmol quanta.m^{-2}.s^{-1}, 12h:12h). Both were fed three times per week, *A.* cf. *pulchella* on newly hatched brine shrimp nauplii and *C. gigantea* on larger brine shrimp nauplii.

Symbiotic algae were isolated from *A.* cf. *pulchella* by the method of Gates et al. (1995), with some minor modifications. Filtered artificial seawater (Tropic Marin™) (FASW) was used instead of natural seawater. For *A.* cf. *pulchella*, the pellet of algae was washed three times, by resuspending it in 2 ml FASW and recentrifuging. After the third wash the algae were resuspended in FASW so as to give a suspension containing about 1.5×10^6 cells.ml^{-1}. In some experiments a sample was taken at this stage for counting of the number of algae present before incubation. Assays were done in Eppendorf centrifuge tubes containing 200 µl of the algal suspension and 200 µl of the test solution. Two replicates were used for each treatment. Two samples (either 25 µl or 50 µl, depending on the experiment) were taken to provide data on the total amount of ^{14}C fixed by the algae. To measure the amount of organic ^{14}C released by the algae in each treatment, the rest of the incubation medium was centrifuged in a microfuge; the supernatant was transferred to a clean tube and recentrifuged, to remove any remaining algae. Duplicate samples were taken for scintillation counting. In some experiments a sample of algae was incubated in each treatment

medium, but without $NaH^{14}CO_3$, and the number of algae remaining in the suspension after the incubation period compared with those present at the start of the incubation period in FASW. It was more difficult to free the algae of *C. gigantea* from host material. These algae were prepared by homogenizing one or two excised tentacles in 2 ml FASW; the initial pellet of algae was resuspended in 6 to 10 ml FASW (depending on the amount of tissue used), and passed through plankton netting (20 µm pore size), washed twice by resuspension and centrifugation (bench centrifuge, approx. 1,000 rpm), homogenized again to break up clumps of algae and host material and washed twice more.

Algae were incubated in FASW, host homogenate (HH) or clotrimazole solution at a final nominal concentration of 50 µM. As clotrimazole is only slightly soluble in water, it was dissolved in ethanol, so that the final concentration of ethanol in the incubation medium was 0.2%. Since the solubility of clotrimazole in water is less than 29 µM (Hoogerheide & Wyka, 1982) the effective concentration for the algae can have been no more than this; the presence of excess clotrimazole (29 µM in solution and the equivalent of 21 µM in suspension at the start of the experiment) ensured that it remained effective throughout the incubation period, even though some of it may have been bound to, or metabolized by, the algae. Controls were incubated in FASW and/or FASW plus 0.2% ethanol. Initial tests showed that the addition of clotrimazole to FASW did not change its pH (8.36) or salinity (34‰).

Results

A number of trends of some potential interest were noticed in the data. These concerned the numbers of cells surviving to the end of the incubation and the effects of the various treatments on the rates of photosynthesis by the isolated algae.

Although cell counts were used in only three experiments with *A.* cf. *pulchella* and two with *C. gigantea*, it was observed that at the end of the incubation period there were fewer algae in the clotrimazole treatment than in the controls or HH treatments. The effect was small, except in Experiment 1 with *C. gigantea*, in which the number of algae remaining in the clotrimazole treatment was about 5% of the num-

Table 1. Results of cell counts (number of cells.ml^{-1} × 10^{-4}).

Experiment number	Initial cell count	Cell counts at end of incubation in:			
		FASW	FASW + 0.2% ethanol	*Aiptasia* host homogenate	Clotrimazole
A. cf. *pulchella*					
2	136	–	162	180	129
3	219	178	186	286	161
4	125	123	118	–	105
C. gigantea					
1	–	143	147	–	8
2	150	126	180	–	126

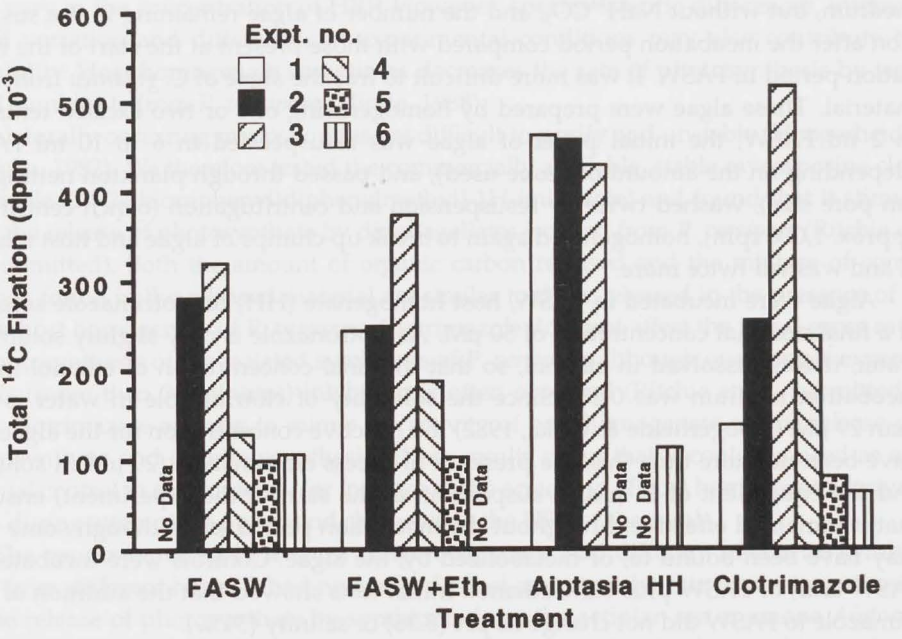

Fig. 1. Rate of photosynthesis by samples of algae isolated from *Aiptasia* cf. *pulchella* (fixed ^{14}C, disintegrations per minute x 10^{-3}) incubated in filtered artificial seawater (FASW), filtered artificial seawater + 0.2% ethanol, (FASW + eth.), host homogenate from *A.* cf. *pulchella* (*Aiptasia* HH) or clotrimazole solution; results of six separate experiments.

ber in the control treatments (FASW and FASW plus 0.2% ethanol) at the end of the incubation period. In contrast, in Experiment 2 with *C. gigantea*, there was little difference in cell numbers, with about the same number remaining in the clotrimazole treatment as in the controls.

In all experiments, except Experiment 5 with algae isolated from *A.* cf. *pulchella*, the rates of photosynthesis were increased by 40% to 80% when algae were incubated in host homogenate (figs 1-2). The addition of ethanol, even at 0.2%, decreased the photosynthetic rate of algae from *C. gigantea*; with algae from *A.* cf. *pulchella* the effects were more variable. Like the *Aiptasia* host homogenate, clotrimazole stimulated photosynthesis (compared with the appropriate controls, in which the algae were incubated in FASW containing 0.2% ethanol) by algae isolated from *A.* cf. *pulchella* (fig. 1). In the case of algae isolated from *C. gigantea*, the effects were more variable: in each experiment total photosynthesis, measured as disintegrations per minute (dpm) ^{14}C fixed per aliquot of algae, decreased by 40% to 90%, compared with the FASW + ethanol controls, when the algae were incubated in clotrimazole (fig. 2). When the rate of photosynthesis per cell was calculated, the clotrimazole treatments showed an increase in fixation rate in Experiment 1 (where cell number was very low) but a decrease in Experiment 2, in which the cell number remained high (data not shown in this form).

Algae isolated from *A.* cf. *pulchella* released about twice as much photosynthate

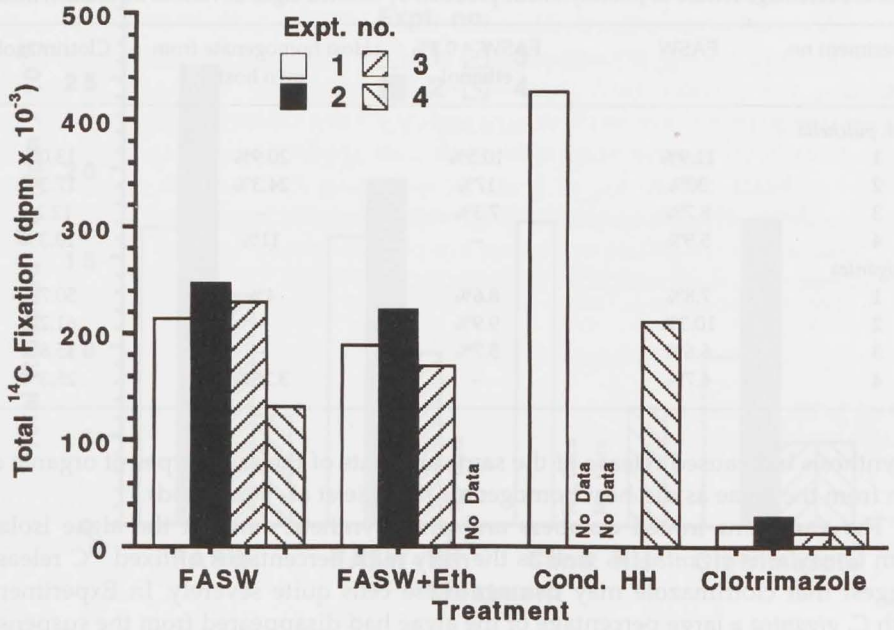

Fig. 2. Rate of photosynthesis by samples of algae isolated from *Condylactis gigantea* (fixed ^{14}C, disintegrations per minute x 10^{-3}) incubated in filtered artificial seawater (FASW), filtered artificial seawater + 0.2% ethanol, (FASW + eth.), host homogenate from *C. gigantea* (*Cond.* HH) or clotrimazole solution; results of four separate experiments.

in the presence of *Aiptasia* host homogenate as in seawater (fig. 3). *Condylactis* host homogenates had little effect on the release of photosynthate by algae isolated from *C. gigantea* (fig. 4).

Clotrimazole gave very different results with the algae from the two species of host. Algae isolated from *A.* cf. *pulchella* released less photosynthate when incubated in clotrimazole than in host homogenate, both in terms of the absolute amounts released (fig. 3) and of the percentage of the total fixed carbon released (table 2). However, for *C. gigantea*, the amounts of photosynthate released in clotrimazole were very similar to those in one or both controls (fig. 4), but the percentage of total photosynthate released in clotrimazole was much higher than in any other treatment (table 2).

Discussion

There was no evidence that clotrimazole had deleterious effects on symbiotic algae isolated from *Aiptasia* cf. *pulchella*. When incubated in clotrimazole or in host homogenate, they fixed more carbon by photosynthesis than did the controls, suggesting that they were not damaged, at least with respect to the uptake of carbonate ions or to photosynthesis. However, they released fixed ^{14}C only at rates similar to those seen in the controls (except in one case). In contrast, when incubated with algae from the coral *Plesiastrea versipora*, clotrimazole usually did not stimulate pho-

Table 2. Percentage release of photosynthetic products by isolated algae in various incubation media.

Experiment no.	FASW	FASW + 0.2% ethanol	Host homogenate from own host	Clotrimazole
A. cf. *pulchella*				
1	11.9%	10.5%	20.9%	13.0%
2	30%	17%	24.3%	17.3%
3	8.7%	7.3%	–	12.25
4	5.9%	–	11%	10.3%
C. gigantea				
1	7.8%	8.6%	4%	50.7%
2	10.3%	9.9%	–	61.2%
3	6.6%	5.7%	–	35.8%
4	4.7%	–	3.25%	25.3%

tosynthesis but caused release of the same amounts of the same types of organic carbon from the algae as did host homogenate (Ritchie et al., submitted).

The variations in cell numbers and photosynthetic rates in the algae isolated from *Condylactis gigantea*, as well as the very high percentages of fixed ^{14}C released, suggest that clotrimazole may damage these cells quite severely. In Experiment 1 with *C. gigantea* a large percentage of the algae had disappeared from the suspension (that is, they had presumably lysed) by the end of the incubation period. In this experiment, the total carbon fixation per algal cell was similar to that in the other

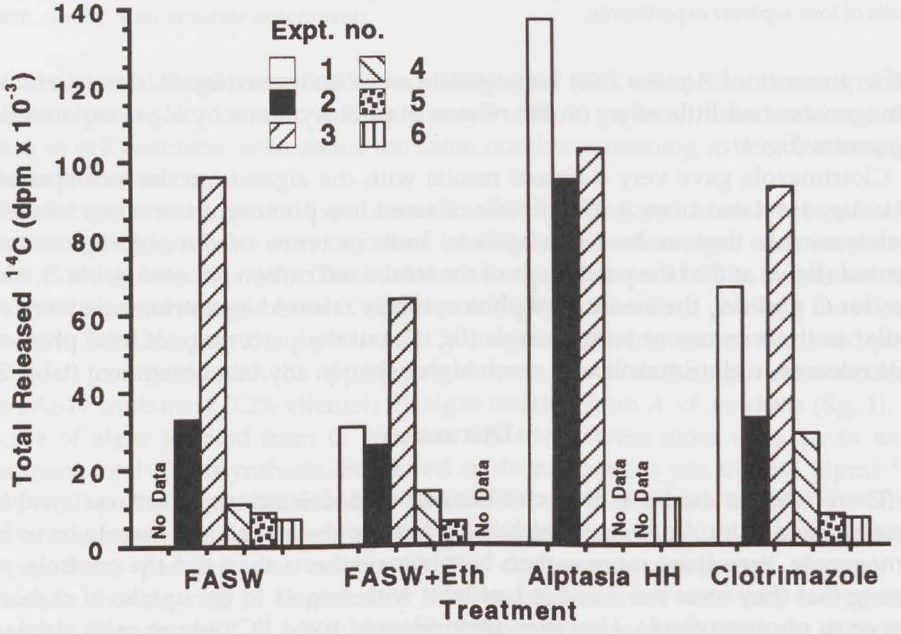

Fig. 3. Amounts of photosynthate released by algae isolated from *Aiptasia* cf. *pulchella* (released fixed ^{14}C, disintegrations per minute $\times 10^{-3}$) in the experiments shown in fig. 1.

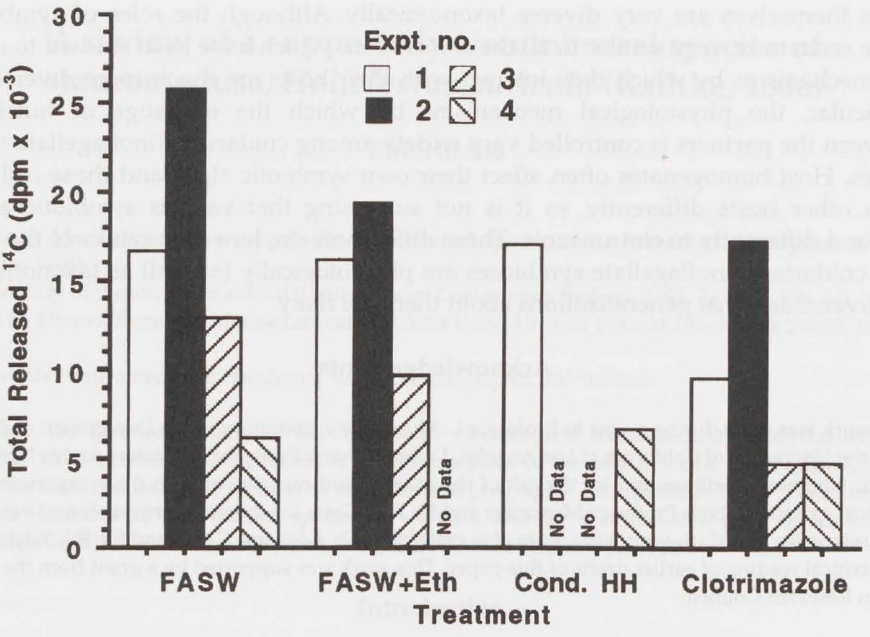

Fig. 4. Amounts of photosynthate released by algae isolated from *Aiptasia* cf. *pulchella* (released fixed ^{14}C, disintegrations per minute $\times 10^{-3}$) in the experiments shown in fig. 2.

treatments, but the absolute amount of ^{14}C fixed was much lower, indicating limited photosynthesis during exposure to clotrimazole (again, this was presumably because the cells were lysing during incubation). In the other experiment in which the algae were counted (Experiment 2), they were not lysed, but both the absolute rate of fixation and the rate per cell were very low (see fig. 2). These data suggest that the cells had been killed or photosynthesis had been strongly inhibited by clotrimazole, even though the cells had not, in this case, lysed. The results from those controls which contained both FASW and ethanol suggest that even 0.2% ethanol had deleterious effects on these algae. However, as algae from *C. gigantea* were difficult to separate from the tissue of the host, some of the observed effects may have been partly due to the method used to isolate them. Thus it is not possible to say whether the effects of clotrimazole on these algae were related to its HRF-like activity with *P. versipora* symbionts.

In summary, clotrimazole did not stimulate the release of photosynthate by the symbiotic algae of *A.* cf. *pulchella*, while with algae from *C. gigantea* it caused release of a large proportion of the photosynthate, but this was probably due to damage to the algal wall, rather than to the specific effects of clotrimazole. These results, which clearly differ from each other, are both different from the results obtained with the symbionts of *Plesiastrea versipora*, in which clotrimazole mimics the effects of the crude host homogenate very closely (Ritchie et al., submitted). These findings emphasize the need for care in generalizing from studies of single cnidarian-alga associations. Both the dinoflagellates found in symbiotic Cnidaria and the animal

hosts themselves are very diverse taxonomically. Although the roles of symbiotic algae seem to be very similar in all the associations which have been studied to date, the mechanisms by which they interact with their hosts are much more diverse. In particular, the physiological mechanisms by which the exchange of nutrients between the partners is controlled vary widely among cnidarian-dinoflagellate symbioses. Host homogenates often affect their own symbiotic algae and those isolated from other hosts differently, so it is not surprising that various symbiotic algae respond differently to clotrimazole. These differences do, however, reinforce the idea that cnidarian-dinoflagellate symbioses are physiologically (as well as taxonomically) diverse and that generalizations about them are risky.

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A review of a supposedly circumboreal species of stauromedusa, *Haliclystus auricula* (Rathke, 1806)

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Key words: Stauromedusae; *Haliclystus*, variation; geographic distribution.

Abstract: The taxonomic status of the supposedly circumboreal stauromedusa *Haliclystus auricula* (Rathke, 1806) is reviewed. Specimens from various parts of the North Pacific and North Atlantic could be classified into four types with characteristic geographic distributions. These types are considered referrable to four distinct species: *H. auricula*, and the resurrected species *H. octoradiatus* (Lamarck, 1816), *H. sanjuanensis* Hyman, 1940, and *H. tenuis* Kishinouye, 1910.

Introduction

With its supposedly circumboreal distribution, *Haliclystus auricula* (Rathke, 1806) is the most widespread species of the scyphozoan order Stauromedusae. Considering that neither its medusae nor its planulae swim (Otto, 1986: 321), it is rather surprising that the species should have such a wide range. Several authors (Uchida, 1927: 228; 1929: 122; Ling, 1937: 10; and Naumov, 1961: 79), considered *H. auricula* a morphologically variable species, and Kramp (1961: 292) regarded *H. octoradiatus* (Lamarck, 1816), *H. tenuis* Kishinouye, 1910, and *H. sanjuanensis* Hyman, 1940, as conspecific with it. A major reason for this was the wide variation in quantitative characters, such as the number of gonadal follicles. Such quantitative characters have often been used in the taxonomy of the group because Stauromedusae possess relatively few attributes that may be used to distinguish species.

The present study was conducted to determine to which extent these characters vary in so-called *H. auricula* and to assess whether it represents a single widespread species or a composite of several geographically more restricted ones.

Specimens were collected and studied at various locations in the Northern Hemisphere, and additional material was examined in collections of several museums.

Results

In the examined material, two important characteristics were recognized at the base of the tentacle clusters. Specimens possess either a shallow internal space with several intertentacular lobules, or a deeper U-shaped space lacking lobules. On the basis of this difference, combined with the presence and distribution pattern of white nematocyst spots, specimens were divisible into four types (fig. 1). Some specimens lacked the white nematocyst spots altogether. Among specimens with white spots, some possessed them only in perradial sectors, others in both perradial and interradial sectors.

Each of the four types showed a restricted geographic range (fig. 2). Type 1 was widely distributed in both the eastern and western North Atlantic. Type 2 occurred also in the North Atlantic, but only in northern Europe and Iceland. The other two types were found in the Pacific, but isolated from each other; Type 3 was distributed along the coasts of the eastern North Pacific and Type 4 in the western North Pacific.

As a rule, not more than one type was found at each location studied, but a few exceptions were noticed in the N Atlantic with regard to Types 1 and 2. In the sample from Reikjavik, Iceland, one specimen assigned to Type 2 was found among 55 specimens of Type 1, and at Grindavik, also Iceland, three specimens of Type 2 were collected among numerous specimens of Type 1. On the other hand, Type 2 specimens dominated in Denmark, accounting for 141 out of 144 specimens from Frederikshavn, and 10 out of 14 from Strandby near Frederikshavn.

Most of the quantitative characters examined showed proportionate increase with increasing size of the calyx (figs 3B, 4), although the number of gonadal follicles was not always significant (fig. 5). All the quantitative characters varied more or less between types. The calyx was much wider in Type 3 than in Type 4 (fig. 3A) and intermediate in Types 1 and 2. The gonads were also widest in Type 3 and became progressively narrower from Type 1, via Type 2 to Type 4 (fig. 3B). The number of tentacles was smallest in Type 4. Among the other types this number varied more between localities than between types (fig. 4). Type 4 differed also strikingly from the other types by its anchors being round to slightly wider than long (fig. 3C), whereas being longer than wide in the other three types.

The number of gonadal follicles differed greatly between types, from a few tens in Types 2 and 4, to over two hundred in Type 3 (in specimens of about 10 mm calyx length). Type 1 was intermediate between the other two groups (fig. 5).

Some characters, such as the ratio of width and length of the calyx, the shape of the anchors, and gonad size, showed little geographic variation (fig. 3). In contrast, the number of tentacles and gonadal follicles varied considerably between remote localities (figs 4-5). The widest variation in the number of follicles was seen in Type 1, with no overlap in the two localities; Type 2 showed a lesser but still obvious variation, and in Types 3 and 4 only little variation was noted between localities which were not very

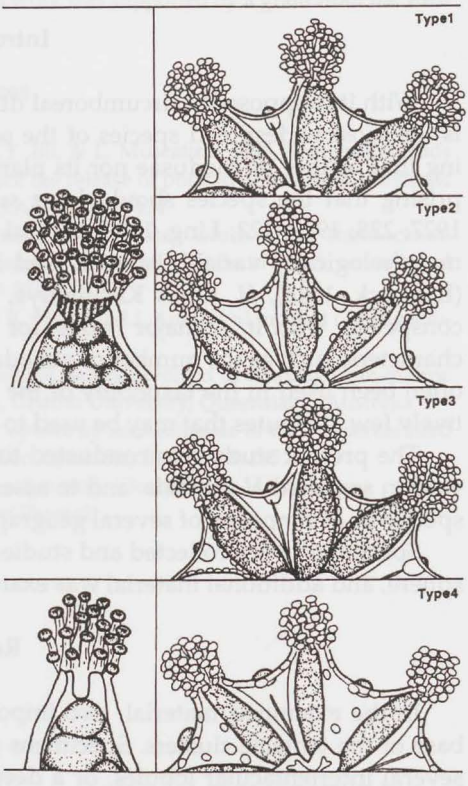

Fig. 1. Diagnostic characteristics of four types of *Haliclystus* recognized in this paper, based on the morphology of the base of the tentacle clusters and on the presence and distribution of white nematocyst spots.

remote from each other. The number of tentacles in Type 2 greatly differed between the two localities, the range of one population being the largest and the other the smallest among the six populations of the three types. The geographic variation of the number of tentacles was moderate in Type 1 (fig. 4).

Discussion

Although Clark (1878: 50) described the intertentacular lobules at the base of the tentacle cluster, this character has seldom been examined and used in taxonomic papers. Descriptions of the white nematocyst spots were given in several papers, including those by Clark (1863: 567), Kishinouye (1910: 4), Uchida (1929: 113) and Gwilliam (1956: 44), but none of these authors regarded them taxonomically important. Yet, the four morphological types recognized in the present paper are easily identified on these two characters, suggesting these to be positively of taxonomic importance. This is confirmed by the characteristic distributional ranges of the four types.

As each type has a distributional range isolated from the others, these types possibly represent subspecies. However, Types 1 and 2 occur sympatrically at the border between their distributional ranges, and I am therefore more inclined to regard these types as species rather than subspecies.

Judging from the descriptions and figures by Rathke (1806) and Clark (1878), *Haliclystus auricula* represents Type 1 of the present study, whereas Type 2 seems referrable to *H. octoradiatus*; Clark(1863: 567) described the white spots from *H. octoradiatus*, not from *H. auricula*. Also, the distribution ranges of Types 1 and 2 substantially agree with those of *H. auricula* and *H. octoradiatus*, respectively, as given by Clark (1863). As a consequence *H. octoradiatus* should in my view be revalidated.

Type 3 seems to be a distinct species as well, referrable to *H. sanjuanensis*.

It is noticeable that the distribution range of *Haliclystus stejnegeri* Kishinouye, 1899, a species agreeing in general morphology with type 3 (including the possession of intertentacular lobules; Hirano, 1986: 189) but differing in the arrangement of the white nematocyst spots , was found close to the local-

Fig. 2. Map showing geographic distribution of Type 1 (●), Type 2 (▲), Type 3 (■) and Type 4 (*). Locations from where the quantitative characters were examined are indicated: a. Lubec, Maine, USA; b. Reykjavik, Iceland; c. Frederikshavn, Denmark; d. Wembury, England; e. Victoria, Vancouver Island, British Columbia, Canada; f. Lopez Island, Washington, USA; g, h. Kitami-Esashi and Otaru, both Hokkaido, Japan.

ities where type 3 was found, so that the ranges of these two species probably overlap in some parts of the northeastern Pacific. At the other end of the range of *H. stejnegeri*, on the coast of Hokkaido in the Sea of Japan, another related, presumably undescribed species, was seasonally found alongside. So far that species has been collected only from the coast of Hokkaido in the Sea of Japan (Hirano, unpublished).

Type 4 is unique among the four types studied in lacking intertentaclar lobules. It is also remarkable in that the anchors are slightly wider than long, and in that the first white spots appear at the anchors (Hirano, 1986: 184), unlike in *H. octoradiatus* and *H. sanjuanensis*, where they appear at the top of the gonads. From these characteristics Type 4 is so clearly distinct from the others that it should not be included in *H. auricula* any longer. Instead, it should be assigned to *Haliclystus tenuis*, a species which is more like *H. borealis* Uchida, 1933, than to *H. auricula*. *Haliclystus borealis* is provided with a U-shaped interspace lacking the intertentacular lobules at the bases of tentacle clusters, with anchors having an almost round margin, and white spots in both perradius and interradius (Hirano, 1986: 186).

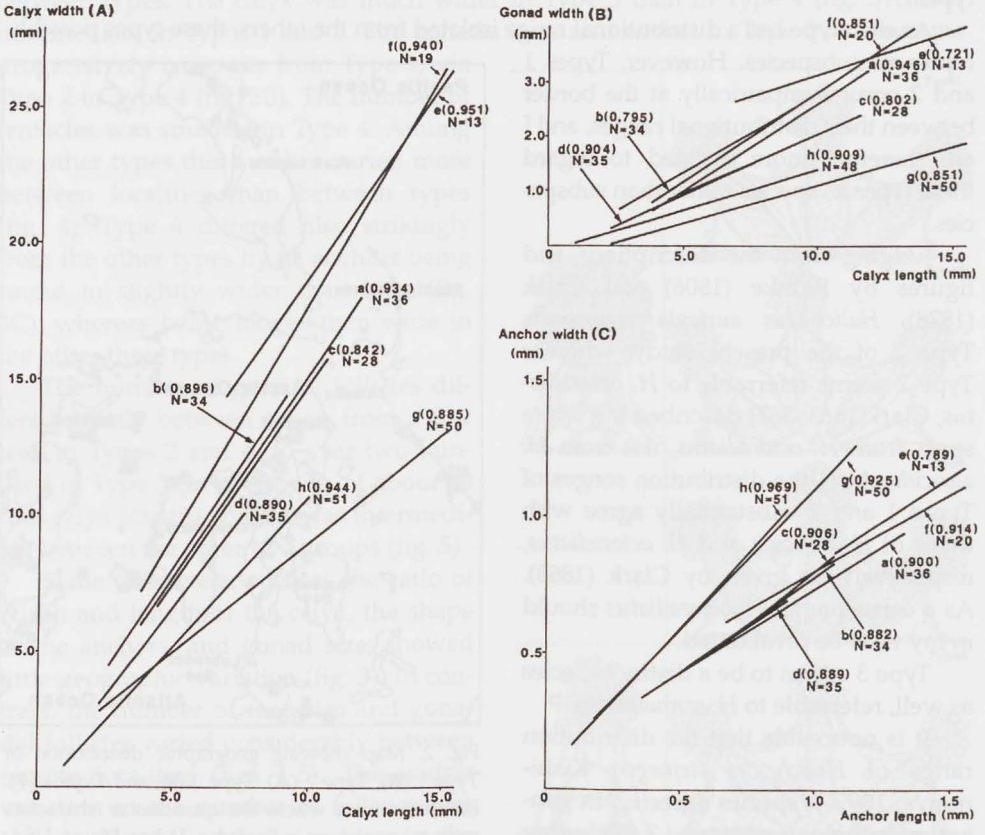

Fig. 3. Comparison of calyx width/calyx length (A), gonad width/calyx length (B) and anchor width/anchor length (C) in eight locations, a-h (a-b, Type 1; c-d, Type 2; e-f, Type 3; g-h, Type 4), shown in fig. 2. The linear regression is significant in all ($P<0.0001$ except for two, $P<0.01$ and $P<0.005$ in B-e and C-e, respectively). The regression coefficients (r) and the numbers of specimens examined (N) are shown.

There is another species of *Haliclystus* which shows resemblance to *H. tenuis*. That species has not been given a nomenclatorially valid name yet, as it was described in an unpublished PhD thesis (Gwilliam, 1956: 57; as *H. californiensis*). Thus, it seems that there are two species complexes, one formed by *Haliclystus tenuis*, *H. borealis* and "*H. californiensis*" and another including *H. auricula*, *H. octoradiatus*, *H. sanjuanensis* and *H. stejnegeri*, which share characteristics distinguishing them from the first three species.

On the basis of the present study a taxonomic revision of the genus *Haliclystus* appears warranted, including species not discussed here, also from the southern hemisphere.

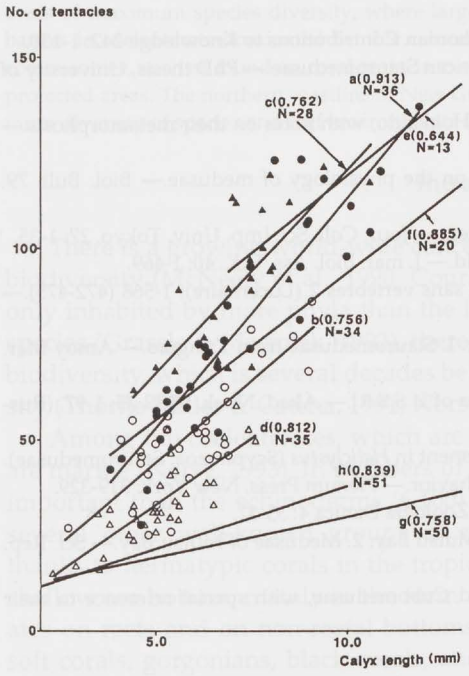

Fig. 4. Comparison of the number of tentacles per cluster in the locations a-h (see fig. 2). Types as in fig. 3. Linear regression is significant in all ($P<0.0001$ except for one $P<0.05$ in e). The regression coefficients (r) and the numbers of specimens examined (N) are shown. Data for specimens of Type 1 (a, b) and Type 2 (c, d) are also shown: a (●), b (○), c (▲) and d (△). To clearly show the difference between the two localities for Type 1 and Type 2, data for Type 3 and Type 4 are not shown in the figure.

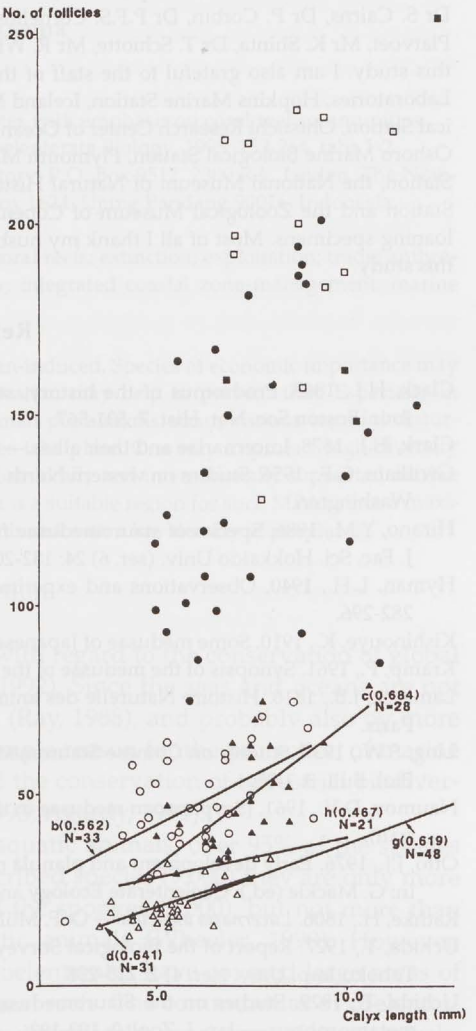

Fig. 5. The number of follicles per gonad in the locations a-h (see fig. 2) compared with calyx length. Types as in fig. 3. The linear regression is significant in b ($P<0.001$), c ($P<0.0001$), d ($P<0.0005$), g ($P<0.0001$), and h ($P<0.05$). The regression coefficients (r) and the numbers of specimens examined (N) are shown. Data for specimens of Type 1 (a, b), Type 2 (c, d) and Type 3 (e, f) are also shown: a (●), b (○), c (▲), d (△), e (■), and f (□). To clearly show the difference between the two localities for Type 2, data for Type 4 are not shown in the figure.

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Conservation problems in coelenterates with emphasis on coral reef communities

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Key words: Coelenterata; conservation; biodiversity; coral reefs; extinction; exploitation; trade; anthropogenic threats; global climate change; sustainability; integrated coastal zone management; marine protected areas; refuges; New Guinea.

Abstract: Most threats to coelenterate species are human-induced. Species of economic importance may become overexploited. Better management and cultivation may help to prevent that. Especially in areas of maximum species diversity, where large human populations occur, various harmful disturbances in coelenterate-dominated benthic communities take place. One way to protect high-diversity communities, such as found on coral reefs, is to establish locally and internationally supported marine protected areas. The northern coastline of New Guinea is a suitable region for such MPAs since a maximum diversity of species and reef environments is combined with a low density of people.

Introduction

There is a growing world-wide concern with regard to the conservation of global biodiversity (McNeely et al., 1990; Courier, 1992). Since the seas and oceans are not only inhabited by more phyla than the land (Ray, 1988), and probably also by more species (Grassle & Maciolek, 1992), there is ample reason for the protection of marine biodiversity, which is several decades behind the conservation of terrestrial biodiversity (Thorne-Miller & Catena, 1991; Norse, 1993; Agardy, 1994).

Among the coelenterates, which are all aquatic animals, over 95% of the species are marine (Norse, 1993). In numbers of described species (9.000) they are only more important than the echinoderms (6.100) and the sponges (5.000), but not more than several other well-known groups of aquatic animals (Wheeler, 1990). However, thanks to hermatypic corals in the tropics, coelenterates have covered large areas of shallow sea bottom, mainly in the form of coral reefs. The communities of coelenterates on reefs and on non-reefal bottoms, predominantly consisting of stony corals, soft corals, gorgonians, black corals, and sea anemones, constitute the richest shallow-water ecosystem of the world.

It is ironic that the coral reefs of SE Asia, which are the most diverse in species of coelenterates, such as corals, sea anemones, and sea pens (Fautin & Allen, 1992; Hoeksema, 1992, 1993; Williams, 1992), are also the most critically threatened (Hatcher et al., 1989; Wilkinson, 1992; Wells, 1993; Wilkinson et al., 1994). Due to their biodiversity and productivity these reefs support the presence of large human populations, which, however, do not exploit them in a sustainable way (White, 1987; Rice, 1991; Bleakley & Mouldoon, 1994; Wilkinson, 1994; Wilkinson & Buddemeier, 1994). In the present paper, threats to coelenterates are shortly reviewed, as well as strategies to conserve them and the communities in which they prevail.

Exploitation and protection of coelenterate species

Like other organisms, coelenterates have several biological enemies. They are subject to predation, parasitic infestations, diseases, and interspecific competition. Present-day species that have succeeded to cope with these biological threats and interactions are now in danger due to human activities. Species that are threatened with extinction are placed on lists to improve public awareness and to regulate their trade (tables 1-2). All these listed coelenterates are benthic. The criteria for their inclusion or exclusion are not always clear. It would be more effective to exclude species that are only locally threatened, and to focus on species that are threatened with global extinction.

For example, the starlet sea anemone *Nematostella vectensis* Stephenson, 1935, is considered vulnerable according to IUCN criteria (table 1). This species is becoming rare in England due to pollution and disappearance of its habitat (Williams, 1976,

Table 1. Status of coelenterates threatened with extinction according to criteria by the IUCN, the World Conservation Union (Wells et al., 1983; Groombridge, 1993).

Taxa	Status	Area
Class Anthozoa		
Order Alcyonacea		
Family Plexauridae		
Eunicella verrucosa (Pallas, 1766)	K	Mediterranean Sea, NE Atlantic
Family Corallidae		
Corallium rubrum (Linnaeus, 1758)	CT	Mediterranean Sea, NE Atlantic
C. elatius Ridley, 1882	CT	NW Pacific
C. japonicum Kishinouye, 1903	CT	NW Pacific
C. konojoi Kishinouye, 1903	CT	NW Pacific
C. secundum Dana, 1846	CT	NW Pacific, Hawaiian Islands
C. spec. nov.	CT	NW Pacific, Hawaiian Islands
Order Actiniaria		
Family Edwardsiidae		
Nematostella vectensis Stephenson, 1935	V	England, W and NE America
Edwardsia ivellii Manuel, 1975	Ex?	U.K.
Order Antipatharia		
Family Antipathidae (c. 150 spp.)	CT	Caribbean, Indo-Pacific

IUCN Threatened species categories:

Ex = Extinct (not encountered in the wild during last 50 years - CITES criterion)
E = Endangered (in danger of extinction, survival unlikely without change in causal factors)
V = Vulnerable (potentially endangered)
R = Rare (restricted geographical area or thinly scattered over a wider range)
I = Indeterminate (known to be E,V, or R)
K = Insufficiently known (suspected to belong to R, V, or E)
T = Threatened (E, V, R, I, or K; taxa comprised of sub-taxa of different status categories)
CT = Commercially threatened (not currently threatened with extinction but threatened as a sustainable commercial resource unless exploitation is regulated; used for marine species of commercial importance that are being overfished in several parts of their ranges)

1983; Manuel, 1988). In northern America, on the other hand, it is widespread along Atlantic and Pacific coasts. Moreover, it appears that it can easily be cultivated (Williams, 1975, 1983; Hand & Uhlinger, 1992).

Coelenterates that are used in the jewel, aquarium and souvenir trade are in serious danger because of their systematic exploitation. Precious red and pink corals (*Corallium* spp.) and black corals (Antipatharia), which are listed by the IUCN (table 1), are traditionally known as valuable merchandise for which special fishing gear has been developed in the Pacific and the Mediterranean (Kosuge, 1993; Cicogna & Cattaneo-Vietti, 1993; Grigg, 1988, 1994a). The awareness of overfishing has stimulated the development of fisheries management (Andaloro & Cicogna, 1993; Caddy, 1993; Grigg, 1994a) and the cultivation of some *Corallium* spp. (Cattaneo-Vietti & Bavestrello, 1993; Ueno et al., 1993). The absence of *Corallium* in CITES (table 2) is due to strong lobbying by the precious coral industry (Anonymous, 1994).

Many species of stony corals represented in table 2 are collected in SE Asia for the international aquarium and souvenir trade (Wells & Alcala, 1987; Wood & Wells, 1988; Wells & Wood, 1989, 1991; Coffey, 1991; Best, 1997). Among dead corals traded, there are many belonging to branching species of *Acropora*, *Pocillopora*, and *Seriatopora*, mushroom corals, such as *Fungia* spp., and the blue octocoral *Heliopora* (Coffey, 1991). The live corals selected for the aquarium trade are usually species with much soft tissue, preferably able to survive detached from a hard substratum, such as those

Table 2. Status of coelenterates potentially threatened with extinction according to criteria by the Convention on International Trade in Endangered Species of Wild Fauna and Flora, CITES (Schouten, 1992; World Conservation Monitoring Centre, 1993).

Taxa	CITES Appendix	Species number
Class Anthozoa		
Order Antipatharia	II	175
Family Antipathidae		
Order Scleractinia	II	1250
All (22) families		
Order Alcyonacea		
Family Tubiporidae	II	1
Tubipora musica Linnaeus, 1758		
Order Helioporacea (= Coenothecalia)	II	1
Family Helioporidae		
Heliopora coerulea (Pallas, 1766)		
Class Hydrozoa		
Order Milleporina	II	15
Family Milleporidae		
Order Stylasterina	II	250
Family Stylasteridae		

CITES Appendices referring to species (fossils excluded):

I = Threatened with extinction; therefore regulated international trade necessary

II = Not necessarily now threatened with extinction, but may become so without regulated international trade

III = Subject to regulation between certain parties

belonging to the scleractinian genera *Goniopora, Heliofungia, Polyphyllia, Trachyphyllia, Cynarina, Euphyllia, Catalaphyllia, Plerogyra, Physogyra,* and *Nemenzophyllia* (Coffey, 1991; Best, 1997; pers. obs.). Apart from these corals, reef-dwelling sea anemones are also collected for the aquarium trade. One reef at SW Sulawesi has been observed to become nearly depleted of large shallow-living sea anemones due to premiums given to local people for caught specimens (Den Hartog, pers. comm.). Export permits are still issued and therefore the regulation through CITES does not necessarily affect international trade (Sullivan et al., 1994; Best, 1997). Exploitation of live anthozoans should be more sustainable by more selective collecting regarding size and species, development of less lethal means of transportation, or by temporary moratoriums in international trade, except for public aquaria and other institutions that try to cultivate them (Yates & Carlson, 1992).

Damage to local coelenterate-dominated communities

Local coelenterate-dominated communities may become damaged by natural catastrophes, such as storms, floods, volcanic eruptions, earthquakes, tsunamis, and extreme low tides (Hughes, 1993; Huston, 1994; Harger, 1995). After replenishment by colonizing species from other areas, small-scale disturbances may lead to mosaics of species assemblages in different successional stages and enrich the species diversity on a larger scale (Karlson & Hurd, 1993). The recovery of coelenterate-dominated communities after such natural catastrophes should not be hampered by human intervention (Cortés et al., 1992; Harger, 1995). It is unclear whether man is not at least partly responsible for mass infestations of predators specialized in benthic coelenterates, such as the Crown-of-thorns starfish, which are harmful to coral reef communities in the Indo-Pacific (Wilkinson, 1990; Wilkinson & Macintyre, 1992).

There is a large variety in the ways human intervention can harm coelenterates. The following anthropogenic threats are usually chronic and wide-spread, and may therefore have serious, long-lasting effects if not managed carefully:

- Excessive sedimentation through human-induced land erosion, harbour dredging and metal mining (Salvat, 1987; Chansang, 1988; Porcher, 1993; Brown et al., 1994; Cortés, 1994; Hodgson, 1994a, 1994b).
- Pollution in the form of household litter, sewage, eutrophication, pesticides and industrial waste is a common problem on reefs near dense human populations (Willoughby, 1986; Marszalek, 1987; Wells & Hanna, 1992; Brodie, 1995). Thermal discharge and radioactive radiation are usually occurring on reefs more remote from human populations (Jokiel & Coles, 1974; Bablet & Perrault, 1987a; Neudecker, 1987; Fan, 1988). Oil spills seem to cause short-term damage to coral communities (Roberts et al., 1994; Vogt, 1995). However, due to lack of data from prior to the pollution, long-term damage may not always be recognized (Loya & Rinkevich, 1987; Guzmán et al., 1991). Chronic spilling by refineries and the use of oil dispersants are clearly harmful (Dodge & Knap, 1994; Eakin et al., 1994).
- Destructive fishing methods, which also damage or kill reef coelenterates, such as blast fishing, the use of `muro-ami' (driving of fish into large nets attached to the reef), use of fish traps, and sodium cyanide fishing, may be prohibited in certain

countries, but this does not prevent their use (Salm & Halim, 1984; Aliño et al., 1985; Alcala & Gomez, 1987; Eldredge, 1987; Gomez et al., 1987; Munro et al., 1987; Randall, 1987; Hingco & Rivera, 1991; Manuputty & Soekarno, 1994; Johannes, 1995). In addition, overexploitation of reef fishes may have an impact on the whole coral reef community, including coelenterates (Bohnsack, 1994).

- Besides during blast fishing, coral fragmentation happens when local people tread on reefs for the collecting of food or building material, or when the recreational activities of tourists result in trampling, anchoring, snorkeling, diving, and boat groundings (Tilmant, 1987; White, 1987a; Wong, 1991; Hawkins & Roberts, 1994; Leão et al., 1994; Auyong, 1995). Furthermore, there are accidental groundings by large vessels that cause local damage to coral reefs (Smith, 1985; Gittings et al., 1994). Nuclear detonations are harmful on a larger scale (Bablet & Perrault, 1987b).
- Construction activities and land reclamation for air fields and hotels does not only ask the sacrifice of reef area but also of coral boulders for building material (Muzik, 1985; Salvat, 1987; White, 1987a, 1987b; Hulm & Pernetta, 1993; Maniku, 1994; Brown et al., 1995). This has lead to the disappearance of some coral reef islands off Jakarta (Ongkosongo & Sukarno, 1986).

In order to maintain the diversity of coelenterate-dominated communities that are presently polluted or over-exploited, i.e., to guarantee them as sustainable marine resources, integrated coastal management plans have to be developed (Best et al., 1992; Wells & Price, 1992; Munro & Munro, 1994; Hotta & Dutton, 1995). This can partly be done by establishing Marine Protected Areas (MPAs), like the Great Barrier Reef and the Thousand Islands off Jakarta (Robinson et al., 1981, Ongkosongo & Sukarno, 1986; Kelleher & Kenchington, 1991; Soekarno, 1991; Djohani, 1996; Yates, 1994). MPAs consist of 'core' and 'buffer' areas, varying in intensity of protection and exploitation, in order to maintain critical ecological processes that are necessary to prevent the disappearance of species (White, 1988; Foster & Lemay, 1989; Agardy, 1994, 1995; Kelleher, 1994; Lassig & Woodley, 1994). Furthermore, community involvement should be developed through education programs and tools made for local villagers, tourists, and decision makers (Wells & Price, 1992). Public awareness can also be improved through parks, public aquaria, and museums (Hopper, 1992; Kelly, 1992; Neudecker, 1992; Yates & Carlson, 1992). Other management options may consist of installing mooring buoys to prevent damage by anchoring (Tilmant, 1987). Transplantation of corals and other coelenterates (preferably from reefs that will become lost) may enhance the recovery of damaged reefs, and may also help to populate artificial substrata (Harriot & Fisk, 1988; Yap et al., 1990; Newman & Chuan, 1994; Clark & Edwards, 1995).

Widespread damage to coelenterate-dominated communities

Mass mortalities of zooxanthellate coelenterates due to large scale events of elevated sea water temperatures, such as the El Niño-Southern Oscillation (ENSO), are becoming increasingly frequent and severe (Glynn, 1990, 1993). The 1982-83 ENSO, which caused widespread mortality among corals and other zooxanthellate coelenterates, was initially believed to have caused the extinction of the hydrocoral *Millepo-*

ra boschmai De Weerdt & Glynn, 1991, endemic to the Pacific coast of Panama (De Weerdt & Glynn, 1991; Glynn & De Weerdt, 1991). This species became very soon rediscovered, but another hydrocoral, *M. platyphylla* Hemprich and Ehrenberg, 1834, has still not been found back alive at the west coast of Panama, although it is still abundant at other Indo-Pacific localities (Glynn & Feingold, 1992; Glynn, 1993). Differences in vulnerability during increased temperatures have also been observed among closely related scleractinian species (Hoeksema, 1991).

There are also other threats related to a global climate change that may have harmful effects on local coral-dominated communities (Buddemeier, 1992; Wilkinson & Buddemeier, 1994). The predicted sea level rise may cause some reefs to become submerged. Changes in rainfall may affect the flux of nutrients and sediments on nearshore reefs. In some areas, a more frequent occurrence of severe storms can be expected. Higher levels of UV-B radiation may disrupt the recruitment of some reef-dwelling animals, which eventually may effect whole communities. Anyhow, the worldwide degradation of coral reefs asks for international action with regard to integrated coastal zone management (Grigg, 1994b; Crosby et al., 1995).

Conservation in refuges of high diversity?

So far, we have proof from the fossil record only that coelenterate species have become globally extinct. Local declines in coelenterate diversity are more obvious, especially in areas with low diversity, like at the Pacific coast of Panama, where species occur at the periphery of their distribution ranges (Glynn, 1993; Veron, 1995). The populations here are more vulnerable than those completely surrounded by neighbouring populations from which repopulation can take place.

One of the possibilities to protect as many species as possible, would be to declare remote reefs as international marine protected areas (Wilkinson & Buddemeier, 1994). Reefs around isolated oceanic islands are too small to host a maximum of species. Furthermore, the right currents and perhaps much time are necessary for larvae to repopulate reefs that need replenishment from there. The northern coastline of New Guinea, however, is not densely populated and it shows a very high diversity of species and reef habitats (Hoeksema, 1992, 1993; Williams, 1992). This may be one of the most suitable regions to establish marine protected areas with international support. If so, their is an urgent need for action because reef exploitation by allochtonous fishermen and erosion from logging has already begun, both in Irian Jaya, Indonesia, and in Papua New Guinea (UNEP/IUCN, 1988; Djohani, 1991; Maragos, 1991; Johannes, 1995).

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The anthozoan neuropeptide Antho-KAamide may be an inhibitory transmitter at neuromuscular junctions in the actinian *Calliactis parasitica* (Couch, 1842)

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Key words: Actinian; *Calliactis parasitica*; neuropeptide; neuromuscular junction; inhibition.

Abstract: The anthozoan neuropeptide, Antho-RWamide I (<Glu-Ser-Leu-Arg-Trp-NH_2) is suspected of being an excitatory transmitter acting on the membranes of sphincter muscle cells of Actiniaria. The neuropeptides Antho-RIamide (L-3-phenyllactyl-Phe-Lys-Ala-NH_2) and Antho-KAamide (L-3-phenyllactyl-Trp-Arg-Ile-NH_2) both inhibit sphincter muscle contractions but their sites of action are not known. Here we have investigated the inhibitory action of these peptides on isolated sphincter muscle cells by comparing their ability to prevent contractions elicited by Antho-RWamide I. Only Antho-KAamide was seen to inhibit the action of low concentrations of Antho-RWamide I. This is the first identified difference between these two inhibitory peptides. Antho-KAamide may be released from synapses near the sphincter muscle cells.

Introduction

To date, immunohistochemical investigations involving several neuropeptides extracted from sea anemones have shown them to be associated with smooth muscle (Grimmelikhuijzen & Graff, 1986: 9819; Graff & Grimmelikhuijzen, 1988a: 355; 1988b: 139; Grimmelikhuijzen et al., 1989: 270). Their physical location and abundance has led to the suggestion that they may be neurotransmitters or neuromodulators. The results of investigations into the effects of neuropeptides on sea anemone preparations are, however, difficult to interpret. In preparations of different muscle groups, e.g. tentacle longitudinals, parietals or circulars, there is a limit to the degree of isolation possible. There are always a large number of nervous connections remaining, each potentially containing different neuropeptides. This means that in muscle preparations there is always the possibility that extracellular application of a neuropeptide may cause the release of other transmitters contained in these remaining nervous connections and that any observed effect is thus not directly due to the initial application. Only in one case, that of the Antho-RWamides, has the action of a peptide at the cellular level been established (McFarlane et al., 1991: 429): both Antho-RWamide I (RW1) and Antho-RWamide II cause contraction of isolated sphincter muscle cells. Two other peptides, Antho-KAamide (KA) and Antho-RIamide (RI), inhibit spontaneous contractions of several muscle groups (McFarlane et al., 1993: 185). Although these two peptides are structurally distinct, we have so far been unable to detect any difference between them either in terms of physiological action or of site of action. The first such difference is reported here: only KA inhibits RW1 induced contraction of isolated sphincter muscle cells.

Material and methods

Synthetic RW1, KA and RI were produced by Bachem (Bubendorf, Switzerland). These peptides were made up in ultra-pure water (pH 8.0) and stored in 500 µl portions at -20°C. The stock concentrations were: RW1 2.3×10^{-3} mol l^{-1}, KA 3.19×10^{-3} and RI 3.17×10^{-3} mol l^{-1}. Specimens of *Calliactis parasitica* (Couch, 1842) were supplied by the Station Biologique Roscoff, France, and the Plymouth Marine Laboratory, UK. They were kept in a marine aquarium at 14-16°C.

Single muscle cells were isolated from the sphincter muscle of *Calliactis* using a mixture of papain and dithiothreitol (Sigma). This method is based on the work of McFarlane et al. (1991: 422). Changes were made as follows: small specimens of *Calliactis* (≈ 1.5 cm to 2.5 cm pedal disc diameter) were bisected horizontally to remove the upper one third of the column. This was trimmed to approximately 0.5 cm^3. The section was then cut into 1 mm slices at 90° to the orientation of the sphincter muscle fibres. These slices were digested for 1 hr at 20-22°C in a mixture consisting of 5mg of papain in 500 µl of ASW and 1.5 mg of dithiothreitol in 1 ml of artificial sea water (ASW). ASW (pH 8.0) consisted of (g/l) NaCl 23.08, KCl 0.74, $CaCl_2$ 1.47, $MgCl_2$ 10.16, Hepes buffer 2.603. This procedure loosened the muscle cells in their mesogloeal tubes from the connective tissue. After 1hr the slices were removed and placed in 3 ml of ASW in a plastic petri-dish. The cells were extracted from the sections by gently pressing the slices against the bottom of the dish.

The first experiment measured the effectiveness of RW1 in producing contraction of the isolated cells. 500 µl portions of the cell suspension were placed in separate wells of a 6-welled culture plate. Each well already contained 1ml of ASW and sufficient RW1 to produce concentrations of either 10^{-5}, 10^{-6}, 10^{-7} or 10^{-8} mol l^{-1}. There was also a control of ASW (N = 7 in each case). The base of each well was placed over a 1 cm^2 square. Random number generation was used to pick grid co-ordinates for one 1 cm grid for each well and the relaxed or contracted appearance of cells was recorded. The cells were generally found aggregated in small clumps. Contracted cells were small spherical balls of 3.9-5.5 µm in diameter, whereas relaxed cells were easily distinguished as structures 20-500 µm long (McFarlane et al., 1991: 429). The cells were examined using a Nikon inverted phase contrast microscope.

The second experiment investigated the ability of the neuropeptides KA (N = 3) or RI (N = 4) to block RW1 induced contractions. To the culture plate wells, which again contained 1ml of ASW, sufficient KA or RI was added to produce a final concentration of 10^{-5} mol l^{-1}; 500 µl of the cell suspension was then added, followed by RW1 to produce the desired concentration (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} mol l^{-1} or a control of ASW). Statistical analysis was performed using a one way ANOVA with Tukey-HSD analysis (Zar, 1984: 186) at a significance level of 0.05.

The third experiment considered the ability of KA and RI to block RW1 induced contractions of whole sphincter muscle preparations. The top third of the column of a *Calliactis* was cut off to produce a ring preparation that consisted primarily of sphincter muscle. The preparation was then left in sea water for 24 hrs to recover before being attached to an isotonic transducer which recorded the pattern of spontaneous contractions. In this type of preparation RW1 is known to cause increased activity (McFarlane et al., 1991: 426). RI or KA were introduced into the bath sur-

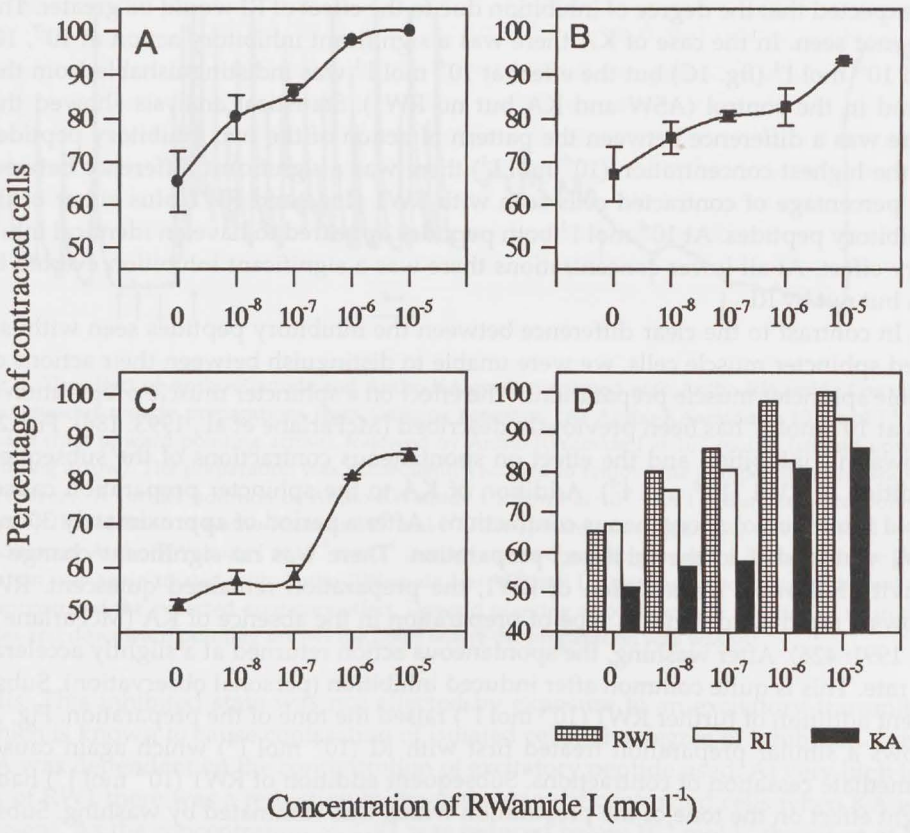

Fig. 1 (A) Partial dose response curve showing the effect of Antho-RWamide I on contraction of muscle cells isolated from the sphincter of *Calliactis parasitica*. (B) The same dose response curve after cells were exposed to 10^{-5} mol l^{-1} Antho-RIamide. (C) The same dose response curve after cells were exposed to 10^{-5} mol l^{-1} Antho-KAamide. Error bars in (A-C) show standard error. (D) Comparison of the excitatory action of Antho-RWamide I alone, and in the presence of Antho-RIamide and Antho-KAamide.

rounding the preparations to produce a concentration of 10^{-5} mol l^{-1}. RW1 (10^{-6} mol l^{-1}) was added after the inhibitory effect of KA or RI became apparent.

Results

KA, but not RI, blocked RW1 induced contractions at low concentrations. The effect of RW1 at various concentrations on the degree of contraction of isolated cells was found to be concentration dependent (fig. 1A). There was a significant difference between the percentage of contracted cells at concentrations above 10^{-8} mol l^{-1} (i.e.. at 10^{-5}, 10^{-6} and 10^{-7} mol l^{-1}) when compared to the control. There was no significant difference between the percentage of contracted cells seen after exposure to RW1 at 10^{-8} mol l^{-1} and that seen in ASW. The effect of 10^{-5} mol l^{-1} RI on the degree of contraction of isolated cells showed a significant inhibition only at the highest concentrations (10^{-5} and 10^{-6} mol l^{-1}) of RW1 (fig. 1B). At the lowest concentrations of RW1 it would

be expected that the degree of inhibition due to the effect of RI would be greater. This was not seen. In the case of KA there was a significant inhibitory action at 10^{-7}, 10^{-6} and 10^{-5} mol l^{-1} (fig. 1C) but the effect at 10^{-8} mol l^{-1} was indistinguishable from that found in the control (ASW and KA but no RW1). Statistical analysis showed that there was a difference between the pattern of action of the two inhibitory peptides. At the highest concentration (10^{-5} mol l^{-1}) there was a significant difference between the percentage of contracted cells seen with RW1 alone and RW1 plus either of the inhibitory peptides. At 10^{-6} mol l^{-1} both peptides appeared to have an identical inhibitory effect. At all lower concentrations there was a significant inhibition exerted by KA but not by RI.

In contrast to the clear difference between the inhibitory peptides seen with isolated sphincter muscle cells, we were unable to distinguish between their actions on whole sphincter muscle preparations. The effect on a sphincter muscle preparation of KA at 10^{-5} mol l^{-1} has been previously described (McFarlane et al., 1993: 186). Fig. 2A shows this inhibition and the effect on spontaneous contractions of the subsequent addition of RW1 (10^{-6} mol l^{-1}). Addition of KA to the sphincter preparation caused rapid inhibition of spontaneous contractions. After a period of approximately 30 min RW1 was added to the inhibited preparation. There was no significant change in activity following the addition of RW1, the preparation remained quiescent. RW1 shows a rapid action on this type of preparation in the absence of KA (McFarlane et al., 1991: 426). After washing, the spontaneous action returned at a slightly accelerated rate. This is quite common after induced inhibition (personal observation). Subsequent addition of further RW1 (10^{-6} mol l^{-1}) raised the tone of the preparation. Fig. 2B shows a similar preparation treated first with RI (10^{-5} mol l^{-1}) which again caused immediate cessation of contractions. Subsequent addition of RW1 (10^{-6} mol l^{-1}) had a slight effect on the tone of the preparation which was eliminated by washing. Subsequent addition of RW1 at 10^{-6} mol l^{-1} produced a marked contraction.

Discussion

Inhibition in sea anemones is important and it seems to occur in at least three general forms. The first is apparently governed by the through conducting nerve net (TCNN) as it can be induced anywhere by direct stimulation of the nerve net, and subsequent stimulation of the muscle itself does not cause contraction (Lawn, 1976: 308). The second is by-passed by direct stimulation of the muscle (Hoyle, 1960: 679) and so must involve inhibition of nervous connections to the muscle rather than inhibition of the muscle itself. There is also a requirement within sea anemone behaviour for a less centralised method of control (the third form of inhibition) which involves inhibition of individual cells or groups of cells at a local level (Pantin, 1935: 136).

The first form of inhibition is thought to be under direct or indirect control of the TCNN. For the sphincter muscle it was suggested that this was co-ordinated by a chemical transmitter released at the neuromuscular junction (Lawn, 1976: 308). This transmitter would be released from either direct or indirect synapses and would inhibit sphincter muscle cells. It appears from our results that KA is a candidate for this inhibitory transmitter as it inhibits contractions of isolated sphincter muscle

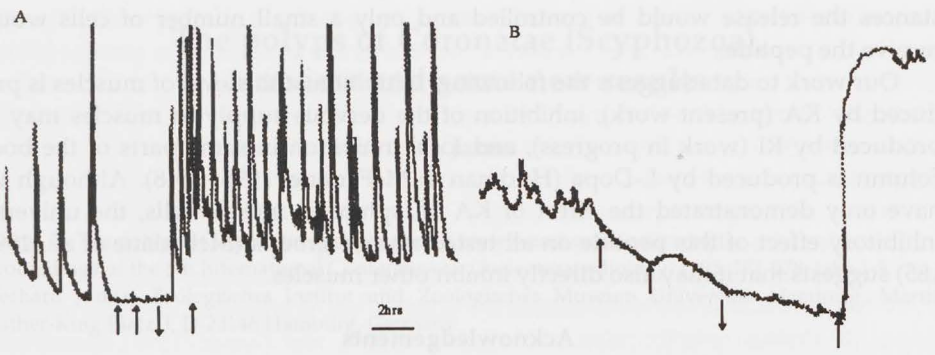

Fig. 2. The effect of Antho-RIamide and Antho-KAamide combined with Antho-RWamide I on circular sphincter muscle preparations from *Calliactis parasitica*. (A) Antho-KAamide at 10^{-5} mol l^{-1} was added (first upward pointing arrow), followed by Antho-RWamide I at 10^{-6} mol l^{-1} (second upward pointing arrow). The preparation was subsequently washed to remove the two peptides and showed a recovery. A further addition of Antho-RWamide I follows at 10^{-6} mol l^{-1} (third upward pointing arrow). (B) Addition of Antho-RIamide 10^{-5} mol l^{-1} (first upward pointing arrow) was followed by the addition of Antho-RWamide I at 10^{-6} mol l^{-1} (second upward pointing arrow). After a wash the preparation was again treated with Antho-RWamide 1 at 10^{-6} mol l^{-1} (third upward pointing arrow) and demonstrated the expected excitatory effect. Upward pointing arrows indicate addition of neuropeptides and downward pointing arrows the point where the preparation was washed.

cells. This inhibited state was not affected by exposure to an excitatory transmitter which is known to cause contraction of isolated cells. The degree of inhibition due to KA was dependent on the concentration of excitatory peptide used. At very high levels of RW1 there was a reduction in the percentage of contracted cells when KA was present. As the concentration of RW1 was reduced below 10^{-6} mol l^{-1} the effect of KA almost eliminated the excitatory effect of RW1. It appears that the activity of the neuromuscular transmitter RW1 is blocked by KA but not by RI. Our results show that at low RW1 concentrations RI does not cause inhibition of isolated cells. There was some inhibition at higher concentrations but we are unable to account for this. Further work using new intracellular techniques (Cho & McFarlane, 1995: 818) may clarify the effect of RI.

When KA or RI were added to whole sphincter muscle preparations they caused inhibition of spontaneous contractions. This state was not affected by the addition of a peptide (RW1) which has previously been shown to act on the muscle directly causing contraction. Thus in a relatively intact preparation, which will therefore still contain the majority of its nervous connections, either of the peptides can inhibit and this inhibition can not be overridden by a known excitatory transmitter. The implication is that RI acts at a different site to KA: it may inhibit neural connections or alternatively it may excite the release of KA.

The third type of inhibition is local i.e. restricted in its spread. This type of inhibition may be associated with L-Dopa containing neurones (Hudman & McFarlane, 1995: 1048). Of course we can not discount the possibility that KA and RI are also associated with local inhibition: in our experiments the inhibitory peptides were applied to every muscle cell in the preparation, whereas in more natural circum-

stances the release would be controlled and only a small number of cells would receive the peptide.

Our work to date suggests the following: inhibition at the level of muscles is produced by KA (present work), inhibition of the nervous supply to muscles may be produced by RI (work in progress), and local inhibition in some parts of the body column is produced by L-Dopa (Hudman & McFarlane, 1995: 1048). Although we have only demonstrated the effect of KA on sphincter muscle cells, the universal inhibitory effect of this peptide on all tested muscle groups (McFarlane et al., 1993: 185) suggests that it may also directly inhibit other muscles.

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The polyps of Coronatae (Scyphozoa), a review and some new results

G. Jarms

Jarms, G. The polyps of Coronatae (Scyphozoa), a review and some new results.
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Key words: Coronatae; review; life cycle; planuloids.

Abstract: A history of the knowledge about polyps of the scyphozoan order Coronatae is outlined, and much of the relevant literature is listed. The polyp stage of *Nausithoe globifera* Broch, 1914, is described for the first time. A new mode of shortened life cycle, by planuloids, is described from observations on eight species referable to the genera *Atorella*, *Linuche*, and *Nausithoe*.

History

The first medusa now classified as a coronate was mentioned by Swartz (1788) as *Medusa unguiculata*, currently known as *Linuche unguiculata* (cf. Eschscholtz, 1829), the thimble jellyfish. The order Coronatae was introduced by Vanhöffen (1892). A detailed history of knowledge about the medusa stage of the coronates was given by Thiel (1936). In this paper I address the history of knowledge about coronate polyps.

The discovery of the polyp stage of coronates actually was made by d´ Orbigny (1839), who described *Tubularia rugosa* from the Patagonian shelf. That species has since been identified as a coronate polyp, although its systematic position is unclear because of inadequate description. Later, Eimer (1872) described a scyphistoma that he considered parasitic. Kowalewsky (1873) and Carter (1873) confirmed that account, both describing cnidarians with a chitinous tube living in sponges. From Antibes, on the southern French coast, Allman (1875) reported a hydrozoan-like cnidarian living in the oscula of some species of Demospongia. Since he found a ring canal and four symetrically arranged radial canals, he stated that it was more medusoid than polypoid, though he did not find a velum, ocelli or even gonads. Thiel (1936) explained this by suggesting that Allman might have seen polyps undergoing strobilation. Allman named this species *Stephanoscyphus mirabilis*, and established the now abandoned order Thecomedusae for it within the class Hydrozoa. Similar coelenterate colonies discovered by Schulze (1877) in sponges such as *Reniera* and *Suberites* were named *Spongicola fistularis* and classified among the class Hydrozoa. However, Schulze mentioned its resemblance to the scyphistoma of the Scyphozoa and stated that elucidating its complete life cycle would be necessary to be certain of its systematic position.

The first to suggest a link between a coronate medusa and the polypoid genus *Spongicola* (= *Stephanoscyphus)* was Metschnikoff (1886). He described the embryology of several hydro- and scyphomedusae, including *Nausithoe marginata* Kölliker, 1853. Some of his planulae of this species were observed to have settled and lost their

cilia, before transforming into a small plate of tissue. After a thin layer of periderm was shed, a chimney-like protuberance appeared in the middle of the plate and four small tentacles eventually appeared. Metschnikoff concluded that the pattern of development resembled that of *Stephanoscyphus mirabilis* and *Spongicola fistularis*. The relation between *Nausithoe* and *Stephanoscyphus* was confirmed by Lo Bianco & Mayer (1890), who observed strobilating polyps of *N. punctata*.

Additional species of coronate polyps were described between 1890 and 1937. Both a colonial species (*Stephanoscyphus allmani*) and a solitary species (*S. simplex*) were studied by Kirkpatrick (1890). Lo Bianco (1903) characterized a solitary species of *Spongicola* which Thiel (1936) named *Stephanoscyphus bianconis*. Another solitary species (*S. striatus*) was founded by Vanhöffen (1910), and Komai (1935) described a third colonial species (named *S. racemosus* by Komai 1936), which he had reared for a long period. Komai (1936), also described another solitary species (*S. corniformis*).

Table 1. Known polyps of Coronatae, after Leloup (1937).

species	author	year	colonial	solitary	internal cusps
Stephanoscyphus mirabilis	Allman	1874	×		1 whorl
= *Spongicola fistularis*	Schulze	1877	×		
= *Nausithoe punctata*	von Kölliker	1853	×		
Stephanoscyphus allmani	Kirkpatrick	1890	×		single
Stephanoscyphus simplex	Kirkpatrick	1890		×	several whorls
Spongicola spec.	Lo Bianco	1903		×	none
= *Stephanoscyphus bianconis*	Thiel	1936		×	
Stephanoscyphus striatus	Vanhöffen	1910		×	none
Stephanoscyphus racemosus	Komai	1936	×		none
Stephanoscyphus corniformis	Komai	1936		×	several whorls
Stephanoscyphus komaii	Leloup	1937	×		1 cupshaped
Stephanoscyphus sibogae	Leloup	1937		×	several whorls

Only Komai (1935) was able to keep specimens (of *S. racemosus*) alive for a long period, and detailed histological work was carried out on them. From then until the late 1960s research on coronate polyps was restricted to preserved material, and important papers were published by Kramp (1951, 1959, 1962) and Naumov (1959).

In the 1960s Bernhard Werner, the scientist most closely associated with our knowledge on coronates, began cultivating many species. He described their relation to the fossil Conulata (Werner, 1966, 1967b), and described life cycles of *Atorella vanhoeffeni* (Werner, 1966), *S. racemosus* (Werner, 1970b), *Linuche unguiculata* and its conspecific *S. komaii* (Werner, 1979) (later described more in detail by Ortiz Corp´s et al., 1987). He also described two new species, *S. planulophorus* (Werner, 1971a) and *S. eumedusoides* (Werner, 1974). Additionally, he significantly advanced knowledge about coronate morphology and the process of strobilation (e.g. Werner, 1967a), about the reduction of metagenesis (Werner, 1970a, 1971a, 1974, 1983, Werner & Hentschel, 1983), the process of regeneration (Werner, 1970b) and the systematics and evolution of the Coronatae and Scyphozoa in general (Werner, 1970a, 1971b, 1973, 1974, 1980).

Meanwhile Kawaguti and Matsuno (1981) founded a new species (*Atorella japoni-*

ca) and later (Matsuno & Kawaguti, 1991) described strobilation of this species in detail.

Jarms (1990) carried on with descriptions of the new species *Nausithoe werneri, N. maculata* and *N. thieli.* He demonstrated that *N. marginata* was valid, contrary to the opinion of Haeckel (1880). The whole life cycle of *N. marginata* is now known (Jarms, 1990), and it is different from that of *N. punctata.* Utilizing taxonomic characters from the tubes of coronate polyps, Jarms (1991) proposed the name stephanoscyphistoma for all polyps of Coronatae whose familial or generic assignment is uncertain.

The polyp of *Nausithoe globifera* Broch, 1914

Living animals collected during a cruise of R/V Meteor in the Atlantic at 25°24'2"N, 16°14'3"W, on 4 February 1982, from 800 m depth, were received from Hj. Thiel . On a single piece of slag were several specimens of stephanoscyphistomae. A specimen of *Nausithoe*, later described as *N. werneri* Jarms, 1990, underwent strobilation. A polyp of *Atorella* next strobilated, its newly liberated ephyrae having six small tentacles (thus differing from *A. vanhoeffeni* Bigelow, 1909). Up to now I have not succeeded in raising it to maturity, although it is probably an undescribed species. A key to coronate polyp identification (Jarms, 1988, 1991) seemed generally reliable for identification of the polyps on this piece of slag, although there were two which did not coincide with any taxa in the key. These two were isolated and cultivated at 8-10°C. Both polyps had very dark periderm. The smaller one was lost but the larger one was still alive when this paper was submitted for publication.

When collected, the measurements and the quotients of diameter and length (Form-quotient, F-q; cf Werner, 1983: 125, 134) of its peridermal tube where as follows: diameter of basal disc 0.44 mm, just above the base 0.12 mm, at 2 mm height 0.24 mm (F-q = 0.12), at 5 mm height 0.64 (F-q = 0.128) and at 6.86 mm (i.e terminally) 0.92 mm (F-q = 0.134). The total number of internal cusp whorls was four, three within the basal 2 mm, and the uppermost one with eight teeth.

The soft body was like that of the stephanoscyphistomae, with a maximum of about 40 tentacles. During strobilation, six to 24 ephyrae were produced, having a diameter of 2.4 mm, eight tentacles, and four gastric filaments already developed. After four weeks, the first suggestion of purple red colour appeared on the manubrium and the gastric filaments, and the central disc began to arch. At this time the diameter of the young medusa was 3 mm. At a diameter of 6 mm, after about two months of culture, the eight gonads appeared as little spots on the interradii. The manubrium and the entire stomach was deep purple-red, but the tentacles and the developing gonads were still unpigmented. The transparent central disc was domed. Two gastric filaments were present in each quadrant. Further growth has not yet been documented. This medusa is referrable to *Nausithoe globifera* Broch, 1914, described again by Russell (1956, 1970), as a valid species distinct from *N. atlantica.* Thus, the species has a metagenetic life cycle. The polyp differs from that of other species of Nausithoidae in its shape, as the Form-quotient does not decrease with length but increases from 0.12 at 2 mm to 0.134 at 6.86 mm (the maximal length recorded). According to Kramp (1959: 176, fig. 5) a dark colour is mainly found in the longest tubes of the species, but this seems not always so. It may occur for all sizes in

both *Nausithoe globifera* and *N. eumedusoides* (Werner, 1974). Kramp may well have had more than one species in his material, because we now know that several species were known by the binomena *Stephanoscyphus simplex* and *S. corniformis* (Werner, 1973). Today, about 40 species of coronate medusae, in 11 genera and six families, are recognized. The life cycles are known of only nine species. We recognize an additional seven polyps with unknown life cycles, and four more species lacking free medusae. Much life cycle work on the group remains to be done.

Table 2. Known polyps of species of Coronatae. * = this paper.

species	author: polyp	year	author: medusa	year
Atorella vanhoeffeni	Werner	1966	Bigelow	1909
Atorella japonica	Kawaguti et al.	1981	Kawaguti et al.	1981
Linuche unguiculata syn: *Stephanoscyphus komaii*	Werner	1979	Swartz	1788
Nausithoe racemosa syn: *Stephanoscyphus racemosus*	Komai	1936	no medusa	
Nausithoe punctata syn: *Stephanoscyphus mirabilis*	Allman	1874	Kölliker	1853
Nausithoe punctata syn: *Spongicola fistularis*	Schulze	1877		
Nausithoe marginata	Jarms	1990	Kölliker	1853
Nausithoe planulophorus	Werner	1971	no medusa	
Nausithoe eumedusoides	Werner	1974	no medusa	
Nausithoe werneri	Jarms	1990	Jarms	1990
Nausithoe thieli	Jarms	1990	Jarms	1990
Nausithoe maculata	Jarms	1990	Jarms	1990
Nausithoe globifera	Jarms	*	Broch	1914
Stephanoscyphus allmani	Kirkpatrick	1890	unknown	
Stephanoscyphus simplex	Kirkpatrick	1890	collective group	
Spongicola spec.	Lo Bianco	1903	unknown	
Spongicola spec. syn: *Stephanoscyphus bianconis*	Thiel	1936		
Stephanoscyphus striatus	Vanhoeffen	1910	unknown	
Stephanoscyphus corniformis	Komai	1936	collective group	
Stephanoscyphus spec.1	Naumov	1959	unknown	
Stephanoscyphus spec. 2	Naumov	1959	unknown	
Stephanoscyphus spec. 3	Naumov	1959	unknown	
Thecoscyphus zibrowii	Werner	1984	no medusa	

Shortened life cycle

Given unfavourable conditions of salinity, temperature, or food supply, the ephyrae of eight species of the genera *Atorella*, *Linuche* and *Nausithoe* undergo an unusual transformation. They first lose motility, the lappets are then bent downwards, and finally only a ball of tissue - a planuloid - is left, without any differentiation except the crystals of the statoliths (visible for a time before they finally disappear). These planuloids have a varied fate in the several species. The bigger the planuloid, the more easily it grows into a polyp. In *Nausithoe globifera* about two weeks elapse in becoming a planuloid and about two months pass before it metamorphoses into a polyp. In *Nausithoe maculata*, only some degenerated ephyrae have been observed to produce a new polyp. It is necessary, in *Nausithoe marginata*, to fuse at least two planuloids to produce a new polyp. If more degenerated medusae are placed close

together, a basal part with two or more polyps, or sometimes a single polyp with eight instead of four septae develop, depending on the planuloids involved. Such polyps have been observed to produce ephyrae with 16 rhopalia and 32 lappets. All polyps formed this way lack the normal basal disc, and possess a globular basal part instead. This has been observed also in nature. A small percentage of collected animals having this type of basal part. An exception is the species *Linuche unguiculata*, the colonial polypoid stage of which develops a basal plate and then a scyphorhiza from which the polyps arise.

Table 3. Species of Coronatae in which young polyps may be formed from degenerated free ephyrae. (ø) = diameter, (–) = no data.

species	ø ephyra	ø planuloid	polyp formation	remarks
Atorella vanhoeffeni	0.8 mm	0.12 mm	never	die within 10 days
Atorella spec.	2.5-3.2	0.28-0.36	–	–
Linuche unguiculata	1.8-2.0	0.24-0.32	some	stolons or plate
Nausithoe marginata	1.0-1.2	0.18-0.22	many	if fusion of 2
Nausithoe maculata	1.1-1.3	0.20-0.24	some	–
Nausithoe thieli	2.0	0.28-0.32	many	–
Nausithoe werneri	2.0-2.2	0.36-0.42	–	–
Nausithoe globifera	2.4	0.24-0.28	some	–
Nausithoe planulophorus	–	0.25-0.30	many	inside tube

These results, though so far incomplete, indicate that a certain quantity of tissue and/or nutrient stored in the planuloid is essential in the formation of a polyp. For strobilation, a sufficient amount of nutrient reserve is necessary, as otherwise the basal residue is unable to regenerate a polyp capable of reaching the terminal end of the tube where it can catch prey and recover (Werner 1979: 94). A paper dealing with laboratory experiments on digestion and distribution of food in coronate polyps is in preparation (Jarms et al.).

In cnidarians, both sexual and asexual reproduction are widespread. There is a tendency in some families of the Hydrozoa for the medusa stage to become reduced. Thus, gonophores may be sessile or even reduced to gonads, as in *Hydra*. Curiously, we have never seen any sexual reproduction during the long term cultivation of *Microhydrula limopsicola* (Jarms & Tiemann, 1996), and we consider it possible that they lack any sexual processes. The capacity to withstand adverse or special environmental conditions, as in interstitial, deep-sea or cave environments by reduction of the metagenetic life cycle, may have some advantages. But reduction of the medusa or the planula restricts the dispersal ability of a species. This is compensated for by the production of other motile stages, such as the creeping frustules of *Microhydrula* (Jarms & Tiemann, 1996). The ability of nearly all Nausithoidae with complete metagenesis to produce planuloids is a prerequisite not to lose too much substance and energy under unfavourable environmental conditions by strobilation into medusae. It enables these species to survive and propagate, at least asexually, even in habitats with low food density or no light, as in caves and the deep-sea. If the medusa stage is suppressed totally, dispersal can be ensured to some extent by motile planuloids, as in *Nausithoe planulophorus* (Werner, 1971a). The short cut of development via planu-

loids avoids the vulnerable medusa stage but prevents any recombination of genes secured by sexual reproduction, and it perhaps leads to evolutionary dead ends. Somatic mutations do occur however, and evolution in apogamous species may go on. Nevertheless propagation by asexual means may be advantageous in some environments. The cave dwelling species *Nausithoe planulophorus* with its apogamous life cycle, has evolved the transformation of ephyrae into planuloids inside the peridermal tube as the regular and only way of propagation and seems to represent the preliminary end-point of this evolutionary line.

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The cnidomes of *Cassiopea andromeda* Forskål, 1775, and *Cassiopea xamachana* Bigelow, 1882 (Cnidaria: Scyphozoa)

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Jensch, F. & D.K. Hofmann. The cnidomes of *Cassiopea andromeda* Forskål, 1775, and *Cassiopea xamachana* Bigelow, 1882 (Cnidaria: Scyphozoa).
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Key words: Cnidaria; *Cassiopea;* nematocysts; development; life cycle.

Abstract: The cnidome of *Cassiopea andromeda* Forskål, 1775, and *C. xamachana* Bigelow, 1882, was investigated in the principal stages of the metagenetic life cycle. Observations by light microscopy of live material and stained semithin sections, as well as scanning electron microscopy of discharged cnidae, was undertaken. Two types of nematocysts were encountered in all examined stages of the life cycle: holotrichous *a*-isorhizas and heterotrichous microbasic euryteles. These were therefore considered as the stable categories. In addition, a third type was recorded for the first time in species of the family Cassiopeidae: the heterotrichous anisorhiza. This type was found to be restricted largely to the medusa stage, but was observed already in the ephyra and even the strobila. It was considered as an unstable category. The first appearance of heterotrichous anisorhizas was observed to be concomitant with the onset of monodiscous strobilation of the scyphopolyp. The nematocyte types were not evenly distributed throughout the tissues, but specific patterns of distribution were found in the epithelia, varying with the stage of development and the different body portions. The distribution pattern of nematoblasts is not identical with that of functional nematocysts which suggests that discharged cnidae are replaced by cells recruited from neighboring areas. The cnidomes of the two *Cassiopea* species were found to be congruent: no significant differences of taxonomic value, useful in distinguishing the two species, were detected.

Introduction

Scyphozoans in general are reported to have a rather uniform complement of nematocysts, and possess three different categories: isorhizas, anisorhizas and euryteles. A majority of species in the order Rhizostomeae in which the nematocyst complement is known, shows only two types of stinging cells (Calder, 1983: table 2, 1190; Werner, 1984: table 2, 32). This appears to apply also to members of the family Cassiopeidae, which do not belong to the dangerous stingers among cnidarians, but no thorough account of the cnidome of *Cassiopea* species is hitherto available.

Taking advantage of developmental studies during which we were able to investigate all stages of the metagenic cycle (for review, see Hofmann et al., 1996), a survey of the nematocyst complement of two rhizostome species was performed: *Cassiopea andromeda* Forskål, 1775, from the Red Sea (an Indo-Pacific species) and *Cassiopea xamachana* Bigelow, 1882, from Florida (a Carribean species). The study aimed at establishing the cnidome of the two *Cassiopea* species at all stages of development, at monitoring the distribution of nematocysts in the epithelia of different body parts, and at identifying the topographic and cellular origin of nematocytes. Furthermore, the study sought to determine whether nematocyst micromorphology would pro-

vide any characters suitable for discrimination of the two species, which have so far proved morphologically indistinguishable.

Material and methods

Adult medusae of *Cassiopea andromeda* were collected from the Red Sea near Eilat (Israel) and those of *C. xamachana* from Florida (USA) in a mangrove area at Grassy Key. Planula larvae were reared from egg masses isolated from brooding female medusae of both species. Mass cultures of polyps were obtained following induction of settlement and metamorphosis of larvae incubated for 48h in a solution of the hexapeptide Z-GPGGPA (10 to 20 µg/ml) (Hofmann & Brand 1987: 109). Cultures were maintained at 23 ± 1°C in aerated glass or plastic aquaria in pasteurized natural seawater from the North Sea. They were fed *Artemia salina* nauplii to repletion at least twice a week and were cleaned about 4h after feeding. Asexual larva-like swimming buds and ephyrae were collected from the cultures before administering food.

The nematocyst complement was studied in squash preparations of unfixed tissues using phase contrast microscopy, as well as in semithin sections of resin-embedded, glutaraldehyde-fixed material stained according to routine methods. Preparations for scanning electron microscopy (SEM) were made from *Artemia salina* nauplii and from pieces of human stratum corneum used as a target for nematocysts when exposed to *Cassiopea* specimens.

The material was fixed in 2,5% glutaraldehyde in 0.05 M collidine buffer at pH 7.5, postfixed in 1% osmium tetroxide in destilled water, dehydrated, and critical point dried. Preparations were viewed and photographed with a Zeiss DSM 950 SEM. Light microscopy was performed using a Leitz Ortholux or a Zeiss Axiophot microscope, equipped with phase contrast and differential interference contrast optics.

Results and discussion

Nematocyst types and their distributions

Not two, as stated by Mariscal and Bigger (1976: 563) but three different types of nematocysts were detected in squash preparations, sections, and SEM micrographs of jellyfish tissues from ephyrae and medusae of both species (terminology of Calder, 1974: 171):

1. Heterotrichous microbasic euryteles.— Capsules (size range: 7.7 × 6.6-9.9 × 7.7 µm) spindle-shaped with operculum; shaft with spines of featherlike appearance; tube provided with helically arranged spines, its diameter (in everted condition) decreasing towards tip and spines becoming concomitantly shorter.

2. Holotrichous *a*-isorhizas.— Capsules smaller (size range: 4.4 × 4.0-6.2 × 5.5 µm) and blunter in shape than type 1, with operculum; tube without shaft, isodiametrical, except for a naked proximal portion provided with spines of uniform size, tightly coiled in the undischarged capsule.

3. Heterotrichous anisorhizas.— Capsules almost spherical (size range: 4.4-6.6 µm in diameter), operculum present; everted tube anisodiametrical, provided with

spines over its entire length; diameter of tube and size of spines decreasing towards distal end. This nematocyst category is here described for the first time from *Cassiopea* species.

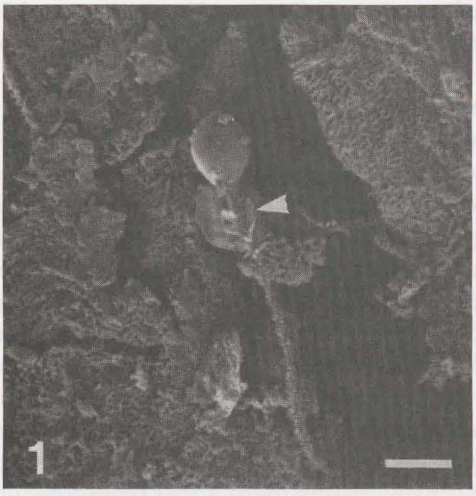
SEM preparations of discharged capsules of all three types of cnidae, with the proximal part of their tubes, are shown in figs 1-6. The distribution of the nematoblast and nematocyte types in the two epithelia of individuals at different stages of development and in different parts of the organism are listed in table 1, showing distinct patterns of distribution. Euryteles were present consistently in the ectoderm of all stages in the body parts investigated. They were the only category also occurring in the endoderm. The *a*-isorhizas were not likewise restricted to the ectoderm, but were not detected in all parts of the polyp. The heterotrichous anisorhizas formed a specific complement of the cnidomes of the ephyra and the medusa. Anisorhizas were detectable in the polyp with the onset of strobilation. However, the exact location and time of the first anisorhizas to appear remains to be established. Surprisingly, some isorhizas were detected in planula larvae, despite the fact that polyps resulting from planulae did not show this type.

The mesogloea was consistently devoid of any stinging cells. Peculiar arrangements of nematocysts were seen on the appendages of the oral arms of the medusae. Those include lappet-like oral lobes, also called oral vesicles, and bands of tentacle-like structures, called digitellae (figs 7-8). Nematocysts, often including particularly large euryteles, are densely packed in the ectoderm of the digitellae, which show enlargement of the epithelial surface in the form of vesicular invaginations. On the oral lobes, groups of ectodermal vesicular structures were found to protrude from the surface (fig. 8). They may detach and swim off upon mechanical disturbance, for instance when egg masses are sampled from the bases of lobes located in the center of the oral disk. These vesicles are propelled by ciliary action and represent swimming "nematocyst bags" with large numbers of nematocysts in the ectoderm. They are probably identical to those described by Smith (1936: 25) in *Cassiopea frondosa* (Pallas, 1774). There are also mesogloeal cells and numerous symbiotic algae in the central part so that they can easily be distinguished from the symbiont-free planula larvae which are about the same size.

Distribution and possible origin of nematoblasts

Nematocytes, the cells producing functional nematocysts, develop from nematoblasts which can be distinguished in stained sections due to their size and different, uniform staining of the capsule rudiment. It is more difficult to discriminate between nematoblasts yielding the different nematocyst types. In the present study, those of the heterotrichous microbasic euryteles were found to differ in shape and size from the other two types. It was not possible, however, to distinguish between the nematoblasts of holotrichous *a*-isorhizas and heterotrichous anisorhizas. A survey of nematoblasts in the tissues has been attempted at different developmental stages (table 1).

Nematoblasts are found in both epithelia of planula larvae and swimming buds. They are, however, limited in the polyps to the ectoderm and endoderm of the calyx and the hypostome. Contrary to this very limited occurrence in the scyphistoma,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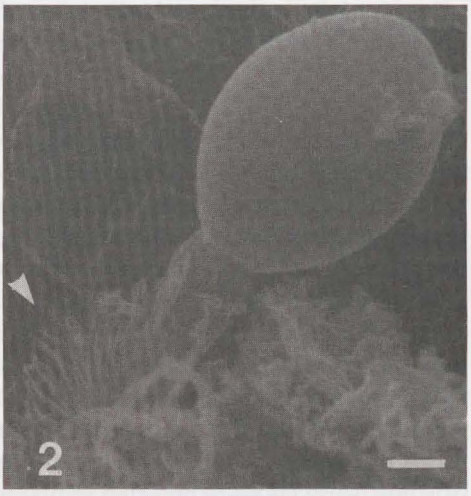
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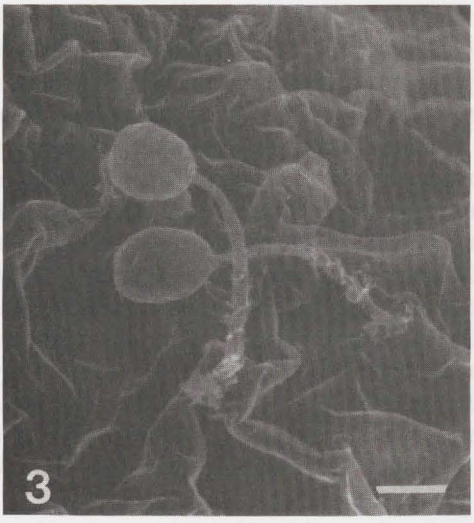
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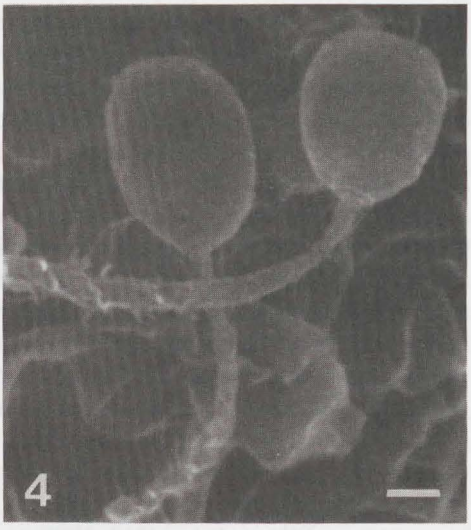
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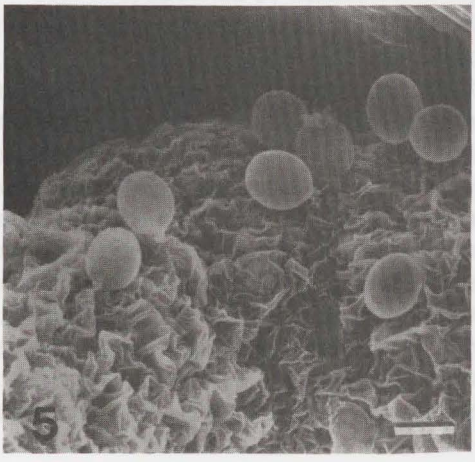
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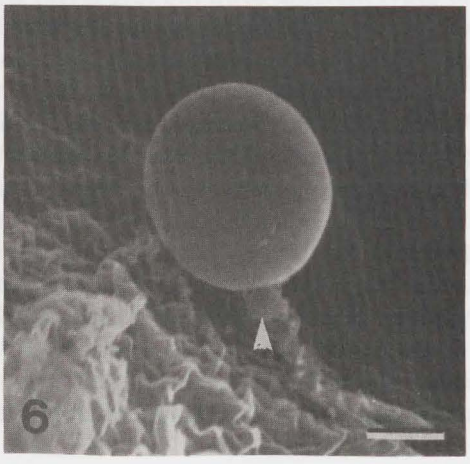
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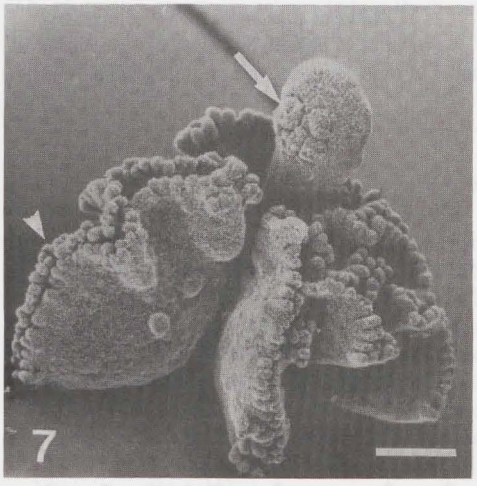

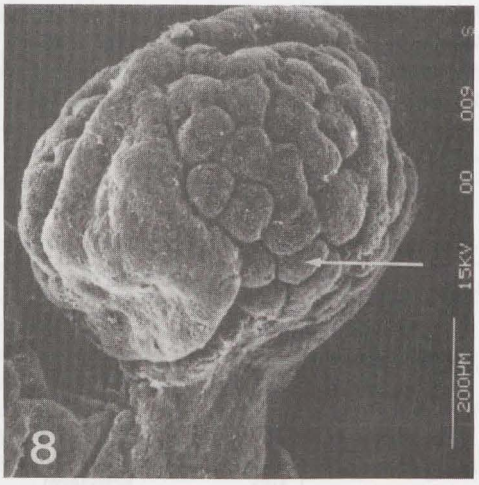

Figs 1-8 *Cassiopea andromeda (C.a.)* and *Cassiopea xamachana (C.x.)*: SEM micrographs of discharged nematocysts and of oral appendages of an adult medusa. Figs 1, 2: *(C.x.)* heterotrichous microbasic euryteles; arrowhead: spines on the shaft. Figs 3, 4: (C.x.) *a*-isorhizas. Figs 5, 6: *(C.x.)* heterotrichous anisorhizas; arrowhead: operculum. Fig. 7: *(C.x.)* appendages of oral arm showing digitellae (arrowhead) and small oral lobe (arrow). Fig. 8: *(C.a.)* close-up of oral lobe with nematocyte bags bulging out from the surface (arrow). For detailed explanation, see text.
Scale bars: fig. 1 = 5 μm; fig. 2 = 2 μm; fig. 3 = 2 μm; fig. 4 = 1 μm; fig.5 = 5 μm; fig. 6 = 2 μm; fig. 7 = 200 μm; fig. 8 = 200 μm.

nematoblasts are more widely distributed in the ephyra and the medusa. Though not recorded in vivo by time lapse cinematography, we infer that nematoblasts may arise at a distance from the body part in which functional nematocysts are mainly required, e.g. in the tentacles of the polyps, and therefore are expected to migrate from "source to sink", i.e. from calyx to tentacles. This is a quite common phenomenon in cnidarians.

Distribution of nematoblasts could be taken as a provisional indication as to where progenitors of the nematocytes are located and possibly even produced. Whereas in hydrozoans nematoblasts are known to originate from pluripotent interstitial cells (I cells; for a review, see Tardent, 1995: 358), the respective progenitor cells have so far not been identified in scyphozoans. Though interstitial cells were reported to occur in planula larvae of *Cassiopea xamachana* (Martin & Chia, 1982: 325), we have not been able to detect cells with staining characteristics similar to those of hydrozoan I cells. The origin of the nematocyte cell line in *Cassiopea* thus still remains to be uncovered.

The cnidome and its taxonomic significance

The cnidome of the two *Cassiopea* species investigated consists of two types that occur in all stages: eurytele and *a*-isorhiza, and an additional type, the anisorhiza, here detected for the first time in the strobilating polyp, and subsequently in the ephyra, the medusa, and the planula. According to Calder (1983: 1186, referring to

Table 1. Distribution of nematocyte and nematoblast types in *Cassiopea andromeda* and *C. xamachana*. Symbols: + = present, +* = very few cells, – = absent, –? = presumably absent, but those of *a*-isorhiza and anisorhiza not distinguishable, (+) = presumably present, but those of *a*-isorhiza and anisorhiza not distinguishable, Nc = nematocyte, Nb = nematoblast.

Life cyle stage	Body part	Epithelium	Nematocyst type					
			Eurypteles		*a*-Isorhizas		Anisorhizas	
			Nc	Nb	Nc	Nb	Nc	Nb
Bud		Ectoderm	+	+	+	(+)	–	–?
		Endoderm	+	+	–	–	–	–?
Polyp	Tentacles	Ectoderm	+	–	+	–	–	–
		Endoderm	–	–	–	–	–	–
	Hypostome	Ectoderm	+	+	–	–	–	–?
		Endoderm	+	+	–	–	–	–?
	Calyx	Ectoderm	+	+	+	(+)	–	–?
		Endoderm	+	+	–	–	–	–?
	Stalk	Ectoderm	+	–	–	–	–	–
		Endoderm	–	–	–	–	–	–
Ephyra/Medusa	Exumbrella	Ectoderm	+	+	+	(+)	+	(+)
		Endoderm	+	–	–	–	–	–
	Subumbrella	Ectoderm	+	+	+	(+)	+	(+)
		Endoderm	+	–	–	–	–	–
	Manubrium	Ectoderm	+	+	+	(+)	+	(+)
		Endoderm	+	+	–	–	–	–
	Digitellae	Ectoderm	+	+	+	(+)	+	(+)
		Endoderm	+	+	–	–	–	–
	Oral lobes	Ectoderm	+	+*	+	(+)	+	(+)
		Endoderm	+	+*	–	–	–	–
Planula		Ectoderm	+	+	+	(+)	+*	(+)
		Endoderm	+	+	–	–	–	–

Weill, 1934 a,b), eurypteles and *a*-isorhizas should be classified as stable categories, whereas the only partially occurring anisorhizas would represent an unstable category. Such a classification has been extended earlier to the rhizostome *Rhopilema verrilli* (Fewkes, 1887) and to some semaeostome species showing similar changes during the life cycle (see Calder, 1983: 1190, table 2, with references of original articles).

Apart from differences in size, minor variations of micromorphological characters within and between individuals, and from occasional malformations of nematocysts, the present study showed the cnidomes of *Cassiopea andromeda* and of *C. xamachana* to be congruent. No differences of taxonomic value, useful in distinguishing between the two species, were detected.

Acknowledgements

Frank Jensch is indebted to Ch. Sommer for valuable advice and help with microscopial techniques.

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Species diversity of hermatypic scleractinian corals: are local communities saturated or regionally enriched?

R.H. Karlson & H.V. Cornell

Karlson, R.H. & H.V. Cornell. Species diversity of hermatypic scleractinian corals: are local communities saturated or regionally enriched?
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 287-293, figs 1-2.
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Key words: Corals; diversity; local saturation; regional enrichment.

Abstract: Although species diversity within ecological communities has been explained in terms of local habitat selection and niche partitioning among competing species, larger scale, regional-historical phenomena can also influence local diversity patterns. In order to determine whether tropical communities of reef-building scleractinian corals are saturated (in accordance with traditional niche theory) or regionally enriched, we have conducted an extensive review of the literature to find 1655 quantitative estimates of species diversity from coral communities at over 150 sites throughout the world. After eliminating diversity estimates which had been pooled across multiple depths and sites, we removed systematic variation due to sampling effort and method from this data to generate residuals for local, within-habitat diversity. Multiple regression analysis of these residuals clearly indicates that the effect of the size of the regional species pool on local, within-habitat diversity was not only detectable, but comparable in magnitude to each of two local variables representing depth and habitat. Thus we conclude that coral communities are regionally enriched and that community structure cannot be completely explained on the basis of conventional niche theory.

Introduction

According to traditional niche theory, highly speciose tropical communities are saturated with species which finely subdivide habitats to limits set by niche overlap and competitive processes (MacArthur, 1965: 531). This paradigm invokes the equilibrial notions of niche differentiation, limiting similarity, and competitive exclusion. Nonequilibrial explanations for tropical diversity patterns include the diversifying effects of disturbances and the effects of history and chance events on the immigration and extinction of species [e.g., among corals (Connell, 1978: 1303; Karlson & Hurd, 1993: 121), coral reef fishes (Sale, 1977: 337), and rain forest trees (Connell, 1978: 1303; Hubbell & Foster, 1986: 314)]. These explanations subordinate competition as the primary process responsible for the organization of natural communities.

The recent resurgence of interest in geographical and historical explanations for local diversity patterns (Cornell & Lawton, 1992: 1; Ricklefs & Schluter, 1993: 1) has motivated us to ask how local and regional processes might simultaneously affect the organization of coral communities. Although local processes are thought to be primarily responsible for their organization (Huston, 1985: 149), evidence for enrichment of local diversity due to speciation events and immigration from regional species pools is accumulating. Hence, we have conducted an extensive review of the coral reef literature to compile estimates of local diversity and to determine if there is

sufficient evidence for the local saturation or regional enrichment of coral communities. Here we present a general description of the overall data set including the within- and between- habitat components of local diversity and the effects of sample size and method. We also note the results of a previous analysis in which we found significant local and regional effects on local species diversity (Cornell & Karlson, 1996: 233).

Methods and results

We restricted our review of the coral literature to publications containing quantitative data on the number of hermatypic scleractinian corals in samples of variable size and method; we modified some of the original diversity estimates to remove hydrocorals and ahermatypic species. We extracted data for individual samples, means of replicate samples taken within habitats, and cumulative totals for species present in multiple samples. In cases in which species diversity was reported for individual samples and as mean values, we only extracted the data for individual samples. We were able to determine cumulative totals as they were reported or by pooling reported data. Although all the data represented local estimates of species diversity, some of the cumulative total data were pooled across multiple depths or sites. We also extracted information on sampling methods, sample size, depth, and habitat type. Sampling methods included quadrat, line transect, and point intercept techniques. Depth was quantified as distance below mean low water and habitats by a ranking of relative distance from shore (i.e., 1 = inner flat, 2 = mid and outer flat, 3 = reef crest and upper slope, 4 = mid-slope, 5 = lower slope).

For our literature search, we used the review by Stoddart (1969: 460) to identify older coral reef publications, the Biological Abstracts for 1970-1993, and the proceedings of the first six International Coral Reef Symposia to find 88 publications containing quantitative local diversity data; most of this data was published after 1970. Estimates of the size of the regional species pool (on a scale of 10^2-10^4 km) matching each local sample were derived from these and an additional 36 publications. In most cases, local and regional estimates of diversity were determined from independent sources. A complete list of these sources will be published elsewhere (Karlson & Cornell, in preparation).

We found 1655 quantitative estimates of local species diversity from coral communities at over 150 sites throughout the tropics and subtropics (fig. 1a). These data include 1329 estimates of within-habitat diversity [10.1 ± 0.3 species (1 standard error)] and 326 estimates of cumulative species diversity [25.2 ± 1.4 species (1 standard error)] determined at larger spatial scales. Estimates of the size of the regional species pools matching the local samples ranged from a minimum of 19 species in the eastern Pacific (Porter, 1972: 89) to a maximum of 411 species in the central Indo-Pacific (Veron, 1993: 1). For a detailed discussion of the effects of error in the estimates of regional diversity on the detection of regional enrichment within local communities, we refer the reader to Cornell & Karlson (in revision).

Although the majority of the local diversity estimates were ≤ 50 species, particularly speciose samples (≥ 100 species) from the cumulative data set indicate the mixing of within- and between-habitat components of diversity in samples taken from

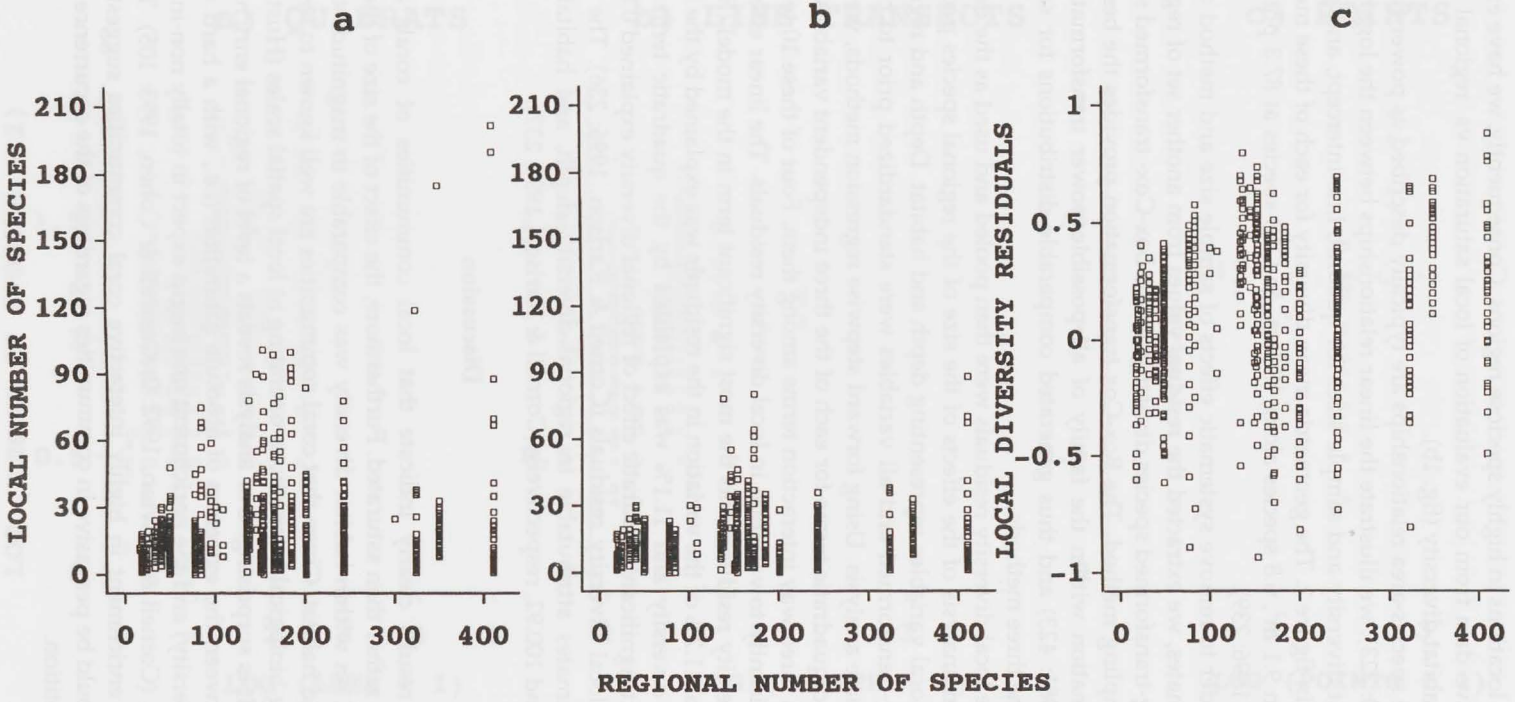

Fig. 1. a) All local diversity data (n = 1655), b) only within-habitat diversity data (n = 1329), and c) standardized local diversity residuals plotted against regional diversity. (After Cornell & Karlson, 1996).

multiple locations in highly speciose regions. Consequently, we have excluded all the cumulative data from our evaluation of local saturation vs. regional enrichment of within-habitat diversity (fig. 1b).

Since species-area relationships are typically described as power functions (Ricklefs, 1990: 723), we illustrate the linear relationships between the logarithms of within-habitat diversity and sample sizes for quadrat, line intercept, and point intercept samples in figure 2. The geometric mean diversity for each of these methods was 7.7 species in 9.1 m^2, 6.8 species along 18.6 m, and 7.3 species at 87.3 points (Cornell & Karlson, 1996: 239).

In order to remove systematic effects of sample size and method on local diversity estimates, we extracted the residual values from another set of regression analyses of log-transformed species diversity versus Box-Cox- transformed sample size for each sampling method. The Box-Cox transformation provides the best normalizing transformation within the family of all possible power transformations (Sokal & Rohlf, 1981: 423) and thus generated comparable distributions for sampling effort among the three methods.

These local diversity residuals were then pooled and used as the dependent variable in an analysis of the effects of the size of the regional species pool (fig. 1c) and the two local variables representing depth and habitat. Depth and regional diversity were log-transformed and all variables were standardized prior to conducting the multivariate analysis. Using forward stepwise regression methods, we evaluated the linear and quadratic terms for each of the three independent variables as well as the two- and three-way interaction terms among them. Four of these 10 terms contributed significantly to variation in local diversity residuals. The linear effect of depth on local diversity residuals was the most significant term in the model (R^2 = 13.8%); an additional 11.2% of the variation in the residuals was explained by the linear term for regional diversity and 11.1% was explained by the quadratic term for habitat; a weak, but significant quadratic effect of regional diversity explained 0.8% of the variation in local diversity residuals (Cornell & Karlson, 1996: 236). The additive variance estimates attributable to regional diversity, depth, and habitat were 110.26, 125.38, and 100.92, respectively (Cornell & Karlson, 1996: 237).

Discussion

Our results clearly indicate that local communities of corals are regionally enriched rather than saturated. Furthermore, the effect of the size of the regional species pool on within-habitat diversity was comparable in magnitude to that of both depth and habitat. Given that coral communities are well known to be characterized by strong biological interactions operating at local spatial scales (Huston, 1985: 154), this result is surprising. Our analysis reveals a level of regional enrichment intermediate between the extremes of absolute saturation (i.e., with a hard upper limit to local diversity) and the enrichment one might expect in totally non-interactive communities (Cornell & Lawton, 1992: 2; Caswell & Cohen, 1993: 105). The significant regional enrichment in highly interactive coral communities suggests that enrichment should be pervasive in communities regardless of the occurrence of interspecific competition.

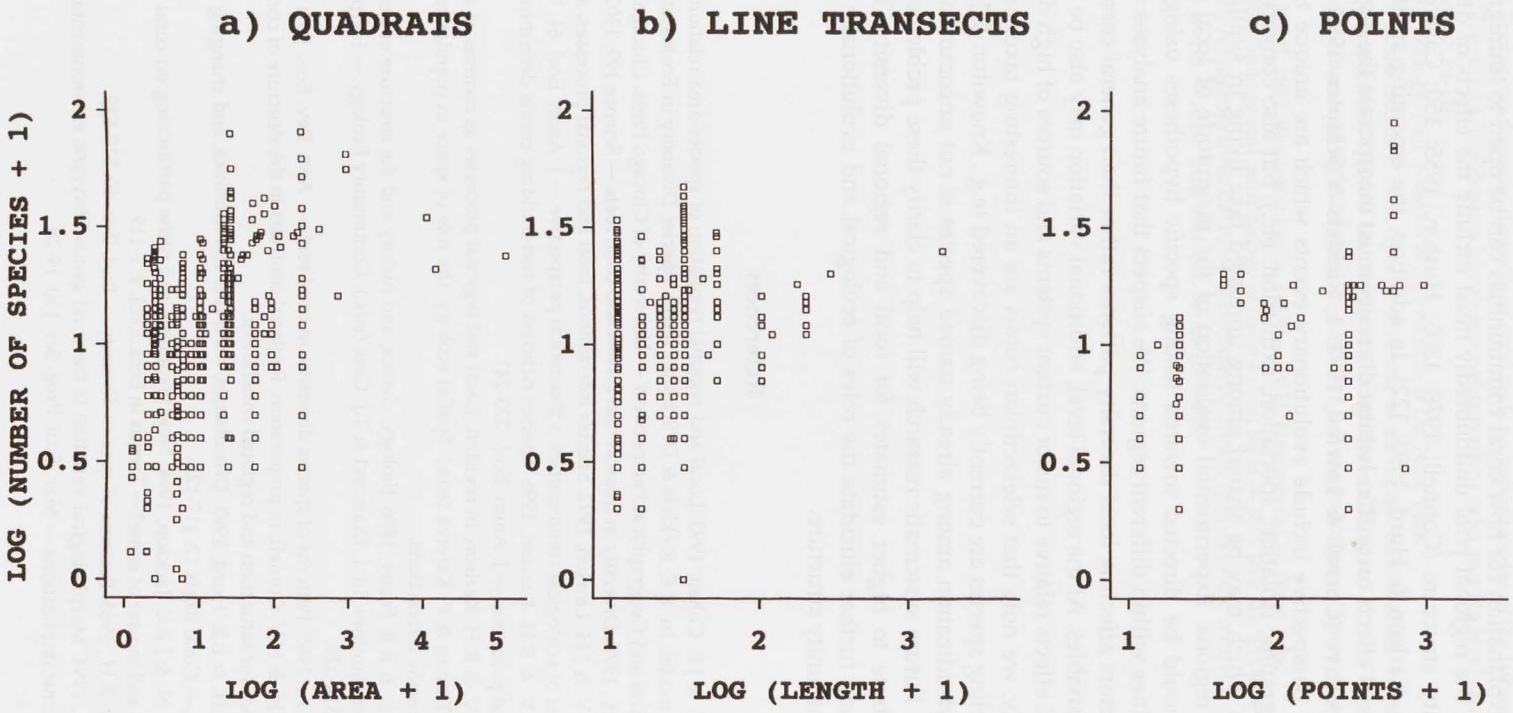

Fig. 2. Local within-habitat diversity plotted against sample size for a) 653 quadrat (area in m^2), b) 542 line transect (length in m), and c) 134 point intercept samples. Redundant values are not plotted.

The explanation for how coral communities can be open to immigration of species from the regional pool undoubtedly must include the effects of disturbance on community structure (Connell, 1978: 1305; Huston, 1985: 150; Caswell & Cohen, 1993: 105; Karlson & Hurd, 1993: 122). In addition, the emerging synthesis of local and regional effects on within-habitat diversity must incorporate the regional- historical perspective (Cornell & Lawton, 1992: 1; Ricklefs & Schluter, 1993: 1). Not only does this perspective include evolutionary events which are unique to any region (e.g., geographic isolation, speciation, extinction, etc.), but also convergent adaptive processes which may be shared among unrelated taxa living in similar habitats in different regions. Experimental evaluation of the magnitude of local and regional effects should be directed towards testing specific hypotheses using comparable communities within different regions. We suspect that future analyses will focus on causal factors affecting local diversity patterns rather than general correlations with habitat variables. At the regional level, additional variation may also be explained by positional effects relative to major current systems and sources of high diversity.

Finally, we note that scleractinian corals are an interesting taxonomic group in which sibling species are currently being discovered (e.g., Knowlton & Jackson, 1994: 7), yet hybridization among already named species is not uncommon (e.g., Miller, 1994: 19). Future systematic research will help to clarify these problems and is likely to contribute to higher estimates for local and regional diversity. This research should help further elucidate the roles of ecological and evolutionary processes on coral community structure.

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Two forms of bivalve-inhabiting hydrozoans that differ in timing of medusa release

S. Kubota

Kubota, S. Two forms of bivalve-inhabiting hydrozoans that differ in timing of medusa release. Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 295-299, figs 1-2.
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Key words: Bivalve-inhabiting hydrozoans; timing of medusa release; *Eutima japonica* forma *intermedia; Eugymnanthea japonica;* sympatry.

Abstract: Two distinct forms of bivalve-inhabiting hydrozoans occurring in Japanese waters (Leptomedusae: Eirenidae) are rarely sympatric. At one place where sympatry occurs, Tsushima Island, Nagasaki Prefecture, the timing of medusa release was studied in *Eugymnanthea japonica* Kubota, 1979, and *Eutima japonica* Uchida, 1925, forma *intermedia* (cf. Kubota, 1992a). In both species, which are commensal with the bivalve *Mytilus edulis galloprovincialis* Lamarck, 1819, a constant pattern of release was detected. Medusae were released from the mantle cavity of the host for several hours a day in the evening, but the peak time of release was different for the two species. Medusae of *Eutima japonica* forma *intermedia* were released earlier in the day than those of *Eugymnanthea japonica*, irrespective of weather conditions.

Introduction

Medusae of *Eugymnanthea japonica* Kubota, 1979, a bivalve-inhabiting hydrozoan in Japan, are released from the mantle cavity of the host *Mytilus edulis galloprovincialis* Lamarck, 1819, for a few hours a day around sunset, coinciding with the maximum shell opening of the host (Kubota, 1996). The present study compares the timing of medusa release with that in another species of bivalve-inhabiting hydrozoan, *Eutima japonica* Uchida, 1925, forma *intermedia* (cf. Kubota, 1992a). In Japanese waters these two species are seldom sympatric (Kubota, 1992b). Therefore, the present study was conducted on Tsushima Island, southern Japan (fig. 1), where both occur.

Material and methods

On 28 June 1995, 77 specimens of *Mytilus edulis galloprovincialis*, 27-69 mm long, were collected from the intertidal zone at Takeshiki (129°E 34°18'N), Asou Bay, Tsushima Island, Nagasaki Prefecture. The shell surface of the mussels was cleaned within 30 minutes after collecting using a knife; epizoic barnacles and polychaetes were completely removed. The mussels were then put into a bag of ca 7 × 5 mm flexible mesh and suspended below a floating mooring-raft at the collecting site, at a depth of 0.5 m (i.e. independent of the tides), inside a vertically-held plankton net of 30 cm across the mouth and 100 cm deep, and ending in a cylindrical plastic bucket 20 mm in diameter covered by a stainless steel lid with a stopcock. Inside the net, made of synthetic resin-processed silk gauze of mesh-size 0.33 mm, any medusae released were trapped.

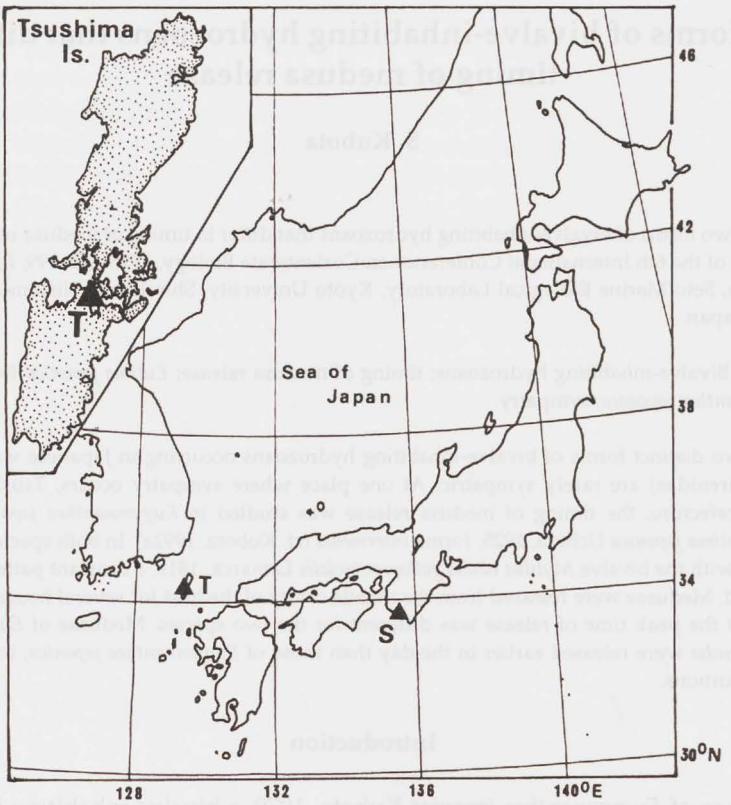

Fig. 1. Location of the study site. T = Takeshiki, Tsushima Island, Nagasaki Prefecture. S = Shirahama, Wakayama Prefecture.

Released medusae were collected from the evening of 28 June till the afternoon of 4 July, 1995, at various intervals. Collections were made 8-13 times per day excepting the first and last days, with a minimum interval of 1 h (fig. 2, upper). On each sampling occasion the net was washed out more than three times to collect all the medusae. These were cooled in an ice-box and brought back to the laboratory within several minutes. The host mussels were removed from the sea during these collecting periods for a few minutes each time. At night, lights were extinguished during sampling. The surface water temperature was measured on every sampling occasion.

The medusae were counted with the aid of a stereo-microscope, either immediately or up to 10 h after collection. The quantity of pseudo-faecal pellets produced by the mussels during each sampling interval was also recorded as a measure of their filter-feeding activity and consequently of the period during which they were opened. Meteorological data from the meteorological station at Izuhara, Tsushima Island, were used. The tide data cited are from the "Tide tables for the year 1995, Iki and Tsushima" published by the Japanese Meteorological Agency.

Results

The number of medusae released from the hosts per day ranged from 290 (2 July) to 955 (30 June) in *Eutima japonica* and from 152 (2 July) to 504 (30 June) in *Eugymnanthea japonica*, excluding the days at the start and the end of the observation period. During the observation period release of 2617 young medusae was recorded in *Eutima japonica* and of 1247 mature medusae of both sexes in *Eugymnanthea japonica*.

On each day most of the medusae of both species were released for several hours, showing clear diurnal periodicity. However, the peak time differed between them. The medusae of *Eutima japonica* were always released in greater numbers earlier in the day than those of *Eugymnanthea japonica*, ca 1-5 h before sunset and around sunset, respectively. This distinction was constant under various weather conditions (fig. 2, upper). During the observation period the weather changed considerably, from fine to rainy as the meteorological data show. Sunrise and tidal change did not apparently influence the timing of release (fig. 2, upper).

The timing of medusa release was not connected with the defaecatory activity of the host mussel since the bivalves discharged many pellets of pseudofaeces throughout the day; there were no intervals in which the host mussels closed their shells completely. It was noted that neither medusae nor hydroids were trapped in the pseudofaeces.

Just before completion of the present observations, the hosts were removed from the sea for approximately the period of the low tide in the morning on 4 July. No medusa release was observed afterwards during daylight hours of that day in either species (fig. 2, upper).

Discussion

The peak time of medusa release of the two species of bivalve-inhabiting hydrozoans were clearly different at the site studied. The cause of this difference is not known. Possibly the species have differing sensitivities to light. It is noteworthy that the peak time for release was maximally different (ca 7.5 h) on 2 July. There was much rain on that day and the number of medusae released was at a minimum in both species (fig. 2, upper). However, the peak time difference between the two species was ca 2 h on 3 July and only 1 h on 29 June. Such a difference of release-time could be related to the reproduction of the medusa; in *Eugymnanthea japonica* the medusa is already mature upon release, capable of rapid sexual reproduction and relatively short-lived, whereas *Eutima japonica* releases immature medusae which have a much longer planktonic life.

The constant pattern of medusa release in *Eugymnanthea japonica* in the intertidal population at the entrance of the Sea of Japan was similar to that found in the subtidal population of Shirahama, Wakayama Prefecture, Middle Japan, facing the Pacific Ocean (see fig. 1, S; Kubota, 1996), supporting the absence of a tidal-related rhythm.

The sea water temperature varied little during the study period; it was mostly between 22-23°C but ranged from 21.9 to 24.1°C (fig. 2, lower). Hence this factor seems not to be the trigger for medusa release as has been assumed in my previous study (Kubota, 1996), and decrease in light intensity is therefore the most plausible factor that triggers medusa release.

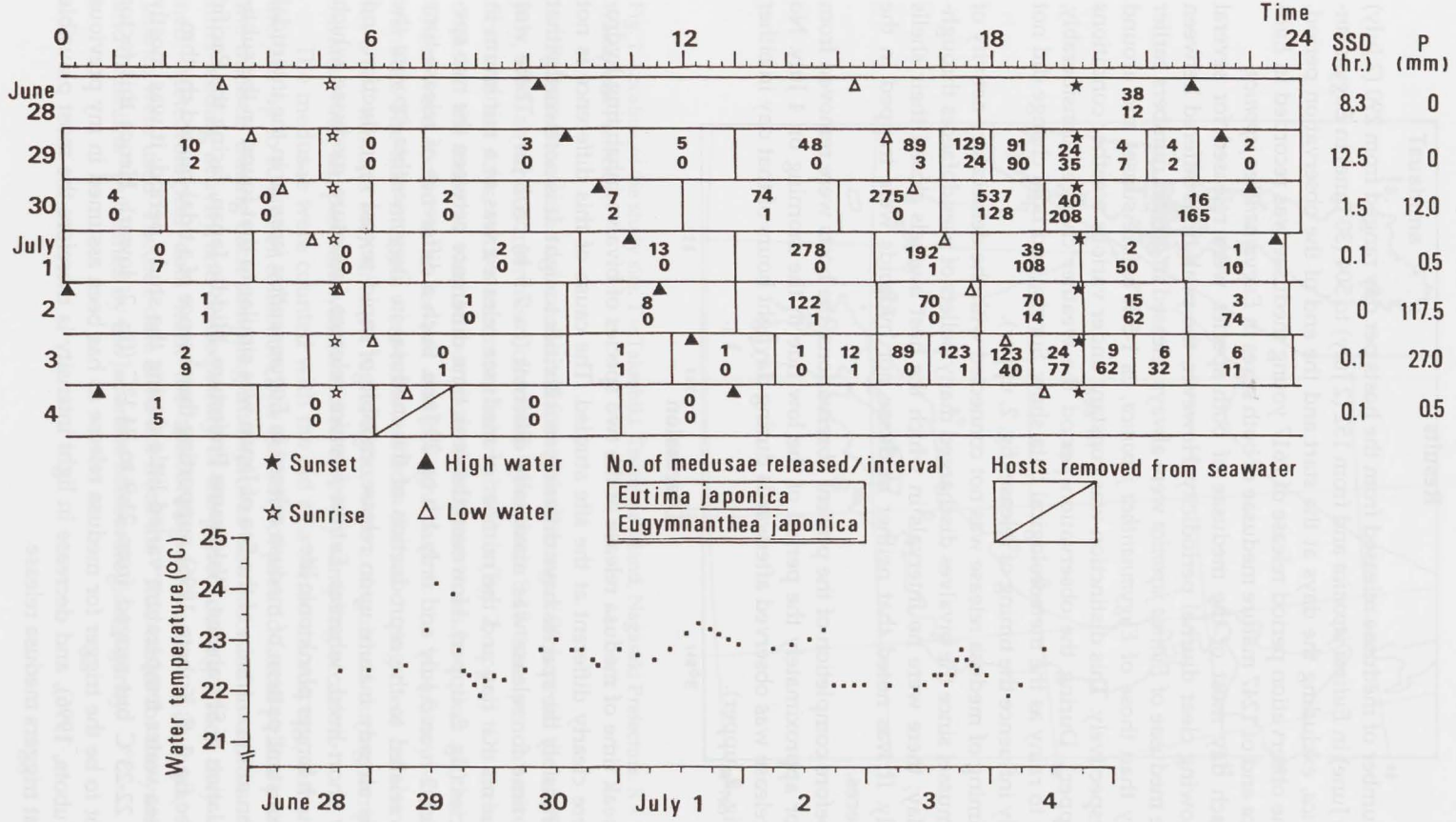

Fig. 2. Upper: Temporal change in the number of medusae released from their hydroids living in the mantle cavity of the host bivalve *Mytilus edulis galloprovincialis* (N=77) from Tsushima Island, Japan, together with astronomical and meteorologial data (SSD = sunshine duration; P = precipitation). Lower: Temporal change of sea surface temperature.

It is also possible that some biological rhythm is operating in this species.

The present hosts were found in the intertidal region. Therefore, they were exposed during low tide. When low tide is in the evening, as it was during the present observation period, the medusae of both species cannot be released at the expected release-time. In such cases it seems plausible that the medusae of the two species will be released at the same time when the tide rises. In the morning of 4 July such a low-tide effect was not observed (fig. 2, upper), perhaps since it was not a normal release-time for the two species. It is noteworthy that on 1 and 2 July a large number of medusae of *Eutima japonica* were released before low tide.

The timing of medusa release was constant in the short observation period, though further study is required to elucidate a possible seasonal change of the release-time, correlated with day-length and/or intensity of solar radiation.

Acknowledgements

I thank the staff of the local meteorological station at Izuhara, Tsushima Island, for the data on sunshine duration and precipitation during the observation period. I wish to express my sincere gratitude to Drs Dale R. Calder, Paul F.S. Cornelius, and J.C. den Hartog for improving the manuscript.

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Distribution of Siphonophora in Lebanese waters (eastern Mediterranean)

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Lakkis, S. & R. Zeidane. Distribution of Siphonophora in Lebanese waters (eastern Mediterranean). Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 301-306, fig. 1, tab. 1.
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Key words: Siphonophora; Physonectae; Calycophorae; Levantine Basin; Lebanese waters.

Abstract: Twenty-eight species of Siphonophora including five physonects and 23 calycophores were found in the Lebanese neritic waters between 1969 and 1994. Of these, 18 species are shared with the Red Sea fauna. *Nanomia bijuga* (Delle Chiaje, 1841) was the most common physonect. The most abundant calycophores were: *Eudoxoides spiralis* (Bigelow, 1911), *Sphaeronectes irregularis* (Claus, 1873), *Chelophyes appendiculata* (Eschscholtz, 1829), and *Diphyes dispar* Chamisso & Eysenhardt, 1821. Of these, *Eudoxoides spiralis* is also common in the southern Adriatic which might indicate that the eastern Mediterranean and the Adriatic are hydrologically connected.

Introduction

Studies to contribute to the knowledge of the composition and the structure of the plankton community in Lebanese waters (Levantine Basin) began in 1969 (Lakkis, 1971; 1982). Concerning the Siphonophora of the Levantine Basin, few data are available. Kimor & Wood (1975) recorded 12 species, Alvarino (1974) mentioned 21 species, and Dowidar & El-Maghraby (1970) mentioned five species from the vicinity of Alexandria. Rottini (1971) listed 21 species from the Ionian and Crete Seas. Most of the species found by these authors in the eastern Mediterranean are also present in the western basin (Ianora & Scotto di Carlo, 1981; Gamulin & Krsinic, 1993).

In spite of the rather high species richness (28 species found, cf. table 1), siphonophores are a less important component of the Levantine gelatinous zooplankton community, occupying the last place in importance after the Scyphomedusae, the Thaliacea and the hydromedusae. The present paper deals with the composition, the seasonal variation and vertical distribution of Siphonophora in Lebanese waters.

Hydrographic conditions

The Levantine Basin is considered as a warm temperate or subtropical sea. Two seasons characterize the annual hydrological cycle: a cool period (December-March) and a long hot and dry period (April-November). During the winter season the surface water temperature drops to a minimum of 16°C due to vertical mixing of water and upwelling currents creating an isothermic condition within the whole water column. The surface water salinity fluctuates between 38.75 and 39.50‰ according to the area, season and the amount of fresh water inflow. The general circulation pattern along the coast of Lebanon is a prevalent northward surface current most of the

year (December-September) with a maximum velocity, due to the local wind stress, of 0.50 m/sec. in February and a minimum of 0.15 m/sec. in August. In the autumn the current is often reversed and less strong. During the summer there is a decrease in the phytoplankton production because of the high water surface temperature (maximum 30°C in August), nutrient depletion and lack of deep water mixing. Temperature differences between inshore shallow water and offshore deep water are negligible. The water is very transparent and the Secchi disk does not disappear at 25 m depth at the offshore stations. Principal component analysis shows that during winter and spring there is a strong relationship between environmental factors (temperature changes, water transparency, chlorophyll content, phosphates and nitrates), and growth and distribution of the plankton community. During the summer, when the plankton biomass is low and productivity very low, these factors have less effect on plankton biomass and distribution of species.

Material and methods

Data are based on plankton samples collected over 25 years (1969-1994) from 22 inshore and offshore stations (fig. 1). Sampling stations covered various environments: harbour area (2 stations), polluted area (2 stations), estuaries (2 stations), neritic stations (6 stations) and deep oceanic waters (10 stations). Three types of net were used: a standard plankton net (diameter 57 cm; mesh size 200 µm) for subsurface sampling and vertical hauls from 50-0 m, a double Bongo-net (diameter 65 cm; mesh size 300 and 500 µm) for oblique vertical tows, and a closing WP2 net (diameter 57 cm; mesh size 200 µm) for deep vertical hauls: 300-50 m and 600-300 m.

Seventy-five percent of the samples were collected in surface and subsurface hauls taken from neritic stations and 15% from estuaries, harbour and polluted waters. The remainder were deep water samples taken from oceanic stations. Most of the sampling was done monthly during the morning time (8h-12h); other series of samples were collected weekly, especially during the plankton bloom periods (spring, autumn). In six stations, sampling was carried out occasionally or seasonally (fig. 1). In all cases hydrographic measurements (temperature, salinity, water transparency, phytoplankton biomass, chlorophyll content and nutrient analysis) were taken simultanuously with the sampling.

More details concerning the hydrological features of stations are reported in previous works (Lakkis, 1982; Lakkis & Zeidane 1988, 1993).

Samples preserved in 4% formaldehyde solution were submitted to qualitative and quantitative analysis. The basic reference works used for species identification were Totton (1954; 1965), Carré (1968), and Pagès & Gili (1992). Identifications and abundance of Calycophorae species were determined on the basis of the eudoxoid stages, and the anterior nectophores.

Results

During the whole period of survey 28 species including 23 Calycophorae and five Physonectae were identified from Lebanese waters (table 1).

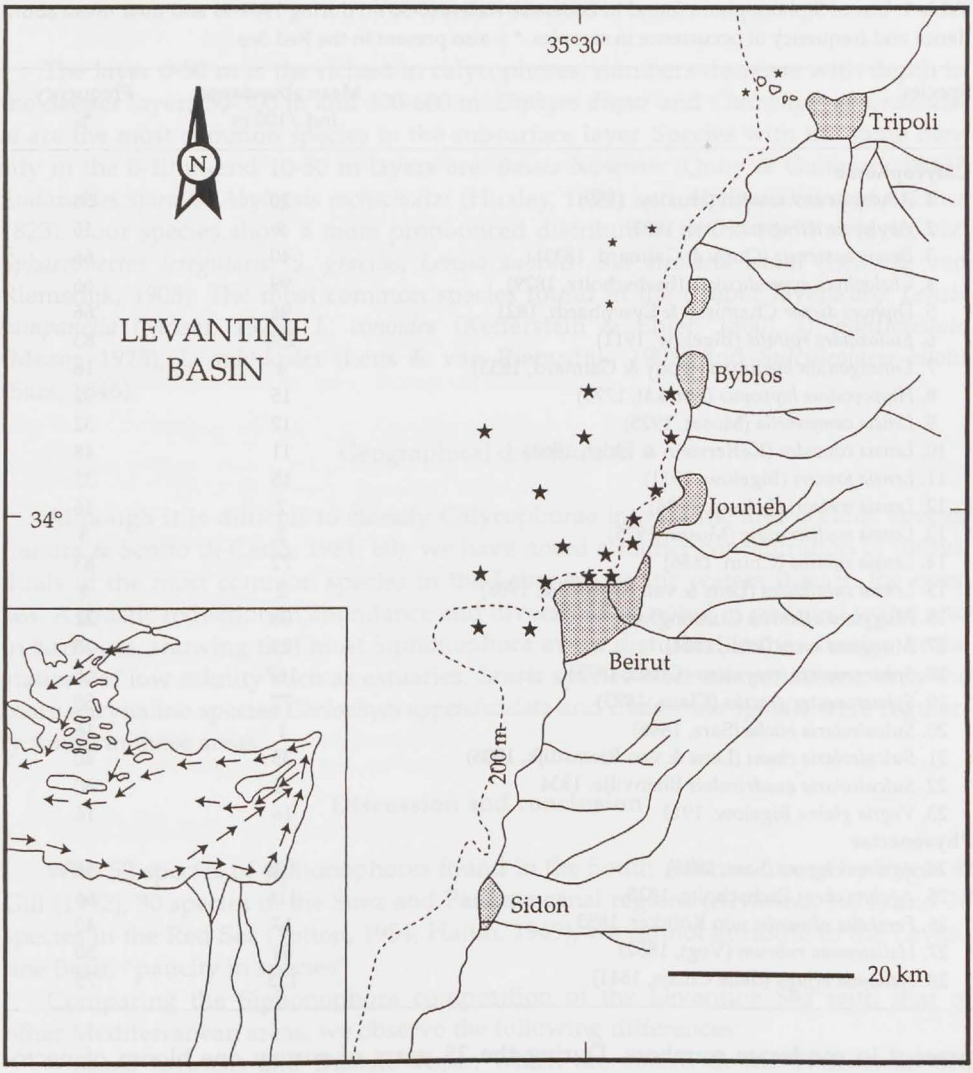

Fig. 1. Stations along the coast of Lebanon sampled during 1969-94. The insert shows the general surface circulation in the Eastern Mediterranean.
★ = stations sampled monthly or weekly; ⋆ = stations sampled occasionally or seasonally.

Physonectae

The five species of Physonectae occur also in the Red Sea (Halim, 1969) and in the Atlantic Ocean (Pagès et al., 1992). The most common species were *Nanomia bijuga* (Delle Chiaje, 1841); *Agalma elegans* (Sars, 1846) and *A. okeni* Eschscholtz, 1825, were found in moderate numbers. *Halistemma rubrum* (Vogt, 1852) and *Forskalia edwardsi* von Kölliker, 1853, were less abundant. All the year round nectophores were

Table 1. List of Siphonophora found in Lebanese waters (0-50 m) during 1969-94 and their mean abundance and frequency of occurrence in samples. * = also present in the Red Sea.

Species	Mean abundance ind./100 m^3	Frequency %
Calycophorae		
* 1. *Abylopsis eschscholtzi* (Huxley, 1859)	20	58
* 2. *Abylopsis tetragona* (Otto, 1823)	9	16
* 3. *Bassia bassensis* (Quoy & Gaimard, 1833)	40	66
4. *Chelophyes appendiculata* (Eschscholtz, 1829)	99	66
* 5. *Diphyes dispar* Chamisso & Eysenhardt, 1821	94	66
6. *Eudoxoides spiralis* (Bigelow, 1911)	250	83
* 7. *Enneagonum hyalinum* (Quoy & Gaimard, 1833)	4	16
8. *Hippopodius hippopus* (Forskål, 1775)	15	32
9. *Lensia campanella* (Moser, 1925)	12	32
10. *Lensia conoidea* (Kefferstein & Ehler, 1860)	11	48
* 11. *Lensia fowleri* (Bigelow, 1911)	15	33
* 12. *Lensia meteori* (Leloup, 1934)	2	16
* 13. *Lensia multicristata* (Moser, 1925)	2	8
14. *Lensia subtilis* (Chun, 1886)	72	83
* 15. *Lensia subtiloides* (Lens & van Riemsdijk, 1908)	2	8
* 16. *Muggiaea atlantica* Cunningham, 1892	58	32
17. *Muggiaea kochi* (Will, 1844)	25	25
18. *Sphaeronectes irregularis* (Claus, 1873)	167	66
* 19. *Sphaeronectes gracilis* (Claus, 1873)	77	50
20. *Sulculeolaria biloba* (Sars, 1846)	1	16
* 21. *Sulculeolaria chuni* (Lens & van Riemsdijk, 1908)	35	40
* 22. *Sulculeolaria quadrivalvis* Blainville, 1834	11	40
23. *Vogtia glabra* Bigelow, 1911	16	16
Physonectae		
* 24. *Agalma elegans* (Sars, 1846)	108	58
* 25. *Agalma okeni* Eschscholtz, 1825	116	66
* 26. *Forskalia edwardsi* von Kölliker, 1853	17	40
* 27. *Halistemma rubrum* (Vogt, 1852)	66	50
* 28. *Nanomia bijuga* (delle Chiaje, 1841)	133	75

present in moderate numbers. During the 25 years of survey one bloom of nectophores was noted at one coastal station in October 1982 (ca 1500 ind./m^3). Physonects show a certain preference for subsurface neritic water (0-10 m).

Calycophorae

The annual mean of calycophores for the whole period of survey ranged from 0.5 to 1 ind./m^3. No bloom was recorded during this period. The maximum population density was recorded between February and June and during December, the maximum number of species (12) in November. Six species contribute up to 95% of the total number of individuals above 50 m; these are by decreasing importance: *Eudoxoides spiralis* (Bigelow, 1911), *Sphaeronectes irregularis* (Claus, 1873), *Chelophyes appendiculata* (Eschscholtz, 1829), *Diphyes dispar* Chamisso & Eysenhardt, 1821, *Sphaeronectes gracilis* (Claus, 1873), and *Lensia subtilis* (Chun, 1886).

Vertical distribution

The layer 0-50 m is the richest in calycophores; numbers decrease with depth in the deeper layers 50-300 m and 300-600 m. *Diphyes dispar* and *Chelophyes appendiculata* are the most common species in the subsurface layer. Species with the same density in the 0-10 m and 10-50 m layers are: *Bassia bassensis* (Quoy & Gaimard, 1833), *Eudoxoides spiralis*, *Abylopsis eschscholtzi* (Huxley, 1859) and *Abylopsis tetragona* (Otto, 1823). Four species show a more pronounced distribution in the 10-50 m layer, viz. *Sphaeronectes irregularis*, *S. gracilis*, *Lensia subtilis*, *Sulculeolaria chuni* (Lens & van Riemsdijk, 1908). The most common species found in the deeper layers are: *Lensia campanella* (Moser, 1925), *L. conoidea* (Kefferstein & Ehler, 1860), *L. multicristata* (Moser, 1925), *L. subtiloides* (Lens & van Riemsdijk, 1908) and *Sulculeolaria biloba* (Sars, 1846).

Geographical distribution

Although it is difficult to classify Calycophorae into neritic and oceanic species (Ianora & Scotto di Carlo, 1981: 60), we have noted a higher concentration of individuals of the most common species in the Lebanese neritic waters than in the open sea. A drastic reduction in abundance and diversity was noted in polluted water and in harbours, showing that most Siphonophora avoid disturbed inshore water. In the stations of low salinity such as estuaries, *Lensia* species were almost absent; only the more euryhaline species *Chelophyes appendiculata* and *Eudoxoides spiralis* were regularly found in these areas.

Discussion and conclusion

With 52 species of siphonophores found in the South Atlantic Ocean by Pagès & Gili (1992), 30 species in the Suez and Panama canal regions (Alvarino, 1974) and 26 species in the Red Sea (Totton, 1954; Halim, 1969), we cannot attribute to the Levantine Basin "paucity in species".

Comparing the Siphonophora composition of the Levantine Sea with that of other Mediterranean areas, we observe the following differences:

– *Bassia bassensis* and *Diphyes dispar*, which are absent in the Tyrrhenean Sea (Gamulin & Krsinic, 1993), are major components of the Levantine Siphonophora; *Bassia bassensis* was reported rare in the Ionian and Crete Seas (Rottini, 1971).

– Whereas the three characteristic species of the western Mediterranean are *Lensia subtilis*, *Muggiaea kochi* (Will, 1844) and *Sphaeronectes gracilis*, five species are characteristic of the Levantine Sea, viz. *Eudoxoides spiralis*, *Lensia subtilis*, *Sphaeronectes irregularis*, *Diphyes dispar* and *Bassia bassensis*.

– *Lensia meteori* (Leloup, 1934), very common in the western Mediterranean, is very rare in the Levantine Basin, and present only in mesopelagic samples.

– *Eudoxoides spiralis*, the most common siphonophore in the Levantine Basin, has also been reported abundant in the southern Adriatic (Gamulin & Krsinic, 1993), which might indicate an hydrological connection between the two areas.

More investigations based on intensive spring and autumn sampling and open sea hauls from deep water (3000-1000 m) are our future target to improve the faunistic

inventary of the Siphonophora and to get more information on Lessepsian migrants from Indo-Pacific origin occurring in the Lebanese waters during the hot season.

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The role of epithelial conduction in the behaviour of *Aglantha digitale* (O.F. Müller, 1776) (Hydromedusae: Rhopalonematidae)

G.O. Mackie & C.L. Singla

Mackie, G.O. & C.L. Singla. The role of epithelial conduction in the behaviour of *Aglantha digitale* (O.F. Müller, 1776) (Hydromedusae: Rhopalonematidae).
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 307-313, figs 1-3.
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Key words: Swimming; jellyfish; *Aglantha digitale*; epithelial excitability; muscle ultrastructure.

Abstract: The exumbrellar epithelium of *Aglantha digitale* (O.F. Müller, 1776) is excitable and conducts impulses that inhibit swimming, but the protective, "crumpling" response elsewhere associated with conducting epithelia is not exhibited and mature specimens lack the radial muscles that are the main crumpling effectors in other medusae. These muscles however are present in juvenile specimens, and it is likely that at this stage crumpling can occur. It is suggested that loss of the crumpling action system in mature medusae is related to the development of giant axon-mediated, locomotory escape behaviour. The exumbrellar epithelium retains its excitability however, functioning as a sensory pathway mediating inhibition of swimming when the rhythmically swimming medusa strikes an object in its path.

Introduction

Most hydroids, hydromedusae and siphonophores have excitable epithelia that function as sensory pathways conveying electrical signals to nerves, muscles or to effectors such as bioluminescent organelles contained within the conducting epithelia themselves. Such systems have evolved in the Hydrozoa (alone among coelenterates) and a sizeable literature has grown up showing how excitable epithelia operate in partnership with nerves in the production of behaviour in this Class (see reviews by Anderson, 1980; Spencer & Schwab, 1982). Recent contributions include a report on the propagation of bioluminescent waves in *Euphysa japonica* (Maas, 1909) (cf. Mackie, 1991) and an analysis of the ionic mechanisms underlying frequency-dependent fatigue of epithelial action potentials in *Polyorchis penicillatus* (Eschscholtz, 1829) (cf. Grigoriev & Spencer, 1995). In only one other group of animals, the pelagic tunicates, can epithelial conduction be said to have comparable behavioural importance (Anderson, 1980).

For more than fifteen years we have been working toward the goal of a complete behavioural analysis of one medusa, *Aglantha digitale* (O.F. Müller, 1776) (Rhopalonematidae). This medusa can perform rhythmical, "slow" swimming like other medusae, but it also has an "escape" swimming response mediated by giant axons. Escape swimming effectively removes the animal from harm's way following tactile or vibrational stimulation. We have now identified the main subsets of nerves involved in escape and non-escape locomotion and associated tentacle movements (Mackie & Meech, 1995a,b; Meech & Mackie, 1995) and hope shortly to present a description of

the pathways involved in feeding behaviour. Near the start of this project (Mackie, 1980) it was noted that *A. digitale* had an excitable exumbrellar epithelium, but at the time the functional significance of this pathway was unclear. In most medusae studied, conducting epithelia form an important component of the action system mediating protective involution ("crumpling"), but as mature *A. digitale* do not show this response, the excitable epithelium seemed of minor interest and was given little attention. However, now that the main features of the central nervous cicuitry are becoming clearer, it seems appropriate to reconsider the role of the conducting epithelium in the behaviour of this animal.

Material and Methods

Some very small, immature medusae (eight tentacles, bell diameters 0.5-1.5 mm) were caught in a 0.5 mm mesh plankton net towed near the surface close to the dock at the Friday Harbor Laboratories, Friday Harbor, Washington, USA during early spring. Their behaviour was not studied, and they were fixed immediately for electron microscopy. Mature medusae were available in abundance during spring and summer and were used for behavioural observations, electrophysiology and electron microscopy. Freely swimming medusae were studied in large tanks and small vessels using a video cassette recorder. Tactile stimuli were applied with a fine metal probe. Paired platinum stimulating electrodes were used to deliver shocks and polyethylene suction electrodes to record electrical responses. Signals were amplified using a Grass P15 preamplifier, displayed on a Tektronix 5113 oscilloscope equipped with a 5D10 wave form digitizer, and photographed from the screen using Polaroid 667 film. During recordings, the temperature was maintained at 10-12°C.

For electron microscopy, specimens were fixed in 3% glutaraldehyde in 0.4 M phosphate buffer for 2 hrs and postfixed in 1% osmium tetroxide in the same buffer for one hour. After dehydration, the material was embedded in Epon 812; subsequently sections were cut, and stained with uranyl acetate and lead citrate.

Results

Impulse propagation in mature medusae

Single electrical shocks evoke bursts of potentials (fig. 1a) that can be recorded extracellularly, spreading in an all-or-nothing manner over the entire exumbrella. These will be termed exumbrellar potentials (EPs) following King & Spencer (1981). The frequency of EPs within bursts is high (40-60 Hz) and the potentials tend to merge, making conduction velocities hard to measure. In the best preparations, the conduction velocity of the first EP in a burst usually lay within the range 25-37 ($\bar{X}$ = 34) cm.s^{-1}. Intracellular recordings were attempted but proved impractical owing to the thinness of the epithelium.

Recordings from the subumbrella also show bursts of EPs following exumbrellar stimulation.

These were originally thought to have spread to, and to be propagated within, the subumbrellar endoderm (Mackie, 1980) but subsequent work has failed to con-

firm this. The potentials recorded on the subumbrellar side are evidently exumbrellar potentials picked up through the mesogloea. The endodermal lamella and radial canals, including those of the peduncle, are inexcitable. There appear to be no conduction pathways traversing the mesogloea.

Cutting through the exumbrellar epithelium blocked passage of EPs but the potentials were capable of propagating through artificially created bridges of tissue as narrow as 0.7 mm.

Propagation was blocked reversibly by treatment with 1.4-1.6 mM heptanol, a gap-junction blocker (Johnston et al., 1980).

Effector correlates of epithelial conduction in mature medusae

Exumbrellar stimulation of quiescent animals had no visible effect. The only observed effect of EP bursts was to inhibit swimming during periods of rhythmic, slow swimming. Rhythmically swimming animals that collided with the water surface or with the walls of the observation tank were often seen to stop swimming briefly. A light touch to the exumbrellar surface with a metal probe during swimming sometimes caused a similar brief pause in the sequence of swimming contractions. Electrical stimulation of pinned specimens with a recording electrode attached to the subumbrellar swimming muscles made it possible to illustrate the inhibitory effect (fig. 1b). In a typical preparation, the mean interval between swimming contractions was initially 2.2 sec. The effect of an evoked EP burst was to increase the interval immediately following the burst to a mean of 4.5 sec (five tests on one preparation). When swimming started again after these brief interruptions, it was resumed at the original rhythmic frequency.

Intracellular recordings from the motor giant and ring giant axons during EP bursts showed no depolarizations or hyperpolarizations attributable to epithelial input.

EP bursts caused no change in the shape of the bell reminiscent of "crumpling" in other medusae. Exumbrellar stimulation of animals in the dark resulted in no light emission.

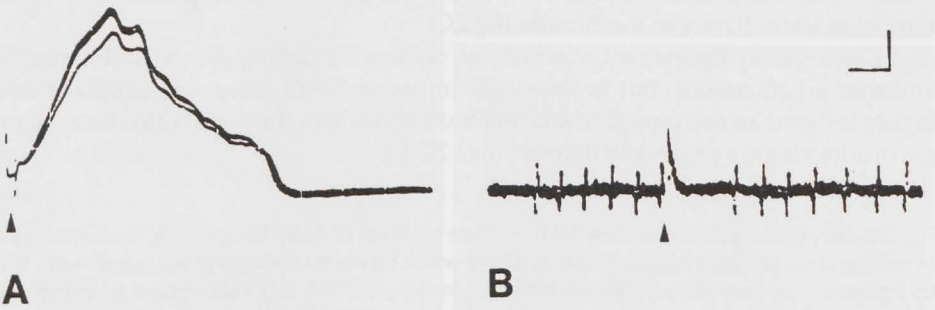


Fig. 1. Electrophysiological recordings. A. A burst of epithelial pulses recorded from exumbrellar epithelium following electrical stimulation (arrowhead) of the epithelium. B. A burst of epithelial pulses evoked by a shock (arrowhead) causes a brief pause in an established swimming rhythm, recorded as an electromyogram from the subumbrellar muscles. Scales: in A, 5 ms, 100 μV; in B, 1 sec, 50 μV. Negative is up in both figures.

Histological observations

a. Mature medusae.— The exumbrellar ectoderm is a nerve-free epithelial monolayer whose cells are interconnected by gap junctions, as described in many other hydromedusae and siphonophores (Spencer & Schwab, 1982). At the margin, the exumbrellar epithelium merges with the thicker, more complex epithelium that envelops bundles of neurons and groups of developing cnidocytes. These epithelial cells are also interconnected by gap junctions and may be assumed to carry signals from the exumbrellar epithelium to groups of ensheathed neurons, as demonstrated in *Polyorchis penicillatus* by Spencer (1981).

In medusae capable of protective involution or crumpling (e.g. species of *Stomotoca, Sarsia, Euphysa*; see Spencer & Schwab, 1982), radially arranged smooth muscle fibres occur in the subumbrellar ectoderm. These muscles are completely lacking in medusae with bell diameters of 5mm or more.

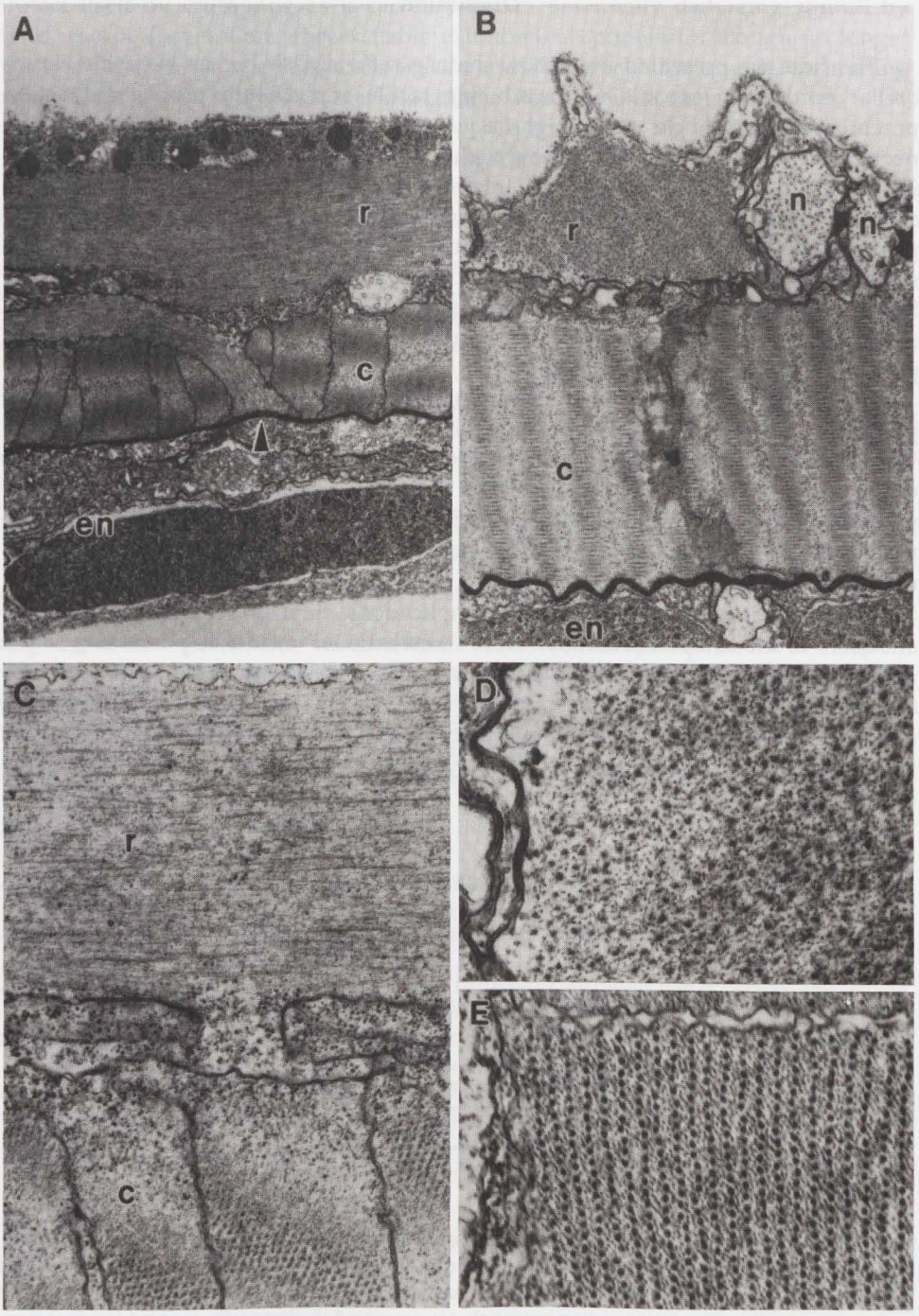
b. Immature medusae.— The actinula larva of *Aglantha digitale* develops directly to the juvenile medusa stage. The specimens examined lacked gonads but showed typical medusan morphology in major respects. Their nervous systems were already well formed, with inner and outer nerve rings and developing giant axons. In the subumbrellar ectoderm the circularly-orientated, striated, swimming muscles showed a comparable degree of differentiation to that seen in mature medusae of *Aglantha digitale.*

Overlying the circular fibres and orientated at right angles to them (i.e. in the radial direction) groups of smooth myofibres were observed (fig. 2A, B). They lie close to the nerve bundles that run up the subumbrellar ectoderm from the margin to the manubrium over the eight, radial canals (fig. 2B). From their location and structure, these muscles appear to be the equivalent of the muscles that bring about protective involution of the margin in numerous other medusae.

The radial muscle fibres are processes of a distinct set of epitheliomyocytes interspersed among the more numerous epitheliomyocytes that form the circular fibre layer. Their radial fibres penetrate the circular muscle layer in places to insert upon the mesolamella (arrowhead in fig. 2A). Where they contact the circular fibres, their membranes fold into those of the latter, an arrangement which presumably secures the radial fibres firmly to the circular (fig.2C).

Histologically the two muscle systems are readily distinguished. Both have thick and thin myofilaments, but in the cicular muscles the filaments are arranged in the highly ordered arrays typical of striated muscle while in the radial muscles they mingle in a less highly organized manner (figs 2C-E).

Fig. 2. Subumbrellar ectodermal muscles in juvenile medusae of *Aglantha digitale*. A. Section cut radially passing through radial muscle fibres (r), one of which inserts on the mesogloea (arrowhead). Circular muscle (c) lies immediately beneath the radial muscle, adjacent to the mesogloeal layer that separates the ectoderm from the endoderm (en) (Magnification × 7750). B. Section cut transversely to a radial muscle fibre (r) and radial nerve bundle (n) overlying circular muscle (c) and endoderm (en) (× 11,000). C. radial section at higher magnification showing interface between radial (r) and circular (c) muscle layers (× 25,000). D. Transverse section through radial muscle showing myofilaments (× 50,000). E. Transverse section through a portion of a striated muscle fibre showing orderly array of thick filaments (× 38,500).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Discussion

The findings presented here show that *Aglantha digitale* has an excitable exumbrellar epithelium capable of transducing tactile stimuli into propagated action potentials. Single shocks evoke high-frequency bursts of action potentials, as in the nectophores of some siphonophores (Mackie, 1978; Mackie & Carré, 1983). On reaching the margin, the action potentials inhibit swimming. Similar responses in *Polyorchis penicillatus* were found to involve direct inhibition (hyperpolarization) of the pacemaker neurons that generate swimming (Spencer, 1981). We assume that the same mechanism applies in *A. digitale* (fig. 3). Mature specimens of *A. digitale* differ however from other medusae in lacking the protective involution (crumpling) response, the effectors that bring it about, and the excitable endoderm that may form part of the pathway transmitting it.

In contrast to mature specimens, juvenile *A. digitale* just past the actinula stage possess radial muscles resembling those involved in crumpling in other medusae. While it has not been possible to record electrically from these tiny medusae and while the muscles might have some other function (e.g. in feeding) it seems highly probable that we are dealing with the typical hydromedusan crumpling effectors and that at this stage, *A. digitale* responds to tactile stimulation of the exumbrella by protective involution.

These findings have some general implications. First, it seems likely that the ancestors of the Rhopalonematidae were "conventional" medusae possessing radial muscles in the subumbrellar and protecting themselves by crumpling. Second, the absence of crumpling behaviour in mature medusae of *Aglantha digitale* suggests that during development, once the giant axon escape circuitry is fully developed and the medusa has developed its slim, streamlined adult morphology, it no longer needs to

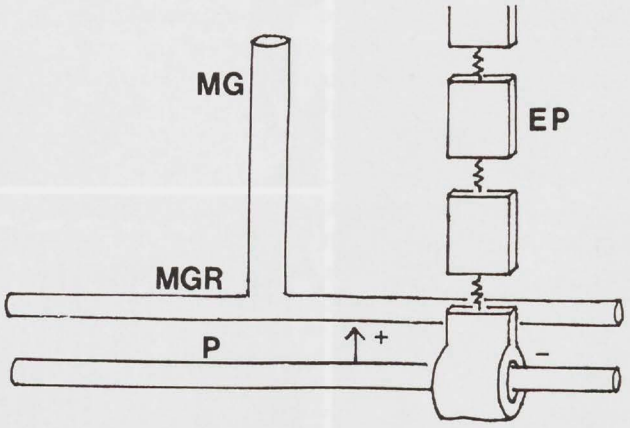

Fig. 3. Summary diagram of proposed swimming inhibition pathway. In normal slow swimming, rhythmic output of pacemaker neurons in the margin (P) excites (+) the rootlet processes (MGR) of the motor giant axons (MG). The motor giants in turn excite the swimming muscles (not shown). Following exumbrellar stimulation, action potentials are generated in electrically coupled (resistor symbol) epithelial cells (EP) and propagate to the margin where they inhibit (-) the pacemaker neurons they ensheath.

protect itself by crumpling as it can respond to potentially damaging stimuli by rapid, escape locomotion. The excitable exumbrellar epithelium, though no longer required for crumpling, continues to serve a useful role by inhibiting the swimming pacemakers when a swimming medusa collides with an object in its path.

Acknowledgements

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The hydroid colony as an organism: regulation of growth in the entire colony

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Key words: Coloniality; hydroids; integration; individuality; modular structure; organism.

Abstract: A hydroid colony has a definite plan of construction which can be conceived as a system of radial 'rays' characterized by a polarized structure. Morphological integration of a colony is expressed as the regulated proportion among the totalities of its different modules. The autoregulation of the structure of a colony is effected through interaction of: 1) branching, 2) growth rate of stolons, and 3) intensity of hydranth resorption. Physiological integration of a colony is achieved through the regular hierarchy of food distribution between the stems composing the colony. The structure of a colony has the advantage of food summation, viz. taking food from different hydranths to support growth in the most important zones. A hydroid colony is essentially a branched, multi-oral, non-centralized type of organism.

Introduction

The classical theory of coloniality has been formulated during the 19th century by E. Haeckel, H. Spencer, J. Geoffroy Saint-Hillaire, E. Perrier and others; it was elaborated theoretically by V.N. Beklemishev (1950; English version, 1970), based on the criteria of comparative anatomy.

The essential thesis of Beklemishev's theory claims that gradual increase in colonial individuality with a simultaneous decrease in individuality of the zooids. The theory finds its reflection in the hypothesis of the origin of multicellular organisms during the evolution of animals and plants. Outside biological science this paradigm has in general been used for the theoretical analysis of development of any primitive system undergoing progressive complication. However, the phenomenon of coloniality has been studied without investigating the overall colonial structure and physiology, being based mainly on its fragments such as the zooids or other characteristic parts of a colony. That method predetermined that results and conclusions were limited.

New methods have been introduced especially for the study of a complete colony, for example: "mapping" of the hydroid colonies; quantitatively controlled feeding of colonies; registering of the movements of hydroplasm in a colony. These methods have enabled us to observe in depth the vital activity of colonial hydroids. The results proved to be much more complicated than the widely accepted notion that certain aggregates of individuals are progressively integrated via compound animal colonies to form a superorganism. We can now confidently argue that the animal, having a compound, colonial body, does not represent an aggregate of individuals but rather has the status of a united organism possessing obvious indications of morphological and physiological integrity (Marfenin, 1993b).

Results

The first "maps" of various hydroid colonies revealed an obvious regularity in their general plan of construction. A hydroid colony can be represented by a system of "rays" (fig. 1), each ray consisting of a stolon with hydranth bearing stems (or stolons with hydranths, if the stems are lacking). The colonial ray has a polarized structure: the age and size of the stem increase progressively from the tip of the stolon towards a central portion of the colony.

In as much as colonies represent modular systems we have tried to study their morphological wholeness by using statistical methods of correlation between various all-colonial quantitative parameters like: number of hydranths (H) or growth tips (B) and length of hydrorhiza (L) or hydrocaulus (M) or whole hydrophyton (C= L+M). The complete colonies of several species were studied using the "mapping method" mentioned above, but most of all *Dynamena pumila* (Linnaeus, 1758), *Gonothyraea loveni* (Allman, 1859), *Laomedea flexuosa* Alder, 1856, *Obelia geniculata* (Linnaeus, 1758), *Cordylophora caspia* (Pallas, 1766) and *Cordylophora inkermanica* (Marfenin, 1983). Each time we found a signi-

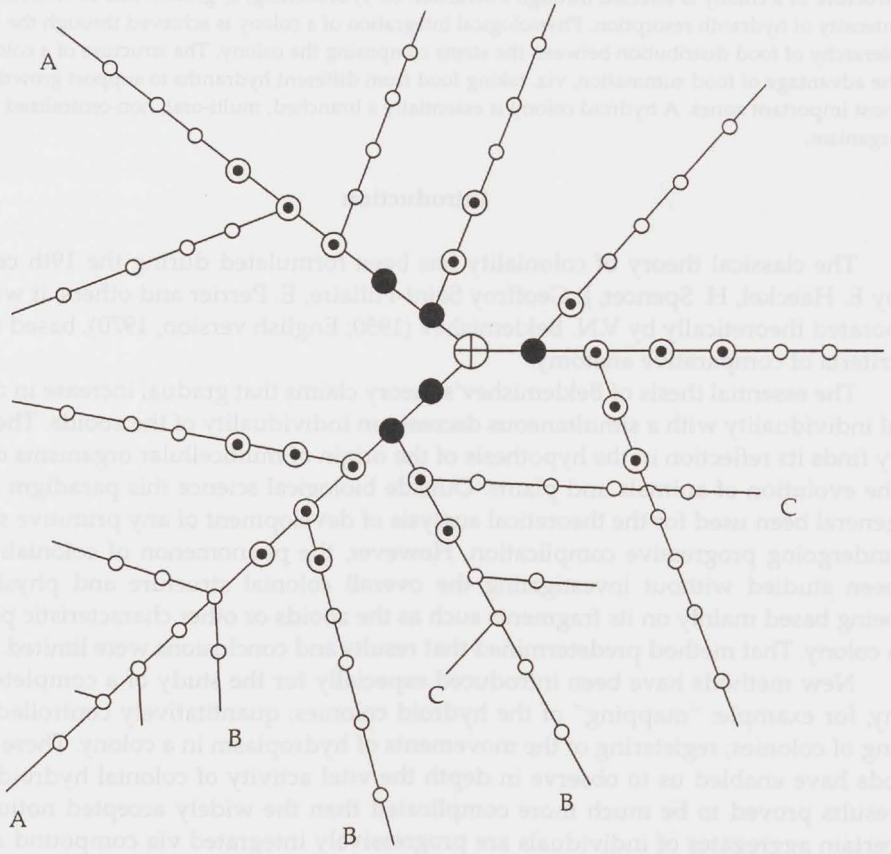

Fig. 1. Framework of a hydroid colony. ⊕ = first stem or hydranth; ● = stalks and hydranth under regression; ⊙ = stalks (or hydranths) with gonangia; ○ = growing stalks (or hydranths) without gonangia. Lines indicate stolons. A, first order rays of colony; B, second order rays; C, third order rays.

ficant correlation between the above named quantitative all-colonial parameters (table 1), taking for study specimens collected from one site and on the same date.

Table 1. Mean coefficients of correlation (CC) between different modules of colonies of *Dynamena pumila*, n = 88; and mean coefficients of variation (CV) for corresponding "pattern parameters; 16 sets from 134 colonies. B = number of growth tips; H = number of hydrants; L = length of hydrorhiza.

Primary parameters		CC	Pattern parameters	CV (%)
H	B	0.913	H/B	20.3
H	L	0.884	H/L	29.4
B	L	0.961	B/L	21.9

Fulton (1960), Braverman (1963) and Simkina (1963) were the first who have drawn attention to the similar effect in *Cordylophora lacustris* Allman, 1844 [= *Cordylophora caspia* (Pallas, 1766)] and *Podocoryna carnea* M. Sars, 1846. Fulton (1963) and Braverman (1978) unsuccessfully tried to make a model of a growing colony based on the assumption of constant rates of growth and branching of all stems and stolons. However, if a colony grows as an aggregate of quite independent components the ration between modules should change continually. In fact the intermodular ratio's are remaining considerably stable for both statistically studied sets and for the dynamics of life in individual colonies. Therefore Marfenin (1977) introduced the "colonial structure (or pattern) parameters" (H/B; H/L; B/L, and so on) for the investigation of the wholeness of a colony its life dynamics, as well as for the comparison of colonies of different sizes. Pattern parameters could be successfully used for the characterization of: 1) the functional state of a colony; 2) reaction of the colony to environmental conditions such as temperature or food availability.

After being artificially altered in experiments the colony pattern parameters soon resumed to their initial value as a sign of self-regulatory processes within the colony (Marfenin, 1977).

Another experiment has permitted the detection of interrelation between morphological and physiological integration of a colony, leading towards the discovery of growth regulation of the entire colony. Using a method of controlled feeding (Marfenin et al., 1979), every colony was provided with a fixed number of *Artemia* nauplii/day. Three rations were chosen: 1, 5, and 10 nauplii/day in an experiment with *Dynamena pumila*. the experiments show that if the colony does not get a sufficient amount of food, only the youngest stems (hydrocauli) continue to grow, although food is still being supplied to the old stems located furthest from the growing tips (fig. 2).

Consequently, distribution of food occurs throughout the entire colony. The tip of an old stem close to the place of feeding will not grow if there is not a sufficient amount of food for the entire colony. The number of non-growing stems in the older part of a colony increases if the amount of food decreases, suggesting that the number of growing stems is proportional to the amount of food. With the lowest ration (1 nauplius/day) only the tip of the stolons and the stem closest to it continue to grow, the stems farther away grow in relation of their distance from the tip as adequate amounts of food for their growth are supplied. The oldest stems in the colony will grow last, when all others have been provided with food.

A colony thus concentrates the food it receives into certain zones that are particu-

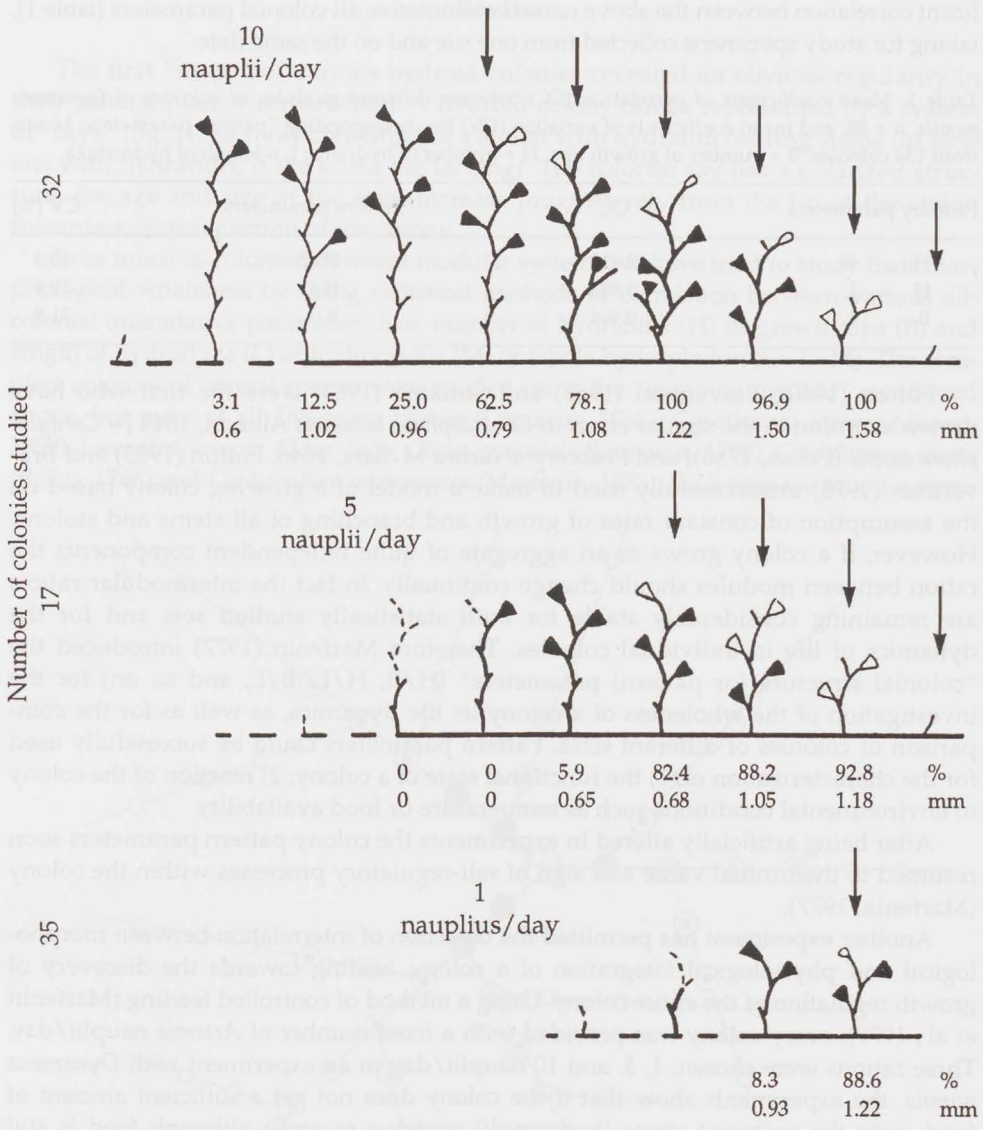

Fig. 2. Growth of *Gonothyraea loveni* colonies under three different, controlled rations: 1, 5, or 10 nauplii/day. Food was given to proximal (oldest) stems. Arrows indicate growing tips. Upper row indicates percentages of growing stems on the corresponding position relative to the growth tip of a stolon; lower row gives mean data of two-days increase of stem (in mm) on that position (data based on number of studied colonies indicated to the left of the three rows). Dotted lines indicate resolved parts of a colony.

larly beneficial for growth. In other words, the structure of a colony has the advantage of "food summation" - taking food from different hydranths to support growth in the most important zones. The transport system is capable of realizing the physiological integration of the entire colony (Marfenin, 1993b). Semi-digested food is col-

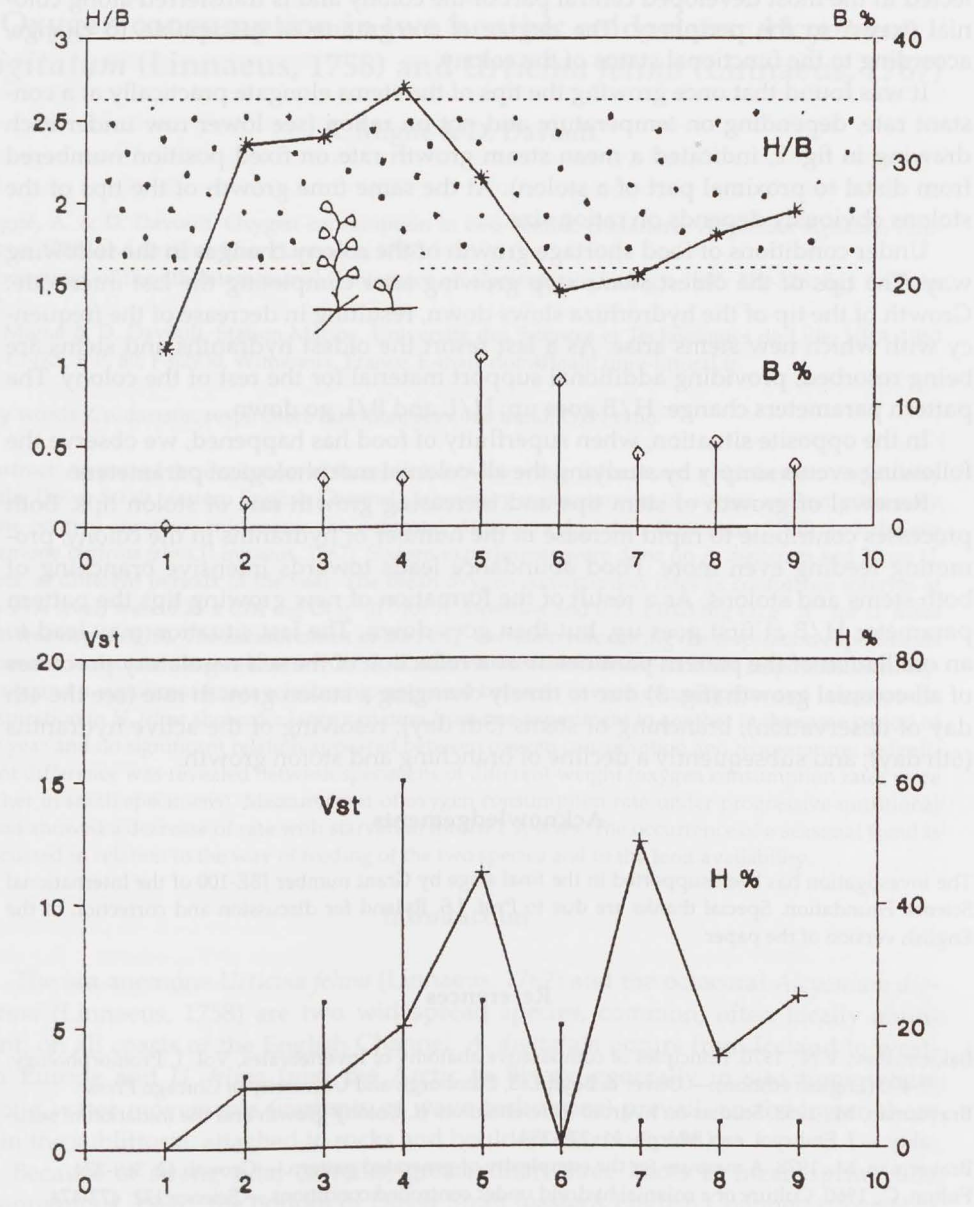

Fig. 3. A case of autoregulation showing the proportional relationship between the parts of a colony of *Obelia geniculata*. Oscillations of the pattern parameters into some narrow limits are ensured by timely reaction of: stolons and stems branching; stolons growth rate; and dedifferentiation of some hydranths. H/B = number of active hydranths (H) to the number of all growing tips in a colony (B); B% = branching rate as a percentage of new branches to all existing before; H% = percentage of active hydranths to all hydranths including resolved one; Vst = mean 4 days increase of stolons in mm; dotted area = normal value of H/B, as the main pattern parameter of colony structure. Abscissa indicates days after beginning of experiment.

lected in the most developed central part of the colony and is transferred along colonial "rays" to the periphery. The degree of integration is susceptible to change, according to the functional status of the colony.

It was found that once growing the tips of the stems elongate practically at a constant rate, depending on temperature and not on ration (see lower row under each drawing in fig. 2, indicated a mean steam growth rate on fixed position numbered from distal to proximal part of a stolon). At the same time growth of the tips of the stolons obviously depends on ration size.

Under conditions of food shortage growth of the colony changes in the following way: The tips of the oldest stems stop growing after completing the last internode. Growth of the tip of the hydrorhiza slows down, resulting in decrease of the frequency with which new stems arise. As a last resort the oldest hydranths and stems are being resorbed, providing additional support material for the rest of the colony. The pattern parameters change: H/B goes up; H/L and B/L go down.

In the opposite situation, when superfluity of food has happened, we observe the following events simply by studying the all-colonial morphological parameters:

Renewal of growth of stem tips and increasing growth rate of stolon tips. Both processes contribute to rapid increase in the number of hydranths in the colony, promoting feeding even more. Food abundance leads towards intensive branching of both stems and stolons. As a result of the formation of new growing tips the pattern parameter H/B at first goes up, but then goes down. The last situation may lead to an oscillation of the pattern parameters as a reflection of the self regulatory processes of all-colonial growth (fig. 3) due to timely changing a stolon growth rate (see the 4th day of observation); branching of stems (5th day); resolving of the active hydranths (6th day); and subsequently a decline of branching and stolon growth.

Acknowledgements

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Oxygen consumption in two benthic cnidarians: *Alcyonium digitatum* (Linnaeus, 1758) and *Urticina felina* (Linnaeus, 1767)

A. Migné & D. Davoult

Migné, A. & D. Davoult. Oxygen consumption in two benthic cnidarians: *Alcyonium digitatum* (Linnaeus, 1758) and *Urticina felina* (Linnaeus, 1767).
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Key words: Cnidarians; respiration; nutrition; seasonal trend; O/N ratio.

Abstract: As part of the evaluation of fluxes between the water column and a rich benthic community of the Dover Strait (eastern English Channel), laboratory measurements of oxygen consumption were done on two common cnidarians: the octocoral *Alcyonium digitatum* (Linnaeus, 1758) and the sea anemone *Urticina felina* (Linnaeus, 1767). Sixteen experiments were done on *A. digitatum* and 20 on *U. felina*, at different periods of the year. The mean oxygen consumption rates (± confidence interval at the 95% level) were 0.21 ± 0.04 mg $O_2.g^{-1}.h^{-1}$ for *A. digitatum* and 0.13 ± 0.03 mg $O_2.g^{-1}.h^{-1}$ for *U. felina* (ash free dry weight). The measurement of the NH_4^+ excretion rate during the respiration experiments allowed to calculate the atomic ratio (O/N). A seasonal trend was revealed in the respiration of *A. digitatum* and a significant linear relationship appeared between oxygen consumption and temperature. Respiration in *U. felina* showed a large variation from one experiment to another in the same period of the year and no significant relation appeared between oxygen consumption and temperature; a significant difference was revealed between specimens of different weight (oxygen consumption rates were higher in small specimens). Measurement of oxygen consumption rate under progressive nutritional stress showed a decrease of rate with starvation for the 2 species. The occurrence of a seasonal trend is discussed in relation to the way of feeding of the two species and to the food availability.

Introduction

The sea anemone *Urticina felina* (Linnaeus, 1767) and the octocoral *Alcyonium digitatum* (Linnaeus, 1758) are two widespread species, common, often locally abundant, on all coasts of the English Channel. *A. digitatum* occurs from Iceland to western Europe and *U. felina* from the Arctic to Biscay, generally in situations where strong water movements (currents or wave turbulence) prevail, on the lower shore or in the sublittoral, attached to rocks and boulders (Manuel, 1981).

Because of strong tidal currents, greater than three knots in mean spring tide (Anonymous, 1988), the bottom of Dover Strait (eastern English Channel) consists of coarse sediment (Larsonneur et al., 1982) which is colonized by a sessile epifauna community (Cabioch & Glaçon, 1975). As part of the evaluation of fluxes between the water column and this community, experimental measurements of oxygen consumption were done on these two species that account for a large part of the biomass. *U. felina* and *A. digitatum* account respectively for 32% and 5% of the mean biomass of 270 $g.m^{-2}$ in ash free dry weight (Migné & Davoult, 1995).

Nothing is known about the minimum need of oxygen of these two species. Since they are sessile and have a low activity, they are supposed to have a relatively low rate of metabolism. A study of oxygen consumption in twelve zooxanthellate species

of Alcyonaria from a coral reef in southern Florida (Cary, 1918) also revealed a very low metabolic rate, though it varied considerably in the different species.

In this study, we intended to determine an order of magnitude of oxygen consumption of the two cnidarians by conducting laboratory measurements, taking into account the influence of season, water temperature, size and nutritional state of organisms. Simultaneous measurement of ammonia excretion allowed to calculate the O/N ratio for the two species.

Materials and methods

Specimens of *Urticina felina* and colonies of *Alcyonium digitatum* were collected (with their substrate) by divers in Dover Strait at 37 m depth. After collection, animals were maintained in the laboratory in circulating sea water. Respiratory rates were measured in the few following days. Measurements were carried out between February 1993 and April 1995.

In each experiment, a specimen of *U. felina* or a colony of *A. digitatum* with its substrate (stripped of other sessile animals) was kept for 6 hours in a tight chamber of 2.5 l capacity, where a magnetic stirrer ensured satisfactory mixing, and placed inside an aquarium with running sea water in order to buffer temperature changes. Continuous registration of the oxygen concentration in the chamber was ensured by means of an oxygen ($\pm 10^{-2}$ mg.l^{-1}) and temperature ($\pm$ 0.1°C) probe (dissolved oxygen meter: YSI model 58) connected to a recorder. Filtered sea water (filtration on glass microfibre filters Whatman GF/C, porosity $\approx$ 1 µm) was used to avoid contamination of the medium by organic matter or bacteria. Temperature in the tight chamber was almost the same as in the field at the time of sampling. To ensure maximum oxygen saturation at the beginning of the incubation, the filtered sea water was aerated. Animals were allowed to acclimate for half an hour during open circuit flushing between the chamber and a tank.

In order to test the influence of nutritional state on respiration rates of the two cnidarians, oxygen consumption rates were examined as a function of progressive nutritional stress. Incubations were performed on laboratory fed specimens the day of nutrition and were repeated 1, 4 and 8 days later. *A. digitatum* was fed with living nauplii of *Artemia* spec. and *U. felina* with fresh mussel.

Sampling of water was done at the beginning and at the end of each experiment to measure the ammonia (NH_4^+) production in order to calculate the atomic ratio of consumed oxygen and excreted ammonia nitrogen which is an indicator of catabolic substrate (Harris, 1959). When an organism is exclusively oxidizing protein, the O/N ratio will be low, less than 7; it will be high when either fat or carbohydrate is oxidized. Ammonia content was measured according to Koroleff's method (1970). For each experiment the O/N ratio was calculated first; the average O/N for each species was subsequently estimated according to the rules for ratio variables (average $O/N = \Sigma O/\Sigma N$; Scherrer, 1984).

Biomass was measured as ash free dry weight (AFDW) as recommended by the Benthos Ecology Working Group of ICES (Anonymous, 1986). Specimens were dried for 96 h at 60°C ($\pm$ 1°C) until stabilization of weight ($\pm$ 0.1 mg), burned for 6 h at 520°C ($\pm$ 20°C) and the ashes weighed. The difference between the weight before and after burning gave the AFDW of the specimen.

Results

The mean oxygen consumption rates (± confidence interval at the 95% level) were 0.21 ± 0.04 mg $O_2.g^{-1}.h^{-1}$ for *Alcyonium digitatum* (n = 16 experiments; table 1) and 0.13 ± 0.03 mg $O_2.g^{-1}.h^{-1}$ for *Urticina felina* (n = 20 experiments; table 2).

A seasonal trend appeared in the respiration of *A. digitatum*: the oxygen consumption rate gradually increased from February (0.09 mg $O_2.g^{-1}.h^{-1}$) to June-July (0.36 mg $O_2.g^{-1}.h^{-1}$), then it decreased to November (0.16 mg $O_2.g^{-1}.h^{-1}$). A sinusoïdal model was fitted to the data (fig. 1), parameters were estimated by the Simplex method:

$$y = 0.207 - 0.097 \sin [(2\pi / 365) x + 1.432]; (n = 16, r^2 = 0.961) \quad (1)$$

where x = Julian days and y = O_2 consumption in $mg.g^{-1}.h^{-1}$.

This seasonal trend followed the temperature variations; there is a significant linear relation between oxygen consumption and temperature:

$$y = 0.016 x + 0.019; (r = 0.669, n = 16, p = 0.005) \quad (2)$$

where x = temperature in °C and y = O_2 consumption in $mg.g^{-1}.h^{-1}$ (fig. 2).

The colonies of *A. digitatum* used in this study had nearly the same weight (AFDW = 1.91 ± 0.31 g; table 1). In one experiment with a small colony (AFDW = 0.31 g), the oxygen consumption rate appeared much higher (0.525 mg $O_2.g^{-1}.h^{-1}$).

No seasonal trend appeared in the respiration rate of *U. felina*. Results showed considerable variation from one experiment to another in a same period of the year (fig. 3) and no significant relation appeared between oxygen consumption and temperature.

Table 1. *Alcyonium digitatum*. Laboratory measurements of oxygen consumption: date of measurement, temperature in experimental chamber (°C), biomass of colony (g of AFDW), oxygen consumption rate (mg $O_2.g^{-1}.h^{-1}$), ammonia production rate (µg $N.g^{-1}.h^{-1}$) and atomic ratio of oxygen consumed to ammonia-nitrogen excreted (O/N).

Date	T°C	AFDW (g)	mg O_2/g/h	µg N/g/h	O/N
22.03.93	9.0	1.11	0.23	9.57	10.56
05.04.93	9.5	1.71	0.14	9.08	6.5
25.06.93	16.0	1.83	0.36	15.43	10.07
25.11.93	10.5	1.76	0.17	8.30	9.12
01.02.94	9.0	2.70	0.11	3.72	12.35
04.02.94	9.0	2.54	0.11	4.40	10.94
25.02.94	7.7	1.83	0.09	3.65	10.91
02.03.94	8.0	1.54	0.22	10.36	9.42
11.03.94	8.7	2.09	0.19	9.03	9.06
08.05.94	12.2	3.42	0.22	22.27	4.26
09.06.94	13.7	1.57	0.31	24.96	5.42
05.07.94	16.9	1.80	0.33	31.86	4.57
15.09.94	17.4	2.06	0.20	8.16	10.45
14.10.94	15.1	1.66	0.26	18.76	6.11
29.11.94	14.0	1.11	0.16	8.60	8.29
10.04.95	11.6	1.82	0.23	14.22	6.98

Table 2. *Urticina felina*. Laboratory measurements of oxygen consumption: date of measurement, temperature in experimental chamber (°C), biomass of specimen (g of AFDW), oxygen consumption rate (mg $O_2.g^{-1}.h^{-1}$), ammonia production rate (µg $N.g^{-1}.h^{-1}$) and atomic ratio of oxygen consumed to ammonia-nitrogen excreted (O/N).

Date	T°C	AFDW (g)	mg O_2/g/h	µg N/g/h	O/N
09.02.93	9.5	7.68	0.07	8.18	3.85
23.03.93	9.1	2.78	0.08	12.52	2.73
24.03.93	9.5	9.85	0.08	7.89	4.55
06.04.93	9.8	5.10	0.06	6.69	3.79
07.04.93	9.6	1.15	0.18	26.49	2.89
28.06.93	16.6	5.38	0.13	7.84	7.42
01.07.93	17.0	5.12	0.09	6.99	5.76
26.11.93	9.8	0.77	0.21	13.41	6.95
29.11.93	9.4	0.77	0.19	12.46	6.74
14.03.94	9.0	9.92	0.05	5.29	3.72
16.03.94	8.8	0.57	0.14	10.59	5.66
07.07.94	17.0	5.00	0.11	12.90	3.63
08.07.94	17.2	9.24	0.09	8.70	4.53
19.09.94	16.3	5.67	0.09	6.97	5.84
20.09.94	16.2	0.93	0.25	12.75	8.54
19.10.94	14.8	0.71	0.34	35.05	4.21
01.12.94	14.4	6.67	0.09	6.42	6.13
02.12.94	14.4	5.22	0.10	7.97	5.71
11.04.95	12.1	3.10	0.15	18.64	3.61
12.04.95	11.4	4.78	0.12	12.45	4.22

For this species, specimens of different weight were used in experiments; in the same period of sampling, the smaller specimens always showed a higher respiration rate (table 2). The mean oxygen consumption rate was 0.094 ± 0.017 mg $O_2.g^{-1}.h^{-1}$ for specimens of AFDW > 2 g (n = 14) and 0.217 ± 0.072 mg $O_2.g^{-1}.h^{-1}$ for specimens of AFDW < 2 g (n = 6). The test of comparison of means (Student's t-test) showed a significant difference at the 0.1% level. Two experiments were done on specimens of AFDW < 0.5 g: the results were 0.635 mg $O_2.g^{-1}.h^{-1}$ for a specimen of 0.17 g and 0.867 mg $O_2.g^{-1}.h^{-1}$ for a specimen of 0.46 g.

Oxygen consumption rate measured on a colony of *A. digitatum* (1.49 g) after it was fed was high (0.423 mg $O_2.g^{-1}.h^{-1}$) and gradually decreased in following days (0.398 mg $O_2.g^{-1}.h^{-1}$ the next day, 0.329 mg $O_2.g^{-1}.h^{-1}$ four days later and 0.255 mg $O_2.g^{-1}.h^{-1}$ eight days later; fig. 4).

In a specimen of *U. felina* (5.04 g) which had been kept in

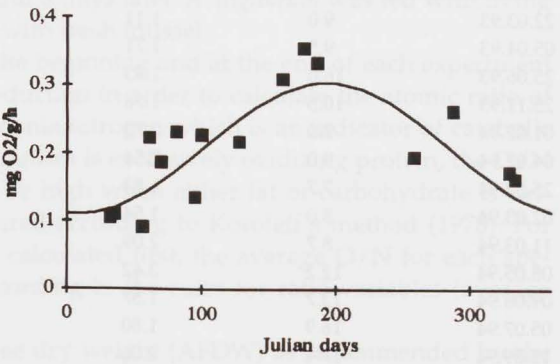

Fig. 1. *Alcyonium digitatum*. Laboratory measurements of oxygen consumption (mg $O_2.g^{-1}.h^{-1}$) at different periods of the year (Julian days) and calculated sinusoïdal function: $y = 0.207 - 0.097 \sin [(2\pi / 365) x + 1.432]$.

laboratory for one month without being fed, the oxygen consumption rate was low (0.096 mg $O_2.g^{-1}.h^{-1}$); it was much higher after the specimen was fed (0.151 mg $O_2.g^{-1}.h^{-1}$), and it decreased in the following days (0.124 mg $O_2.g^{-1}.h^{-1}$ the next day, 0.123 mg $O_2.g^{-1}.h^{-1}$ four days later and 0.119 mg $O_2.g^{-1}.h^{-1}$ eight days later; fig. 4).

Measurement of the NH_4^+ excretion rate during the respiration experiments allowed the calculation of the average atomic ratio (O/N), which was 6.47 for *A. digitatum* and 4.42 for *U. felina*.

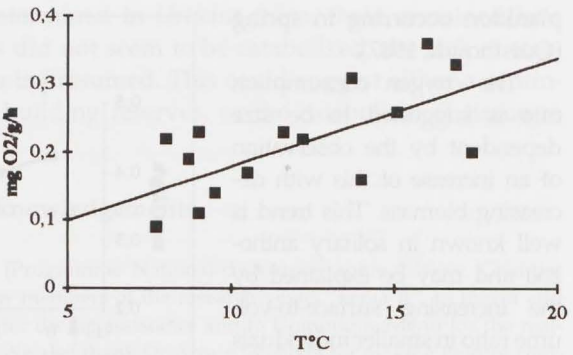

Fig. 2. *Alcyonium digitatum*. Laboratory measurements of oxygen consumption (mg $O_2.g^{-1}.h^{-1}$) plotted against temperature (T°C), and regression line: $y = 0.016 x + 0.019$.

Discussion

The present survey allows an estimate of the mean oxygen consumption rate for the two cnidarians *Alcyonium digitatum* and *Urticina felina*. These rates are probably underestimated because measurements were made a few days after sampling, i.e. after a few days of starvation. Decrease of oxygen uptake with starvation has already been mentioned for cnidarians (Svoboda & Porrmann, 1980).

Being a suspension feeder, *A. digitatum* feeds almost continuously and it may therefore be assumed that the nutritional state of collected colonies was the same. Unlike *A. digitatum*, *U. felina* does not only feed on plankton, but captures preys discontinuously (Sebens, 1981). Thus, the heterogeneity observed in measurements (not always correlated with variation of size) may in the latter species be explained by variations in nutritional state of collected specimens.

Repetitive measurements through the year showed a seasonal trend in oxygen consumption rate, correlated with temperature, for *A. digitatum*. This seasonality (with a maximum occurring in summer) could also be related to variations in food availability. Though it has been proven capable to feed on phytoplancton (Roushdy & Hansen, 1961), *A. digitatum* mainly feeds on zooplankton (Pratt, 1905; Matthews, 1917) and the greatest concentrations of zooplankton (Le Fèvre-Lehoërff et al., 1983) follow the great concentrations of phyto-

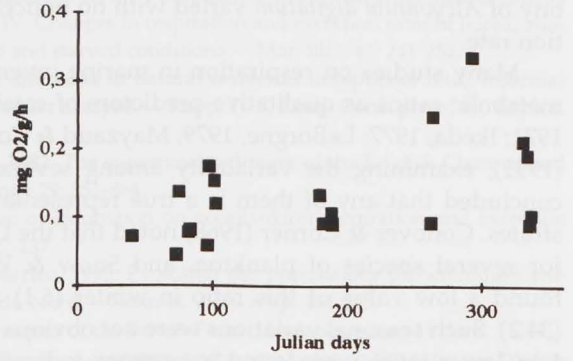

Fig. 3. *Urticina felina*. Laboratory measurements of oxygen consumption (mg $O_2.g^{-1}.h^{-1}$) at different periods of the year (Julian days).

plankton occurring in spring (Quisthoudt, 1987).

The oxygen consumption rate is suggested to be size dependent by the observation of an increase of this with decreasing biomass. This trend is well known in solitary anthozoa and may be explained by the increasing surface-to-volume ratio in smaller individuals (Svoboda & Porrmann, 1980). Variation in the rate of oxygen consumption by unit of weight between small and large colonies is also noticed in pennatulids (Brafield & Chapman, 1965; Brafield & Chapman, 1967; Chapman, 1972) and may be explained by a higher number (biomass) of polyps compared with the living rest of colony (coenenchym which is probably less active in metabolism) in the small colonies.

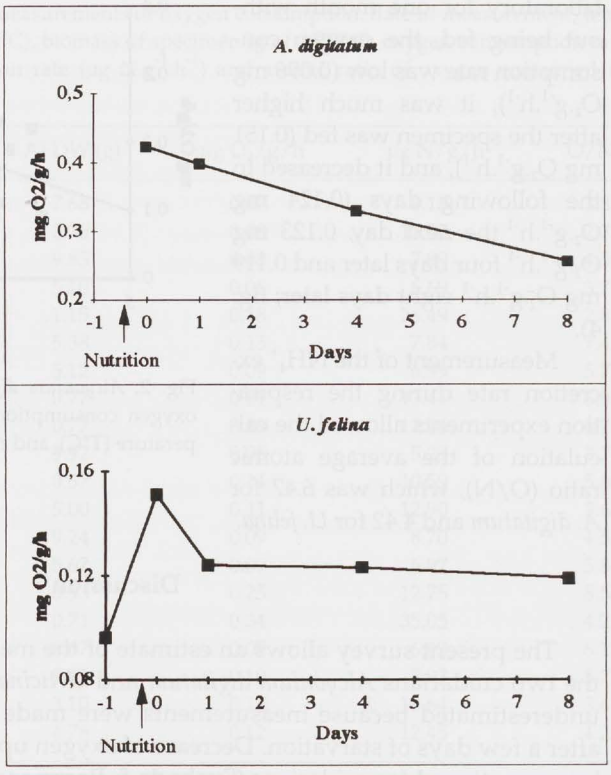

Fig. 4. *Alcyonium digitatum* and *Urticina felina*. Laboratory measurement of influence of feeding on oxygen consumption (mg $O_2.g^{-1}.h^{-1}$).

In Alcyonaria, the surface in contact with water varies greatly with the state of contraction and expansion of the colony, and as a consequence one would expect variation in oxygen consumption rate (Brafield & Chapman, 1965 ; Brafield & Chapman, 1967; Chapman, 1972). However, during the 6 hours of our experiments, the state of contraction/expansion of the colony of *Alcyonium digitatum* varied with no noticeable effect on the oxygen consumption rate.

Many studies on respiration in marine invertebrates have used excretion-based metabolic ratios as qualitative predictors of catabolic substrates (Snow & Williams, 1971; Ikeda, 1977; LeBorgne, 1979; Mayzaud & Conover, 1988). Nevertheless, Hatcher (1991), examining the variability among several excretion-based metabolic ratios, concluded that any of them is a true representation of the nature of catabolic substrates. Conover & Corner (1968) noted that the O/N ratio varies on a seasonal basis for several species of plankton, and Snow & Williams (1971), studying a prawn, found a low value of this ratio in winter (6.1) and a high value in early summer (34.2). Such seasonal variations were not obvious in our experiments. But in *A. digitatum*, lower ratios were found in summer, indicating that protein is probably catabolized during this period, and higher ratios in winter, suggesting that a part of the reserves as either carbohydrates or fat or both is being utilized. No seasonal trend

was revealed in the O/N ratios determined in *Urticina felina*; these remained low, nearly always less than 7. As lipids did not seem to be catabolized, the lack of storage processes to build lipid reserves is presumed. This could suggest either a continuous food supply and no need of building reserves, or the catabolism of tissue to survive starvation.

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Anthozoan bleaching on the southeastern coast of Brazil in the summer of 1994

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Key words: Cnidaria; Anthozoa; *Mussismilia hispida*; *Palythoa caribaeorum*; bleaching; temperature stress; Brazil.

Abstract: The first bleaching event observed in the southeastern coast of Brazil occurred in the summer of 1994. Most of the colonies of the scleractinian coral *Mussismilia hispida* (Verrill, 1868) and of the zoanthid *Palythoa caribaeorum* (Duchassaing & Michelotti, 1860) became white or pale. Completely discoloured colonies of *M. hispida* were tagged and monitored. Except for one dead colony, all colonies had recovered (61.7%) or were in the process of recovery (36.2%) in June; the latter had completely recovered by November. Part of the bleached colonies of *P. caribaeorum* died, but most regained colour, with no sign of bleaching by late November. The bleaching occurred after some days of high sea surface temperature, 2.4°C above the long term average (1980-1993) for February.

Introduction

Cnidaria bleaching, the loss of endosymbiotic zooxanthellae, has been recorded frequently and increasingly in all tropical oceans, especially since the middle 1980s (Williams & Bunkley-Williams, 1990; Goreau & Hayes, 1994). There is strong evidence that prolonged seawater warming is the primary cause of bleaching (Glynn & D'Croz, 1990; Goreau & Hayes, 1994). The resulting mortality of the bleached animals may lead to a significant reduction in species number and density (Warwick et al., 1990).

During February 1994, shallow water cnidarians from the southeastern coast of Brazil, between Parati (23°11'S 44°36'W), Rio de Janeiro State, and Bertioga (23°49'S 45°30'W), São Paulo State, bleached suddenly, after a period of high sea surface temperature (SST). The species affected were the scleractinians *Mussismilia hispida* (Verrill, 1868) and *Madracis decactis* (Lyman, 1859), and the zoanthids *Palythoa caribaeorum* (Duchassaing & Michelotti, 1860), *Zoanthus sociatus* (Ellis, 1767) and *Z. solanderi* (Le Sueur, 1817). This paper describes this event and the recovery of *Mussismilia hispida*.

The phenomenon in Brazilian waters seems to be recent and not extensive. Bleaching was also observed in early 1994 on the coast of Pernambuco (Elga Mayal personal communication) and in the Abrolhos, Bahia (Clovis B. Castro, personal communication; see also Goreau & Hayes, 1995).

Materials and Methods

Observations were mainly made at the São Sebastião Channel between February, shortly after bleaching was noticed, and December 1994. Occasional observations were

also made in other areas, viz. on Ilha de São Sebastião and in the vicinity of Bertioga, Ubatuba and Parati (fig. 1).

In São Sebastião, tides are semi-diurnal, with a mean range of about 0.6 m. The climate is tropical, with a dry season in winter. Mean annual rainfall is 2,500 mm and mean air temperature is 24.4°C. The São Sebastião Channel is lined with small sandy beaches and rocky shores of large boulders and rock faces, with smooth or moderately steep slopes down to 4-10 m.

Mussismilia hispida, Palythoa caribaeorum, Zoanthus sociatus and *Z. solanderi* are common in the entire area. *Madracis decactis* is abundant in Parati, but absent or scarce in the other sites mentioned. *P. caribaeorum* covers considerable areas of the rocky coast, from the infralittoral fringe down to about 3 m. *M. hispida* occurs from 1 to 10 m deep, not covering significant areas of rocky substrate (personal observation).

On the southern portion of Ponta do Jarobá, all colonies of *Mussismilia hispida* more than 3 cm in diameter were recorded according to the following categories: 1) normal; 2) pale (lighter in colour than 'normal' or with different degrees of discolouration, including patches of white); 3) white (living tissue uniformly colourless), and 4) recently dead (with a bare skeleton and almost no overgrowth). Forty-seven white colonies of *M. hispida*, more than 5 cm in diameter, at Ponta do Jarobá and Ponta do Baleeiro, were tagged and monitored at about fifteen day intervals, from March to September 1994, and monthly from October 1994 to March 1995, and classified

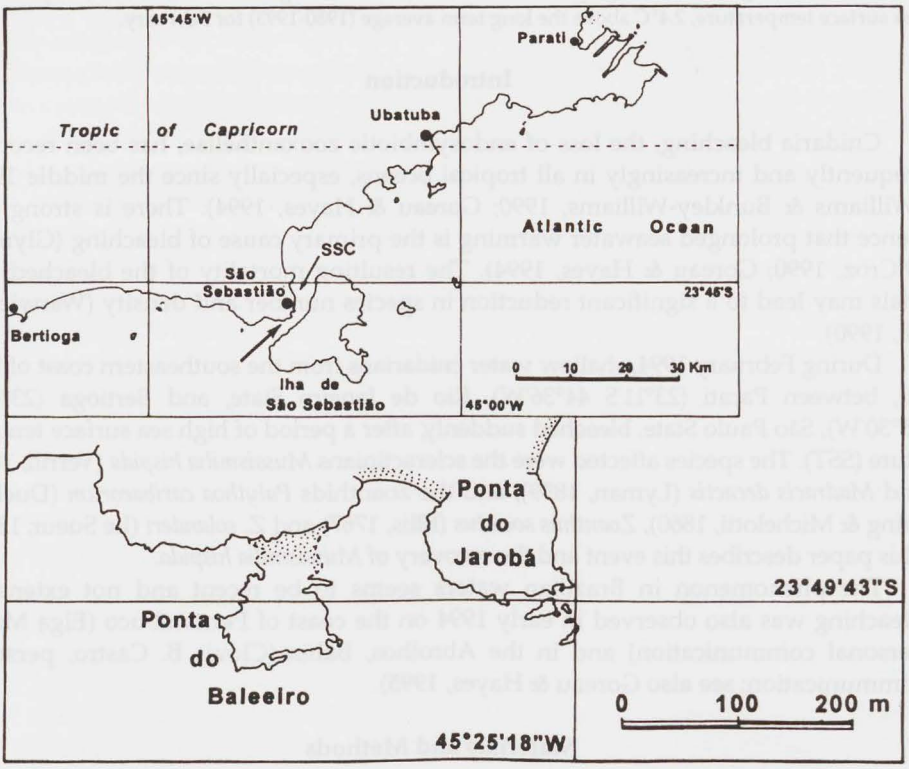

Fig. 1. The study site and the entire area affected by the bleaching event (inset) in the southeastern coast of Brazil. The arrow (inset) indicates the study site location. SSC = São Sebastião Channel.

according to the above categories. Bleaching extent in *Palythoa caribaeorum* was estimated, as percentage cover, with a 1 m^2 quadrate placed in areas occupied by these colonies. In situ sea surface temperature (SST) records are those from the Centro de Biologia Marinha, from Ponta do Jarobá, available since 1979; the data set consisted of 3-4 readings per week until 1985, after which it became daily. In situ data were compared to mean monthly SST, from maps issued by the National Oceanic and Atmospheric Administration-NOAA, based on data from satellite and oceanographic vessels (partially published by Goreau & Hayes, 1995).

Results

In the São Sebastião Channel, bleaching was first observed by the end of February. The species most affected were *Mussismilia hispida* and *Palythoa caribaeorum*; *Zoanthus sociatus* and *Z. solanderi* bleached, but much less. *Madracis decactis* was seen bleached only in São Sebastião. Bleaching occurred suddenly and simultaneously; in the case of *M. hispida* and *P. caribaeorum* it did not progress after the beginning of the observations in pale specimens. There were bleached and unbleached colonies of both species in shaded or lighted areas, as well as in shallow (near tidal level) and deeper water (3-5 m).

Mussismilia hispida.— Of the 118 colonies found in the Ponta do Jarobá census, 95 (80.5%) were affected, of which 62 (52.5%) were completely white and 33 (28%) pale. Only one colony was found recently dead. Mortality or necrosis were not seen at this time in bleached colonies of adjacent areas.

The first sign of recovery appeared at the end of March: one of the 47 tagged colonies regained colour and two were reacquiring normal colour (fig. 2). Recovery increased rapidly in the following months. In June there were no white colonies: 29 (61.5%) had fully recovered and 17 (36.2%) were recovering; one dead colony was found in May. By July, 74.5% of the surviving colonies had recovered. From this month on recovery rates decreased, and it was not until November that all colonies were again normal.

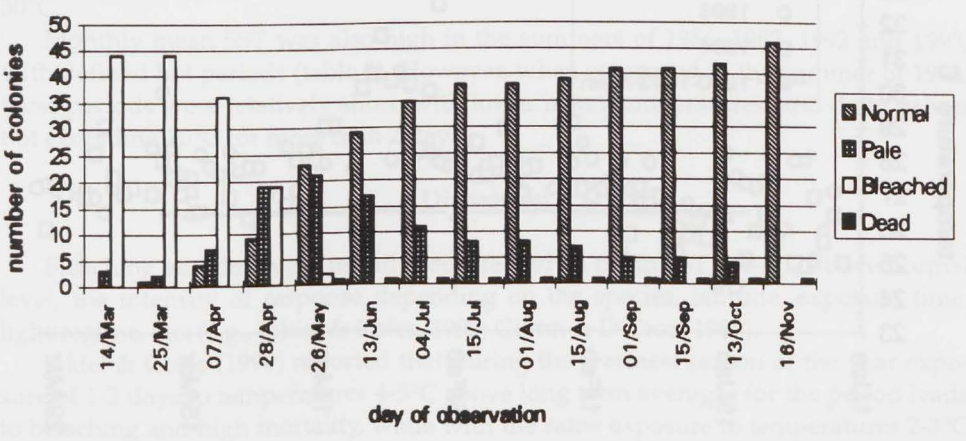

Fig. 2. Recovery of tagged bleached colonies of *Mussismilia hispida* from the São Sebastião Channel, after the bleaching in February 1994 on the southeastern coast of Brazil.

Recovery usually progressed from the periphery of the colony towards the centre; in one colony pigmentation appeared simultaneously on the lateral and upper surfaces. Of all the tagged colonies, only two had suffered partial necrosis, noticed in the beginning of August, each having lost c. 30 and 10% of their tissues. One month later, the bare parts were overgrown by algae and the dead surface area had increased to c. 40 and 20%, respectively.

Palythoa caribaeorum.— About 60% of the colonies at the São Sebastião Channel were affected; most were blotched with white and normal areas in a mosaic pattern. Some colonies bleached more in the centre, remaining normal in the periphery, and vice-versa, but there were also white and entirely normal colonies. The affected polyps usually had a thin layer of sediment, and were often retracted and not as turgid as the healthy ones.

Necrosis was observed in the beginning of April, usually in the peripheral parts of large bleached portions. At this time, signs of recovery were also seen in the periphery of the affected areas. In July, bleached portions with live polyps were seen torn off from rocks after storms.

Large bleached portions were present in the colonies until September/October, but most had recovered by the end of December. A small, partially bleached colony, collected on 14 April and kept in running seawater aquarium under diffuse day light and ambient temperature (24-27°C), recovered completely within 15 days.

Most of the colonies from the infralittoral fringe of Ponta do Baleeiro did not bleach, but a considerable number of bleached colonies were seen just below the infralittoral fringe, clearly showing the boundary between the intertidal and sublittoral areas. Temperatures of three intertidal colonies were monitored during exposure at low spring tides in March and April. Due to the protected condition of the site, and the calm weather, the animals were only wet by splash water for a few minutes between emergence and submergence. In this period, marked by relatively cloudless days, the exposed animals were subject to strong and direct solar radiation, for 1.5-2 hours, approximately between 9:30-12:30 h, depending on the tidal cycle. During

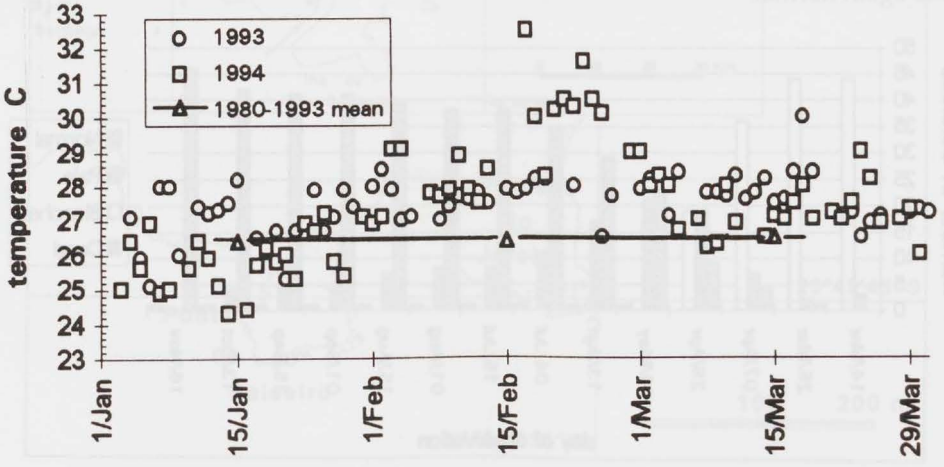

Fig. 3. Record of sea surface temperatures (SST) at Ponta do Jarobá, São Sebastião, SP, for 1993 and 1994 and monthly averages for 1980-1993 (cf. table 1).

Table 1. Mean, maximum and minimum sea surface temperatures (SST) (°C) from Ponta do Jarobá, São Sebastião, Brazil, for January, February and March (cf. fig. 3). Data from Centro de Biologia Marinha, Universidade de São Paulo.

Year	January			February			March		
	mean	max	min	mean	max	min	mean	max	min
1985	25.3	27.5	21.0	28.7	30.0	27.0	28.4	29.0	27.0
1986	27.5	30.0	25.0	26.6	28.5	25.0	27.4	28.5	25.0
1987	28.9	31.0	27.0	26.2	30.0	21.0	26.8	29.0	25.0
1988	26.7	28.0	24.0	26.1	29.0	25.0	25.7	27.0	23.0
1989	23.7	27.0	16.5	26.5	29.0	25.0	26.5	27.0	26.0
1990	24.9	27.0	20.5	24.6	26.0	23.0	25.4	28.0	24.0
1991	25.6	28.0	24.0	26.4	30.0	25.0	26.5	27.0	26.0
1992	27.5	30.0	26.0	25.5	29.0	22.0	25.5	29.0	22.0
1993	26.6	28.2	28.2	27.5	28.5	26.3	27.4	30.0	26.1
1994	25.4	27.2	23.2	28.7	32.5	25.7	27.1	29.0	24.8
1980-93	26.4	31.0	16.5	26.4	30.0	21.0	26.5	30.0	22.0

exposure, temperatures on the surface of the colonies reached 28.5 to 33.4°C, i.e. 1.5-6.1°C above local SST (26.5-28.5°C). These colonies showed no sign of bleaching until the end of the present study.

Temperature records.— In 1994, during the hot period between 3 February and 4 March, SST reached 32.5°C at Ponta do Jarobá, the maximum value ever recorded (table 1). This was also the hottest warm period recorded so far for the region, with mean SST of 28.7°C, i.e., 2.3°C above the long term average (1980-1993) for February (26.4°C). This warm period, initially characterized by several days with temperatures between 27.5 and 29.0°C, reached its maximal value on 17 February (32.5°C). Temperatures remained above 30°C for 9 more consecutive days (until 25 February) (fig. 3). After 4 March the temperatures decreased to levels around 27°C. Coincidental with the SST hot period of February 1994, the weather was calm, cloudless, and the air temperature was also high, with maxima from 28-34°C, and minima from 27-30°C.

Monthly mean SST was also high in the summers of 1986, 1987, 1992 and 1993, with defined hot periods (table 2). However, when compared to the summer of 1994, these periods were relatively short, with lower mean temperatures, and daily values not exceeding 30°C for more than 2 days.

Discussion

Bleaching and death are usually reported when mean SST is 1-4°C above normal level, the intensity of response depending on the species, latitude, exposure time, light regime, etc. (e.g., Jokiel & Coles, 1990; Glynn & D'Croz, 1990).

Jokiel & Coles (1990) reported that during the warmest season of the year exposure of 1-2 days to temperatures 4-5°C above long term averages for the period leads to bleaching and high mortality, while with the same exposure to temperatures 2-3°C above long term averages bleaching is gradual and less extensive, producing lower mortality. The relatively rapid and almost complete recovery of the species observed

Table 2. Number of days with sea surface temperatures (SST) exceeding 28, 29, 30, 31 and 32°C in the period 1986-1994 (Ponta do Jarobá, São Sebastião). Hot period arbitrarily defined as more than 5 consecutive days of SST ≥ 27.5°C.

Year	Number of days (°C)					Hot Period
	≥ 28	≥ 29	≥ 30	≥ 31	≥ 32	
1986	27	5	0	0	0	
1987	27	20	5	0	0	23-30 Jan. (χ = 28.0); 17-23 Mar. (χ = 27.9)
1988	7	1	0	0	0	
1989	1	0	0	0	0	
1990	1	0	0	0	0	
1991	4	1	1	0	0	
1992	26	12	2	1	0	2-16 Jan. (χ = 27.8); 24 Jan.-4 Feb. (χ = 28.2)
1993	16	1	1	0	0	10 Feb.-19 Mar. (χ = 27.7)
1994	21	13	8	2	1	3 Feb.-4 Mar. (χ = 28.8)

in the present study indicates that the thermal stress was not too long and/or strong enough to cause mortality (see Bunkley-Williams et al., 1991; Glynn & D'Croz, 1990). However, after temperatures returned to normal, no data were collected about possible subsequent damage on reproduction and growth. The fact that in pale specimens the bleaching process did not progress after the beginning of the observations, points to the acute nature of the stressor.

The observed variation in bleaching pattern could be due to different physiological tolerances of the association zooxanthellae-host (see Buddemeier & Fautin, 1993). Droller et al. (1994) concluded that "hermatypic corals have multiform reactions to stress", which "depend on genetic variations within and among species".

In situ data show that the mean SST between 3 February and 4 March was 2.4°C above long term average for this period. The mean SST for ocean water near Ilha de São Sebastião obtained from satellite data for February 1994 (Thomas Goreau personal communication; Goreau & Hayes, 1995) is much lower, however, than in situ records reported here; also, the maximum temperature recorded in February was higher in 1993 (26.4°C) than in 1994 (26.0°C). Although Goreau & Hayes (1995) considered both summers abnormally warm, the positive anomaly detected for February 1994 (0.5°C above average) alone could hardly explain the bleaching episode. They remarked, however, that the satellite data reflect open ocean surface temperature, and that shallow coastal waters can be considerable hotter or cooler, due to local circulation patterns and cloudiness (Goreau & Hayes, 1995).

The present data emphasize the importance of very short and very hot periods in triggering bleaching, given that 1987 had more days above 29°C than 1994 (see table 2). In these cases of brief and intense hot periods, the use of monthly mean values from satellite data are likely to underestimate the actual temperature related stress or even not detect it (Goreau & Hayes, in press).

Indeed, in 1994 positive thermal anomalies associated with bleaching episodes were detected in several locations of the Pacific, Indian and Atlantic oceans, after a period of 3 years in which no mass bleaching was recorded anywhere in the world (Goreau & Hayes, in press).

While the present data strongly suggest that unusually high SST triggered the

bleaching event, the effects of other potential multiple concurrent stresses, as pollution, sedimentation, and solar radiation (especially UV), can not be discarded (Goreau, 1992). Apparently, light was not an important factor in the present study, since no bleaching pattern was related to light exposure. For the past two decades, the coast of São Sebastião has suffered from several kinds of pollution, such as sewage outfalls, dredging, terrigenous sedimentation due to erosion, oil spills, etc. (Schaeffer-Novelli, 1990). *Mussismilia hispida* and *Palythoa caribaeorum* occur throughout the São Sebastião Channel in areas with various degrees of sedimentation and turbidity, being also common near sewage outfalls. The region has been intensively studied by researchers and students and bleaching was never reported before, even after episodes of petroleum and fuel oil spills. Goreau (1992), in a survey of bleaching stress factors in Jamaica, observed, however, that corals chronically subject to local anthropogenic stresses are less susceptible to mass bleaching, and that "standard local anthropogenic stresses are not a cause of bleaching".

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Protection of the symbiotic shrimps *Periclimenes pedersoni, P. yucatanicus,* and *Thor* spec. from fish predators by their host sea anemones

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Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 337-343, fig. 1.
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Key words: Sea anemone; anemoneshrimp; cleaner; predation; symbiosis; commensalism; mutualism; parasitism; *Periclimenes; Thor.*

Abstract: Anemoneshrimps live with impunity among the tentacles of sea anemones. Shrimps may receive protection from predation, but no studies have tested this hypothesis experimentally. This paper examines predation by fishes on three species of symbiotic shrimps: *Periclimenes pedersoni* Chace, 1958, *P. yucatanicus* (Ives, 1891), and *Thor* spec. Field observations (of *Thor* spec.) and laboratory trials showed that isolated shrimps were readily consumed by fishes. In the laboratory, however, shrimps living with anemones survived significantly longer than shrimps without anemones. This study shows that anemones provide protection for their shrimp associates, including *P. pedersoni,* which is a known "cleaner" and heretofore presumed to need little, if any, protection from fish predators.

Introduction

Symbioses involving organisms living with sea anemones are commonly categorized as either commensalistic or mutualistic, with one or both partners benefitting. A benefit common to many organisms living with anemones (or other cnidarians) is protection as a result of nematocyst discharge (Fautin & Allen, 1992). For example, anemonefishes (*Amphiprion* spp.) avoid predators by seeking shelter among the anemones tentacles (Davenport & Norris, 1958; Mariscal, 1970). Additionally, hydroids (*Hydractinia* spec.)(Grant & Pontier, 1973; Brooks & Mariscal, 1985) and anemones (*Calliactis* spp.) (Ross, 1971; McLean & Mariscal, 1973; McLean, 1983; Brooks, 1988) living as symbionts on gastropod shells protect some hermit crabs from predatory crabs and octopods. Hermit crabs can reciprocally protect these same cnidarians from predatory asteroids and polychaete worms (Brooks & Gwaltney, 1993).

Mahnken (1972) suggests associations between shrimps (*Periclimenes* spp. and *Thor* spp.) and anemones exist primarily to provide protection to the shrimp. It is possible, however, that *Periclimenes* spp. are recognized as "cleaners" by many reef fishes and, thus, may need little protection. For example, Stanton (1977) found that predation by fishes on several species of *Periclimenes* "cleaners" was minimal in the Bahamas, but predation on the "non-cleaner" *Thor amboinensis* (De Man, 1888) was high. He did not discuss whether the difference in predation on the two shrimp genera was related to cleaning behaviour.

In the present study, we looked at whether *Periclimenes pedersoni* Chace, 1958, a known cleaner, *P. yucatanicus* (Ives, 1891), a possible cleaner, and Thor spec., a non-cleaner, are protected from fish predation by living with sea anemones.

Materials & Methods

Collection and maintenance

Anemone shrimps *Periclimenes pedersoni*, *P. yucatanicus*, and *Thor* spec. (complexity of characters within this genus precluded identification of species), and sea anemones *Stichodactyla helianthus* (Ellis, 1767), *Condylactis gigantea* Weinland, 1860, and *Bartholomea annulata* Le Sueur, 1817, were collected in shallow waters (<15 m depth) off southeast Florida and the Florida Keys. Specimens of three species of predatory fishes, viz. *Balistes capriscus* Gmelin, 1789 (Gray triggerfish), *Sphaeroides testudineus* (Linnaeus, 1758) (Checkered puffer), and *Lutjanus griseus* (Linnaeus, 1758) (Gray or Mangrove snapper) were obtained at the same sites where shrimps were collected. Animals collected in the Florida Keys were stored temporarily at the Keys Marine Laboratory, Long Key, Florida. All animals were eventually kept in 38 l closed-system aquaria in the laboratory at Florida Atlantic University and were fed weekly on a diet of frozen *Artemia*. Fish were not fed within 72 h of testing to standardize satiation levels. Animals were used in laboratory trials within two weeks of collection.

Laboratory predation trials

Trials were performed in 9.5 1 aquaria without gravel. For *Periclimenes* spp. a single shrimp was added to the aquarium in the controls and a single shrimp with an anemone in the treatments. In trials for *Thor* spec., three shrimps were added to the aquarium in the controls and treatments, to simulate shrimp densities observed in the field. Shrimps in aquaria with the anemone present were allowed to associate with the host 24 h prior to testing. One fish was added to each aquarium with the shrimps. Sizes of individual prey and predators within a species did not vary more than 5 mm for shrimps and 20 mm for fishes.

Anemone hosts.— Observation times varied depending on how quickly fish consumed shrimp prey. For most trials, observations were made during the first five minutes and at 30 min intervals for 180 min. Survival times for *Thor* spec. were recorded in the same manner, except that if any of the three individuals remained at a 30 min. interval, the whole group was considered to have survived. Because the Checkered puffer and Gray snapper rarely consumed *Periclimenes* spp. shrimp in 180 min., trials involving these fish/shrimp combinations were run for 24h. The data were analyzed based on whether shrimps survived the 24 h trials. Average survival times for each shrimp species with and without an anemone were calculated and analyzed using Wilcoxon Rank Sum test (Zar, 1974). The following combinations of shrimp and anemones were used: (1) *P. pedersoni* with *Condylactis gigantea*, (2) *P. yucatanicus* with *Stichodactyla helianthus*, and (3) *Thor* spec. with *Bartholomea annulata*. These combinations were the most frequently observed in the field (Mihalik, 1989).

Limited availability of shrimps precluded testing other combinations that occur less frequently (for discussion on host specificity with these shrimps, see Gwaltney & Brooks, 1994).

Substitute host.— Trials using a 7.5 × 3.0 cm brown glass bottle as a substitute host were conducted for *Thor* spec. with all three predatory fish species to determine whether shrimp could receive protection by hiding near or on a dark object, rather than by nematocyst discharge from anemones. The coloration of *Thor* spec. matched well the shade of the bottle. The experimental procedure and analysis were identical to those described previously. Limited availability of shrimps of the two *Periclimenes* spp. shrimp precluded their use in these substitute host trials.

Results

Collection and field observations

Various species of fish were found in areas where shrimps and anemones were collected, and some fish were observed foraging around the tentacles of the anemone *Bartholomea annulata*. It was not known whether the fish were eating the tentacles or shrimp. Therefore, several individuals of *Thor* spec. were removed from *B. annulata* in the field and were eaten immediately by snappers and grunts. Preliminary laboratory trials also showed that all three species of fish ate the shrimps. *Octopus* spec. was also observed consuming anemone shrimps, but because of limited availability experiments were not conducted in the lab with this predator.

Shrimp survival rates in predation trials with fish predators

Gray triggerfish.— Four controls (no anemone) and four treatments (anemone present) were run with this fish predator. Shrimps with anemones all survived more than 170 min., while shrimps without anemones survived less than 5 min. ($p<0.05$ for each test) (fig. 1A). The survival rate of *Thor* spec. with the brown bottle was significantly lower than the rate observed with an anemone ($p<0.05$).

Checkered puffer.— Five controls and five treatments were run with this fish predator. Four out of five *Periclimenes pedersoni* with the anemone *Condylactis gigantea* survived for 24 h, but all five without the anemone were eaten by the Checkered puffer ($p<0.001$). All five *Periclimenes yucatanicus* with the anemone *Stichodactyla helianthus* survived for 24 h with the fish, while none of the five shrimps without the anemone survived the 24 h period ($p<0.001$). *Thor* spec. survived longer with *Bartholomea annulata* than without the anemone ($p = 0.004$) (fig. 1B). The survival rate of shrimps with the brown bottle was significantly less than the survival rate with the anemone ($p = 0.028$).

Gray snapper.— Five controls and five treatments were run with this fish predator. Four of the five *Periclimenes pedersoni* and *P. yucatanicus* with their respective anemones survived for 24 h in the presence of the snapper, but no shrimp for either species survived the 24 h without an anemone ($p<0.001$). Individuals of *Thor* spec. with an anemone survived significantly longer than those without an anemone ($p = 0.004$) (fig. 1C). The survival rate for *Thor* spec. with the bottle was significantly less than with the anemone host ($p = 0.028$) (fig. 1C).

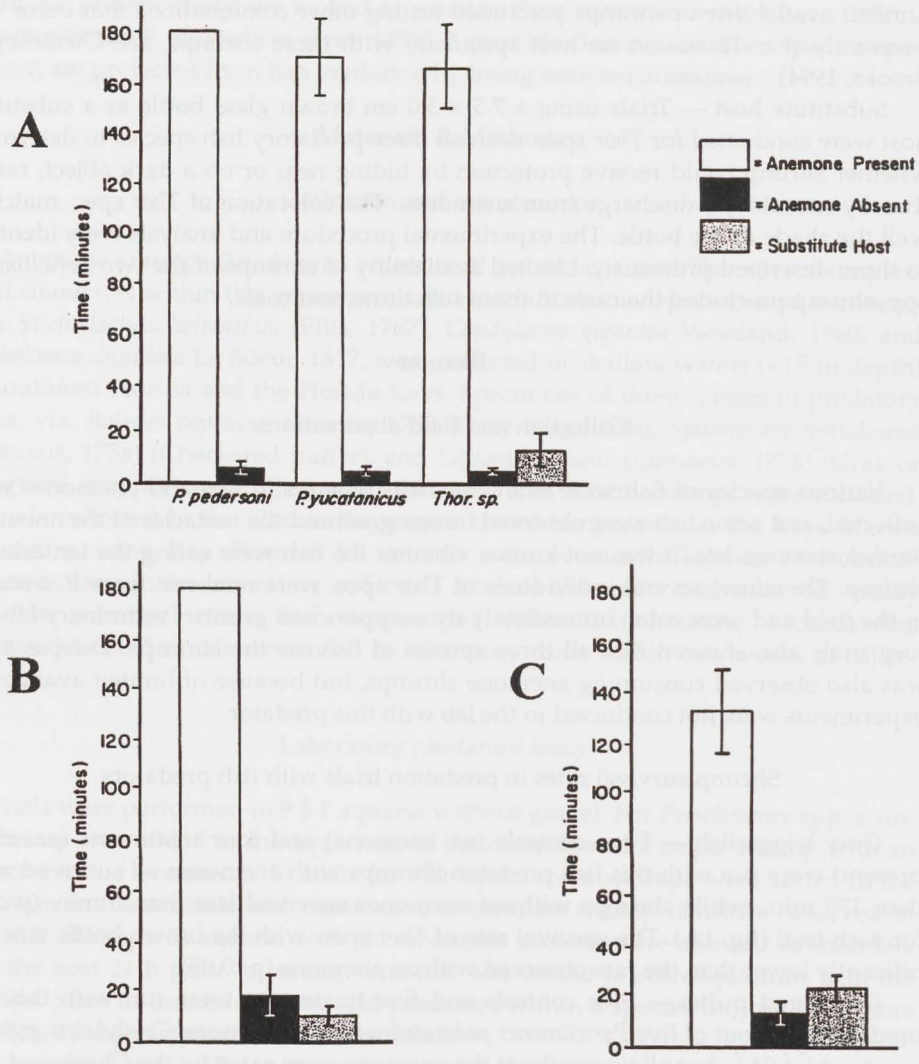

Fig. 1. Histograms showing survival times for shrimp with fish predators.
A. *Periclimenes pedersoni*, *P. yucatanicus*, and *Thor* spec. with and without anemones in the presence of the fish *Balistes capriscus* (Gray triggerfish). The following shrimp/anemone combinations were used: (1) *Periclimenes yucatanicus* with *Stichodactyla helianthus*, (2) *P. pedersoni* with *Condylactis gigantea*, and *Thor* spec. with *Bartholomea annulata* and a dark glass bottle as a substitute in place of anemone host. Bars indicate standard deviations.
B. *Thor* spec. with and without the anemone *Bartholomea annulata* and the bottle substitute host in the presence of the fish *Sphaeroides testudineus* (Checkered puffer).
C. *Thor* spec. with and without the anemone *Bartholomea annulata* and the bottle substitute host in the presence of the fish *Lutjanus griseus* (Gray snapper).

Behaviour of shrimp and fish in predation trials

Periclimenes pedersoni was generally found on the tentacles of *Condylactis gigantea* when the fish were present. In one instance, when an anemone was absent *P. pedersoni* jumped on a fish and began to pick and eat material from the predator. The shrimp was then eaten immediately.

Periclimenes yucatanicus was generally found directly on the oral disc or underneath on the column of the anemone *Stichodactyla helianthus* when a fish was present. Only one *P. yucatanicus* was eaten with an anemone present. In this case, the shrimp was actually plucked off the host and eaten by a triggerfish. The shrimp, however, survived for 150 min. In general, when an anemone was present, the fish usually stayed at the opposite end of the tank. In one instance, however, a triggerfish contacted *S. helianthus* and responded as if it had been stung.

Thor spec., when associated with *Bartholomea annulata*, was often found near, under, or on the tentacles. Sometimes the shrimps were found in a vertical position (with either head or tail up) on the column of the anemone. Those shrimps that were eaten, however, were often 10-15 cm from the anemone. In general, the fish stayed at the opposite end of the tank when an anemone was present. In one instance, a Gray triggerfish was observed nipping at the tentacles of *B. annulata*. When individuals of *Thor* spec. were given access to the brown bottle as a substitute host, they were found on the bottle in the same vertical position as seen on the column of the anemones.

Discussion

These laboratory results show clearly that shrimps with anemones gain at least some degree of protection from predatory fishes. Additionally, being a "cleaner shrimp" did not deter fish predation. *Periclimenes pedersoni* was eaten even while cleaning a fish, which illustrates the potential risks if hosts are unreceptive to cleaning services. Hobson (1971) suggests that predation by host fishes on cleaner fishes is partially related to satiation levels of the potential hosts. Our study supports this assertion, as fish were not fed within 72h of trials. In the field, predation on cleaner shrimps may also be related to the feeding state of host anemones.

Protection from fish predation was not absolute, as some shrimps associated with anemones were consumed. Factors such as anemone morphology (e.g., relative tentacle length) and nematocyst toxicity may influence whether predators contact and subsequently avoid anemones and their shrimp. Camouflage may also be important. All three shrimp species are transparent with pigment spots, which makes them cryptic among anemone tentacles. It would be of interest to determine whether predation is increased when shrimps associate with anemones with contrasting colour patterns.

Thor spec. was tested with a brown bottle, which served as a crude mimic for a sponge or rock which the shrimp could potentially use for shelter from predators. Results of these trials showed clearly that the object provided no protection to *Thor* spec. This is fully in accordance with the idea that anemones provide active protection (presumably through cnida discharge), rather than a passive hiding place.

Our study shows that certain shrimps do receive the benefit of protection by associating with sea anemones. The impact of the shrimp on the anemone, however,

is unclear. *Periclimenes* spp. consume anemone mucus and tissues (Suzuki & Hayashi, 1977; Turnbull, 1981; Bruce & Svoboda, 1983; Mihalik, 1989; Fautin et al., in press), which suggests the shrimps are parasites. Fautin et al., (in press), found that the shrimp *P. brevicarpalis* (Schenkel, 1902) consumed tentacles of its symbiotic anemone *Entacmaea quadricolor* (Rüppell & Leuckart, 1828) in laboratory trials, but reported finding no anemones in the field with comparable damage. Svoboda & Svoboda (1975), however, found that *Periclimenes amethysteus* (Risso, 1827) and *P. sagittifer* (Norman, 1861) consumed host anemone tentacles in both the laboratory and field.

These associations may also represent mutualism, because shrimp can remove sand, sediment, and inorganic particles from the anemone (Herrnkind et al., 1976) as well as organic debris, such as excess mucus or possibly anemone parasites (e.g., copepods) as suggested for fishes that associate with anemones (cf. Mariscal, 1970). Another benefit anemones may receive from shrimps, aside from a "cleaning service", is protection from predation. Smith (1977) observed the snapping shrimp *Alpheus armatus* Rathbun, 1902, defending its host anemone *Bartholomea annulata* against the predatory polychaete *Hermodice carunculata* (Pallas, 1766). Based on the present study and previous reports, it is likely that all three categories of symbiotic associations involving shrimp and sea anemones exist in nature.

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What molecular data tell us about the evolution of the 'Coelenterata'

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Key words: Phylogeny; Cnidaria; *Acropora*; zootype.

Abstract: Molecular data have so far failed to provide clear answers to the 'big questions' about animal evolution, particularly the origins of the Metazoa and the relationships between the animal phyla. Molecular information can provide the 'big answers', but to do so the focus must change from the comparison of ribosomal RNA sequences on to qualitative molecular data, looking for specific genes and gene hierarchies which are likely to be good indicators of major evolutionary events. We believe that an extended version of the zootype hypothesis provides the means by which to address the major issues in animal evolution, and that the same kind of approach could be applied to deep phylogeny within the other kingdoms. Our aim is to use zootype principles to evaluate relationships within the Cnidaria and between the various diploblastic phyla. Here we describe progress in characterizing zootype genes from the anthozoan cnidarian *Acropora*.

Introduction.

Two interrelated factors are central to the failure of quantitative molecular approaches to understanding deep phylogenetic relationships. The first of these is the nature of major evolutionary change itself. The most interesting periods in evolution are characterized by sudden radiations of variations on a theme, followed by long periods of fine tuning, during which no major changes occur. The most familiar example of this is the Cambrian explosion, the sudden appearance in the fossil record of all the major animal body plans in the period around 540 million years ago. Since then, no new body plans have emerged; most extant animal phyla correspond to body plans established during that period, and are well defined. However, because the radiation at the base of the Cambrian occurred during a narrow time window and a long time ago, there are major problems in defining the relationships between the phyla. In a few cases candidate fossil missing links have been found, but the implied relationships are often controversial (see, e.g., Conway Morris, 1994: 1).

The second factor is the limited resolution provided by ribosomal RNA sequence data. To establish patterns of animal phylogeny, we require a means of defining the orders of a series of branches occurring within a period which may have been as

brief as 5 million years (Bowring et al., 1993: 1293). The precision of rRNA sequence analyses simply does not approach that required. For example, Philippe et al. (1994: 15) have estimated the resolving power of the 18S rRNA, widely used to address deep phylogenetic relationships, as ± 40 million years. Thus even sequences for entire ribosomal transcription units cannot provide the resolution required to unravel the Cambrian explosion. Clearly other approaches are needed, and these will be based on other molecules.

What we know and what we suspect

Metazoans are a monophyletic group.— There is now compelling evidence for monophyly of the Metazoa. This has come not from analyses of rRNA sequences, which have been equivocal at best, but rather from qualitative molecular data. Metazoan monophyly is well supported by the identification of a number of molecular synapomorphies, gene families found only in animals. Such markers of animalness include α-type (replication-dependent) histone genes (see, e.g., Miller et al., 1993: 245) and 'animal-type' homeobox genes (see below) as well as collagen genes (Exposito & Garrone, 1990: 6669).

The diploblasts are probably not a monophyletic group.— Fig. 1 summarizes the implications of several recent ribosomal RNA sequence analyses for the phylogeny of diploblasts. As fig. 1a indicates, some of these studies suggest that diploblasts diverged from a common ancestor after the split between diploblasts and triploblasts (Philippe et al., 1994: 15). This implies that two metazoan sub-kingdoms exist, and that these may be the products of distinct radiations. Although consistent with the

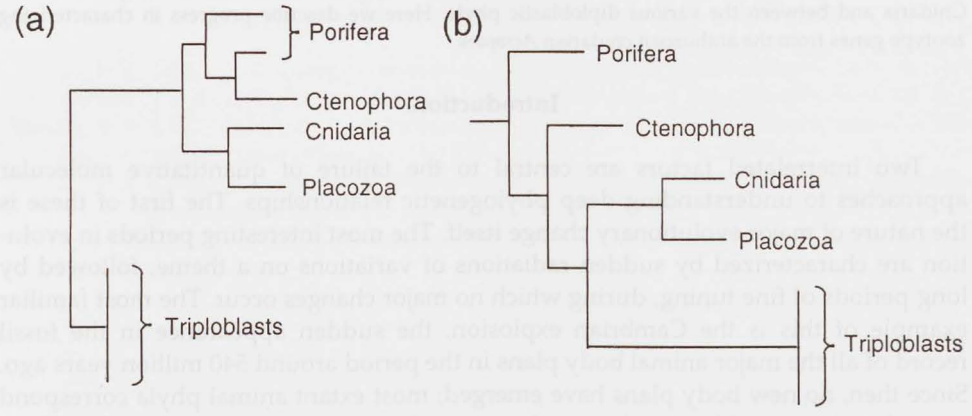

Fig. 1. The implications of recent rRNA sequence analyses for the phylogeny of diploblastic animals. The topology shown as fig. 1(a) is based on Philippe et al. (1994), whereas that in fig. 1(b) is from Wainwright et al. (1993) ; branch lengths are not accurately represented and many taxa have been removed. Although both trees were based on analyses of complete 18S rRNA sequences, the topologies differ in several important respects. In (a) the diploblasts are monophyletic, however this may be a consequence of the lack of appropriate outgroups. In Wainwright's study (b), several outgroups (including chaonoflagellates) were included, resulting in the tree becoming rooted and indicating that the diploblasts are paraphyletic.

view that the (earlier) Vendian and Cambrian 'explosions' correspond to distinct radiations of diploblastic and triploblastic animals respectively (see Conway Morris, 1993: 219), we believe that this topology is likely to be a consequence of the absence of appropriate outgroups. Inclusion of choanoflagellate sequences, for example, would enable trees to be unambiguously rooted. Fig. 1b summarizes the findings of Wainwright et al. (1993: 340) relevant to the issue of diploblast relationships. In this study, a number of non-metazoan eukaryotes were included. The result is that the diploblasts are now paraphyletic, the choanoflagellates forming the sister group of the Metazoa. Note that in both of these trees the 'Coelenterata' are paraphyletic, and in both cases the Placozoa, rather than the Ctenophora, are the sister group of the Cnidaria. Low bootstrap support, however, indicates that many aspects of the phylogeny of diploblastic organisms remain equivocal.

Within the Cnidaria, the Anthozoa are likely to be the basal Class.— One of the key features of metazoans is the presence of a compact (14-42 kb) and circular mitochondrial genome. In this respect, anthozoan cnidarians and ctenophores are typical metazoans, whereas the other classes of cnidarians are characterized by possession of linear mitochondrial genomes (Bridge et al., 1992: 8750). The linear type of mitochondrial genome is assumed to be a derived character state. Thus this single molecular character implies that within the Cnidaria the Anthozoa are basal.

Where do we go from here?

As is clear from the above, there are major difficulties in applying strictly quantitative molecular analyses to these questions of deep phylogeny, and definite answers are more likely to come from qualitative molecular characteristics. The fine structure of the mitochondrial genomes of diploblasts (Pont-Kingdon et al., 1994: 387) will have major implications for the issue of diploblast relationships, and data for sponges are urgently required. Qualitative information about ribosomal RNAs and the genes which encode them are also likely to be phylogenetically informative. For example, secondary structure data, and information on positions of insertions in the rRNA genes are potentially powerful tools for investigating deep phylogenetic relationships.

Whilst there can be no doubt as to the usefulness of these other kinds of qualitative molecular data, we believe that the zootype hypothesis (Slack et al., 1993: 490) provides the most powerful means of addressing phylogenetic relationships at the Phylum and Class level. The basic premise of the zootype hypothesis is that we define an animal by a particular spatial pattern of gene expression (the zootype), and that this is most clearly expressed at the phylotypic stage for each taxon. This hypothesis suggests that it is possible to make informative comparisons across the whole of the Metazoa, and should allow: (1) establishment of the evolutionary position of 'borderline' organisms, such as sponges and mesozoans (if they show the zootype, then they are in, if not, they are out), (2) the identification of the 'real' phylotypic stage, which will have major implications for understanding the evolution of some phyla (see below), and (3) definition of relationships between and within the phyla. This last point requires elaboration: if all animals have the zootype, then the next

'layer' of control genes is likely to represent the next level of control of body patterning, and should correspond to the next level of phylogeny. Thus it may be possible to define the 'deuterotype', the 'echinotype', and so on.

What constitutes the zootype?— The zootype hypothesis was founded on some remarkable similarities in the details of molecular embryology of the mouse and the fruit fly (*Drosophila melanogaster* Meigen, 1830). Clusters of genes (the HOX clusters) in mammals are not only clearly structurally homologous to the HOM-C complex of *Drosophila*, but also have strikingly similar domains of expression. This discovery led to the idea that the DNA-binding motif (the homeodomain, encoded by the homeobox) present in the products of all of these genes could be the Rosetta stone of developmental biology, enabling direct comparisons to be made between animals with diverse developmental programs.

Based on the mouse/fly comparison, Slack et al. (1993: 490) originally suggested that the zootype comprised six HOX genes. Recent studies on the nematode *Caenorhabditis elegans* (Maupas, 1900) (cf. Wang et al., 1993: 29), however, imply that there may have been only three or four HOX genes in the common ancestor of all animals. The HOX genes, together with homologs of the *Drosophila* genes *empty-spiracles* (*ems*), *orthodenticle* (*otd*) and *even-skipped* (*eve*), constitute the zootype as originally proposed. In both the fly and mouse, the first two of these classes of genes (there are two homologs of both *ems* and *otd* in mammals) are involved in patterning the extreme head-end, whereas the HOX genes define much of the rest of the anterior-posterior body axis. The original function of *eve*-class genes is unclear, as three distinct roles have been described.

The phylotypic stage and its significance.— The phylotypic stage is defined as the stage in development when all the major body parts are represented by undifferentiated cell masses at their final positions. It is also the stage at which all of the members of a phylum are most similar. For most animal groups, the phylotypic stage is clearly defined; in the insects, for example, it is the fully segmented germ band stage, and for vertebrates the tailbud stage. For other groups, including the Cnidaria, the phylotypic stage is less obvious. Most cnidarians have motile larvae, but these come in a bewildering array of shapes (e.g. planula, ephyra, actinula), and larval morphologies do not correspond completely with class distinctions based on adult characteristics. For the Cnidaria, it is not yet evident whether the larva or the developing adult is the phylotypic stage (nor, if the former, which type of larva). Evidence from other phyla indicates that the zootype must be displayed at the phylotypic stage, therefore examination of the zootype in cnidarians can tell us which stage is likely to be phylotypic, and hence which stage should be compared with other phyla. The zootype should therefore provide the definitive verdict on the debate over polypoid or medusoid ancestry, and Class-level relationships of the Cnidaria. Comparison of the next level of control genes should provide insights into higher level relationships.

Which zootype genes are present in diploblastic animals?— In 1992, our group (Miles & Miller, 1992: 159) and Galliot's (Schummer et al., 1992: 1815) independently reported sequences for zootype genes from cnidarians. Amongst four *Hydra* homeo-

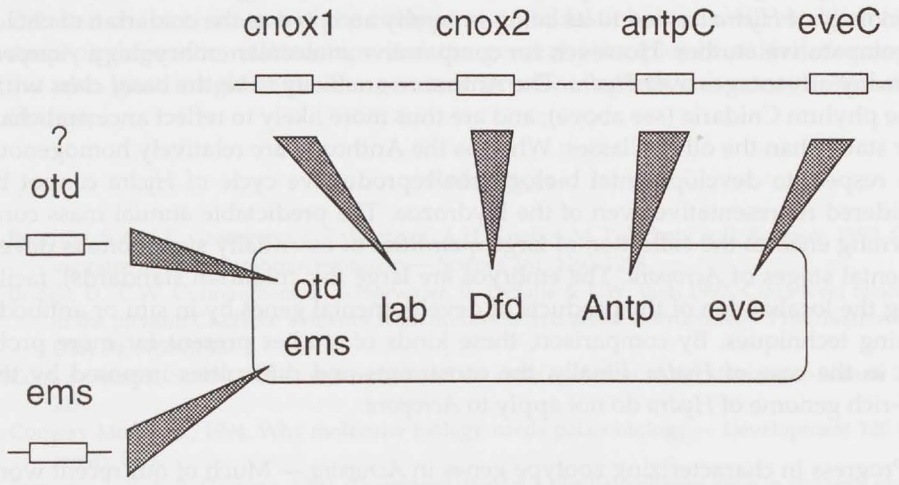

Fig. 2. Progress towards characterizing zootype genes in *Acropora*. The enclosed box represents the zootype ; outside the box the genes so far identified in *Acropora*. It is likely that *antpC* and *eveC* (which are known to be linked) constitute the 5'-end of the cnidarian HOX cluster, likely to also include *cnox1* and *cnox2*.

box genes cloned by Galliot were two candidate zootype genes, *cnox1* and *cnox2*, were tentatively identified as homologs of the *Drosophila* HOX genes *labial* and *Deformed*, respectively. Our group cloned an *eve* homolog from the scleractinian coral *Acropora formosa* (Dana, 1846), which we called *eveC*. Subsequently, we showed that *eveC* is linked to a HOX-class gene which did not correspond to either *cnox1* or *cnox2* (Miller & Miles, 1993: 215). We concluded that this gene was likely to correspond to the *Drosophila* gene *Antennapedia* (*Antp*), hence we called it *antpC*. Expression data for *cnox2* (Shenk et al., 1993: 657) are consistent with a role in axis formation in *Hydra*.

Consideration of the existing data led us to suggest that the HOX gene cluster in *Acropora* consists of four genes, as shown in fig.2. These kinds of studies have general implications beyond establishing the basis of cnidarian phylogeny and *Acropora* development. For example, the linkage between an *eve* class gene and a HOX gene has only previously been detected in vertebrates. This common feature of evolutionarily distant taxa suggests that the linked state is ancestral, and that the 'primitive' function of *eve* class genes is in axis formation, rather than in segmentation or neurogenesis. Another interesting point is that *Acropora* apparently lacks a homolog of *Abdominal-B* (*Abd-B*), which is often considered to have diverged early from other HOX genes. This implies either that in *Acropora* there has been secondary loss of an *Abd-B* cognate, or else this gene class evolved later than has previously been assumed.

For diploblastic animals other than cnidarians, very little zootype data are available. Nothing is known about the zootype complement of ctenophores and, although sponges do contain homeobox genes (Seimiya et al., 1994: 219), all (three) of those known are unlikely to correspond to zootype genes.

Acropora as a model cnidarian.— We believe that *Acropora* is a particularly useful

experimental organism for comparative molecular embryology. The well-established cell biology of *Hydra* has led to its being generally accepted as the cnidarian of choice for comparative studies. However, for comparative molecular embryology, *Acropora* has many advantages over *Hydra*. The Anthozoa are likely to be the basal class within the phylum Cnidaria (see above), and are thus more likely to reflect ancestral character states than the other Classes. Whereas the Anthozoa are relatively homogenous with respect to developmental biology, the reproductive cycle of *Hydra* cannot be considered representative even of the Hydrozoa. The predictable annual mass coral spawning enables the collection of large quantities of essentially synchronous developmental stages of *Acropora*. The embryos are large (by cnidarian standards), facilitating the localization of the products of developmental genes by in situ or antibody staining techniques. By comparison, these kinds of studies present far more problems in the case of *Hydra*. Finally, the constraints and difficulties imposed by the (AT)-rich genome of *Hydra* do not apply to *Acropora*.

Progress in characterizing zootype genes in *Acropora*.— Much of our recent work has been directed towards characterization of zootype genes in *Acropora*, and exploring the evolutionary implications of these data. The HOX gene which we initially identified was only distantly related to those found in *Hydra* by Galliot's group, therefore we set out to clone the *Acropora* homologs of the *Hydra* genes *cnox1* and *cnox2*. Based on the predicted homeodomain sequence, we have certainly cloned the latter gene (Dodd et al., in preparation). We believe that we have also cloned *Acropora cnox1*, although similarity with the *Hydra* gene is not as convincing in this case, the *Acropora* gene is more like *labial* (the archetype of this class) than is the *Hydra* gene. Clarification of the identity of these genes awaits expression data, which we are presently undertaking. In addition to these genes, we have cloned the *Acropora* homolog of *empty-spiracles* as well as another homeobox gene which corresponds to either *orthodenticle* or a *paired*-class gene. Thus we are near to having characterized the *Acropora* homologs of all of genes included in the original description of the zootype.

Summary and speculations

A number of recent molecular analyses have important implications for understanding relationships between the diploblastic phyla. It is probable that the diploblasts are not a monophyletic group, and that the 'Coelenterata' may have no real status as the 'Coelenterata' appear to be paraphyletic and the Placozoa, rather than the Ctenophora, are likely to be the sister group of the Cnidaria. Many aspects of the early evolution of the Metazoa remain equivocal, and are likely to remain intractable to quantitative molecular analyses. We believe that the zootype hypothesis provides a powerful approach with which to address issues of deep phylogeny. A 'bonus' from applying zootype principles to phylogenetic analysis is that we stand to gain insights into mechanisms which permit or drive major evolutionary change. The Cambrian explosion is characteristic of the pattern of major evolutionary change - the angiosperms and the vertebrates (for example) show the same pattern. This kind of change is almost certainly driven by the discovery of novel genetic principles - new genes and new ways to use old genes. The zootype genes have clearly played a central role

in animal evolution, but the extent to which they have driven change is unclear. Detailed characterization of the zootype of diploblastic animals will establish not only deep phylogenetic relationships and the general principles of animal development, but also give insights into the genomic processes which drive major evolutionary change.

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Population genetic studies of antipatharian black corals from Doubtful and Nancy Sounds, Fiordland, New Zealand

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Abstract: Early studies of the New Zealand endemic *Antipathes fiordensis* Grange, 1990, showed no evidence of male gametes, and more recent histological examination suggests male colonies may be rare. We used allozyme electrophoresis to gauge the extent of sexual and asexual reproduction in *A. fiordensis* populations and also to examine genetic relatedness between sites as a measure of larval dispersal. Preliminary results from Fiordland, New Zealand, suggest complex reproductive patterns occur in this population. Various statistics indicate allele frequencies are significantly different among sites, and some sites do not conform to Hardy- Weinberg equilibrium. Genotypic diversity was significantly lower than expected at some sites suggesting some asexual reproduction, but clones were found among sites and between fiords (distances of up to 60 km) suggesting dispersal of asexual planulae rather than fragmentation. However, almost half of the 193 individuals screened had unique genotypes suggesting genetic recombination due to sexual reproduction is also important. There were no significant genetic differences between fiords, so larvae may be widely dispersed, or there may be few selective processes for genetic divergence in this unique ecosystem. Our preliminary findings indicate reproduction in *A. fiordensis* occurs asexually as well as sexually in the New Zealand fiords, and that dispersal of larvae is likely to be complex. These results emphasise the need for further studies on the reproductive ecology of antipatharian black corals.

Introduction

Antipatharian black corals are a poorly known group because they usually live at depths inaccessible to SCUBA divers. Despite numerous studies world-wide that have estimated distributions and biomass to assess the sustainability of black coral harvesting (e.g. Grigg, 1976; 1977; Mailer, 1983; Dept. Lands & Survey Tonga, 1985; Grange, 1985), only one study has been published on reproduction in black corals (Grigg, 1977), and only anecdotal information exists on larval biology and ecology in the Antipatharia (Grigg, 1965; Grange and Singleton, 1988; Oakley, 1988). However, an understanding of reproductive processes is essential for proper management of sustainable black coral resources. One species in which reproduction can be more easily studied is *Antipathes fiordensis* Grange, 1990, an endemic of southern New Zealand. Populations of *A. fiordensis* in New Zealand fiords differ from other black coral populations as most of the biomass occurs in shallow water (15-25 m depth; Grange, 1985).

Reproduction in *A. fiordensis* takes place over the austral summer (Nov-Feb; Grange, 1988; Grange & Singleton, 1988). Colonies are gonochoric and ripe female

colonies are easily distinguished by their pink/orange colour caused by the presence of eggs in the polyps (Grange, 1988). Until recently, male colonies had not been reported in the New Zealand fiords. However, histological studies have shown male colonies are present in Doubtful Sound (N. Parker, unpublished data). It is unknown whether fertilisation occurs internally or externally in black corals, and broadcast spawning of gametes in *A. fiordensis* has been an assumption based on the disappearance of pink colouration in female colonies. There has been one sighting of an *A. fiordensis* colony "spawning" (R. Grace, personal communication) but whether gametes or planulae were released was not reported. It is also unknown whether larvae are long-lived active swimmers, whether they are positively or negatively buoyant or whether they have the potential for long-distance dispersal.

Asexual reproduction is suspected in *Antipathes fiordensis* populations. It is common for large *A. fiordensis* colonies to be surrounded by smaller colonies (personal observation). This distribution pattern may result from larvae settling close to parent colonies (e.g. the crawling benthic larvae of *Balanophyllia elegans* Verrill, 1864 (cf. Gerrodette, 1981) or alternatively from fragmentation with limited dispersal. Fragmentation is a mode of asexual reproduction found in other coral groups (e.g. scleractinians, Highsmith, 1982; octocorals, Brazeau & Lasker, 1990) and has been reported in the black coral *A. dichotoma* Pallas, 1766 (cf. Grigg, 1976). It is possible that fragmentation occurs naturally in *A. fiordensis*, as fragments generated experimentally are capable of re-attaching to the fiord walls (K. Grange, unpublished data).

Allozyme electrophoresis is a useful tool for addressing questions of sexual and asexual reproduction, clonality in populations and genetic differentiation between geographic regions (Richardson et al., 1986). It has been applied successfully to questions of asexual reproduction and clonality in scleractinian corals (e.g. Stoddart, 1984; Willis & Ayre, 1985; Hunter, 1985), octocorals (Brazeau & Harvell, 1994), and other marine invertebrates (e.g. starfish: Johnson & Threlfall, 1987; bivalves: Tyler-Walters & Crisp, 1989). We have begun to use electrophoresis in an attempt to understand more about the dynamics of *A. fiordensis* populations throughout New Zealand, particularly the relative contribution of sexual and asexual reproduction in fiord populations. This paper presents results from an electrophoretic survey of *A. fiordensis* in two fiords in southern New Zealand.

Methods

Samples of *Antipathes fiordensis* were collected from seven sites in Doubtful Sound and two sites in Nancy Sound, Fiordland, New Zealand (fig. 1). Small branches (50-100 mm long) were removed from colonies and placed in individual bags under water. Care was taken to choose branches that were free of epibionts. At the surface, samples were frozen immediately in liquid nitrogen, and later transferred to a -80° freezer for storage. Where possible, 25 colonies were sampled at each site.

Allozyme electrophoresis was used to determine the 12 locus genotype of each colony. Starch gels (12% w/v) were stained for nine different enzymes: malate dehydrogenase (MDH: EC 1.1.1.37), glucose-phospate isomerase (GPI: EC 5.3.1.9), phosphoglucomutase (PGM: EC 2.7.5.1), hexokinase (HK: EC 2.7.1.1), malic enzyme (ME: EC 1.1.1.40), esterase (EST: EC 3.1.1.1), isocitrate dehydrogenase (IDH: EC 1.1.1.42),

leucyl-alanine-peptidase (LA: EC 3.4.11/13), and xanthine oxidase (XO: EC 1.2.3.2). MDH, GPI, PGM and HK were assayed on gels using a Tris-EDTA-citrate buffer, pH 7.4 (modified from Goodall & Stoddart, 1989) run at 50mA for 4 hours. ME, EST and IDH were assayed on gels using a Tris-citrate buffer, pH 8.0 (TC2, Selander et al., 1971) run at 60mA for $4^1/_2$ hours. LA and XO were assayed on gels using a Tris-EDTA-Borate buffer, pH 8.4 (TEB, Selander et al., 1971) run at 60 mA for $4^1/_2$ hours.

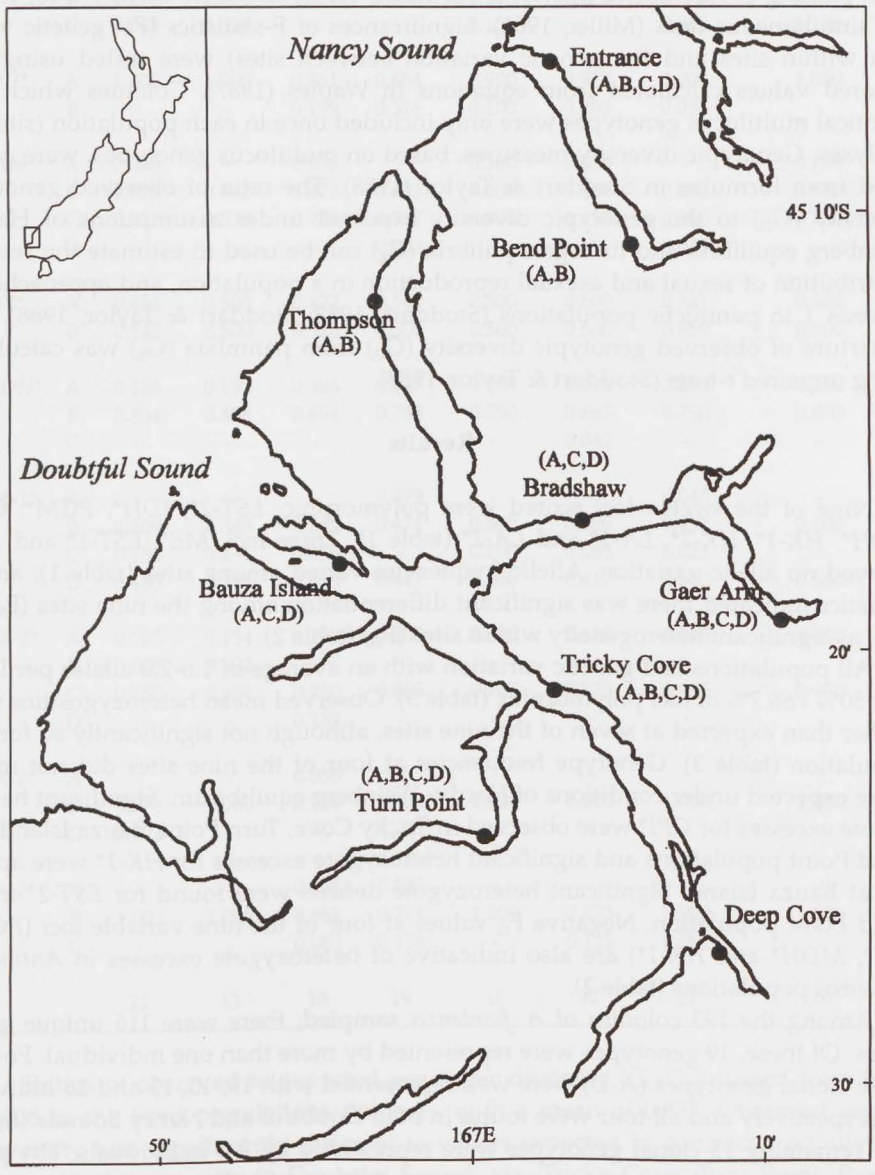

Fig. 1. Location of sampling sites in Doubtful and Nancy Sounds, Fiordland, New Zealand. Letters denote the presence of the four most common genotypes at each site (genotypes A, B, C and D; n = 19, 10, 13 and 16 respectively).

Stains were based on recipes from Allendorf et al. (1977) and applied using agar overlays. Alleles and loci were numbered consecutively according to relative mobility from the origin.

Allele frequencies and population genetic statistics were calculated using BIOSYS (Swofford & Selander, 1981). It was assumed that black corals are diploid organisms. Conformity to Hardy-Weinberg equilibrium was tested with exact tests with pooling of rare alleles (Elston & Forthofer, 1977), and were corrected for multiple simultaneous tests (Miller, 1966). Significances of F-statistics (F_{IS}: genetic variation within sites and F_{ST}: genetic variation between sites) were tested using chi-squared values calculated from equations in Waples (1987). Colonies which had identical multilocus genotypes were only included once in each population (site) for analyses. Genotypic diversity measures, based on multilocus genotypes, were calculated from formulae in Stoddart & Taylor (1988). The ratio of observed genotypic diversity (G_O) to the genotypic diversity expected under assumptions of Hardy-Weinberg equilibria and linkage equilibria (G_E) can be used to estimate the relative contribution of sexual and asexual reproduction in a population, and approaches or exceeds 1 in panmictic populations (Stoddart, 1983; Stoddart & Taylor, 1988). The departure of observed genotypic diversity (G_O) from panmixia (G_E) was calculated using unpaired t-tests (Stoddart & Taylor, 1988).

Results

Nine of the twelve loci scored were polymorphic: *EST-2**, *IDH**, *PGM**, *GPI**, *MDH**, *HK-1**, *HK-2**, *LA-1** and *LA-2** (table 1). Three loci, *ME**, *EST-1** and *XO**, showed no allelic variation. Allelic frequencies varied among sites (table 1), and F-statistics indicated there was significant differentiation among the nine sites (F_{ST}) as well as significant heterogeneity within sites (F_{IS}) (table 2).

All populations had genetic variation with an average of 1.6-2.0 alleles per locus and 50% - 66.7% of loci polymorphic (table 3). Observed mean heterozygosities were higher than expected at seven of the nine sites, although not significantly so for any population (table 3). Genotype frequencies at four of the nine sites did not match those expected under conditions of Hardy- Weinberg equilibrium. Significant heterozygote excesses for *GPI** were observed in Tricky Cove, Turn Point, Bauza Island and Bend Point populations and significant heterozygote excesses for *HK-1** were apparent at Bauza Island. Significant heterozygote deficits were found for *EST-2** in the Bend Point population. Negative F_{IS} values at four of the nine variable loci (*PGM**, *GPI**, *MDH** and *HK-1**) are also indicative of heterozygote excesses in *Antipathes fiordensis* populations (table 2).

Among the 193 colonies of *A. fiordensis* sampled, there were 116 unique genotypes. Of these, 19 genotypes were represented by more than one individual. Four of these clonal genotypes (A-D) were well represented with 19, 10, 13 and 16 individuals respectively and all four were found in both Doubtful and Nancy Sounds (fig. 1). The remaining 15 clonal genotypes were represented by 2-4 individuals. The probability of the clonal genotypes ocurring more than once in the population by chance alone was calculated to be $<3 \times 10^{-4}$. Hence colonies with identical nine-locus genotypes were considered to be the result of asexual reproduction.

Table 1. Allele frequencies at variable loci in populations of *Antipathes fiordensis* from nine sites in two fiords, Fiordland, New Zealand.

		Doubtful Sound							Nancy Sound	
Locus/allele		Tricky Cv	Deep Cv	Turn Pt	Bauza Is	Gaer Arm	Bradshaw	Thompson	Entrance	Bend Pt
*EST-2**	A	0.136	0.462	-	-	-	0.125	0.182	-	0.271
	B	0.818	0.500	1.000	1.000	0.900	0.875	0.773	0.900	0.729
	C	0.045	0.038	-	-	0.100	-	0.045	0.100	-
*IDH**	A	1.000	1.000	0.861	0.684	0.900	0.917	0.909	1.000	0.958
	B	-	-	0.139	0.316	0.100	0.083	0.091	-	0.042
*PGM**	A	0.045	-	0.278	-	0.100	0.083	-	0.150	0.104
	B	0.523	0.462	0.472	0.605	0.600	0.458	0.455	0.650	0.604
	C	0.432	0.538	0.250	0.342	0.300	0.458	0.500	0.200	0.292
	D	-	-	-	0.053	-	-	0.045	-	-
*GPI**	A	0.455	0.500	0.472	0.500	0.500	0.500	0.341	0.400	0.500
	B	0.545	0.500	0.528	0.500	0.500	0.500	0.659	0.600	0.500
*MDH**	A	0.136	0.192	0.306	0.237	0.250	0.292	0.250	0.200	0.208
	B	0.864	0.808	0.694	0.763	0.750	0.667	0.750	0.800	0.792
	C	-	-	-	-	-	0.042	-	-	-
*HK-1**	A	-	-	-	0.079	-	-	0.045	-	-
	B	0.409	0.385	0.361	0.474	0.500	0.500	0.432	0.500	0.333
	C	0.136	0.231	0.056	-	0.100	0.083	0.023	-	-
	D	0.455	0.385	0.583	0.447	0.400	0.417	0.500	0.500	0.667
*HK-2**	A	0.205	0.154	0.111	0.105	0.300	0.125	0.045	0.050	-
	B	0.045	0.192	-	-	-	-	-	-	-
	C	0.750	0.654	0.750	0.895	0.700	0.875	0.955	0.950	1.000
	D	-	-	0.139	-	-	-	-	-	-
*LA-1**	A	-	-	0.056	-	-	-	-	-	-
	B	0.977	1.000	0.944	1.000	1.000	1.000	1.000	1.000	0.979
	C	0.023	-	-	-	-	-	-	-	0.021
*LA-2**	A	-	-	0.056	0.132	-	-	-	-	-
	B	1.000	1.000	0.861	0.711	1.000	0.875	0.909	1.000	0.583
	C	-	-	0.083	0.158	-	0.125	0.091	-	0.417
n		22	13	18	19	10	12	22	10	24

Ratios of observed to expected genotypic diversity (G_O:G_E) ranged from 0.556 to 1.008 in the fiord populations (table 3), with a mean of 0.837. Observed genotypic diversity was significantly different to values expected in panmictic populations at four out of seven sites in Doubtful Sound, viz. Tricky Cove, Turn Point, Bradshaw Sound and Bauza Island (table 3).

Nei's unbiased genetic distance (D) was low between sites (0-0.047) and between

Table 2. Summary results from F-statistic estimates of within population variation (F_{IS}) and between population variation (F_{ST}) at nine loci for *Antipathes fiordensis* from nine sites in Doubtful and Nancy Sounds. *** $p < 0.001$, ** $p<0.01$, * $p<0.05$.

Locus	No. of alleles	df	F_{IS}	F_{ST}
*EST-2**	3	16	0.231**	0.156***
*IDH**	2	8	0.618***	0.114***
*PGM**	4	24	-0.103	0.045*
*GPI**	2	8	-0.884***	0.012
*MDH**	3	16	-0.080	0.016
*HK-1**	4	24	-0.472***	0.030
*HK-2**	4	24	0.187***	0.094***
*LA-1**	3	16	0.542***	0.033
*LA-2**	3	16	0.479***	0.176***
Mean			-0.179*	0.061**

Table 3. Genetic variation (± SE) in *Antipathes fiordensis* populations from nine sites in Doubtful and Nancy Sounds. N_I is the number of individual colonies at each site. N_C is the number of genotypes observed at each site. G_O and G_E are observed and expected genotypic diversity, respectively. * $p<0.05$, ** $p<0.01$, ***$p<0.001$.

Site	mean no. of alleles/locus	% loci polymorphic	Mean heterozygosity		N_I	N_C	G_O:G_E
			Observed	H-W expected			
Doubtful Sound							
Tricky Cove	1.9 (0.3)	58.3	0.254 (0.091)	0.224 (0.071)	29	22	0.728*
Deep Cove	1.8 (0.3)	50.0	0.308 (0.108)	0.261 (0.082)	13	13	1.008
Turn Point	2.0 (0.2)	66.7	0.250 (0.097)	0.264 (0.070)	25	18	0.817*
Bauza Island	1.8 (0.2)	58.3	0.298 (0.104)	0.258 (0.071)	25	19	0.813*
Gaer Arm	1.8 (0.2)	58.3	0.292 (0.112)	0.243 (0.072)	11	10	0.874
Bradshaw	1.9 (0.2)	66.7	0.292 (0.108)	0.254 (0.069)	25	12	0.566***
Thompson	2.0 (0.3)	66.7	0.250 (0.094)	0.231 (0.065)	25	22	0.889
Nancy Sound							
Nancy Entrance	1.6 (0.2)	50.0	0.267 (0.106)	0.183 (0.066)	15	10	0.879
Bend Point	1.8 (0.2)	66.7	0.250 (0.096)	0.240 (0.068)	25	24	0.961

fiords (0-0.038) (table 4), and was within the expected range between populations of a single species. There was no clustering of sites by fiord or relative to their geographic separation within fiords based on Nei's D (fig. 2). Hierarchical analysis of standardised genetic variation (F_{ST}) combined across loci for *A. fiordensis* indicated that higher levels of variation existed among sites within fiords than existed between Doubtful and Nancy Sounds (F_{ST} among sites in fiords = 0.043, F_{ST} among sites total = 0.039, F_{ST} between fiords total = -0.005).

Discussion

Allozyme electrophoretic results suggest *Antipathes fiordensis* populations repro-

Table 4. Nei's unbiased genetic distance (D) for populations of *Antipathes fiordensis* between sites in Doubtful and Nancy Sounds.

SITE	1	2	3	4	5	6	7	8
Doubtful Sound								
1. Tricky Cove	-							
2. Deep Cove	0.005	-						
3. Turn Point	0.010	0.038	-					
4. Bauza Island	0.019	0.047	0.007	-				
5. Gaer Arm	0	0.018	0	0.007	-			
6. Bradshaw	0	0.012	0	0.003	0	-		
7. Thompson	0.004	0.014	0.012	0.012	0.007	0	-	
Nancy Sound								
8. Entrance	0.002	0.029	0.002	0.012	0	0	0.004	-
9. Bend Point	0.026	0.038	0.022	0.021	0.031	0.013	0.016	0.020

duce both sexually and asexually. It was previously thought that male colonies were extremely rare in Fiordland and that asexual reproduction may be the dominant reproductive mode in *A. fiordensis*. However, histological studies have shown that male colonies are present in Doubtful Sound (N. Parker, unpublished data) and recent surveys suggest them to be relatively common (K. Miller, unpublished data). Natural spawnings of black corals have never been observed. However, artificial spawnings of male and female black coral colonies produce externally fertilised eggs which develop into ciliated planulae over a 36 hour period (K. Miller, unpublished data). These observations suggest that sexual reproduction does occur in *A. fiordensis* (probably with external fertilisation and larval development) and the presence of large numbers of unique genotypes in both Doubtful and Nancy Sounds is consistent with the occurrence of sexual reproduction in the population.

The high frequency of four multilocus genotypes (A-D) in both Doubtful and Nancy Sounds is thought to reflect clonality in *A. fiordensis*, and this assumption is supported by significant G_O:G_E values at four of the nine sites. The mean value of G_O:G_E in *A. fiordensis* is relatively high (0.837) compared to scleractinian corals which are known to be primarily asexual (e.g. *Pocillopora damicornis* (Linnaeus, 1758) mean G_O:G_E = 0.27, Stoddart, 1984; *Pavona cactus* (Forskål, 1775) mean G_O:G_E = 0.35, Ayre & Willis, 1988) and asexually reproducing anemones (mean G_O:G_E = 0.52, Ayre et al., 1991). However, G_O:G_E in *A. fiordensis* is similar to populations of scleractinian coral that are predominantly sexual but have some asexual recruitment (*Montipora digitata* (Dana, 1846) mean G_O:G_E = 0.79/0.86, Stobart & Benzie, 1994; *Seriatopora hystrix* Dana, 1846, mean G_O:G_E = 0.75, Ayre & Dufty, 1994). The number of clones found in *A. fiordensis* is also considerably higher than in the entirely sexual scleractinian coral, *Platygyra*, in which only two out of 148 colonies had identical 9-locus genotypes (Miller 1994). We suggest that recruitment to *A. fiordensis* populations will be predominantly sexual but that some asexual reproduction also occurs. The wide range of G_O:G_E values in *A. fiordensis* populations (0.566-1.008) most likely reflects the differing contributions of sexual and asexual reproduction to recruitment at each site.

Interestingly, clones were found in both fiords (fig. 1) which suggests that asexual

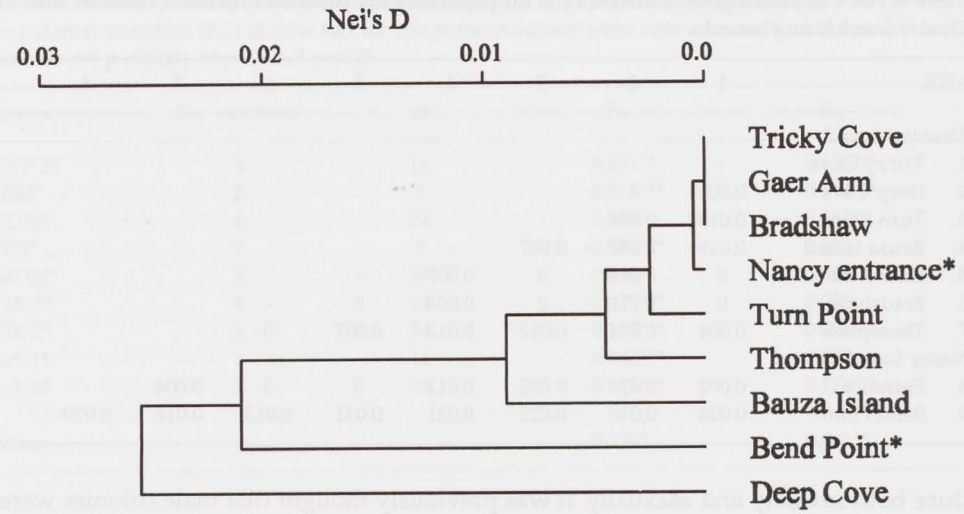

Fig. 2. Cluster diagram of sites using UPGMA based on Nei's unbiased genetic distance (D). * denotes sites in Nancy Sound, all other sites are in Doubtful Sound.

propagules can be widely dispersed. The mode of asexual reproduction in *A. fiordensis* is unknown. Fragmentation with high dispersal is unlikely as fragments are negatively buoyant and will fall downwards. In addition, the New Zealand fiords are highly stable environments with weak currents and restricted water movement (Stanton & Pickard, 1981) which are unlikely to aid in the dispersal of large coral fragments. The spatial distribution of clones within sites (unpublished data), as well as between sites, is also inconsistent with the hypothesis that fragmentation with limited dispersal may be occurring.

Modes of asexual reproduction that have been observed in other anthozoans include parthenogenesis of spawned eggs in the gorgonian *Plexaura* A (Brazeau & Lasker, 1989) and asexually produced brooded planulae (produced independently of the gonads) in the scleractinian corals *Pocillopora damicornis* (cf. Stoddart, 1984), *Tubastrea diaphana* Dana, 1846, and *T. coccinea* Lesson, 1829 (cf. Ayre & Resing, 1986). While brooded planulae have never been observed in the polyps of *A. fiordensis*, insufficient studies of the reproductive cycle of these corals have been completed to rule out this possibility.

A type of "polyp bail-out" (Sammarco, 1982) has been observed in *A. fiordensis* colonies (N. Parker, personal communication; personal observation). Under stressful conditions in aquaria, parts of the polyp (presumably the tentacles) will drop off the colony forming large, ciliated and highly mobile "planulae". Conditions associated with high levels of rainfall or plankton blooms may stress colonies in natural populations and induce "polyp bail-out". This phenomenon would be an effective mode of asexual reproduction and dispersal but it is not known if it occurs naturally.

There was significant structuring within sites (F_{IS}) and genotype frequencies in *A. fiordensis* did not match those expected under conditions of Hardy-Weinberg equilib-

rium (i.e. random mating). This is not surprising in a population which we suggest is reproducing both sexually and asexually. Heterozygote excesses may result from a variety of mechanisms including assortive mating and positive selection. However we suggest that in *A. fiordensis*, the heterozygote excesses found at *HK-1** and *GPI** may occur as a result of asexual reproduction. Similarly, the significant heterogeneity within sites (F_{IS}; table 2) could result from differential asexual reproduction of clones.

The pattern of gene flow in *A. fiordensis* appears complex. There was significant genetic variation among sites within fiords (F_{ST}, table 2) suggesting some localised restriction in larval dispersal. Laboratory reared sexual planulae of *A. fiordensis* are negatively buoyant, weak swimmers (K. Miller, unpublished data) and seem unlikely to be transported any distance. Similarly, water movement both within and between fiords is restricted due to the presence of shallow sills at their entrances and strong estuarine circulation (Stanton & Pickard, 1981). However, no significant genetic differences were found between Doubtful and Nancy Sounds (table 4, fig. 2) which suggests that gene flow does occur between the two fiords. Clustering of sites by genetic distance showed no clear geographic patterns as might be expected if gene flow was restricted (ie sites that are separated by up to 60 km are no more genetically distinct than sites which are within 10 km of each other). In addition, the presence of clones between fiords suggests that dispersal of asexual propagules may take place over large distances. It may be that *A. fiordensis* has at least two reproductive modes: an asexually produced, widely dispersed larva and a sexually produced limited dispersal larva. Such a reproductive strategy would be unique in anthozoans which generally have a sexually produced dispersal phase with asexual recruitment occurring on a local scale (e.g. scleractinian corals, Harrison & Wallace, 1990; octocorals, Beneyahu & Loya, 1985).

The genetic structuring observed within populations (sites) may be the result of a combination of factors, including asexual reproduction, dispersal and overlapping generations. The lack of genetic differentiation between the two fiords may also be due to the absence of selection in this unique and relatively stable environment or to similar selection acting in both fiords. Long generation times of black corals (Grange, 1985) confound the interpretation of dispersal patterns as long lived genotypes with relatively low dispersal may still reach wide geographic areas (Potts & Garthwaite, 1991; Hughes et al., 1992). In addition, the distribution of *A. fiordensis* in areas outside the mouths of the fiords, and the potential contribution of these colonies to the gene pool within fiords is unknown.

From the results of this study we have inferred that two modes of reproduction occur in the antipatharian black coral *A. fiordensis*. Similarly, the pattern of geographic variation suggests that dispersal patterns in these corals will be complex. Preliminary laboratory studies of reproduction in *A. fiordensis* support these findings. However, interpretation of many of our results is confounded by a lack of detailed knowledge covering many fundamental aspects of black coral biology, and highlight the need for further research on the reproductive ecology in the Antipatharia.

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Hydroids from the vicinity of a nuclear power plant site (CNAAA-Unidade I) at Angra-dos-Reis, Rio de Janeiro, southeastern Brazil

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Key words: Cnidaria; Hydroida; faunal list; Brazil.

Abstract: An inventory of anthoathecate and leptothecate hydroids was carried out in areas adjacent to the Central Nuclear Almirante Álvaro Alberto-Unidade I (CNAAA-Unidade I) nuclear power plant at Angra-dos-Reis, Rio de Janeiro State, SE Brazil. Undertaken prior to completion of the plant, collecting was limited to shallow depths (1 m below the *Chthamalus* zone). In all, 38 species were collected, referrable to 24 genera. Two genera (*Tubiclava, Filellum*) and three species (*Tubiclava* spec., *Filellum serratum* (Clarke, 1879), *Halecium lightbourni* Calder, 1991) are reported from Brazil for the first time.

Introduction

Utilization of atomic energy for peaceful purposes, including the generation of electric power, has been overshadowed by the portentous applications of nuclear technology. Moreover, the safety of nuclear powered electric plants has sometimes been questioned. Environmental assessments are therefore routinely conducted to determine the impact and potential hazards of such projects. Thus, an environmental impact assessment was carried out in 1980/81 in the vicinity of the power plant site at Angra-dos-Reis, a location also important as tourist destination, prior to completion of the first nuclear plant located in the state of Rio de Janeiro.

To determine the level of biological diversity in the region, collecting was carried out between 1976-1979 while the power plant was still under construction. A delay in completion of the plant, due to technical difficulties, provided an opportunity to commence a larger scale integrated study of the area. The resulting project ("Análise Biológica da Flora e Fauna Marinhas da Região sob influência da Central Nuclear Almirante Álvaro Alberto-Unidade I (CNAAA-Unidade I), Angra-dos-Reis, Rio de Janeiro") was undertaken from January 1980 to March 1981. The project constituted the most extensive and systematic inventory carried out on the biota of the Brazilian rocky coast up to that time. It also represented the first large-scale environmental impact study to be undertaken with support by private organizations (Furnas Centrais Elétricas S.A., in conjunction with Fundação Universitária José Bonifácio) by a Brazilian university (Universidade Federal do Rio de Janeiro). Three departments within the Instituto de Biologia (UFRJ) took part. Animal communities and abiotic factors were studied and/or analysed by the staffs of the Departamento de Zoologia

and Departamento de Biologia Marinha, while the algal flora was investigated by members of the Departamento de Botânica.

The nuclear power plant, with a pressurized water cooling system, was built at Itaorna Inlet, Angra-dos-Reis. Water used to cool the system is taken from an intake at Itaorna and discharged through an outlet at Piraquara de Fora, Baía da Ribeira. Such water circulation systems often create ideal conditions for the development of rich encrusting assemblages, making use of biocides or antiencrustants necessary (Mayr et al., 1989: 47). Radioactive materials inside the reactor are treated and isolated. Effluent from the plant, elevated in temperature and in chloride content, is also monitored for radionuclides as it is discharged.

The purpose of this report is to provide a baseline list of the species of anthoathecate and leptothecate hydroids occurring in the vicinity of the nuclear power plant CNAAA-Unidade I.

Material and Methods

The study area, located 120 km south of Rio de Janeiro, Baía da Ribeira (fig. 1), with the inlets Piraquara de Fora and Piraquara de Dentro, is sheltered by the nearby Serra do Mar mountain range and is characterized by calm waters. The coastline is predominantly rocky with numerous islets. The climate is humid and tropical. Rainfall is usually highest in summer (December to March), while the winter season is relatively dry.

Collecting was undertaken at 67 locations on the rocky coast of Piraquara de Fora and Piraquara de Dentro. Hydroids were present in 34 of these locations. Samples were collected monthly from January 1980 to February 1981 from eight stations, chosen randomly. The stations were sampled by snorkeling from a boat. Material collected from a horizontal band 1 m below the *Chthamalus* zone was anaesthetized using menthol crystals and fixed in a 10% seawater-formalin solution. Permanent slides were mounted basically following Mayal's method (Mayal, 1973). Specimens collected during this investigation have been deposited in the Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (DZ-IB-UFRJ). The hydroid classification followed here is largely that of Bouillon (1985).

Results

In 1980, the annual range of water temperature at the study area was 22-30°C, being fairly constant from February to April, decreasing 5-7°C from May to September, rising again in November. The salinity range was 28-36‰ through the year. It was higher from June to September, and lower when rainfall was heaviest. Marked differences in temperature and salinity were not observed between the surface and 5 m depth (Nogueira et al., 1991: 3224).

The hydroids collected were referrable to 38 species and 24 genera in 14 families (table 1). Most of the species were collected on algae, mainly *Sargassum* spec. but also *Amphiroa fragilissima*, *Gelidium pusillum* and *Dictyopteris delicata*. Some specimens were found growing on the telestacean anthozoan *Carijoa riisei* (Duchassaing & Michelotti, 1860), as well as on Zoanthidea, Mollusca, Ectoprocta and Porifera. Five species (*Filellum serratum* (Clarke, 1879), *Hebella scandens* (Bale, 1888), *Clytia hemi-*

sphaerica (Linnaeus, 1767), *Sertularia turbinata* (Lamouroux, 1816) and *Obelia dichotoma* (Linnaeus, 1758) were observed living epizootically on other hydroids.

Among the 38 species collected, fertile specimens were found of only seven of them: *Eudendrium carneum* Clarke, 1882, with male gonophores; *Aglaophenia latecarinata* Allman, 1877, with a female corbula; *Coryne pusilla* Gaertner, 1794; *Halopteris diaphana* (Heller, 1868); *Sertularia marginata* Kirchenpauer, 1864; *Sertularia distans* (Lamouroux, 1816); and *Sertularia turbinata* (Lamouroux, 1816).

Two of the genera (*Tubiclava* Allman, 1863, and *Filellum* Hincks, 1868) and three of the species (*Tubiclava* spec., *Filellum serratum* (Clarke, 1879), and *Halecium lightbourni* Calder, 1991) are here recorded for the first time from Brazil.

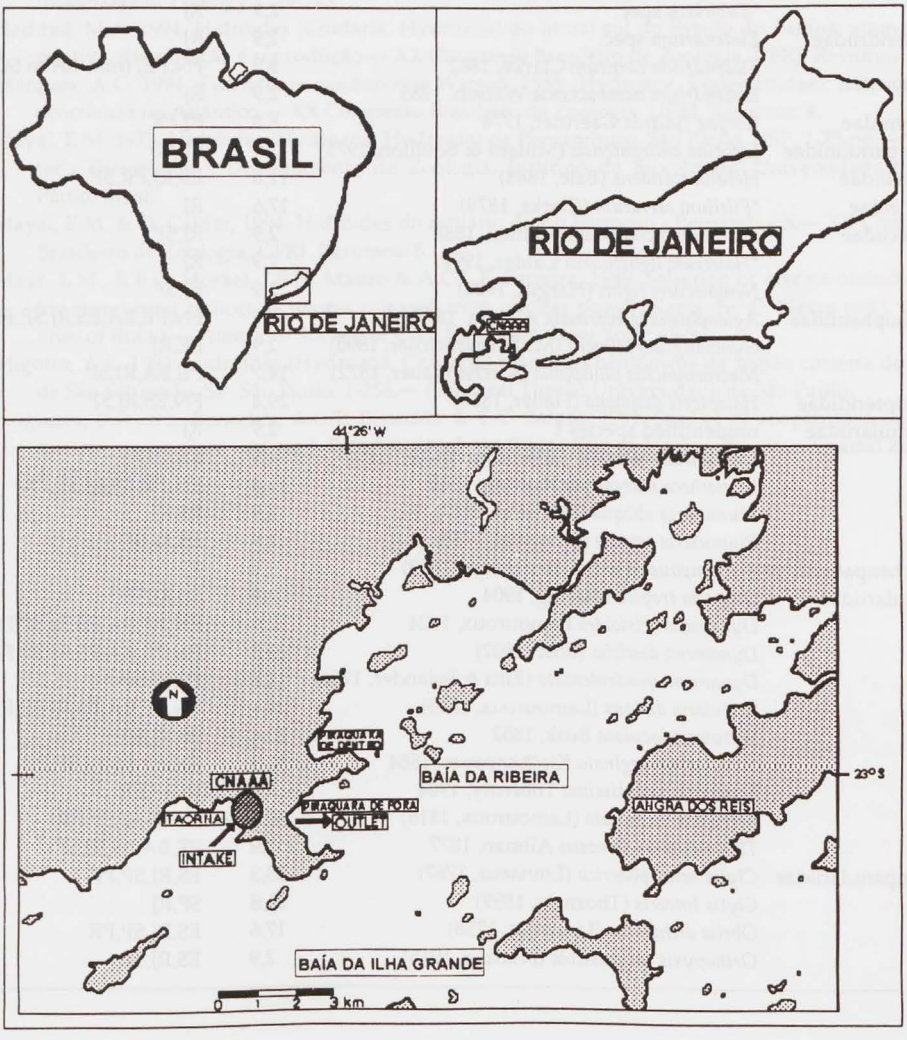

Fig. 1. Map of Baía da Ribeira region, showing location of the CNAAA-Unidade I nuclear power plant at Angra-dos-Reis, Rio de Janeiro, Brazil. Sea-water intake and outlet sites are at Itaorna and Piraquara de Fora, respectively. (Modified from Mayr et al., 1989).

Table 1. Hydroid species and their frequencies of occurrence in the 34 qualitative collections from Baía da Ribeira, Angra-dos-Reis, Rio de Janeiro, Brazil; and their known ranges along the Brazilian coast (after Migotto, 1993; Haddad, 1994; Marques, 1994; and Mayal & Calder, 1994); * = genus/species recorded from Brazil for the first time.
Abbreviations: FN = Fernando de Noronha Island; PE = Pernambuco; BA = Bahia; ES = Espirito Santo; RJ = Rio de Janeiro; SP = São Paulo; PR = Paraná; SC = Santa Catarina.

Family	Species	Frequency (%)	Distribution
Bougainvilliidae	*Bougainvillia ?rugosa* Clarke, 1882	2,9	SP,RJ
Clavidae	unidentified species 1	2,9	RJ
	unidentified species 2	2,9	RJ
	**Tubiclava* spec.	2,9	RJ
Eudendriidae	*Eudendrium* spec.	2,9	RJ
	Eudendrium carneum Clarke, 1882	5,9	FN,PE, from BA to SC
	Eudendrium nambuccense Watson, 1985	2,9	RJ
Corynidae	*Coryne ?pusilla* Gaertner, 1774	8,8	RJ,PR
Campanulinidae	*Lafoeina amirantensis* (Millard & Bouillon, 1973)	2,9	PE,RJ
Hebellidae	*Hebella scandens* (Bale, 1888)	11,8	ES,RJ,PR,SP
Lafoeidae	**Filellum serratum* (Clarke, 1879)	17,6	RJ
Haleciidae	*Halecium dichotomum* Allman, 1888	2,9	RJ,SP
	**Halecium lightbourni* Calder, 1991	2,9	RJ
	Nemalecium lighti (Hargitt, 1924)	2,9	RJ,SP
Aglaopheniidae	*Aglaophenia latecarinata* Allman, 1877	47,1	FN,PE,BA,ES,RJ,SP,PR
	Gymnangium allmani (M.-Turneretscher, 1890)	2,9	PE,RJ
	Macrorhynchia philippina (Kirchenpauer, 1872)	14,7	PE,BA,RJ,SP
Halopteridiidae	*Halopteris diaphana* (Heller, 1868)	29,4	FN,ES,RJ,SP
Plumulariidae	unidentified species 1	2,9	RJ
	Dentitheca bidentata (Jäderholm, 1920)	2,9	PE,ES,RJ
	Monotheca margaretta Nutting, 1900	11,8	FN,PE,ES,RJ,SP,PR
	Plumularia obliqua (Johnston, 1847)	2,9	ES,RJ
	Plumularia setacea (Linnaeus, 1758)	2,9	ES,RJ,SP
Kirchenpaueriidae	*Kirchenpaueria halecioides* (Alder, 1859)	2,9	RJ
Sertulariidae	*Diphasia tropica* Nutting, 1904	2,9	ES,RJ,SP
	Dynamena crisioides Lamouroux, 1824	2,9	FN,PE,BA,ES,RJ,SP,PR
	Dynamena disticha (Bosc, 1802)	23,5	FN,PE,BA,ES,RJ,SP,PR
	Dynamena quadridentata (Ellis & Solander, 1786)	11,8	ES,RJ,SP
	Sertularia distans (Lamouroux, 1816)	29,4	FN,PE,BA,ES,RJ,SP,PR
	Sertularia loculosa Busk, 1852	20,6	ES,RJ,SP
	Sertularia marginata Kirchenpauer, 1864	52,9	FN,PE,ES,RJ,SP,PR
	Sertularia rugosissima Thornely, 1904	8,8	RJ,SP,PR
	Sertularia turbinata (Lamouroux, 1816)	50,0	PE,ES,RJ,SP,PR
	Thyroscyphus ramosus Allman, 1877	5,9	PE,BA,ES,RJ,SP
Campanulariidae	*Clytia hemisphaerica* (Linnaeus, 1767)	35,3	ES,RJ,SP,PR
	Clytia linearis (Thornely, 1899)	11,8	SP,RJ
	Obelia dichotoma (Linnaeus, 1758)	17,6	ES,RJ,SP,PR
	Orthopyxis sargassicola (Nutting, 1915)	2,9	ES,RJ,SP

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The puzzle of the polyp phase of *Bougainvillia superciliaris* (L. Agassiz, 1849) (Hydroidea: Athecata) in the White Sea

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Orlov, D.V. The puzzle of the polyp phase of *Bougainvillia superciliaris* (L. Agassiz, 1849) (Hydroidea, Athecata) in the White Sea.
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Key words: Hydroidea; *Bougainvillia superciliaris*; planula; behaviour; settlement; metamorphosis; White Sea.

Abstract: Although medusae of the metagenetic hydroid *Bougainvillia superciliaris* (L. Agassiz, 1849) are abundant in the White Sea, its polyp phase had been found there only once, in 1980. In the period 1991-1994 an attempt was made to forecast the possible benthic location of the colonies, based on a study of larval settlement in the laboratory. In vitro observations demonstrated that after release from the medusae, planulae of *B. superciliaris* sink to the bottom, and, showing positive geotaxis and schizotaxis, creep into the substratum, to settle gregariously, especially on bivalve shells and stones. In the White Sea, at depths below 25 m, these last-named types of substrate are present in abundance, protruding through soft sediments and thus forming a potential niche of the polyp phase. Field research in August 1995 indeed confirmed the presence of colonies of *B. superciliaris* in the predicted niche, supporting the idea that the benthic distribution of *B. superciliaris* is determined by the specific behaviour of settling planulae. Similar studies of larval settlement in the laboratory may also prove useful in predicting unknown habitats of other sessile organisms.

Introduction

Medusae of the athecate, metagenetic hydroid *Bougainvillia superciliaris* (L. Agassiz, 1849) are abundant in arctic seas, in the Norwegian Sea, in the White Sea and the Sea of Japan (Hartlaub, 1911; Rees, 1938; Russell, 1953; Werner, 1961; Uchida & Nagao, 1960; Margulis & Karlsen, 1985). The polyp phase was found for the first time by Hartlaub (1911) in the Norwegian Sea. During the following 84 years hydroid colonies were recorded only three times: twice in the Sea of Japan on the shell of the gastropod *Neptunea* spec. (Uchida & Nagao, 1960; Nagao, 1964) and once in intertidal zone of the White Sea, on rhizoids of the alga *Laminaria saccharina* and on the carapace of an amphipod (Margulis & Karlsen, 1985). As the colonies of *B. superciliaris* were never found in Russian seas prior to 1980, Naumov (1960) mistakenly gave the description of *Bougainvillia ramosa* (Van Beneden, 1844) as that of *B. superciliaris* in his monograph on hydroids of Russian seas. The colony from the Kuril Islands described as *B. superciliaris* by Antsulevich, 1987, differs from those described by previous authors. The polyp phase of *B. superciliaris*, raised from larvae released by medusae under laboratory conditions, was described by Rees (1938), Uchida & Nagao (1960) and Werner (1961). The process of larval settlement was not studied, and the only information available was that the planulae settled laterally on substrate surfaces in tanks.

The importance of larval settlement in relation to spatial distribution of benthic

sessile forms has been reported for various marine invertebrate taxa (Crisp, 1984; Hadfield, 1986; Roughgarden et al., 1988), including hydroids (Orlov, 1996a, 1996b) and other cnidarians (Chia & Bickell, 1978). The present paper reports on a laboratory study of larval settlement in *Bougainvillia superciliaris* undertaken with the object to find indications about the natural habitat of the polyp phase in the White Sea.

Material and methods

Observations and experiments were conducted in 1991-1994 at Velikaja Salma Bay, White Sea. In search of colonies of *Bougainvillia superciliaris* different substrata at 0-25 m depth were carefully examined in the period from May-September, using SCUBA equipment.

Medusae with mature larvae were collected in the bay and placed in glass vessels. At two hour intervals newly liberated planulae were transferred by pipette from the bottom to experimental dishes.

Shells of molluscs and cirripeds, crab carapaces, stones, and sand were placed in two illuminated and two dark vessels. One hundred planulae were placed into each vessel. The number of planulae having settled on each of proposed substrata was recorded at the end of each experiment. Intraspecific reactions of planulae to the presence of conspecific larvae, juvenile polyps and developed colonies were observed while conducting the experiments.

The reactions of settling planulae to environmental factors such as light, gravity, types of natural substrate, inclination and roughness of the substrate surface, and water flows (ranging from 2 mm/s. to 160 mm/s.) were studied using methods described in detail in previous papers (Orlov, 1996c; Orlov & Marfenin, 1994, 1995).

The reactions to light were studied in dark petri dishes 80 mm across with transparent areas of two types, a circle 5 mm in diameter, and a strip 3 mm wide and 80 mm long, both in the centre of the dish, illuminated from below by a day-light lamp.

The influence of substrate inclination was studied on plastic plates inclined at angles of 5°, 10°, 15°, 20°, 30°, 45° and 60°. The influence of substrate roughness was investigated in 40 mm wide channels created by placing small petri dishes (diameter 80 mm) inside larger ones (diameter 160 mm). The bottom of each channel was covered by a plastic ring divided into 28 (4 groups of 7) equal segments of varied roughness, arranged in a regular pattern. In each group one segment was untreated (smooth), whereas the other six were rubbed with sand paper of different grain size, varying in diameter from 0.02-0.1 mm to 1.3-1.5 mm. The influence of substrate relief was investigated using similar ring-shaped channels with paraffin wax on the bottoms, in which crevices and depressions were carved in with a scalpel.

Substrate preference experiments were also done in petri dishes with microbial films washed from the various substrates tested. In each experiment were used nine dishes with, and nine control dishes without such film; in six dishes with a film the water was removed by pipette once a day, in three dishes for one hour and in the other three for three hours. Into each of these dishes 20 planulae were placed. Water removal in the dishes was conducted to imitate intertidal exposure during low tides both in the upper and lower parts of littoral. The statistical significance of variations in larval settlement on different substrata was determined with the chi-square test.

General illumination in laboratory was diffuse and rather dim throughout day and night. The temperature in the laboratory was maintained at 10-14°C, i.e. about 2-4°C higher than the surface temperature in the bay.

Results

Settlement and metamorphosis

Larvae of *Bougainvillia superciliaris* are usually released from medusae during the last two weeks of June. Strong contractions of the medusan velum detach planulae from the manubrium and push them into the subumbrellular cavity, and then out to the water. At liberation the colourless, cylindrical, ciliated planulae are 0.3 mm long (N = 50; SD = 0.08 mm) and are slightly swollen apically. They sink to the bottom and start to metamorphose three or four days after the liberation. The settling planula attaches to a substrate laterally and looses its cilia. The apical part buds a primary polyp with four tentacles. A stolon can develop from the tail end, from both ends or simultaneously from the ends and the middle. Twisted and bifurcated stolons create a branching network of hydrorhizal fibres (fig. 1).

On natural substrata such as sand, stones, molluscan and cirripedian shells, as well as on microbial films covering their surfaces, settlement of 600 planulae in total started at the age of three to four days, and the settlement of 280 planulae at the age of five to six days. In the control dishes free of hard substrata or microbial films, 480 larvae metamorphosed within 8 to 14 days after liberation, while the settlement of 90 planulae was delayed for three weeks. The intensity of planula settlement decreased in the following order of natural and artificial substrates: shells of bivalves, stones, gastropod shells, cirriped shells, empty crab carapaces, sand, plastic and glass (fig. 2).

On smooth substrata such as shells of bivalves, plastic and glass, planulae of more than three days of age showed positive thigmotaxis: several planulae approached each other, orientated and metamorphosed simultaneously. The stolons of such aggregated specimens grew tightly together in one direction. Initially, groups of planulae accumulated around organic or sand particles, in crab carapaces and in hydrothecae of hydroids, in hollows or even at the lowest point of an inclined substrate. Later on larvae settled around or even on those initial colonies, their growing stolons covering the initial specimens, forming giant aggregations with numerous polyps with interlaced stolons. Several aggregated colonies reached maximum of two hundred individuals.

Responses of planulae to environmental factors

The distribution of 80 planulae in four partly lighted petri dishes was random during the whole period of activity; larvae were moving in all directions irrespective of light and dark, and brightness of illumination. There was also no difference in larval settling behaviour between dark and illuminated vessels (fig. 2).

Observations of settling planulae demonstrated positive geotaxis: although planulae could move up a surface inclined at a maximum angle of 15°, all larvae (N = 150) placed on plastic plates oriented at angles between 5° and 60° accumulated at

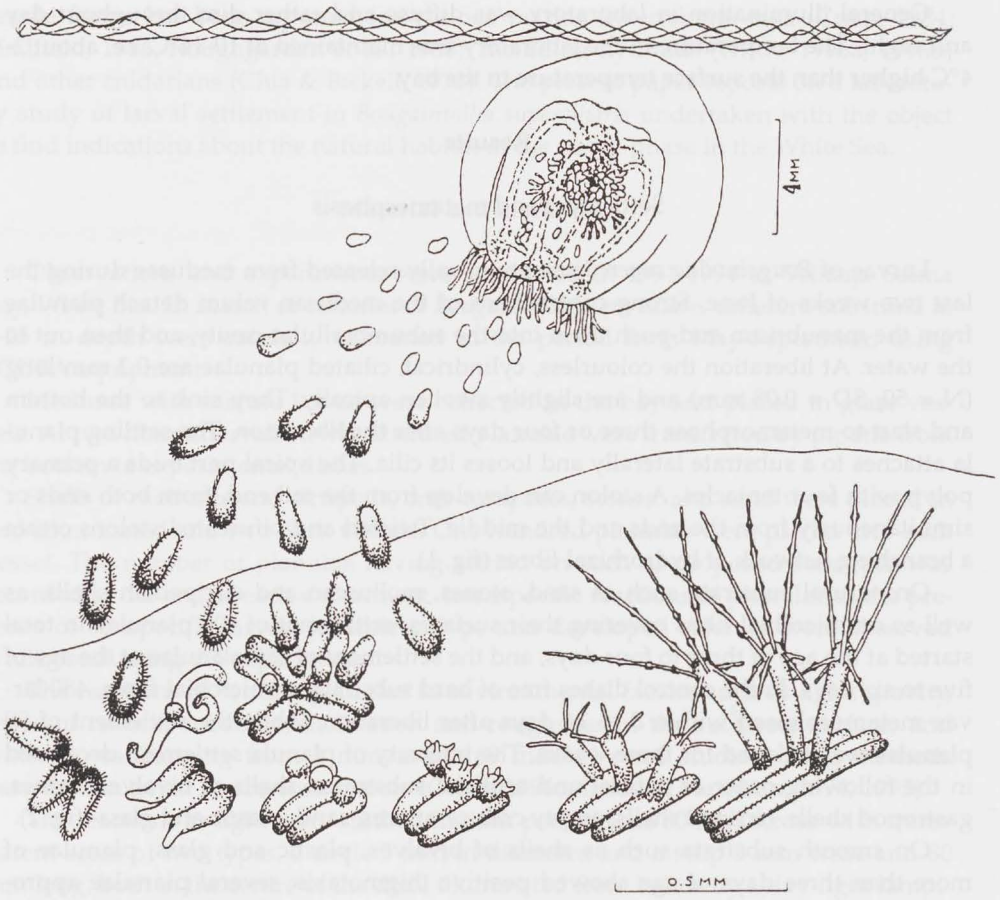

Fig. 1. Larval settlement of *Bougainvillia superciliaris*: liberation from medusa, contact with bottom, orientation, attachment and metamorphosis.

the bottom of the vessels, i.e. at the lowest surface. Planulae of all ages moved regularly to the lowest part of a substrate regardless of its inclination. Planulae settled only on or under horizontal surface and did not attach to inclined substrata. They (N = 110) performed schizotaxis by creeping into substrate depressions, crevices, holes, cracks, hollow hydrothecae of hydroids, crab and some other crustacean carapaces, cirriped shells, under stones, between or under sand grains, and even by settling as regular rows in the growth rings of bivalve shells. Planulae of *Bougainvillia superciliaris* (N = 1206) distributed and settled on surfaces of varied roughness without preference for smooth or rough surfaces (table 1).

The planulae did not move if the velocity of the water flow exceeded 8 mm/s. (N = 110); in flows ranging from 3 mm/s. to 8 mm/s. the planulae moved in various directions but did not settle (N = 150); the larvae metamorphosed only if the current was slowed down to less than 3 mm/s. (N = 90).

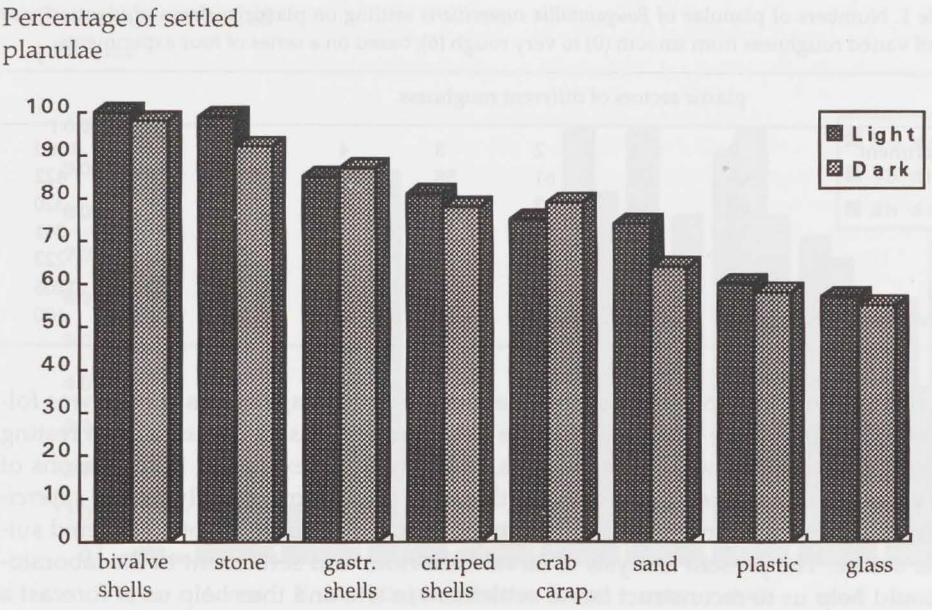

Fig. 2. Planula settlement of *Bougainvillia superciliaris* on various substrata in light and darkness: bivalve shells, stones, gastropod and cirriped shells, crab carapaces, sand, plastic and glass.

Response to algae and microbial films

Of the 200 planulae placed on the surface of brown and red algae only ten planulae settled: two on *Rhodymenia palmata* and eight on *Laminaria saccharina;* the remaining larvae decomposed on thalli of brown algae (*Ascophyllum nodosum, Fucus vesiculosus, F. inflatus* and *F. serratus;* (N = 50 for each species).

The rate of larval settlement on microbial films washed off various substrata decreased in the following sequence: stones, shells of bivalves, *R. palmata,* sand, *L. saccharina,* plastic with a film of seawater, *F. inflatus, A. nodosum, F. serratus.* Not a single planula settled on the films of *F. vesiculosus* and *Ulva* spec. The planulae started metamorphosing on the films of hard substrata at the age of five days, on the films of brown algae at the age of eight days. Temporary exposure to air slightly activated larval settlement on films washed off mollusc shells, thalli of *L. saccharina, A. nodosum,* and *F. vesiculosus;* three-hour exposure facilitated the settlement on films of *F. inflatus* and *F. serratus.* Exposure to air suppressed settling of planulae on films of stones, sand, and thalli of *R. palmata* (fig. 3).

Discussion

Why has the polyp phase of *Bougainvillia superciliaris* so rarely been found in nature while its medusae release numerous planulae in the sea every year? Antsulevich (1987: 20) explained this by the small size of the colonies. However, many colonial hydroids are known in various areas that are even smaller than *B. superciliaris.*

Table 1. Numbers of planulae of *Bougainvillia superciliaris* settling on plastic surfaces of identical size but of varied roughness from smooth (0) to very rough (6); based on a series of four experiments.

	plastic sectors of different roughness							
Experiment	0	1	2	3	4	5	6	total
1	48	76	61	58	64	57	58	422
2	60	46	43	56	30	35	50	320
3	35	55	32	30	27	16	46	241
4	52	28	24	34	24	28	33	223
total	195	205	160	178	145	136	187	1206
Percentage	16	17	13.3	14.7	12	11.5	15.5	100

For this reason it was considered possible that the larval stage of this species was followed by an immobile cyst stage and the development was continued after a resting period. Benthic shallow water substrates, however, were examined in all seasons of the year, but no colonies were found. It therefore seemed more likely that *B. superciliaris* inhabited niches not examined so far, or lived on a substrate not considered suitable before. The present analysis of larval behaviour and settlement in the laboratory could help us to reconstruct larval settlement in situ and thus help us to forecast a suitable niche for the colonies in Velikaja Salma Bay.

In nature, planulae of *B. superciliaris* are released near the water surface and sink to the bottom during the following three to four days or more (this is the minimal "precompetent" period of the planula, during which it can not metamorphose). Dispersal of planulae takes place by tidal currents. When reaching the bottom, they creep between various hard structures into hollows, cracks and wrinkles of the surface; the positive geotaxis and the schizotaxis thus lead the larvae to sites where the diminished water movement will not prevent the planulae from settling. Planulae of hydroids from the tidal zone usually are positive phototactic, but the larvae of *B. superciliaris* in accordance with those of subtidal hydroids have no photoreaction. Temporary exposure to air did not facilitate metamorphosis as it did in tidal species (Orlov, 1996a, 1996c). Polyps of *B. superciliaris*, therefore, most probably inhabit the subtidal zone. The suppression of planula settlement by algae indicates that macrophytes are unlikely to form a substrate for the species. Among the natural substrata that were tested, shells of bivalves and stones as well as microbial films washed off their surfaces, were optimal substrates inducing 100% planula settlement. Bivalve shells and stones therefore are to be considered potential natural substrates of *B. superciliaris*.

The role of positive thigmotaxis and gregarious settlement in the ecology of the colonies is not directly evident, because smooth substrata that in our experiments facilitated gregarious behaviour of planulae, are not abundant in the White Sea. However, larvae falling on the soft bottom, might aggregate on protruding stones and mollusc shells (covered with a specific fouling community), which characterize the "landscape" of the White Sea bottom below 25 m depth, and this might represent the habitat of the polyp phase.

Postscript (added 22 May 1996).— In August 1995, four colonies of *Bougainvillia superciliaris* (identical to those reared in the laboratory) were found in Velikaja Salma Bay on empty shells of the gastropods *Buccinum elatior* (von Middendorf, 1849) (3 ×)

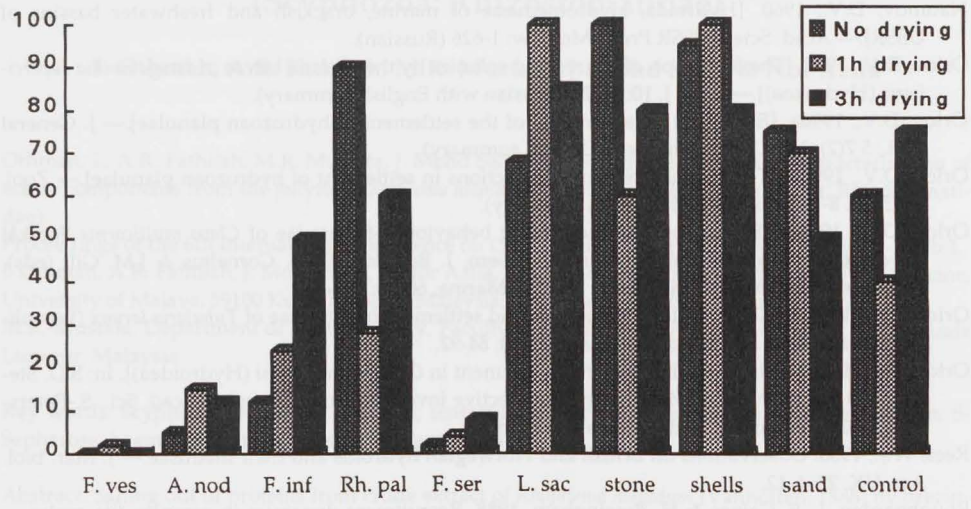

Fig. 3. Planula settlement influenced by the temporary exposure to air, on the surface microbial films washed off hard substrata and thalli of the algae: F. ves = *Fucus vesiculosus*, F. inf = *F. inflatus*, F. ser = *Fucus serratus*, A. nod = *Ascophyllum nodosum*, Rh. pal = *Rhodymenia palmata*, L. sac = *Laminaria saccharina*; shells = bivalve shells.

and *Neptunea despecta* (Linnaeus, 1758) (1 ×) on the bottom slope composed of fine sand and soft sediment between 22 and 30 m depth, at a site characterized by almost complete darkness, slow water movement, the absence of tidal currents, a temperature of 1°C, and a salinity of 26‰.

Two colonies were situated mainly in the sutures and surface depressions of the gastropod shells. The other two covered most of the surface of the shells as a network of branching and anastomosing stolons.

These records confirm the character of the natural habitat of *B. superciliaris* as predicted on the basis of an experimental study of larval settlement. Therefore such studies could also be useful in research of other species.

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Isolation and characterization of active components from the jellyfish *Rhopilema hispidum* (Vanhöffen, 1888) (Scyphozoa: Rhizostomatidae)

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Othman, I., A.R. Fathilah, M.R. Mustafa, J. Mohd Saad, & Nor Azila. Isolation and characterization of active components from the jellyfish *Rhopilema hispidum* (Vanhöffen, 1888) (Scyphozoa: Rhizostomatidae).
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Key words: Scyphozoa; *Rhopilema hispidum*; tentacular extract; ammonium sulphate precipitation; S-Sepharose; haemolytic activity; vascular-relaxant activity.

Abstract: Salting out of proteins from crude extract of *Rhopilema hispidum* (Vanhöffen, 1888) by precipitation with solid ammonium sulphate at 70% saturation, isolated proteins that exhibited the highest vascular relaxant and haemolytic activities. Fractionation of the proteins on S-sepharose showed the presence of one unbound peak (designated Peak I) and 3 bound protein peaks (designated Peak II, III, & IV). The highest specific haemolytic activity was exhibited in Peak III. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of Peak III showed the presence of only one band with a molecular weight of 37.5 kd. Peak III was found to exhibit a strong relaxant effect on the phenylephrine-induced contraction of the rat aorta indicating that the toxin may have an effect on the mechanism involved in the relaxation of smooth muscles, including cyclic guanosine monophosphate (cGMP) systems.

Introduction

Two comprehensive reviews on the chemistry, biological activities, toxicology, immunology and treatment with respect to Coelenterata, including Scyphozoa, have been made by Burnett and Calton (1977; 1987). In these reviews, no mention is made of the jellyfish *Rhopilema hispidum* (Vanhöffen, 1888). This jellyfish is of significant interest in SE Asia since it is among the few jellyfish that are consumed. The umbrella section, due to its large size provides a significant amount of material that can be used in cooking various local dishes. Similarly, there are other species of jellyfish, including *Rhopilema esculentum* Kishinouye, 1891 (Huang et al., 1985) and *Stomolophus meleagris* L. Agassiz, 1862 (Huang, 1988) reported to be eaten by people in China and Korea.

Even though the umbrella of *Rhopilema hispidum* is edible, contact with its tentacles can cause severe stinging pain and burning sensation. In this paper, we report on the isolation and characterization of haemolytic and vascular relaxant components isolated from the tentacles of *R. hispidum* which may be responsible for some of the clinical symptoms observed.

Methods

Extraction of crude venom extract.— Tentacles of *Rhopilema hispidum* were removed and frozen at -20°C until used. Semi-thawed specimens were homogenized in a blender-mixer followed by an Ultra-Turrax homogenizer. The homogenates were filtered through muslin cloth before sonication. The sonicated homogenate was centrifuged at 15,000 rpm at 4°C for 20 min. The supernatant was taken as the crude extract, divided into aliquot parts and stored at -20°C.

Ammonium sulphate precipitation.— The crude extract (100 ml) was fractionated using solid ammonium sulphate precipitation method. Proteins salted out at 70% saturation were pelleted down at 15,000 rpm at 4°C for 20 min. The pellet was diluted with Tris-HCl buffer and used in further purification steps.

Ion-exchange chromatography.— The diluted 70% fraction was placed onto a S-Sepharose ion-exchanger column using a stop-flow method. The unbound protein was washed out with 50 mM Tris-HCl buffer, pH 7.4. A gradient of 1M NaCl (100 ml) was applied after two bed volumes wash. Fractions of 2.5 ml per tube were collected at a flow rate of 20 ml/h and absorbance read at 280 nm to determine the protein content.

Haemolytic activity and specific haemolytic activity.— Specific haemolytic activity was determined according to the method of Azila et al. (1991), a modified version of the method described by Jorgensen et al. (1983). Specific haemolytic activity is expressed as Unit/mg protein. One Unit is defined as the amount of protein (mg) that is required to elicit 50% haemolytic activity in a 1% rabbit erythrocyte suspension at 28°C for 30 min.

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE).— SDS-PAGE using 10% gel was conducted according to the method of Laemli (1970). Protein detection in the polyacrylamide gels was carried out using a sensitive silver staining method by Wray et al. (1981).

Pharmacological studies.— The thoracic aorta of male Sprague-Dawley rats weighing approximately 250 g was dissected into rings of 2-3 mm and incubated in 5 ml Krebs buffer containing: 136.9 mM NaCI, 5.4 mM KCI, 5.5 mM glucose, 23.8 mM $NaHCO_3$, 1.5 mM $CaCI_2$, 1 mM $MgCI_2$, and 0.01 mM EDTA, for 30 minutes, aerated with 95% O_2 and 5% CO_2. The contractile tension of the smooth muscle was recorded isometrically using a force-displacement transducer connected to a polygraph.

Results and discussion

Extraction of active components of *Rhopilema hispidum* was carried out on whole tentacles as described by Azila et al. (1991). Initial crude extract obtained exhibited a heterogeneous population of proteins, with molecular weights range of 44.1 to 121.3 kd (fig. 2) on SDS-PAGE, has a toxicity of 110.11µg/ml (Finney Probit Analysis Pro-

gramme) on brine shrimp and a haemolytic specific activity of 27.2 U/mg protein using rabbit erythrocytes.

Initial fractionation of crude extract using gel filtration on Sephadex G-25 and ion-exchange chromatography on S-Sepharose, produced low recovery and lost of toxicity. In addition, peak fractions obtained showed heterogeneous band patterns on SDS-PAGE. The haemolytic activity was retained, albeit with lower specific activity. An alternative method using solid ammonium sulphate was employed in fractionating the active components of *R. hispidum*. Fraction obtained at 70% saturation exhibited the highest amount of protein recovered, highest specific haemolytic activity, and was partially purified as indicated by SDS-PAGE. This fraction was designat-

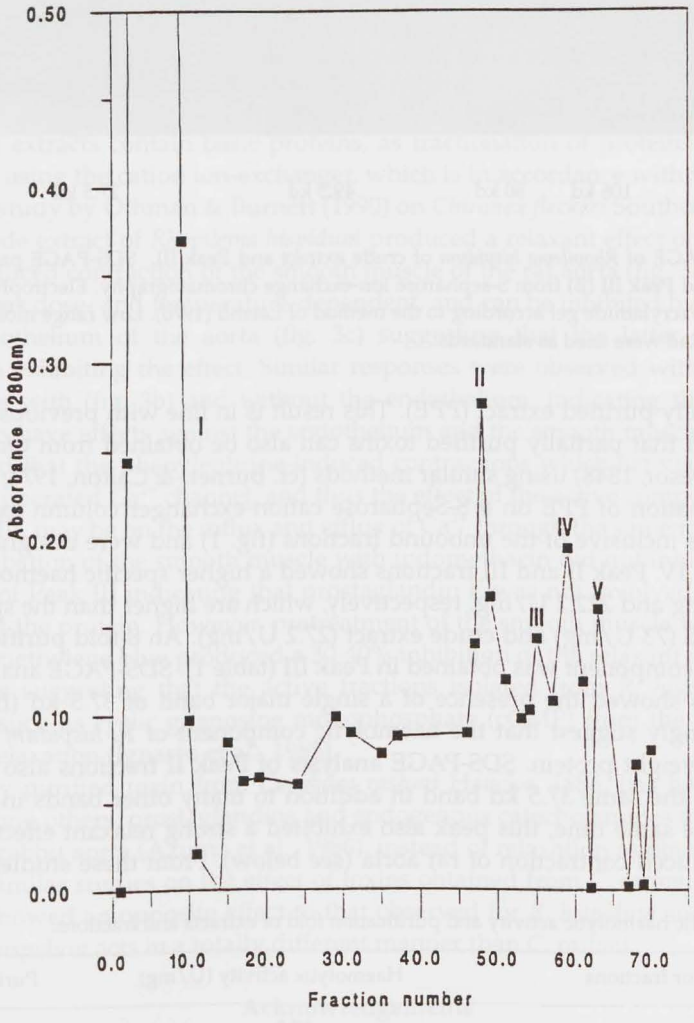

Fig. 1. Ion-exchange chromatography of partially purified extract (PPE) on S-Sepharose column. PPE was subjected to a S-Sepharose column (6 × 1.8 cm) using the stop-flow method. The column was pre-equilibrated with 50 mM Tris-HCl (pH 7.4) buffer before loading of sample.

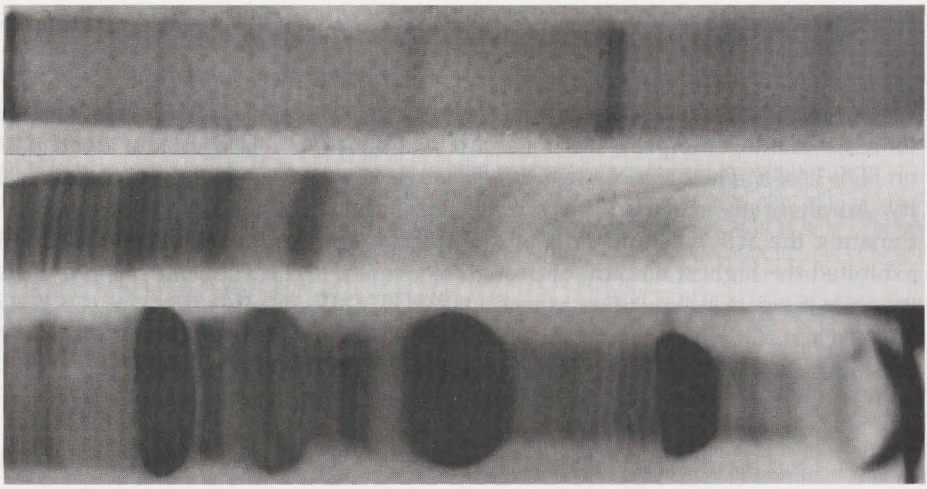

Fig. 2. SDS-PAGE of *Rhopilema hispidum* of crude extract and Peak III. SDS-PAGE patterns of crude extract (A) and Peak III (B) from S-sepharose ion-exchange chromatography. Electrophoresis was run on a 10% polyacrylamide gel according to the method of Laemli (1970). Low range molecular markers (S) from Bio-Rad were used as standards.

ed as partially purified extract (PPE). This result is in line with previous studies that have shown that partially purified toxins can also be obtained from *Chrysaora quinquecirrha* (Desor, 1848) using similar methods (cf. Burnett & Calton, 1974; 1976).

Fractionation of PPE on a S-Sepharose cation-exchanger column exhibited four major peaks inclusive of the unbound fractions (fig. 1) and were designated as Peak I, II, III and IV. Peak II and III fractions showed a higher specific haemolytic activity of 83.6 U/mg and 222.2 U/mg, respectively, which are higher than the specific activities for PPE (73 U/mg) and crude extract (27.2 U/mg). An 8-fold purification of the haemolytic component was obtained in Peak III (table 1). SDS-PAGE analysis of Peak III fractions showed the presence of a single major band of 37.5 kd (fig. 2). These results strongly suggest that the haemolytic component of *R. hispidum* is a 37.5 kd molecular weight protein. SDS-PAGE analysis of Peak II fractions also showed the presence of the same 37.5 kd band in addition to many other bands in the gel patterns. At the same time, this peak also exhibited a strong relaxant effect on phenylephrine induced contraction of rat aorta (see below). From these studies, it appears

Table 1. Specific haemolytic activity and purification fold of extracts and fractions.

	Extract or fractions	Haemolytic activity (U/mg)	Purification fold
1.	Crude extract	27.2	–
2.	Partially purified extract (PPE)	73	2.7
3.	Peak II	83.6	3.1
4.	Peak III	222.2	8.2

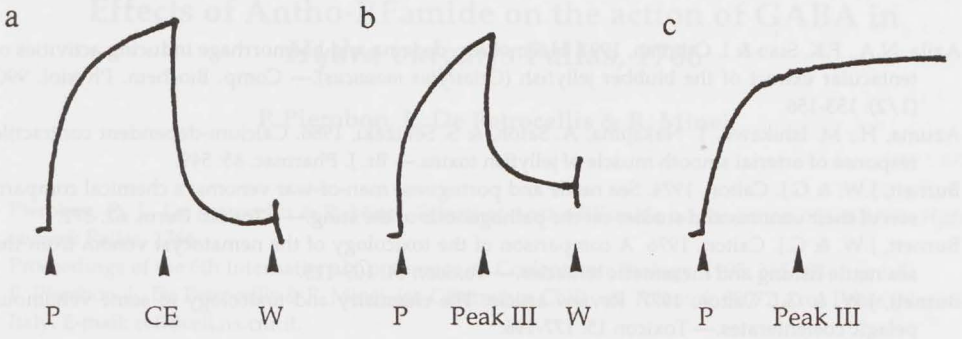

Fig. 3. Relaxant effect of crude extract and Peak III on phenylephrine-induced contraction of the smooth muscle of rat aorta with endothelium (a, b) and without endothelium (c).

R. hispidum extracts contain basic proteins, as fractionation of proteins can only be carried out using the cation ion-exchanger, which is in accordance with the results of a previous study by Othman & Burnett (1990) on *Chironex fleckeri* Southcott,1956.

The crude extract of *Rhopilema hispidum* produced a relaxant effect on the phenylephrine-induced contraction of the smooth muscle of the rat aorta (fig. 3a). The relaxant effect was dose- and temperature-dependent, and can be inhibited by the removal of the endothelium of the aorta (fig. 3c) suggesting that the latter was directly involved in exhibiting the effect. Similar responses were observed with Peak III on both tissues with (fig. 3b) and without the endothelium, indicating that the active components have effects against the endothelium and the smooth muscle. It has been documented that the phenylephrine-induced contractions which act via the opening of receptor operated Ca^{2+} channel, and thus the effect of the active components isolated in Peak III, may be on the influx and efflux of Ca^{2+} through the same mechanism.

Preincubation of the smooth muscle with indomethacin did not inhibit the relaxant effects of Peak III indicating that prostaglandin E was not involved in the relaxant effect of the protein. However, pretreatment of the smooth muscle with endothelium with methylene blue produced a 30-40% inhibition of the relaxant effect of Peak III fractions suggesting that the active fractions isolated from *R. hispidum* release mediators such as cyclic guanosine monophosphate (cGMP) from the endothelium to induce relaxation (Ignarro et al., 1985).

Partially purified toxin from *Carybdea rastoni* Haacke, 1886, has been shown to release among others, prostaglandins and endogenous catecholamines to induce contraction in rabbit aorta (Azuma et al., 1986), instead of relaxation as observed with *R. hispidum*. Similar studies on the effect of toxins obtained from *C. rastoni* on rat aorta, however, showed an opposite effect to that observed for *R. hispidum* suggesting that *Rhopilema hispidum* acts in a totally different manner than *C. rastoni*.

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Effects of Antho-RFamide on the action of GABA in *Hydra vulgaris* Pallas, 1766

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Pierobon, P., L. De Petrocellis & R. Minei. Effects of Antho-RFamide on the action of GABA in *Hydra vulgaris* Pallas, 1766.
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Key words: *Hydra*; RFamide peptides; arachidonic acid; GABA.

Abstract: We have studied the effects of two peptides, pGlu-Gly-Arg-Phe-NH_2 (Antho-RFamide) and Phe-Met-Arg-Phe-NH_2 (FMRFamide) on the action of GABA on the feeding response in *Hydra vulgaris* Pallas, 1766. Antho-RFamide, which is present in many cnidarian neurons, suppresses the GABA induced enhancement of the response at 1 µM and 10 µM concentrations with a dose dependent effect. 1-10 µM Antho-RFamide also reduces duration of the response in the absence of exogenous GABA; the maximal effect is already obtained at 1 µM levels. The effects of Antho-RFamide are suppressed by PLA_2 inhibitors, indicating that release of arachidonic acid is necessary for peptide action. FMRFamide, the molluscan cardioexcitatory peptide, produces a small but significant increase in duration of the feeding response at 1 and 10 µM concentrations; the effect does not increase with peptide concentration. Simultaneous GABA and 1-10 µM FMRFamide administration do not significantly modify duration of the response with respect to GABA, indicating an independent mode of action of the two molecules.
We conclude that the opposite effects of the two peptides are obtained by different interactions with the GABAergic system. A possible metabolic pathway involved in peptidergic modulation of the response is discussed.

Introduction

The feeding response induced by reduced glutathione (GSH) in the absence of living prey in *Hydra* represents a well established model for the study of chemoreception (Lenhoff & Bovaird, 1961: 486). While GSH association to its receptor has been extensively studied (Grosvenor et al., 1992: 120), the mechanisms by which termination of the GSH-induced response is achieved and its modulation by the nerve net are still poorly understood. We have recently shown that gamma-aminobutyric acid (GABA), the inhibitory neurotransmitter, increases the duration of the feeding response in *Hydra vulgaris* Pallas, 1766 (Pierobon et al., 1995: 1485). The effect of GABA is potentiated by benzodiazepines and suppressed by specific antagonists such as bicuculline and picrotoxin, suggesting that a population of $GABA_A$-like receptors is involved in the regulation of the feeding response. Moreover, Cl^- channel ligands such as barbiturates, the anesthetic steroid alphaxalone and the cage convulsant t-butylbicyclophosphorothionate (TBPS) respectively prolong or decrease response duration, indicating that GABA-gated Cl^- channels are involved (Concas et al., in press).

Recently much attention has been focused on the ability of free unsaturated fatty acids to directly or indirectly regulate the activity of chloride ion channels, including

$GABA_A$ receptor-coupled Cl^- channels (Schwartz & Yu, 1992: 405). Arachidonic acid (AA) and other free unsaturated fatty acid metabolites are involved in the control of pattern formation, tentacle regeneration, budding and metamorphosis in freshwater and marine hydrozoans (De Petrocellis & Di Marzo, 1994: 215). AA and its lipoxygenase metabolites significantly reduce duration of the feeding response and inhibit the action of exogenous GABA in *Hydra vulgaris* (Pierobon et al., 1997).

Peptides of the RFamide family have been shown to modulate muscular contraction in anthozoans (McFarlane & Grimmelikhuijzen, 1991: 669). RFamide-like peptides have been localized in specific neuronal populations of all cnidarian species examined (Grimmelikhuijzen et al., 1992: 1) including *Hydra*, where they are thought to be involved in neuromuscular transmission (Koizumi et al., 1989: 17). More direct electrophysiological evidence to support a neurotransmitter role of these peptides has been hard to obtain owing to the difficulty to develop suitable cell preparations, while a modulatory role of neuronal activity is rather suggested by their biphasic activity in vivo (for a concise review, see Spencer, 1991: 565). On the basis of this information we have undertaken a study of the effects of two peptides, pGlu-Gly-Arg-Phe-NH_2 (Antho-RFamide) and Phe-Met-Arg-Phe-NH_2 (FMRF-amide) on the action of GABA on the feeding response. Here we report the effects of Antho-RFamide and its possible association to the arachidonic acid cascade in the modulation of the GABAergic system in *Hydra*.

Materials and methods

Polyps of *Hydra vulgaris* were cultured in our laboratory in physiological solution CN (Lenhoff, 1983: 29). The feeding reaction was studied by the procedure described elsewhere (Pierobon et al., 1995: 1485); Antho-RFamide, FMRFamide, oleyl-oxyethyl-phosphoryl-choline (OOPC) and 4-bromophenacyl bromide (BB) were examined in a 1-100 μM concentration range. The test was started by adding GSH at different concentrations (1-10 μM); 6-12 animals were tested per group and per GSH concentration. Scoring of mouth opening and closing was performed on a cold light stereo microscope Wild and times recorded by independent observers. In every experiment a control series was effected; for each substance tested the experiments were repeated several times. All the experiments were carried out in a conditioned environment at 22°C.

Since duration of the response varied with the animals' physiological state, data from duplicate experiments were expressed as percentages of maximal values obtained in each experiment. GSH concentrations/(Tf-Ti) corresponding times ratios were then calculated and plotted as a function of GSH concentration. Differences between control and treated groups were analyzed by linear regression. All the reagents were purchased from Sigma except OOPC which was purchased from Biomol.

Results and discussion

Administration of 1 μM Antho-RFamide significantly reduced duration of the feeding response by shortening times of mouth closing (Tf); times of mouth opening (Ti) were not modified. The difference between treated and control animals was hig-

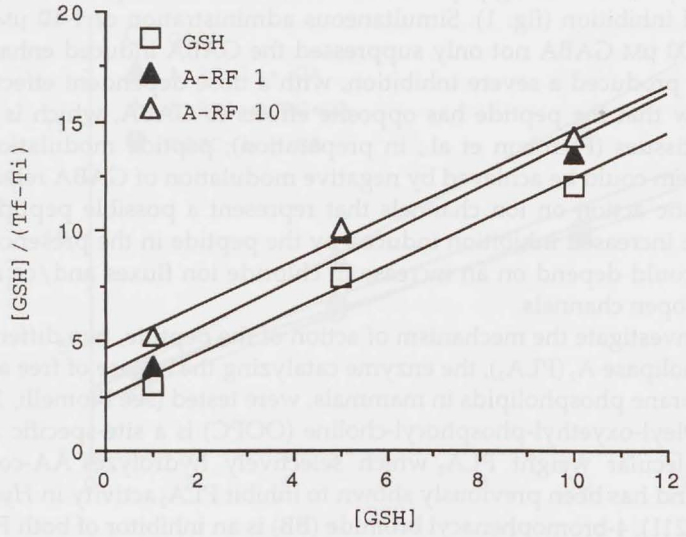

Fig. 1. Effects of 1 and 10 μM Antho-RFamide (A-RF) on the feeding response after exposure to 1, 5 and 10 μM GSH. The experiments were repeated three to five times and data were fitted by linear regression curves.

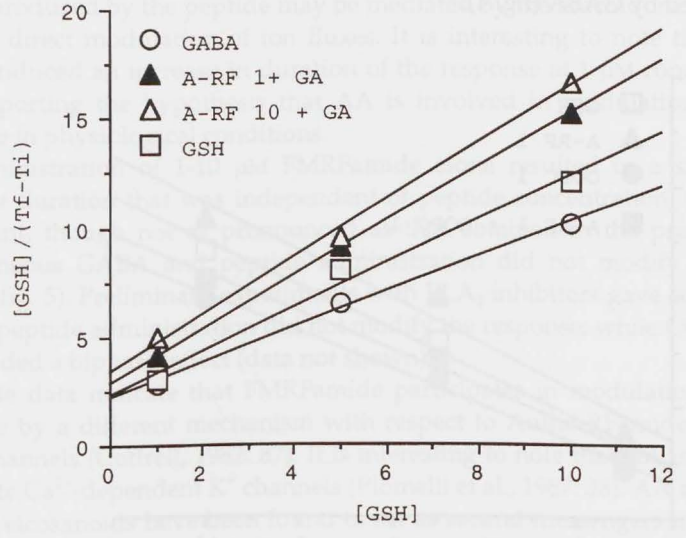

Fig. 2. Effects of simultaneous 1 and 10 μM Antho-RFamide (A-RF) and 100 μM GABA (GA) administration at 1, 5 and 10 μM GSH.

hest at 5 μM GSH with about 30% reduction in response duration (13-15 min versus 18-20 min of control). Increasing peptide concentrations did not produce significant further levels of inhibition (fig. 1). Simultaneous administration of 1-10 μM Antho-RFamide and 100 μM GABA not only suppressed the GABA induced enhancement of response but produced a severe inhibition, with a dose dependent effect (fig. 2). These data show that the peptide has opposite effects to GABA, which is endogenous in *Hydra* tissues (Pierobon et al., in preparation); peptide modulation of the GABAergic system could be achieved by negative modulation of GABA release or by direct antagonistic action on ion channels that represent a possible peptide target. Accordingly, the increased inhibition induced by the peptide in the presence of exogenous GABA could depend on an increase in chloride ion fluxes and/or an increased number of open channels.

In order to investigate the mechanism of action of the peptide, two different inhibitors of phospholipase A_2 (PLA_2), the enzyme catalyzing the release of free arachidonate from membrane phospholipids in mammals, were tested (see Piomelli, 1993: 274 for a review). Oleyl-oxyethyl-phosphoryl-choline (OOPC) is a site-specific inhibitor of the low molecular weight PLA_2 which selectively hydrolyzes AA-containing phospholipids and has been previously shown to inhibit PLA_2 activity in *Hydra* (Borrelli et al., 1995: 211). 4-bromophenacyl bromide (BB) is an inhibitor of both PLA_2 and phospholipase C, which also catalyzes AA release in combination with a diacylglycerol-lipase (Van den Bosch, 1980: 191). Simultaneous administration of 1 μM Antho-RFamide and equimolar doses of either PLA_2 inhibitor suppressed the effect of the peptide, restoring physiological mouth closing times (fig. 3). In these conditions the peptide also failed to antagonize the effect of GABA: administration of 1 μM Antho-RFamide in the presence of the inhibitors did not modify the increase in response duration obtained by GABA (fig. 4).

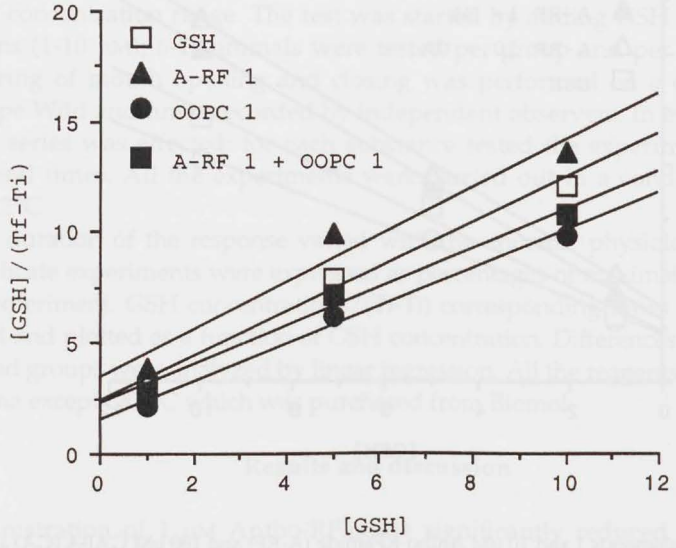

Fig. 3. Effects of 1 μM OOPC on 1 μM Antho-RFamide (A-RF) at 1, 5 and 10 μM GSH.

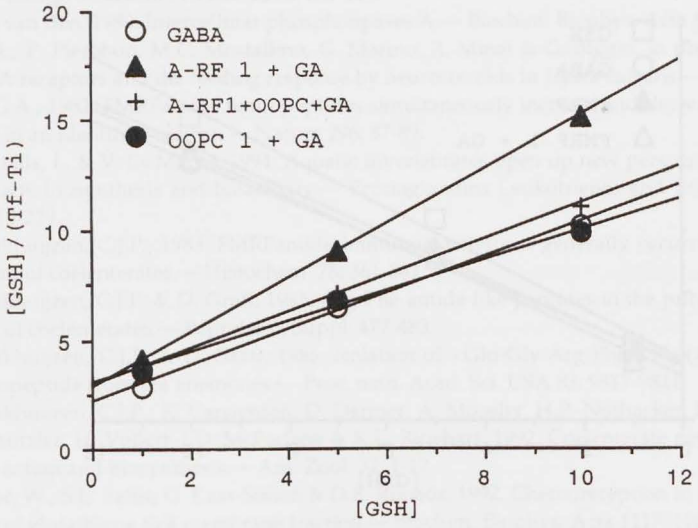

Fig. 4. Effects of 1 μM OOPC on simultaneous 1μM Antho-RFamide (A-RF) and 100 μM GABA (GA); 1 μM OOPC did not affect the response to GABA.

These experiments suggest that the release of AA is a necessary step in the response to Antho-RF-amide. The data also suggest that inhibition of the effects of GABA produced by the peptide may be mediated by the arachidonate cascade rather than by direct modulation of ion fluxes. It is interesting to note that both BB and OOPC induced an increase in duration of the response at 1 μM concentrations, further supporting the hypothesis that AA is involved in modulation of the feeding response in physiological conditions.

Administration of 1-10 μM FMRFamide alone resulted in a small increase in response duration that was independent of peptide concentration; the increase was significant, though not as pronounced as that obtained in the presence of GABA. Simultaneous GABA and peptide administration did not modify the response to GABA (fig. 5). Preliminary experiments with PLA_2 inhibitors gave conflicting results: BB and peptide administration did not modify the response, while OOPC administration yielded a biphasic effect (data not shown).

These data indicate that FMRFamide participates in modulation of the feeding response by a different mechanism with respect to Antho-RFamide, possibly acting on K^+ channels (Cottrell, 1982: 87). It is interesting to note that AA is also reported to modulate Ca^{2+}-dependent K^+ channels (Piomelli et al., 1987: 38). AA and lipoxygenase derived eicosanoids have been found to act as second messengers in the modulation of K^+ channel activity by FMRFamide in molluscs (Bahls et al., 1992: 165; Schacher et al., 1993: 1079). The lack of an effect on the action of GABA, though not conclusive, is in agreement with this hypothesis. Further work is in progress in order to investigate the mechanism of action of the peptide.

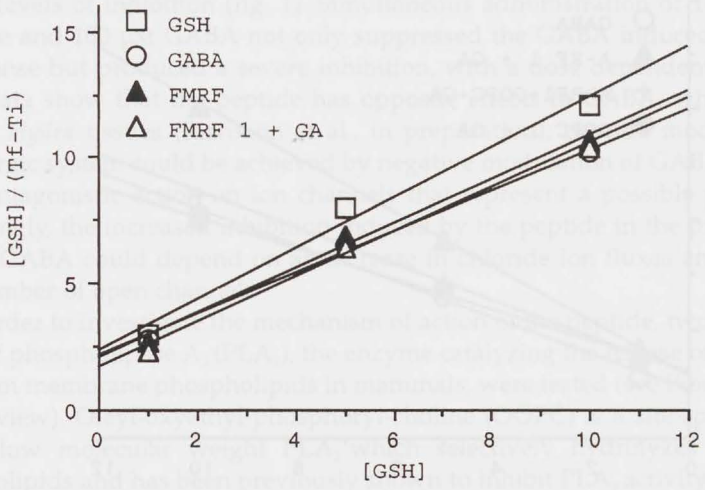

Fig. 5. Effects of 1 µM FMRFamide on 1, 5, 10 µM GSH and 100 µM GABA (GA) administration.

Conclusions

Antho-RFamide has been found in Anthozoa (Grimmelikhuijzen & Graff, 1986: 9817); in *Hydra* successful staining with anti-FMRFamide antisera has led to the conclusion that FMRFamide may be present (Grimmelikhuijzen, 1983: 361; Koizumi and Bode, 1986: 407). However, the finding that antisera raised against the Arg-Phe-amide fragment stained much better (Grimmelikhuijzen & Graff, 1985: 477) has modified that assumption, suggesting that a peptide with a RF residue at the carboxyterminus may be the native peptide. The difficulty of conducting direct electrophysiological studies at the cellular level in *Hydra* has discouraged so far extensive investigation of the nature of the active peptides and of their mode of action. Our data suggest that Antho-RFamide could be physiologically involved in the modulation of responses to chemical stimulation and that arachidonic acid may act as the signal transduction pathway for the peptide.

Acknowledgements

We are indebted to Dr V. Di Marzo for helpful suggestions and critical readings of the manuscript. We also wish to thank S. Piantedosi for excellent photography and art work, and G. Marino for skillful technical assistance.

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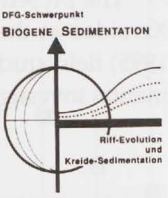
Different modes of adaptation to light conditions in Red Sea Xeniidae reflected by their depth distribution patterns (Octocorallia, Alcyonacea)

G.B. Reinicke



Reinicke, G.B. Different modes of adaptation to light conditions in Red Sea Xeniidae reflected by their depth distribution patterns (Octocorallia, Alcyonacea).
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 393-402, figs 1-5, tab. 1.
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Key words: Red Sea; Octocorallia; Xeniidae; depth distribution; symbiosis; photo-adaptation; zooxanthellae; chlorophyll; peridinin.

Abstract: Benthic coral reef communities characterised by a high abundance of Xeniidae (Octocorallia, Alcyonacea) were recorded at the species level by repetitive transect assessments on reef slopes south of Aqaba (Jordan, northern Red Sea). A sequence pattern of preferred depth ranges of predominating xeniid species was found at different locations.
An in situ translocation experiment with five abundant species (viz., *Xenia macrospiculata* Gohar, 1940; *X. obscuronata* Verseveldt & Cohen, 1971; *X. faraunensis* Verseveldt & Cohen, 1971; *X. benayahui* Reinicke, 1995; and *X. novaecaledoniae* Verseveldt, 1974) revealed four different modes of photo-adaptation to varying light conditions. Under experimentally reduced light conditions, mean colony sizes of four species (not *X. faraunensis*) increased by the extension of polyps and tentacles. Furthermore, after one year, changes in densities of zooxanthellae in polyp tissues and concentrations of photosynthetic pigments (chlorophyll-a, -c_2 and peridinin) were measured. The experiment revealed four different modes of photo-adaptation, which are reflected in the bathymetric zonation pattern of the five species.

Introduction

Abundance of the zooxanthellate soft corals of the family Xeniidae, mainly belonging to the genera *Xenia* Lamarck, 1816, *Anthelia* Lamarck, 1816, and *Heteroxenia* von Kölliker, 1874, can reach values of about 25-56% absolute area coverage, representing 40-70% of the living benthic coelenterates coverage in certain habitats of coastal and off-shore fringing reefs in the Red Sea. These figures illustrate the possible significance of Xeniidae species as space competitors in Red Sea reef communities (Schuhmacher, 1975). On the Great Barrier Reef, Xeniidae generally reach only 5-15% of the living cover, with *Efflatounaria* Gohar, 1934, as the dominant genus (Dinesen, 1983; Fabricius, 1995). As competitors for space in light, they can prevent settlement and growth of scleractinian coral larvae (e.g., Maida et al., 1988). However, ecological studies on soft corals (Alcyonaria) are scarce due to taxonomic problems: distribution

Note added in proof (21 December 1996): the species referred to in this paper as *Xenia novaecaledoniae* Verseveldt, 1974, appears to represent another species to be described as *X. gohari* spec. nov. in vol. 16 of Fauna of Saudi Arabia (expected in 1997).

records presented by Benayahu & Loya (1977), Tursch & Tursch (1981), Dinesen (1983), Dai (1988) and Fabricius (1995) do not indicate the complete depth ranges of species, or considered data only at the generic level.

The present study, however, presents experimental and field data on the ecology of Xeniidae at the species level. Based on a taxonomic inventory of the group (Reinicke, 1995) field studies on depth distribution patterns were carried out together with experimental investigations on the photo-adaptive features of five abundant species.

Material and methods

Distribution pattern.— From 1990 to 1992, transect mappings were carried out to detect depth distribution patterns of 12 common xeniid species in the coastal fringing reefs of Saudi-Border Bay, 20 km south of Aqaba, northern Gulf of Aqaba, Red Sea (fig. 1). Transects of 10 m length at depths of 10, 15, 20 and 30 m, were used (cf. Loya, 1977). Sites with a high abundance of Xeniidae were selected. Surface cover was recorded under the transect lines to the nearest cm, during the autumns of 1990 and 1991, and the spring and autumn of 1992. The recordings were complemented by visual estimations from 5-10 and 30-40 m depth. The cover percentage of the categories sand, dead coral rock, scleractinians, species of Xeniidae, other octocorals, other invertebrates and algae was assessed. Mean and relative abundance of xeniid species were determined in relation to depth. Intensities of photosynthetic active radiation (PAR) were measured from the surface to 40 m depth using a LI-QOR Li-192SA sensor (fig. 2b).

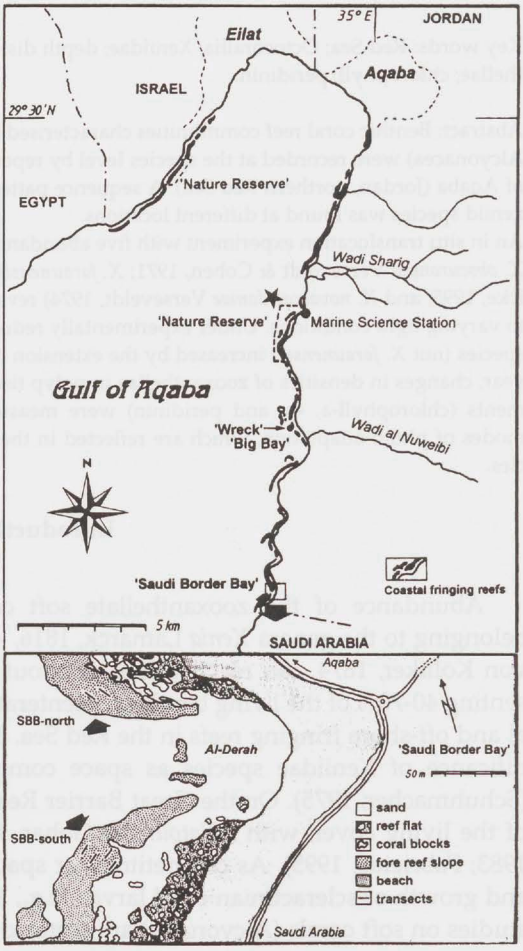

Fig. 1. Sites of experiments (★) and transects (arrows) south of Aqaba (Jordan, Gulf of Aqaba).

Adaptation to light levels.— Animals of five dominating species were used in a translocation experiment to investigate the dependence of the distribution pattern on available light intensities (viz., *Xenia macrospiculata* Gohar, 1940; *X. obscuronata* Verseveldt & Cohen, 1971; *X. faraunensis* Verseveldt & Cohen, 1971; *X. benayahui* Reinicke, 1995; and *X. novaecaledoniae* Verseveldt, 1974). Colonies from a

single clone of each of the five species were translocated to each of a series of six PVC-trays exposed to various light intensities (fig. 2a). Tray nos 1, 5 and 6 were placed horizontally at 12, 18 and 25 m depth, respectively. Tray nos 2-4 were mounted with inclination angles towards the surface of 45°, 90°, 135°, respectively, at 12-13 m depth, thus receiving reduced direct radiation and increased proportions of diffuse radiation (varying from 212 ± 156 to 7 ± 5 µE/m²s PAR, i.e. approximately 20 to 1% surface irradiance). Initially, 10 to 35 colonies (depending on colony size) of each species were fixed on the trays to cover 15-20% of the tray area (fig. 5a). The trays were photographed in order to map the colony distributions at the beginning of each experiment and after 352 days of exposure. Colonies were counted and their mean sizes calculated. Tissue samples were taken to measure the densities of zooxanthellae and to analyse pigment contents. Three complete polyps were removed from two replicate colonies of each species on every tray. Each set of three polyps was homogenized as one sample in 41.5% NaCl and frozen for transportation and subsequent processing.

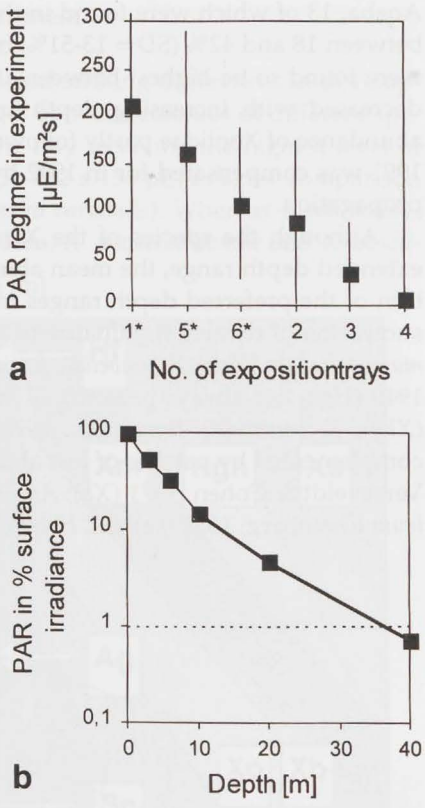

Fig. 2a. Mean photon fluxes (PAR) measured perpendicularly on the exposed trays in the translocation experiment at different times of the day (n = 10), * = trays exposed horizontally. Fig. 2b. Relative light intensities (orientation SSW) at reef slopes south of Aqaba, Jordan (April-June 1992, n = 6; broad line represents range of experiment).

Densities of zooxanthellae.— Concentrations of zooxanthellae were measured using a Thoma counting chamber (n = 10 replicates for each sample). Protein contents were determined according to Lowry et al. (1951) using ovalbumin as standard. A phycobiont/zoobiont protein ratio of 35/65 was used (Schlichter & Kremer, 1985) for calculation of the protein contents.

Pigmentanalysis.— Chlorophylls were qualitatively and quantitatively determined according to Hiscox & Israelstam (1978) using Dimethylsulfoxyd (DMSO) for extraction and the equation of Jeffrey and Humphrey (1975). Peridinin contents were determined according to Jeffrey & Haxo (1968). Concentration values were referred to the respective mean light intensities on the experiment trays and tested using the Spearman rank correlation test.

Results

Mapping of benthic communities

A total of 15 species of Xeniidae was observed to be abundant on the reefs near

Aqaba, 13 of which were found in the transects. The mean cover of Xeniidae ranged between 18 and 42% (SD = 13-51%; mean SD at Saudi-Border Bay 41%). Fluctuations were found to be highest between 10 and 20 m depth at a northern reef slope, and decreased with increasing depth and more southerly exposition. Fluctuations in abundance of Xeniidae partly followed seasonal changes; decrease during the winter 1991 was compensated for in 1992 by the summer recruitment and rapid vegetative propagation.

Although the species of the Xeniidae generally were observed spread over an extended depth range, the mean abundance of each species revealed a zonation pattern of the preferred depth ranges in the transects down to about 40 m (fig. 3). The succession of relative dominance of species follows the depth gradient (fig. 4): *Xenia macrospiculata* (*Xm*); *X. umbellata* Lamarck, 1816 (*Xu*); *Heteroxenia ghardaqensis* Gohar, 1940 (*Hgh*, not always present); *X. obscuronata* (*Xo*); *X. faraunensis* (*Xf*); *X. benayahui* (*Xby*); *X. verseveldti* Benayahu, 1990 (*Xv*); *X. novaecaledoniae* (*Xnc*). This pattern is complemented by patches of less abundant species or single colonies, viz., *X. biseriata* Verseveldt & Cohen, 1971 (*Xb*); *Anthelia glauca* Lamarck, 1816 (*Ag*); *Sympodium caeruleum* Ehrenberg, 1834 (*Sc*) and *Heteroxenia fuscescens* (Ehrenberg, 1834) (*Hf*).

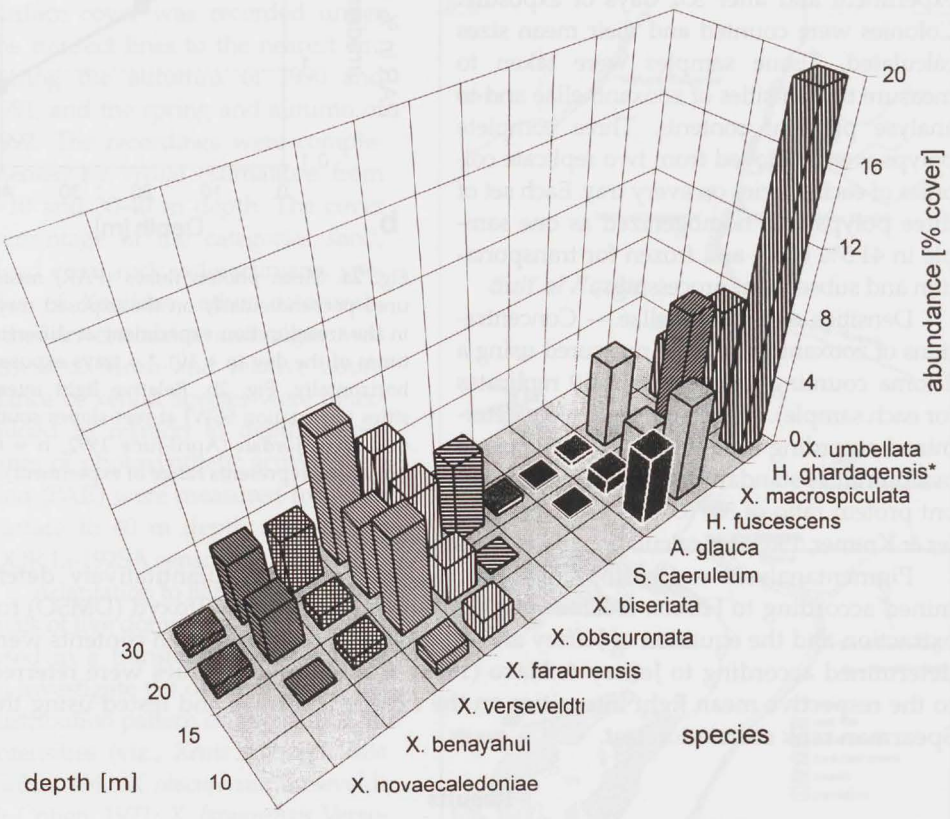

Fig. 3. Mean abundances (n = 4 transect inventories) of 12 species of Xeniidae at four depths on the reef slope in Saudi-Border Bay, Aqaba, 1990-1992. (*: in one case *Heteroxenia ghardaqensis* occupied extended space among *Xenia umbellata* and *X. macrospiculata*).

Translocation experiment

After 352 days, the colonies on the trays showed striking differences in development. Aggressive interference (e.g. tissue damage) between colonies of different species was in no case observed. Colonization of the tray receiving the highest level of illumination is shown in figs 5a-b (tray no 1; PAR: 212 ± 156 µE/m²s; for comparison of figs 5a and b, note position of colony of *Acropora variabilis*). Whereas *Xenia macrospiculata* and *X. fraunensis* expanded in colony area, *X. novaecaledoniae* and *X. obscuronata* hardly maintained their space. The small colonies of *X. benayahui* became restricted to the spaces between those of *X. macrospiculata* and *X. fraunensis*. On tray 4 (figs 5c-d; PAR: 7 ± 5 µE/ m²s) colony numbers decreased in all five species, but the surviving colonies generally tended to expand their surface area by growth/expansion of their polyps and tentacles for optimal exposure of zooxanthellae under reduced light conditions. While only one colony of *X. macrospiculata* survived and *X. fraunensis* and *X. obscuronata* hardly maintained their space, colonies of *X. novaecaledoniae* and of *X. benayahui* extended the area covered.

Table 1 presents a survey of statistically significant correlations of adaptive changes of algal densities and pigment contents of five xeniid species by means of an experimental light regime. Negative correlations were found for symbiotic algal densities of *X. benayahui* and *X. novaecaledoniae* with prevailing light conditions. *Xenia macrospiculata* showed negative correlations of chlorophyll contents with the mean light levels, whereas *Xenia benayahui* and *X. novaecaledoniae* showed positive correlations of chlorophyll-c_2 with the mean light levels on experimental trays. *Xenia fraunensis* showed negative correlations of peridinin contents.

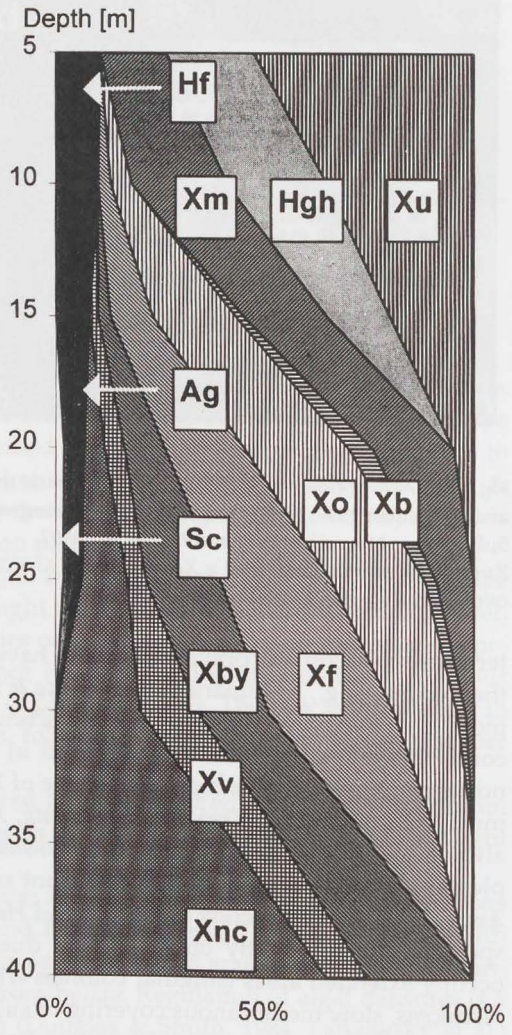

Fig. 4. Scheme of the zonation sequence of relative dominance of Xeniidae species down from 5-40 m depth in coastal fringing reefs at Saudi-Border Bay (this figure is based on data from fig. 3 and further observations at 5-10 m and 30-40 m depth; for abbreviations see text).

Discussion

Light dependent distribution pat-

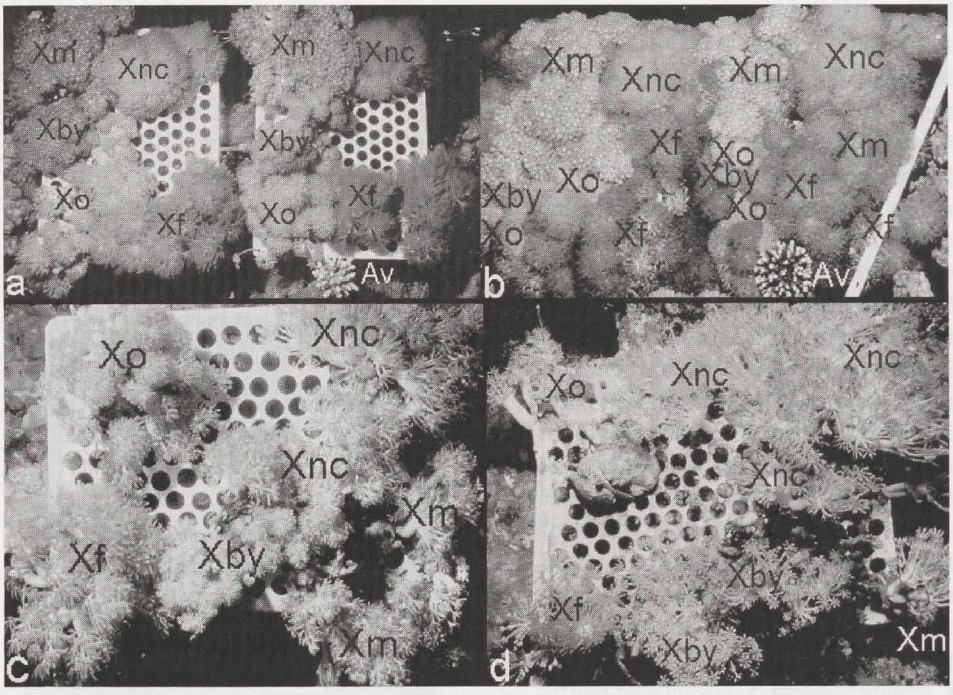

Fig. 5a-b. Arrangement of colonies on experimental tray 1 (PAR: 212 ± 156 µE/m^{2}s) in November 1991 and November 1992, respectively. Fig. 5c-d. Arrangement of colonies on experimental tray 4 (PAR: 7 ± 5µE/m^{2}s) in November 1991 and November 1992.
Xm = *Xenia macrospiculata*, Xo = *X. obscuronata*, Xf = *X. faraunensis*, Xby = *X. benayahui*, Xnc = *X. novaecaledoniae*, Av = *Acropora variabilis*.

terns of scleractinian coral assemblages have been reported by various authors from the Red Sea (e.g., Kühlmann, 1983; Fricke & Schuhmacher, 1983) and other reef provinces (e.g., Dinesen, 1982; Fricke & Meischner, 1985; Titlyanov & Latypov, 1991). Soft corals, however, had not been included in such investigations. At reef sites in the northern Red Sea with a high abundance of Xeniidae, four to seven xeniid species are most prominent (viz., *Xenia macrospiculata, X. umbellata, Heteroxenia ghardaqensis* (not always present), *X. obscuronata, X. faraunensis, X. benayahui, X. novaecaledoniae*), supplemented by five to seven less abundant species (viz., *Xenia verseveldti, X. biseriata, Anthelia glauca, Sympodium caeruleum* and *Heteroxenia fuscescens*). The two last-named species were frequently observed, but due to different life strategies they do not occupy extended areas (singular colonies without rapid vegetative propagation in *H. fuscescens*, slow membranous covering of substrate in *S. caeruleum*).

The most abundant xeniid species show a sequence of preferred depth ranges (see fig. 4) over the entire gradient of light intensity down to the 1% surface irradiance level (PAR; compare fig. 2b). According to the results of the translocation experiment, these depth ranges reflect different strategies of the symbiotic coral-algae complex to adapt to varying levels of prevailing light conditions. For the species in the experiment they are the following:

Tab. 1. Positive (+) and negative (–) correlations of densities of zooxanthellae and pigment contents with mean values of relative light intensities (PAR) on experimental trays: Spearman rank correlation, levels of significance = 1% (++/– –) and = 5% (+/–); level of significance = 10 % is placed between brackets (due to the limited database); *Xm = X. macrospiculata; Xo = X. obscuronata; Xf = X. faraunensis; Xby = X. benayahui; Xnc = X. novaecaledoniae.*

Correlations with PAR [% I_0]	Xm	Xo	Xf	Xby	Xnc
mean colony size	–	[–]		–	–
zooxanthellae			[–]		– –
chlorophyll-a	[–]				
chlorophyll-c_2	–			+	+
chlorophyll-a + -c_2	–			[+]	+
chlorophyll-c_2 /-a			++	+	++
peridinin			–		
peridinin / chl-a			– –		

1. Zooxanthellae of *Xenia macrospiculata* adapt to high light levels by reducing the number of photosynthetic units (PSU) in the cells (photo-acclimatization). This type of adaptation is a common feature described for many light-absorbing organisms to prevent unfavourably high rates of photosynthesis (oxygen-toxicity; Prezelin & Alberte, 1978). Within the group of reef-dwelling *Xenia* species, *X. macrospiculata* is dominant in the upper part of the zonation pattern.

2. *Xenia obscuronata* showed no relation between either density of zooxanthellae or pigment contents and the prevailing light conditions in the experiment. However, frequent observation of contracted colonies on horizontally exposed substrate surfaces during noon hours of high irradiance levels indicates an adaptive behaviour which prevents unfavourably high rates of photosynthesis. The species dominates in the central range of the zonation pattern, followed by *X. faraunensis* ranging deeper than *X. obscuronata*.

3. Colonies of *Xenia faraunensis* showed a negative correlation of algal peridinin content, thus increasing the ratio of antenna pigment in PSU's with decreasing light intensity (Falkowsky et al., 1990).

4. *Xenia benayahui* and *X. novaecaledoniae* showed significant negative correlations between zooxanthellae concentrations and the experimental light conditions. With decreasing light levels, these species increase the surface area of tentacles and pinnules, thus providing more space for zooxanthellae. Regulation of algal densities is a common feature in symbiotic organisms (Douglas & Smith, 1984, Falkowsky et al., 1990). The adverse modification, a reduction of algal densities under low light conditions as reported for several scleractinian species (cited from Kaiser et al., 1993: table 3), is explained by the prevention of self-shading due to the multi-layer arrangement of zooxanthellae in the two-dimensionally exposed tissues. This may not be relevant in *Xenia* species due to the overall exposure of the translucent pinnule tissues,

allowing the species to colonize deeper reef slopes with relatively low light intensities. They dominate the lower range of the zonation pattern.

The positive correlations of chlorophyll-c_2 in zooxanthellae of *X. benayahui* and *X. novaecaledoniae*, however, contradict the common mode of photo-adaptive increase of the chlorophyll-c_2/chlorophyll-a ratio (Falkowsky et al., 1990; Kaiser et al., 1993), and cannot be explained on the basis of the available data.

An increasing amount of evidence over the last 15 years demonstrates that "*Symbiodinium microadriaticum* Freudenthal, 1962" includes a wide variety of different types (species) within the genus *Symbiodinium* (e.g., Blank & Trench, 1985a, 1985b; Trench & Blank, 1987; Rowan & Powers, 1992; Blank, 1992). The ecological significance of these differentiations are under investigation. Kampmann (unpublished data) studied photosynthetic rates and efficiency of isolated symbionts from the scleractinian corals *Mycedium elephanthotus* (Pallas, 1766) and *Acropora squarrosa* Ehrenberg, 1834, after experimental translocations to different depths. The results indicate different strains of *Symbiodinium* as symbionts of different coelenterate genera and different modes of adaptation to depth-light regimes (e.g., Chang et al., 1983).

Acquisition of zooxanthellae differs in xeniid species. Juvenile primary polyps of *Heteroxenia fuscescens* become infested at a very early stage of growth after external brooding of the larvae (Gohar, 1940; Benayahu et al., 1989). The internally brooded larvae of *X. umbellata* and *X. macrospiculata* receive their symbionts in the course of planula ontogenesis in the brooding pouches of parental colonies (Benayahu et al., 1988; Achituv et al., 1992). This has also been described for other symbiotic species (Benayahu et al., 1992). Furthermore, oocytes of various coelenterates have been reported to possess algal symbionts (e.g., Krupp, 1983; Dinesen, 1985; Babcock et al., 1986). Thus, the transmission of specific symbionts with the respective photoadaptive features from parental colonies to the offspring is evident.

Different reproductive modes such as gonochorism and hermaphroditism (Benayahu et al., 1992), however, might be linked with the presence or absence of species-specific adaptive features of the symbiotic algae. Namely, gonochorism might thus be a key feature of symbiotic species for the colonization of habitats characterized by relatively extreme conditions. However, reproductive modes of only a few species of Xeniidae are known to date. Of these species, only *X. macrospiculata* was studied during the present experiment.

The photo-adaptive modes described for *Xenia macrospiculata, X. faraunensis, X. benayahui* and *X. novaecaledoniae* enable these species to disperse over a wide range of light intensities and available habitats. In contrast, species without these symbiont specific features seem to be restricted to a certain intermediate range of light intensities (*X. obscuronata, H. fuscescens*). Neighbouring colonies of different species of Xeniidae grow without aggressive interference, though competition is fact. Local dominance in competitive coexistence results from differences in life history traits and initially different responses to accumulative favourable abiotic conditions, especially light.

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Plesiastrea versipora (Lamarck, 1816), the white rat for coral research?

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Key words: Scleractinian coral; *Plesiastrea versipora*; regeneration; polyp bail-out.

Abstract: *Plesiastrea versipora* (Lamarck, 1816) is a hardy scleractinian coral that can be maintained for long periods in the laboratory even without feeding. After removal of tissue, it shows considerable powers of regeneration and, following recovery, can be reused in later experiments. The rates of respiration, photosynthesis and translocation of photosynthate from algae to animal recover to normal levels and the regenerated animal tissue has host release factor activity. We have also shown that small pieces broken off the colony will survive and grow slowly to form clones of the parent colony. These properties make *P. versipora* an ideal subject for laboratory experiments. Our qualitative observations and preliminary data on regeneration and fragmentation are presented here.

Introduction

The zooxanthellate ("hermatypic") scleractinian coral *Plesiastrea versipora* (Lamarck, 1816) has a broad range of environmental tolerances. It occurs throughout much of the Indo-Pacific, in both tropical and temperate waters and has been recorded from the entire mainland coastline of Australia, from both coral reef and non-reef environments (Veron, 1986). Over its range it is exposed to a wide range of temperatures and grows, in many areas, at low light intensities and in locations where there are periodic influxes of silt. The southern ecotype was originally described as *Plesiastrea urvillei* Milne Edwards & Haime, 1849. In the temperate part of its range (including Sydney), colonies are normally encrusting; in the tropics, colony form is more diverse (Veron, 1986).

Kevin & Hudson (1979) showed that *P. versipora* not only survived but continued to grow in the laboratory, in tanks with a continuous flow of filtered, re-circulated seawater. In fact colonies survived even when kept in the dark for 134 days, although they expelled their symbiotic algae after about 48 days. Normal densities of zooxanthellae were maintained when the corals were kept under fluorescent lights (12 μmol.quanta.m^{-2}.s^{-1}, 12h light:12h dark) for 134 days, regardless of whether or not they were fed during this period. They fed freely on live, freshly hatched brine shrimps (*Artemia* spec.), ground commercial fish food and freshly ground crab meat (Kevin & Hudson, 1979).

We have used *P. versipora* in the study of coral-algal symbiosis for some years, as it is the only hermatypic scleractinian coral which is abundant near Sydney and is particularly easy to keep in the laboratory. We have found that it can be kept under even less stringent conditions than those used by Kevin & Hudson (1979). We have

also found that it can be fed a semi-defined diet, has considerable powers of regeneration, shows interesting survival strategies in response to deleterious conditions, and will reproduce in the laboratory. In addition, it is possible to generate multiple small colonies of *P. versipora* from any colony artificially. These qualitative observations and some preliminary data relating to them will be described here in the hope that they will be of use to others doing laboratory studies of corals; detailed experimental work on these phenomena is continuing.

Materials and methods

Plesiastrea versipora was collected from Fairlight, Port Jackson (N.S.W., Australia). The coral occurs at about 4 to 9 m depth, below a band of dense stands of the kelp *Ecklonia radiata*. The water at this site is usually turbid, with maximal light intensity at 6 m in September (Spring) reaching about 180 μmol.quanta.m^{-2}.s^{-1} at noon (Stewart, 1986).

Colonies were removed from the rock with a chisel. Most collections were of small colonies (1 to 10 cm in diameter); where pieces from larger colonies were required they were broken off carefully with the chisel. The corals were taken to the laboratory in plastic bags of seawater and transferred into glass aquaria, usually within 2h of collection. Aquaria were kept on the laboratory bench near north facing windows, so that they received natural light, plus laboratory lighting (from fluorescent tubes) during the day; thus they were exposed to variable light intensities and photoperiods, depending on the weather and the season. The temperature in the laboratory was normally maintained at 18-20°C, but sometimes went as low as 15°C in winter and as high as 28°C for brief periods in summer. The maximum water temperature at the collection site ranges from 15°C in winter to 22°C in summer. Seawater was not recirculated, but was changed at intervals of 7 to 14 days; at the same time the aquarium walls were brushed clean. Between water changes the salinity was checked at intervals with a field refractometer and, if necessary, adjusted by slowly adding deionized water to the tanks while stirring. The volume of water in the aquarium was always at least 25 times the volume of the coral. In some cases colonies were fed every two weeks with *Artemia*, *Tubifex* or ground commercial dry cat food (Whiskettes®, Uncle Ben's of Australia, Wodonga, Victoria, Australia). Many colonies have lived for two years in the laboratory and one colony for over three years.

For experiments on the physiology of symbiosis the tissue of the coral was removed with a toothbrush, by brushing gently until the skeleton appeared bare. The chlorophyll content of the zooxanthellae remaining in colonies which had been brushed was measured by soaking them in 90% acetone; the resulting extract was used to measure the concentration of chlorophyll by the method of Jeffrey & Humphrey (1975). The chlorophyll content of zooxanthellae in the slurry of tissue which had been removed from the skeleton was measured by adding 100% acetone (to give a final concentration of 90%); after extraction the mixture was centrifuged to clarify it before readings were taken. Isolated algae were counted using a haemocytometer. Rates of respiration and photosynthesis by whole colonies were measured in an oxygen electrode (Rank Bros, Bottisham, UK). Isolated algae were incubated in seawater or in coral host homogenate and $^{14}CO_2$ was used to measure their rates of photosyn-

thesis and of release of photosynthate, by the methods of Ritchie et al. (1993).

After brushing, the remains of the corals were rinsed thoroughly with seawater and replaced in aquaria. In some instances, brushed corals were returned to the field (after a few days in aquaria) by attaching them to bricks or directly to the sandstone substratum with Vepox CC57 epoxy resin (Vessey Chemicals Pty. Ltd., Sydney).

Clones were produced by pinching off small pieces of coral tissue from colonies, separating them from both the skeleton and the rest of the colony's tissue.

Results

Maintenance of corals.— When larger colonies of *Plesiastrea versipora* were broken during collection it was observed that the tissue of the coral quickly grew over the exposed edges of the broken skeleton. Both the collected pieces and the colonies from which they had been cut survived the disturbance.

Colony fragments survived for many months in the laboratory, regardless of whether they were fed. In the laboratory, polyps were often extended during the day, particularly when the corals had been kept without food for some time and in bright sunlight; there was some variation between colonies in their tendency to remain extended during the day. In the field, polyp extension was rarely observed in the daytime. *Plesiastrea versipora* accepted, and apparently digested, brine shrimps, *Tubifex* and the cat food.

Regeneration of colonies.— Comparisons of the chlorophyll contents of the slurry of tissue brushed off colonies and of the tissue left on the skeleton showed only 30% to 50% of the algae were removed (depending on the pressure used). Microscopic observations showed that during brushing the polyps were damaged but withdrew into the calices, and that what was removed was mainly the intercalicular tissue. When such brushed corals were replaced in sea water, the animal tissue regenerated, eventually forming fully differentiated polyps with apparently normal coenosarc between them. Immediately after brushing the skeleton sometimes became covered with thick, sticky white material, which appeared to consist of extruded mesenterial filaments and mucus. By about seven days after scrubbing, this material had disappeared from the colonies, which were observed ingesting it, and the regenerating polyps had developed tentacles. In some cases patches of polyps were completely destroyed during brushing, leaving bare patches of skeleton among the regenerated polyps; if the bare area was small it was eventually overgrown by the surrounding coral tissue. In the laboratory a complete cover of tissue, of normal thickness, was regenerated in the first two to four weeks. Due to the time that would have been required, it was not possible to make equivalent detailed observations for colonies regenerating in the field, but almost all regenerating corals which were returned to the field regenerated well and survived for at least one year.

Regenerated corals showed normal rates of photosynthesis (P) and respiration (R) and retained host release factor activity (Ritchie et al., unpublished data). Their populations of symbiotic algae also returned to normal levels ($\geq 5 \times 10^5$ cells.cm^{-2}; Grant et al., unpublished data) within three weeks. In preliminary experiments with oxygen electrodes, freshly brushed corals showed abnormally high rates of respiration and very low rates of photosynthesis (no net O_2 evolution in the light). By the

second day after brushing the net rate of photosynthesis was positive again, although lower than normal, while after seven days the rates of both photosynthesis and respiration were in the normal range and the P:R ratio had returned to the value found in freshly collected coral (approximately 2) (Ritchie et al., submitted).

Isolated algae from regenerated colonies also fixed $^{14}CO_2$ photosynthetically and host homogenates prepared from the regenerated corals stimulated the release of photosynthetically fixed carbon from the isolated algae. Rates of both photosynthesis and release (in seawater and in host homogenate) fell within the normal ranges. For example, the rate of carbon fixation, in seawater, by algae isolated from a coral which had been brushed several weeks before the experiment and had regenerated in the laboratory was 244 nmol carbon per 4×10^5 algal cells, which was within the normal range for freshly collected corals (mean = 260.9 ± 87.7 nmol C per 4×10^5 algal cells; n = 36). The rate of release of photosynthate, from these algae, induced by homogenate from the same coral was equivalent to 28% of total fixed carbon, compared with 1% for algae incubated in seawater, indicating that both algae and coral tissue were responding in a normal manner (mean values for freshly collected corals were 25.4% in host homogenate (n = 46) and 3.2% in seawater (n = 36)). In a second experiment, in which the coral had been brushed and returned to the field 10 months before the experiment, the algae fixed carbon at a rate of 140 nmol carbon per 4×10^5 cells and the host homogenate induced release of 40% of the total fixed carbon (controls released 5%) (Grant et al., unpublished data).

Polyp bail-out.— Colonies of *Plesiastrea versipora* kept under adverse conditions sometimes underwent polyp bail-out, released "free" polyps formed by budding, or produced planulae. Polyp bail-out occurred when colonies (or parts of them) were dying. In these colonies the coenosarc thinned progressively and contracted towards the calices until the polyps were completely detached from each other, as described by Sammarco (1982) for *Seriatopora hystrix* Dana, 1846. The polyps then rounded up into spherical masses and detached themselves from the skeleton. Budding occurred when corals were subjected to physiological stress (high temperature, lowered salinity) but were otherwise healthy. This occurred without changes in the coenosarc; instead, the polyps produced a smaller polyp (which had a gastrovascular cavity but no tentacles) or a "bud" (a flattened, ciliated structure with neither gastrovascular cavity nor tentacles). After bail-out or budding the polyp balls differentiated into polyps which survived in glass dishes in the laboratory for three weeks or more, but did not secrete a skeleton. In some stressed corals polyps divided, giving rise to daughter polyps with gastrovascular cavities, mouths and tentacles. These polyps separated from the parent colony, settled and, within two to four weeks of release, started to secrete a skeleton. We have also observed the production of planulae directly by "healthy" colonies of *P. versipora* without obvious change to the polyps. We do not know whether these are produced sexually or asexually, but we have also observed release of eggs and sperm from polyps in the laboratory.

Artificial production of clones.— Viable clones were produced artificially by pinching off small pieces of tissue (without skeletal material) from colonies, with forceps. The separated tissue organized itself into flattened, ciliated, motile bodies. Within four weeks of their separation from the parent colony (and sometimes within days) these differentiated into polyps, settled and began to secrete skeletons. They would settle on the softer glass of McCartney bottles but not on Pyrex glass.

Discussion

Collection and maintenance of corals.— It is not clear whether the extension of the polyps often seen in the laboratory is a response to lack of food or to light, or even whether it is abnormal. Certainly it ensures that all the symbiotic algae have maximal exposure to light, since the host's tissue is stretched out thinly and the tentacles spread widely.

Preliminary data on the carbon budget (Stewart, 1986) suggest that this coral may be heterotrophic under the conditions in Port Jackson. However, its survival and growth in the laboratory without macroscopic food suggests that it may require only the products of its symbionts under these conditions. It is possible, though, that it can feed on micro-organisms or use dissolved organic matter to supplement the phototrophy by the symbionts, and that in the aquaria, in which the water was changed once every seven to 14 days, it used these sources of nutrients in addition to phototrophy. Since the polyps ingested and, apparently, digested commercial cat food, it would be possible to base a fully defined diet on analysis of this food. This would be of great value in studies of coral nutrition.

Regeneration of colonies.— Since Kevin & Hudson (1979) showed that there are more symbiotic algae in the upper parts of the colonies than in the lower parts, it is likely that although 30% to 50% of the algae were removed by brushing, a smaller proportion of the coral tissue was actually removed. Although the colonies were routinely brushed until no more tissue was visible, we observed that the polyps withdrew deep into the calices, which provided residual tissue for regeneration.

Our preliminary data suggest that pieces of *Plesiastrea versipora* that have been brushed in this way can recover completely if rinsed, returned to seawater and kept in the light. The corals appear normal after a period of several weeks. As the coral tissue regrows the symbiotic algae multiply, so that the population density of the algae soon becomes normal again. All physiological functions which have been measured on these corals have returned to normal ranges after two to four weeks; that is, the respiratory rate of the whole association, the photosynthetic rate of the algae in hospite and in vitro, the ability of host tissue to stimulate release of photosynthate from the algae and the ability of the algae to respond to the active constituents of the host release factor, all return to the normal range.

Polyp bail-out.— The responses to stress seen in the laboratory (polyp bail-out or budding and release of polyps) have not been observed in the field, but the frequency with which they occur in the laboratory suggests they are likely to occur in the field too. Although asexual reproduction by fragmentation has been reported from many corals, it is more common in branching than in massive and encrusting species. The planulae found in the laboratory may have been produced sexually or asexually. Again, it is not known whether this occurs in the field. If any of these means of reproduction do occur in natural populations, they would have an important influence on the distribution and genetic structure of *P. versipora*. A field study of reproduction in this coral is in progress (Withers, unpublished). The laboratory data show clearly that *P. versipora* can recover from damage involving loss of up to 50% of the coral tissue and algae, and disruption of the structure of the polyps, either by repair of the existing colony (regeneration) or by the asexual formation of various sorts of

propagule. These processes can occur throughout the year, unlike sexual reproduction, which appears to be seasonal (Withers, unpublished).

Although we have not yet grown any planulae, buds or bailed-out polyps beyond colony sizes of four polyps, methods to do so could probably be developed. Many polyp balls settled, without any special treatment, on pieces of brick or dead coral or on softer forms of glass, but not on Pyrex glass. They were not fed, and it is possible that they would grow faster if fed at this stage of development. In these early stages the symbiotic algae grow very fast and probably release much less organic carbon, making the coral more dependent on prey capture than at later stages.

These results, and the production of the artificial clones by pinching off tissue from the colonies, mean that large numbers of genetically identical small colonies could be produced from single colonies of *P. versipora* and reared under identical, standard conditions. Such clones would be very valuable in studies of coral physiology, which have often been hampered by the lack of genetically related replicate colonies with similar histories. This is a particular difficulty with massive and encrusting species, from which it is usually difficult to cut a number of similar pieces, and which are less likely to reproduce by fragmentation than branching forms. This lack of uniform material is probably a major cause of variability in studies of corals. The ability to use the same corals for more than one experiment, by allowing them to regenerate in the laboratory or in the field, and the potential to grow them in laboratory "culture", will lessen the pressure on field populations of this species. It is still not known how regeneration generally occurs in scleractinian corals. The great ability of *P. versipora* to recover from damage and to reproduce asexually when stressed may be related to its very wide geographic range or its ability to live in temperate waters, where conditions are more variable than in the tropics, and might not be common.

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Factors affecting the orientation of growth of *Sertularia perpusilla* Stechow, 1919 (Hydrozoa: Sertulariidae) on leaves of *Posidonia oceanica*

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Key words: *Sertularia perpusilla; Posidonia oceanica;* oriented growth.

Abstract: *Sertularia perpusilla* Stechow, 1919, an obligate epiphyte of the seagrass *Posidonia oceanica* colonises leaves by stolons from which the new hydrorhizae grow preferentially downward onto the younger leaf surfaces. This downward growth could be in response to gravity (positive geotropism), light (negative phototropism) or to some age-related feature of the leaf surface. In situ experiments in spring and summer were conducted in which sections of leaves of different ages were grafted onto leaves on which *S. perpusilla* was growing. Natural downward growth was curtailed by grafting old leaf sections below the growing tip of the hydrorhizae, but not by grafting young leaf sections. Unnatural upward growth was stimulated by grafting young leaf sections above *S. perpusilla* hydrorhizae, but not by grafting old leaf sections. Scraping the epiflora and epifauna from above *S. perpusilla* did not result in increased upward growth. These results indicate that the natural downward orientation of the growth of *S. perpusilla* is in response to some age-related feature of the leaf surface, rather than to the epibiota, and not to gravity or light. There was no relationship between growth rates and size. Hydrorhizal growth rates were varied but less than 2mm.d^{-1}, and may be limited by the rate of cellular proliferation in the tip of the hydrorhiza.

Introduction

Seagrasses are often abundant in shallow waters from temperate to tropical regions where their leaves provide plentiful substrate for epiphytic organisms. *Posidonia oceanica* plants are abundant in the Mediterranean Sea and consist of discrete leaf bundles each of approximately 6 parallel leaves. The plants are perennial and produce new leaves from basal meristems in the centre of the bundle, each of which may live for only a few weeks (Ott, 1980). The growth strategies of hydroids that are obligate epiphytes on *Posidonia* leaves were studied by Hughes, Johnson & Smith (1991), who described three different patterns in three species. *Aglaophenia harpago* Von Schenk, 1965, showed no orientation of growth and was most common on the distal, oldest parts of the leaves, a distribution typical of facultative epiphytes. *Campanularia asymmetrica* Stechow, 1919, grew mostly down the leaves and was found on all leaf surfaces. *Sertularia perpusilla* Stechow, 1919, grew down the leaves also, but resorbed its oldest tissues and consequently was found only on the proximal, youngest, leaf surfaces. Kaehler & Hughes (1992) found three similar growth patterns in *Ventromma halecioides* (Alder, 1859), *Symmetroscyphus intermedius* (Congdon, 1907) and *Tridenta tumida* (Allman, 1877) respectively on *Thallasia testudinum* in the Caribbean Sea.

Downward growth onto the younger leaf surface is an adaptive behaviour as it maximises the residence time on a leaf, facilitates transfer of the colony to adjacent young, short leaves and reduces competition with other epiphytes (Hughes, Garcia Rubies & Gili 1991). Colonisation of other leaves is achieved by the production of long stolons from the hydrorhizae, which attach to adjacent leaves where a new colony develops before the stolon breaks from the parent colony (von Schenck, 1962; Boero, 1981). Downward growth of the hydrorhiza may be in response to gravity (positive phototropism), to light (negative geotropism), to an age-related feature of the leaf surface or to the presence of other epiphytes. The downward growth of *S. perpusilla* was studied further by Hughes, Garcia Rubies & Gili (1991) who concluded that gravity and light were probably not important, since when leaves were inverted growth continued upward onto young leaf surfaces. They considered that an age-related feature of the leaves might be important in determining orientation of growth.

This study investigated the hypothesis that the orientation of growth of *Sertularia perpusilla* is related to the age of the substrate by in situ experiments in which sections of leaves of different age were grafted onto *Posidonia* leaves on which the hydroids were growing and by observing the effects on the growth rates of the hydroids.

Methods

The experiments were conducted at two sites on Cap Creus (northeastern Spain) at Cadaques, at a depth of 2 m, and at Port de la Selva at a depth of 10 m. There was no difference in the results from these two sites and they have been combined. There were two similar series of experiments, one in spring and one in summer. Each series consisted of seven treatments, six experimental treatments and control treatments, with ten replicates of each. The six experimental treatments, shown diagrammatically in fig. 1, were:

(1) distal graft of a section of young leaf above the upper tip of the hydroids, (2) distal graft of a section of old leaf above the upper tip of the hydroids, (3) distal graft of a section of young leaf above the upper tip of the hydroids and proximal graft of a section of old leaf below the lower tip of the hydroids, (4) proximal graft of a section of young leaf below the lower tip of the hydroids, (5) proximal graft of a section of old leaf below the lower tip of the hydroids (6) no leaf grafts but scraping the distal leaf surface with a sharp blade to remove the epibionts at, and above, the distal tips of the hydroids. This procedure should remove most of the epiflora and fauna to mimic the relatively clean surfaces of young leaf sections.

Leaves with only one hydroid colony were used. Where leaf grafting did not occur the upper and lower extremities of the tissue-filled hydrorhiza of each hydroid were marked by snipping the leaf edge with scissors. Where grafting occurred the leaves were cut horizontally across the distal and/or proximal tips of tissue-filled hydrorhiza, removing a small amount of hydroid tissue. Young or old sections of other leaves were grafted under these cut ends by use of two metal staples (of the sort usually used to attach sheets of paper). The staples were positioned on either side of the hydrorhiza and parallel to it, and fixed the two leaf surfaces so that the leaf with the hydroid overlaid the grafted section. At the end of the experiments the amount of upward and downward growth of hydrorhizae was measured, and the number of new hydrocauli and hydranths were counted.

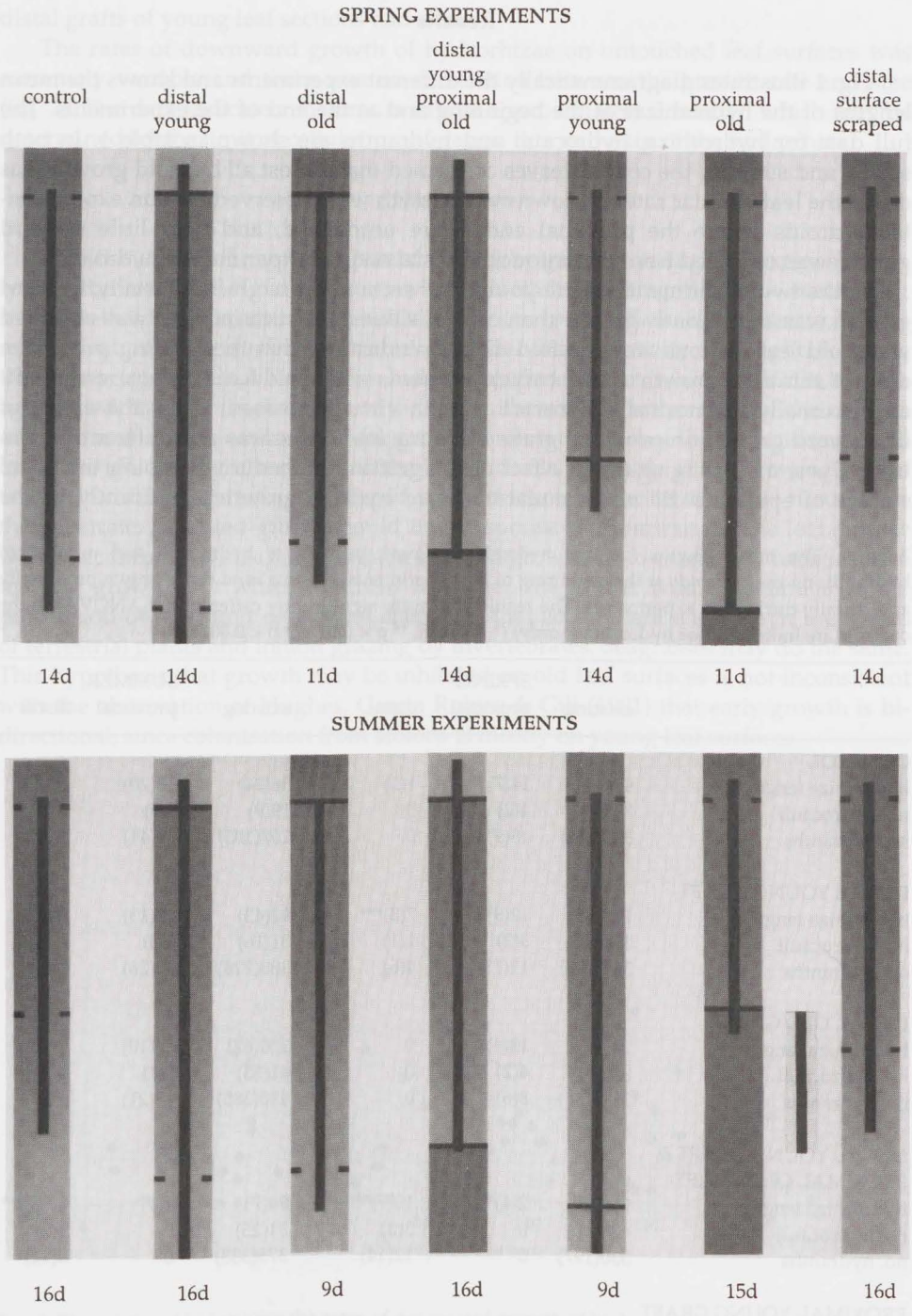

Fig. 1. Diagrammatic representation of the experiments showing the mean lengths of hydrorhizae (longitudinal bars). The small horizontal bars indicate where the leaf was marked at the beginning of the experiments at the proximal and/or distal tip of the hydrorhizae. The complete horizontal bars indicate the junction of a leaf graft. The duration of each experiment in days is shown. The graduated grey coloration indicates the relative ages of the leaf surfaces (darker = older). Scale bar hydrorhizae = 2 cm.

Results

Fig. 1 illustrates diagrammatically the different experiments and shows the mean lengths of the hydrorhizae at the beginning and at the end of the experiments. The full data for hydrorhiza, hydrocauli and hydranths are shown in table 1. In both spring and summer the control leaves confirmed that almost all hydroid growth was down the leaf. Similar rates of downward growth were observed also on experimental hydroids where the proximal ends were untouched, and very little upward growth was observed from the untouched distal ends of experimental hydroids.

In the two experiments where young leaf sections were grafted distally, upward growth was significantly greater than control values. No such increase was observed when old leaf sections were grafted distally, indicating that the grafting procedure did not stimulate growth. In the two experiments where old leaf sections were grafted proximally the normal downward growth virtually ceased, while the extent of downward growth on proximal grafts of young leaf tissue was not different to control values, indicating again no effect of the grafting procedure. Scraping the distal surface of epibionts did not stimulate upward hydroid growth significantly, as the

Table 1. The mean lengths (s.d.) of hydrorhizae (mm), number of hydrocauli and number of hydranths on the hydroids at the beginning of the experiments (existing) and those grown proximally and distally during the experiments. The results that were significantly different (by ANOVA) to the controls are indicated (for hydrorhizae only) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

	SPRING			SUMMER		
	existing	proximal	distal	existing	proximal	distal
CONTROL						
hydrorhiza length	99(37)	14(7)	1(1)	56(24)	33(20)	2(4)
no. hydrocauli	35(14)	4(2)	0	19(9)	9(5)	1(1)
no. hydranths	383(256)	15(9)	0	207(105)	60(41)	8(17)
DISTAL YOUNG GRAFT						
hydrorhiza length	111(53)	12(9)	7(3)***	92(43)	23(13)	8(4)
no. hydrocauli	35(17)	3(2)	1(1)	31(16)	6(3)	1(1)
no. hydranths	366(190)	11(13)	4(6)	380(228)	30(26)	4(6)
DISTAL OLD GRAFT						
hydrorhiza length	93(49)	12(4)	0	100(67)	12(10)	0
no. hydrocauli	31(19)	4(2)	0	41(33)	3(4)	0
no. hydranths	324(236)	8(6)	0	410(385)	18(21)	0
DISTAL YOUNG GRAFT & PROXIMAL OLD GRAFT						
hydrorhiza length	96(30)	2(4)**	10(7)**	94(71)	1(1)*	10(5)***
no. hydrocauli	42(16)	0	3(3)	31(25)	0	3(2)
no. hydranths	330(197)	0	12(14)	375(333)	0	8(12)
PROXIMAL YOUNG GRAFT						
hydrorhiza length	70(36)	13(7)	2(2)	114(60)	12(5)	1(2)
no. hydrocauli	26(13)	3(2)	1(1)	41(21)	3(2)	0
no. hydranths	242(135)	10(11)	2(2)	442(257)	10(10)	0

distal grafts of young leaf sections had done.

The rates of downward growth of hydrorhizae on untouched leaf surfaces was extremely variable and there was no correlation between the growth rate and size (fig. 2). The data indicate a maximum rate of approximately 2 mm.d^{-1}, which may be determined by the limit of cellular proliferation rate in the tip of the hydrorhiza.

Discussion

The stimulation of unatural upward growth by grafting young leaf sections distally, and the cessation of natural downward growth by grafting old tissue proximally, indicates that the orientation of growth of *Sertularia perpusilla* is in response to an age-related feature of the surface of the leaves, rather than to gravity or to light. Scraping the leaf surface to remove epibionts had no significant effect, which indicates that the age-related feature is a character of the leaves themselves rather than of the epiflora and fauna. A simple explanation for growth orientation is that the hydroids respond to, or are affected by, chemicals on the leaf surfaces, probably at the growing tips of the hydrorhizae before the perisarc forms and insulates the hydrorhizal tissue from the substrate. Oriented growth could be a response to chemicals on the leaf surface which accumulate with age and inhibit hydroid growth, or to chemicals that stimulate hydroid growth, but which diminish with age. The former would be similar to the production of toxic and distasteful chemicals that accumulate with age in the leaves of terrestrial plants and inhibit grazing by invertebrates. Seagrasses may do the same. This hypothesis that growth may be inhibited on old leaf surfaces is not inconsistent with the observation of Hughes, Garcia Rubies & Gili (1991) that early growth is bi-directional, since colonisation from stolons is mostly on young leaf surfaces.

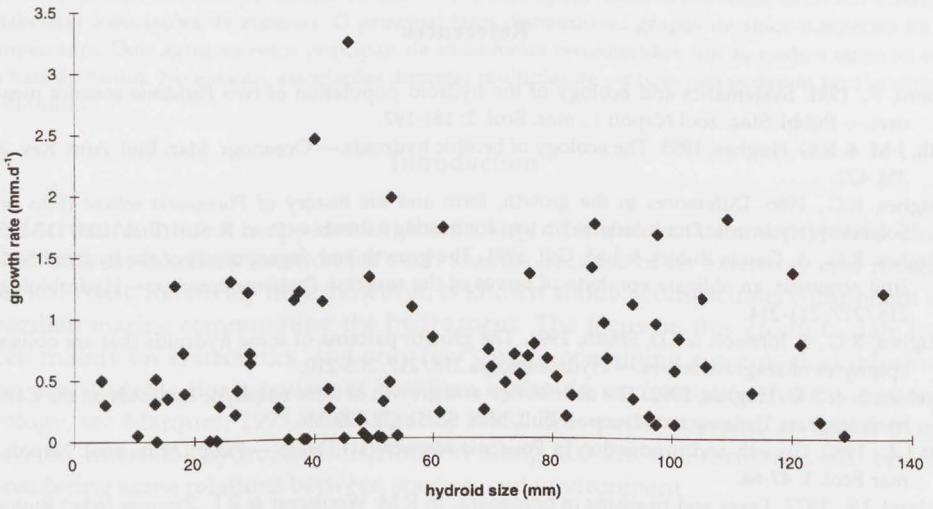

Fig. 2. The relationship between the rates of downward growth of hydrorhizae on untouched leaf surfaces and hydroid size. The data are from the spring experiments and are from hydroids with one growing tip, except the two outlying data points [in parentheses] in which the hydrorhizae had branched and in which there were two growing tips.

Hughes, Garcia Rubies & Gili (1991) observed that the growth of small colonies from attached stolons was oriented upward (temporarily) and downward and postulated that this initial bi-directional growth would more rapidly span leaf surfaces of different age to allow an assessment of which was the direction of the youngest tissue. This hypothesis requires that information regarding the substrate, which is probably detected at the growing tips, is somehow transmitted and integrated somewhere in the colony. This is unlikely in these structurally simple organisms.

While the results of this study indicate that responses to light or to gravity have no influence on the orientation of growth of established hydroids, the initial orientation of hydrorhizal growth from attached stolons, which is up and down and not sideways, may be in response to light or gravity (Hughes, Garcia Rubies & Gili, 1991), since any effect of leaf tissue age would operate equally in all directions at the single point of stolon attachment. This could be investigated by an experiment in which colonisation by stolons of leaves held horizontally is facilitated.

There are other examples of oriented growth of marine invertebrates on their hosts. The growth of the hydroid *Plumularia setacea* (Linnaeus, 1758) is down onto the younger parts of its host *Nemertesia antennina* (Linnaeus, 1758) (see Hughes, 1986) and bryozoans may grow toward the younger or older parts of their hosts (Ryland, 1977). What stimulated these behaviours was not investigated. The ability of hydroids to respond to specific chemicals produced by their host is known for a few species in which the planula shows substrate specificity (for a review, see Gili & Hughes, 1995).

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Patterns of intertidal hydrozoan distribution along the coast of São Paulo State, southeastern Brazil

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Key words: Hydrozoa; geographical distribution; canonical analysis; Brazil.

Abstract: Hydrozoan assemblages in the intertidal zone along the coast of São Paulo State, SE Brazil, were investigated by semi-quantitative methods. Their tolerances to ranges of variation in hydrodynamics, light incidence, and elevation in the intertidal zone, were also assessed. Canonical correspondence analysis was applied to classify sites, delimit zones, and establish species associations. The main factor distinguishing sites and species groups was temperature. Two main site clusters were recognized, one to the north and other to the south of Santos Bay. Multiple discrete species associations, however, could not be clearly distinguished.

Resumo: Associações de hidrozoários na zona entremarés ao longo da costa do estado de São Paulo, SE do Brasil, foram pesquisadas com métodos semi-quantitativos. Suas tolerâncias às amplitudes de variáveis hidrodinâmicas, incidência de luz e posicionamento na zona entremarés foram também inferidas. Análise de correspondência canônica foi aplicada para classificar os sítios, delimitar zonas, e estabelecer associações de espécies. O principal fator distingüindo grupos de sítios e espécies foi a temperatura. Dois agrupamentos principais de sítios foram reconhecidos, um ao norte e outro ao sul da baía de Santos. No entanto, associações discretas múltiplas de espécies não puderam ser claramente dintintas.

Introduction

Increasing interest is apparent in the ecology of tropical marine ecosystems. Brazil affords an excellent location for such studies because of its extensive and mostly tropical coast. Relatively little, however, is known about a conspicuous component of Brazilian marine communities: the hydrozoans. The focus on this group to date has been mainly on systematics, and only few studies containing autecological information are available (for a review of Brazilian hydroids, on faunistics, systematics and ecology, see Marques, 1993, and Migotto, 1993). This paper presents an initial overview on intertidal hydrozoan distribution along 400 km of Brazilian coast, briefly considering some relations between species and environment.

Methods

The hydroids and abiotic factors were investigated during the winters of 1991 and 1992, from intertidal rocky shores during low spring tides, along the coast of São

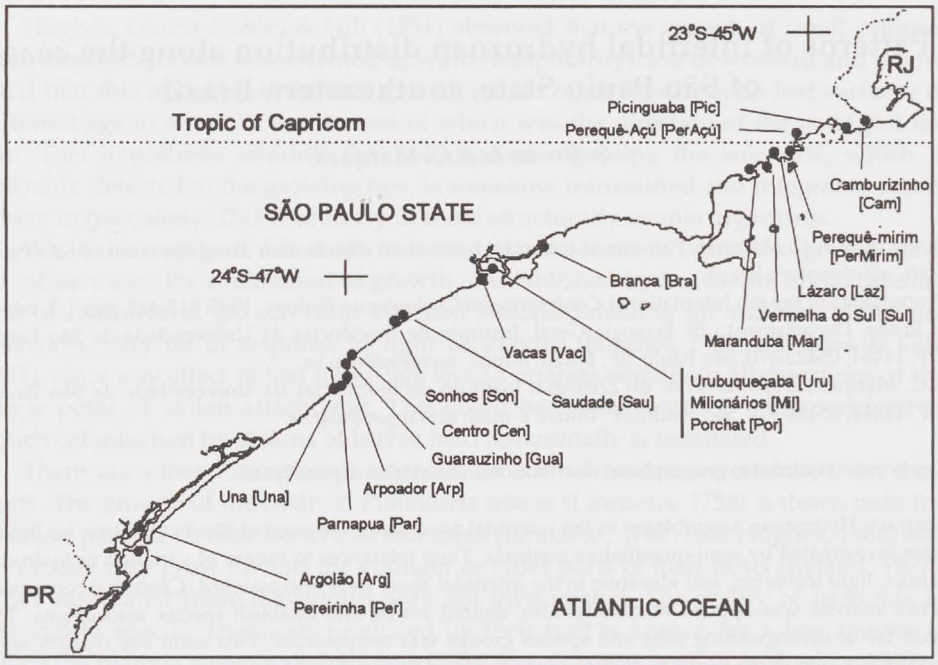

Fig. 1. Sites sampled along the coast of São Paulo State, Brazil.

Paulo State, SE Brazil (23°22'S 44°43'W to 25°13'S 48°03'W; see fig. 1). Each species was assigned in the field to one of three classes of abundance: rare, common, and dominant, corresponding respectively to the values 1, 10, and 100. This assured an initial downweight of more inconspicuous species. Specimens were later identified in the laboratory.

An analysis of field data led to ecological classification of species in relation to their habitats. Species occurring only in sheltered areas were termed "steno-dynamic", those living also in exposed places, were identified as "eury-dynamic". By analogous criteria, "steno-topic" (lower intertidal), "steno-photic" (shadow), and the eury- alternatives were defined.

Canonical Correspondence Analysis (CCA) was carried out after a preliminary screening of data to select the environmental variables to be included. Based on evidence from an exploratory phase, salinity was omitted from the treatment due to its very low significance ($p = 0.27$), high correlation with river proximity, and weak importance explaining variance (0.1 of the overall 1.3).

Apart from the elimination of salinity, no other manipulations such as additional weighting of samples or species were performed. No covariables were defined. From CCA, a species-environment biplot was prepared with the environment canonical vectors component drawn as a detail in a scale proportional to that of the main plot. Sample scores, based both on strictly environmental variables and on these variables together with species data, were plotted apart but at the same scale as species scores in the biplot. In a search for statistically significant species- environment correlations, t-values for species (main plot; $\alpha = 0.05$, $n > 20$) and environmental variables were

Table 1. Species studied [abbreviations for names used in the figs shown in brackets], collecting sites and habitat (A: steno-dynamic/eury-topic/steno-photic, B: eury-dynamic/eury-topic/eury-photic, C: steno-dynamic/steno-topic/steno-photic, D: eury-dynamic/steno-topic/eury-photic, E: steno-dynamic/eury-topic/eury-photic).

Species	Sites (see fig. 1)	Habitat
Bougainvillia spec. [Boug]	Sau	C
Bimeria vestita Wright, 1859 [Bimvest]	Pic Par	C
Bimeria spec. [Bimsp]	Cen Gua Par Arp Per	D
Turritopsis nutricula McCrady, 1859 [Turr]	Par Per	C
Eudendrium carneum Clarke, 1882 [Eucar]	Pic PerMirim Laz Sul Bra Por Vac Son Gua Per	D
Eudendrium glomeratum Picard, 1951 [Euglo]	Laz Mar Per	C
Eudendrium pocaruquarum Marques, 1995 [Eupoca]	PerAçú	C
Eudendrium ramosum (Linnaeus, 1758) [Euram]	Pic	C
Halocordyle disticha (Goldfuss, 1820) [Haloc]	Laz Bra Por Gua Par Arp Per	D
Ectopleura warreni (Ewer, 1953) [Ectwarr]	Mil Por Vac Sau Son Cen Gua Par Arp Una Arg Per	D
Hebella scandens (Bale, 1888) [Hebe]	Pic	D
Halecium dyssymetrum Billard, 1929 [Hale]	Sul	C
Aglaophenia latecarinata Allman, 1877 [Agla]	Pic Laz Sul	C
Lytocarpia tridentata (Versluys, 1899) [Lyto]	Bra Por	C
Halopteris diaphana (Heller, 1868) [Halo]	Mar Bra	C
Kirchenpaueria halecioides (Alder, 1859) [Kirch]	Arg Per	D
Monotheca margaretta Nutting, 1900 [Mono]	Laz	C
Plumularia floridana Nutting, 1900 [Plum]	PerMirim Vac Per	D
Dynamena crisioides Lamouroux, 1824 [Dynacr]	PerAçú PerMirim Sul Bra Vac Gua Arp	E
Dynamena disticha (Bosc, 1802) [Dynady]	Cam Pic Mar Bra Par	E
Idiellana pristis (Lamouroux, 1816) [Idiel]	Per	C
Sertularia distans (Lamouroux, 1816) [Serdis]	Laz Mar Bra Vac Cen Arp	C
Sertularia marginata Kirchenpauer, 1864 [Sermarg]	Cam Pic PerMirim Laz Sul Mar Bra Por Vac Son Cen Gua Par Arp Una	B
Sertularia rugosissima Thornely, 1904 [Serrug]	Bra	B
Sertularia turbinata (Lamouroux, 1816) [Sertur]	Pic PerMirim Laz	C
Thyroscyphus ramosus Allman, 1877 [Thyr]	Pic PerMirim Laz	A
Campanulariidae [Campan]	Cam	C
Clytia hemisphaerica (Linnaeus, 1767) [Clyhemi]	Pic PerMirim Laz Sul Mar Uru Por Gua Par	C
Clytia linearis (Thornely, 1899) [Clylin]	Pic	C
Obelia dichotoma (Linnaeus, 1758) [Obel]	Cam PerAçú PerMirim Bra Uru	D
Orthopyxis sargassicola (Nutting, 1915) [Orth]	Mil Por Vac Sau Cen Gua Par Per Son	D

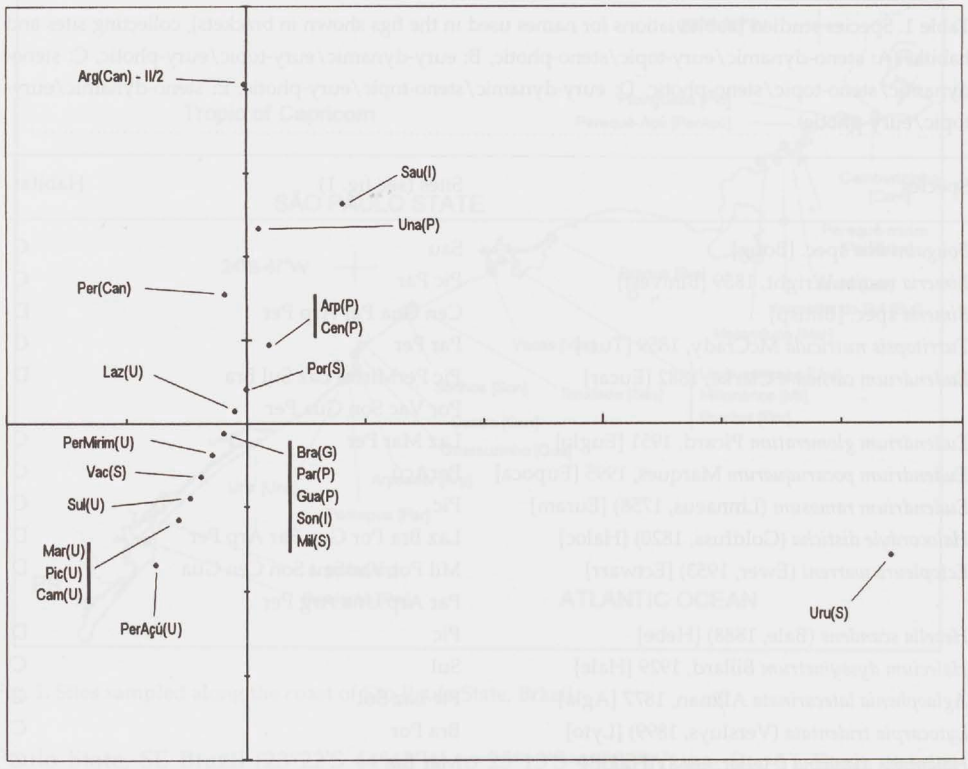

Fig. 2. Sites ordination diagram (based strictly on abiotic data).

plotted in a third diagram. Projections of environmental canonical vectors on the direction of each species vector, when falling outside the interval ± species-arrow length, showed significance of the relation between the corresponding environmental factor and species (ter Braak, 1990: 28).

Results and Discussion:

Initial results came from an exploratory phase. Along with salinity, mangrove proximity also showed little significance ($p = 0.58$) and explanatory value (0.14 of 1.3). River proximity and muddy deposits were better descriptors, significant at the 0.73 level and accounting for 0.2 and 0.25 out of the overall variance. The latter two factors were not omitted, due to their important effect on particular sites/species. The best environment descriptors, significant at 0.85 and 0.96 levels and explaining 0.22 and 0.38 of the full 1.3 variance, were temperature and unidirectional water flow respectively.

The results related to mode of occurrence of species are presented in table 1. For *Turritopsis nutricula* McCrady, 1859, the habitat information coincides with that of Mammen (1963: 36, the species growing in shadowed zones) but disagrees with Bandel & Wedler (1987: 39). The eury-topic habitat of *Dynamena crisioides* Lamouroux,

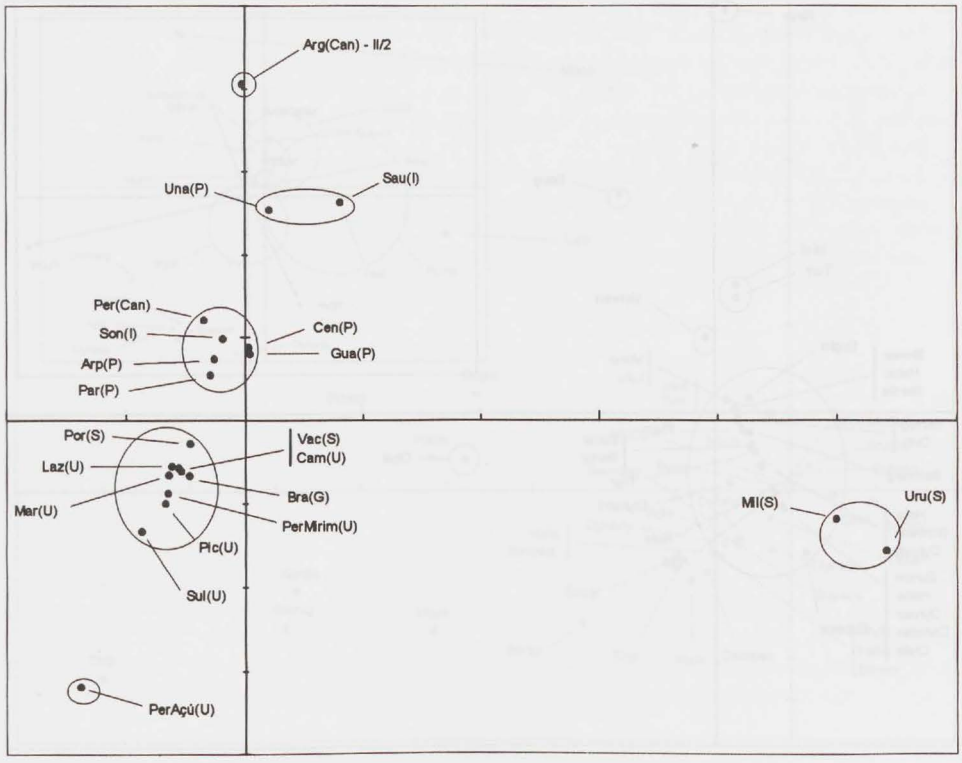

Fig. 3. Sites ordination diagram (based on species and abiotic data).

1824, is well known from the literature (Bandel & Wedler, 1987: 42; Calder, 1991a: 2072; 1991b: 2998; Migotto, 1993: 142), and is also common to its congeneric species, *Dynamena disticha* (Bosc, 1802). *Idiellana pristis* (Lamouroux, 1816) was only recorded in the Pereirinha, a calm water site, in concordance with Migotto's (1993: 155) observations.

Considering strictly environmental factors (fig. 2), almost all sites were regularly ordered from south to north in the direction of the temperature canonical vector (see frame in fig. 4). Only four points appeared displaced from the temperature gradient, one with strong unidirectional current (Urubuqueçaba Island), one near extensive mangrove areas and presenting muddy deposits (Argolão), and in a lesser degree two others near mangroves (Pereirinha and Una). A fifth point (Saudade), with very low salinity, is displaced along the river proximity vector (direction coincident with that of the temperature).

Considering species together with environment, most sites and species (figs 3, 4) were clearly ordinated in the temperature direction; the sites grouped in two main clusters (those of Santos Bay in an intermediary position between sites of northern and southern areas). Outside the main cluster were two species-poor sites in Santos Bay (Milionários and Urubuqueçaba Island), dominated by *Obelia dichotoma* (Linnaeus, 1758), and even more dispersed, the remaining sites. Una, Saudade, and Argolão were

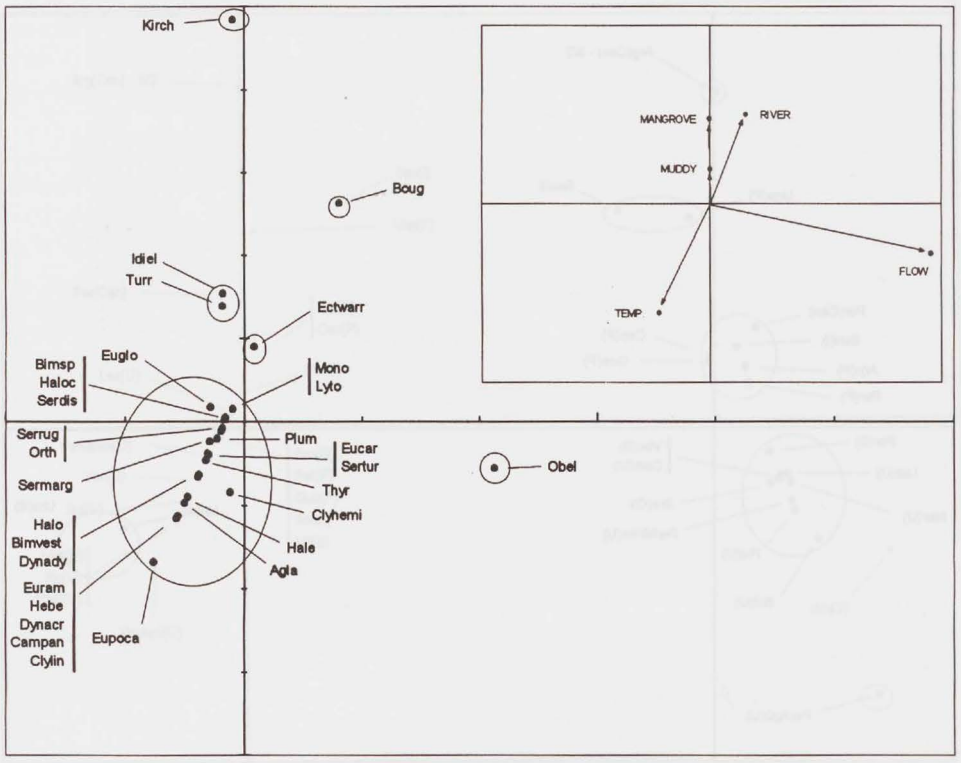

Fig. 4. Species ordination diagram.

mangroves or fresh water inflows, the later very poor in species. Near mangrooves, *Kirchenpaueria halecioides* (Alder, 1859) and *Ectopleura warreni* (Ewer, 1953) occurred in very small quantities in these areas, while Calder (1991a: 2068), sampling in various substrata, found 48 species at Twin Cays, Belize. Areas of fresh water inflow were also poor in species, but strongly dominated by *Ectopleura warreni* (which has a very well defined winter season of recruitment and occurrence; Migotto & Marques, unpublished data). At the extreme of the ordination, Perequê-Açú stood alone, strongly dominated by *Dynamena crisioides*. The species *Ectopleura warreni*, *Sertularia marginata* Kirchenpauer, 1864, *Bimeria* spec. and *Halocordyle disticha* (Goldfuss, 1820) were well represented in at least some of the sites of Peruíbe and Itanhaém. *Eudendrium carneum* Clarke, 1882, *Dynamena crisioides* and again *Sertularia marginata* were important species in most of the places from Santos Bay to Ubatuba (São Paulo State). Discrete species associations, however, could not be clearly distinguished.

Only four species appeared with some statistically significant ways with environmental factors (fig. 5). The abundance of *Obelia dichotoma* was correlated with unidirectionally flowing water, the main factor distinguishing Urubuqueçaba Island. The great abundance of the species also in Milionários, where strong unidirectional flow was not observed, suggests it is a response to some other non-measured factor which would be highly typical for both Milionários and Urubuqueçaba Island. *Ectopleura*

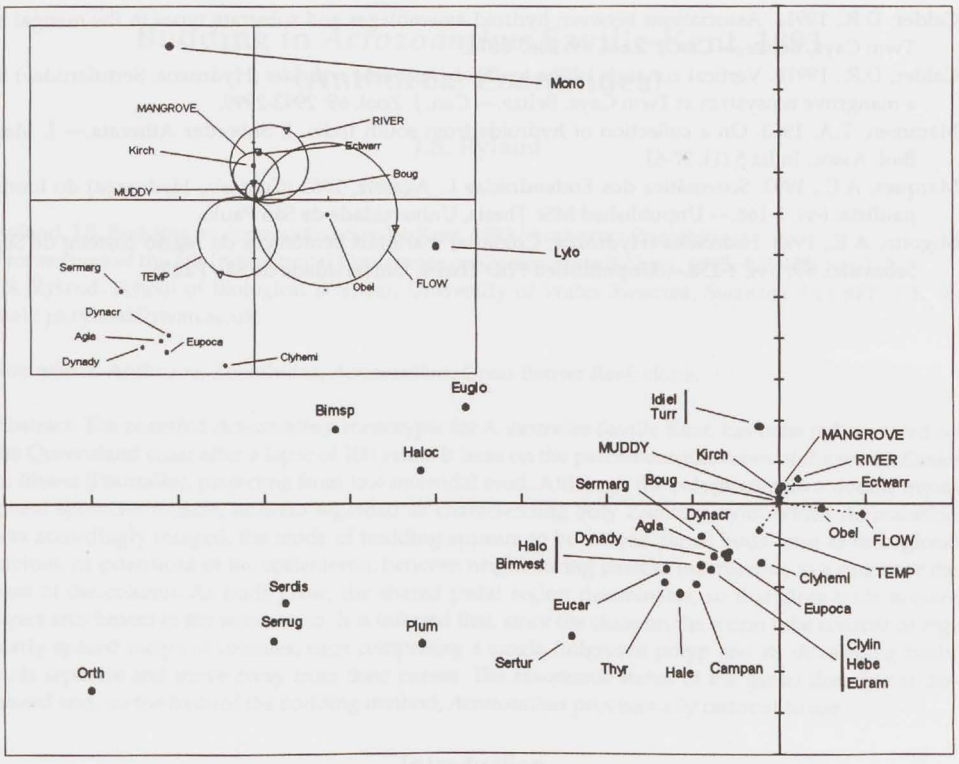

Fig. 5. T-values biplot. The circles in the frame indicate significance limits.

warreni occurs in habitats near river or mangroves, although it exhibited a significant and negative relation to water temperature (in the biplot of fig. 5, the point corresponding to the species is located in an area symmetrically opposite to the delimited one for the factor; see ter Braak, 1990, for details on diagram interpretation). *Kirchenpaueria halecioides* was correlated with mangrove proximity, and *Bougainvillia* spec. with river proximity.

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Budding in *Acrozoanthus* Saville-Kent, 1893 (Anthozoa: Zoanthidea)

J.S. Ryland

Ryland, J.S. Budding in *Acrozoanthus* Saville-Kent, 1893 (Anthozoa: Zoanthidea).
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Key words: Anthozoa; Zoanthidea; *Acrozoanthus*; Great Barrier Reef; clone.

Abstract: The zoanthid *Acrozoanthus*, monotypic for *A. australiae* Saville-Kent, has been rediscovered on the Queensland coast after a lapse of 100 years. It lives on the parchment-like tubes of the worm *Eunice* cf. *tibiana* (Pourtalès), projecting from low intertidal mud. Although the polyps contain a double mesogloeal sphincter muscle, hitherto regarded as characterizing only *Zoanthus*, with which *Acrozoanthus* was accordingly merged, the mode of budding appears to be unique. Small buds arise as mesogloeal cavities, or extensions of the coelenteron, between neighbouring pairs of macrosepta, in a ring near the base of the column. As buds grow, the shared pedal region delaminates, so that large buds acquire direct attachment to the substratum. It is inferred that, since the clone on the worm tube consists of regularly spaced incipient colonies, each comprising a single fullgrown polyp and its developing buds, buds separate and move away from their parent. The taxonomic status of the genus *Zoanthus* is discussed and, on the basis of the budding method, *Acrozoanthus* provisionally restored to use.

Introduction

The genus *Acrozoanthus* was created by Saville-Kent (1893: 154) for the single species *A. australiae* from the Great Barrier Reef of Australia. The new genus was based on the interpretation that the erect colonies were supported by an axial skeleton of their own secretion, as was known in the distantly related genus *Gerardia* Lacaze-Duthiers, 1864. However, Haddon (1895), to whom Saville-Kent had sent a specimen, pointed out that the supporting structure was actually a polychaete tube of the type described by Ehlers (1887) as being constructed by *Eunice tibiana* (Pourtalès, 1867). Haddon then proposed "to abolish the genus *Acrozoanthus*, and place Mr. Saville-Kent's form under the genus *Zoanthus*", with which *A. australiae* agreed in [unspecified] morphology. There appear to have been no further reports of this zoanthid in the succeeding century and it was, therefore, exciting to rediscover *A. australiae* on the Queensland coast in March 1995. The find has made possible a reexamination of the species and an evaluation of the status of *Acrozoanthus*. Saville-Kent (1893) described the species well enough for instant recognition but gave neither histological information nor any account of the budding process, which appears to be unique in the order Zoanthidea.

Locality, methods, and depository of material

The specimen was found on 17 March 1995 at Cape Hillsborough, near Mackay, Queensland, at low water of a spring tide (0.5 m) in a rocky causeway, exposed only on low spring tides, at Wedge Island. The locality has rocky shores, with scattered

corals and other zoanthids, and comprises part of reef 20-275 in the Mackay/Capricorn section of the Great Barrier Reef Marine Park (Great Barrier Reef Marine Park Authority, 1988). The site is very sheltered from the prevailing southeasterly winds by a protective arc of headland, island and islet, and mud had accumulated between the boulders. The tube of the worm, *Eunice* cf. *tibiana*, projected some 20-25 cm from the mud. The apical portion of about 10 cm, with part of the zoanthid clone on it, was removed, photographed, and then preserved on site in 95% ethanol (the visit was part of extended fieldwork to collect zoanthids for DNA extraction: no histological fixative was available).

On return to Swansea the preserved and retracted specimens were further photographed (fig. 1) and a few polyps with buds were removed for sectioning. Specimens were wax embedded, sectioned in longitudinal and transverse planes at 7 µm, and stained with Mallory's trichrome though, owing to preservation in ethanol, neither the sections nor the staining were of exceptional quality.

The colony, in which the nematocysts are being studied, and histological preparations are retained in my collection at Swansea, registered number JSR 543.

Description of *Acrozoanthus australiae* Saville-Kent, 1893

The zoanthid clone comprised a series of discrete polyps on an erect worm tube of approximately 25 cm free length (which may extend up to 45 cm according to Saville-Kent or even more (J.C. den Hartog, personal communication)); clustered near the apex of the tube, well separated lower down (fig. 1). Well grown polyps 11 × 8 mm, obovoid or hot air balloon shaped in contraction, with the basal disk slenderer than the middle column. Each large polyp supports a developmental series of (up to six) buds, in a circle, near the base of the column (figs 1, 2A); the largest bud on some polyps, nearly as large as the parent, having made independent contact with the surface of the worm tube. The tube being slightly but distinctly zigzag, with openings at the angles, the discrete polyps, with their buds, are positioned at the angles (fig. 1A-C). Outer surface of polyps smooth, soft, not encrusted with sand.

Large polyps have 24-26 macrosepta and about 50 tentacles. Septa with a large canal (fig. 2E) near their base. Sphincter muscle mesogloeal, in two annuli separated by a cleft; the inner annulus much the shorter and thicker (fig. 2F). Mesogloea of column thick, with numerous canals (fig. 2E). Endoderm of tentacles deep, packed with zooxanthellae. Ectoderm with moderately abundant spirocysts. Buds arising above the pedal disk (fig. 2A), first as a pocket of the coelenteron into the mesogloea, between two macrosepta (fig. 2E); then bulging from the surface and lengthening (fig. 2B-D). No gonads are present.

Colour: oral disk and tentacles grey brown; column light fawn grey, paler toward its base, with flecks of brown and a vivid emerald green ring at about the position of the sphincter (agreeing closely with chromo plate 3 in Saville-Kent, 1893).

The worm tube belongs to *Eunice* cf. *tibiana* (P.A. Hutchings, personal communication). Its substance is parchment like and the outer surface heavily colonized by small algae, bryozoans and hydroids.

Saville-Kent referred to the occurrence of *Acrozoanthus australiae* in the Great Barrier Reef from the vicinity of Mackay to Torres Strait, and also from Darwin (where it is still found, P. Alderslade, personal communication), noting its restriction to "some-

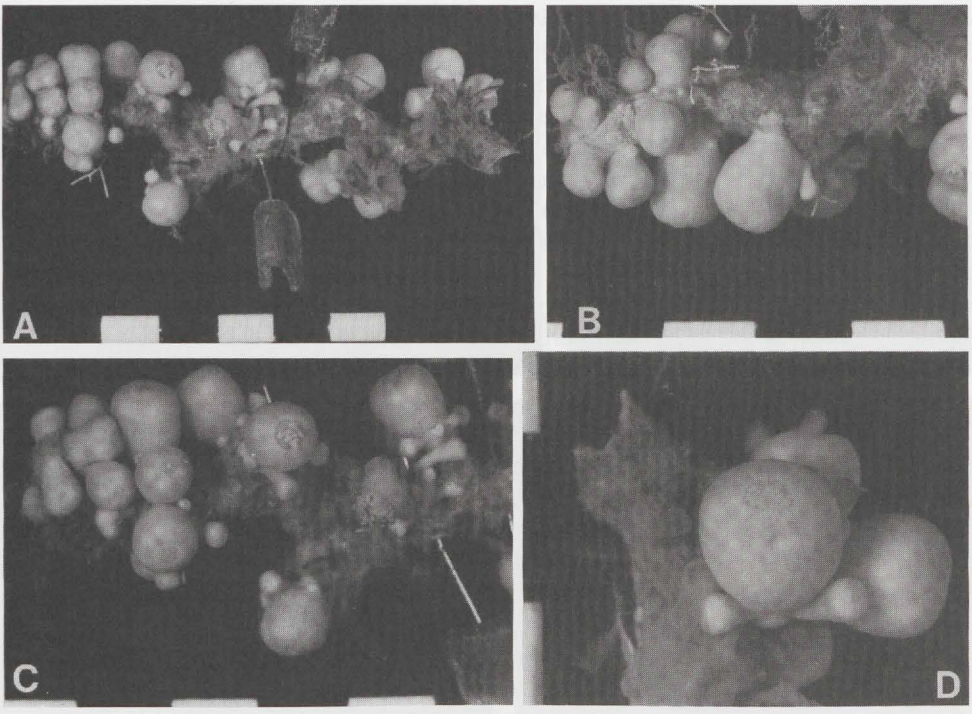

Fig. 1A-D. *Acrozoanthus australiae*. Photographs of preserved clone on part of tube of *Eunice* cf. *tibiana*, Cape Hillsborough, Queensland. Scale bar in cm.

what muddy shores". The association with muddy shores may explain the lack of recent records: reef researchers currently pay little attention to such sites. Outside Australia, *A. australiae* is known from Indonesia, with specimens in the Nationaal Natuurhistorisch Museum, Leiden, from Ambon in the Moluccas, collected by the Rumphius Biohistorical Expedition 1990 (RMNH 23405-7), and from the Spermonde Archipelago in southwest Sulawesi, collected in 1990 and 1994 (RMNH 23408-11).

Discussion

Acrozoanthus australiae is readily recognizable both for its unusual habitat and the striking coloration, well depicted by Saville-Kent (1893, chromo pl. 3). The generic placement, however, is open to debate. Certain morphological characters, such as the particle free mesogloea in combination with the double, mesogloeal sphincter, are diagnostic for *Zoanthus* Lamarck, 1801, as currently used. The type species of *Zoanthus* is *Actinia sociata* Ellis & Solander, 1786 (which see, p. 5, pl. 1) from the Caribbean. A closely similar species from the Great Barrier Reef and Fiji is *Z. coppingeri* Haddon & Shackleton, 1891 (now recognized as an older name for the species described as *Z. mantoni* Carlgren, 1937, by Ryland & Muirhead (1993)). These two species form colonies of polyps united by flat stolons or thin spreading coenenchyme; buds, and hence polyps, arise at intervals from the coenenchyme, not from the column of polyps.

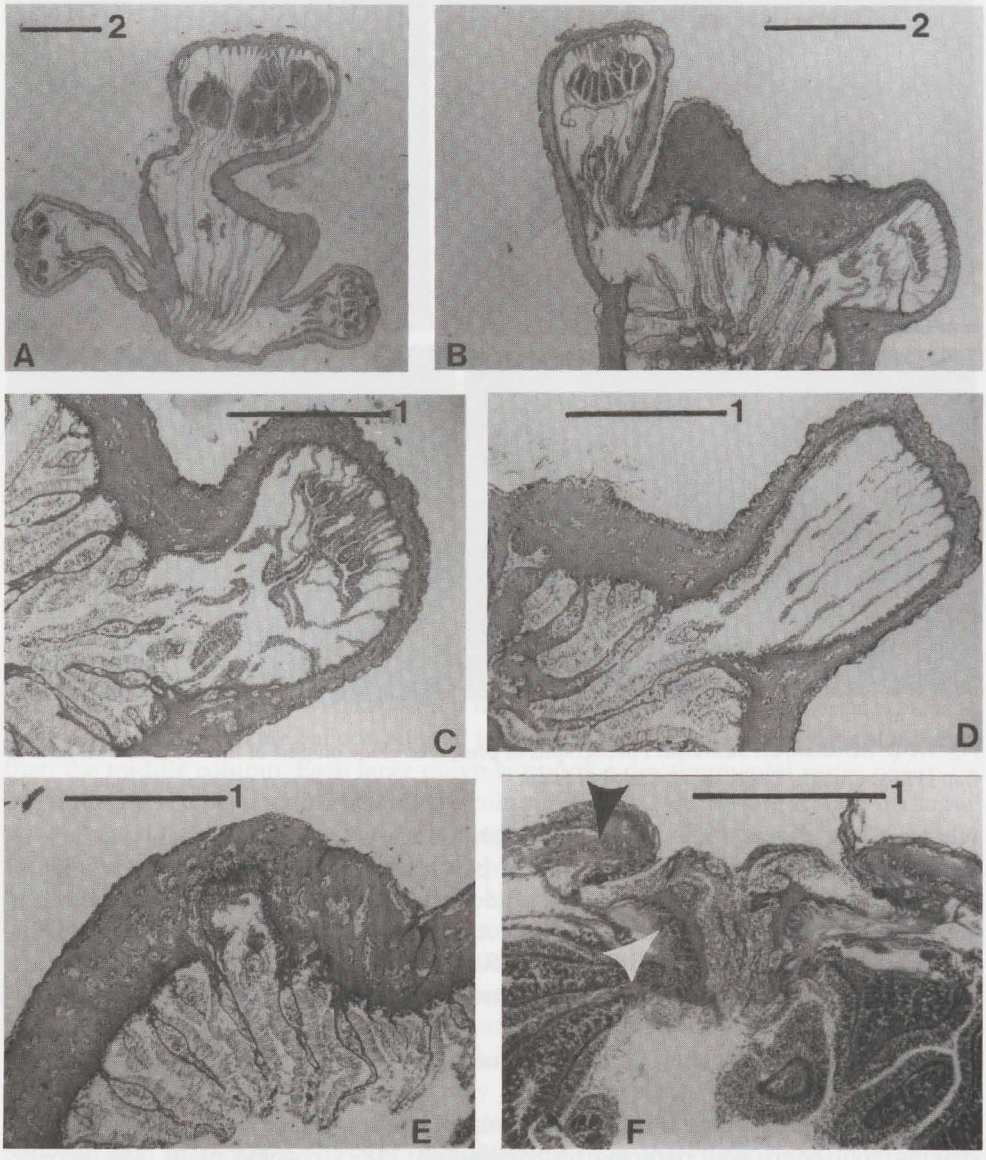

Fig. 2A-F. Sections through *Acrozoanthus australiae*. A, Nearly median longitudinal section through polyp with two buds; B, Transverse section through part of polyp with two buds; C, Section through developing bud (transverse section with reference to parent polyp); D, Transverse section through polyp wall showing incipient bud; E, Transverse section through part of column showing all septa with a large basal canal, the mesogloeal canals, and an incipent bud between two macrosepta as an evagination of coelenteron into the mesogloea; F, Median longitudinal section to show the sphincter muscle, the two parts indicated with black and white arrowheads. Scale bar 1 = 1 mm; 2 = 2 mm.

A different kind of colony occurs in *Zoanthus vietnamensis* Pax & Müller (1957), also redescribed by Ryland & Muirhead (1993), in which polyps are essentially immersed in thick continuous coenenchyme (this also being the principal character of *Palythoa* Lamouroux, 1816, sensu stricto used by those authors, most recently Ryland & Muirhead (1993), who separate *Protopalythoa* Verrill, 1900, from *Palythoa*). Since recent work (Burnett et al., 1997) has shown that the genetic distance between *Z. coppingeri* and *Z. vietnamensis* well exceeds what is usual in congeners, the broad concept of *Zoanthus* as including all soft bodied zoanthids with a double sphincter muscle seems untenable.

The method of budding from the column, giving rise to a clone of virtually separate polyps made miniature colonies only by the developing buds, provides such a strong contrast to the budding method and colony form of *Zoanthus sociatus* and *Z. coppingeri*, that it seems to justify acceptance of Saville-Kent's (1893) genus, albeit with changed diagnosis.

Cnidom characters have not been considered, though included in taxonomic papers in the past. Muirhead & Ryland (1985) found them unhelpful when revising the related genus *Isaurus* Gray, 1828, and their traditional method of use, as practised by the measurement of a small sample of capsules, appears of questionable value and a contributary factor in the past creation of several spurious species of both *Zoanthus* and *Palythoa* (Burnett et al., 1997). Nevertheless, differences in cnidom do occur between zoanthid taxa, and a comparative study based on the rigorous statistical analysis of large populations of nematocysts is in progress. The cnidom of *Acrozoanthus australiae* will be compared with those of *Z. coppingeri* and *Z. vietnamensis*.

Genetic evidence of the relationship of *Acrozoanthus australiae* with *Zoanthus coppingeri* and *Z. vietnamensis* will also be sought. The genus *Acrozoanthus* (Akros, Gr., high or tall) is meanwhile provisionally retained, contrary to the view of Haddon (1895) only recently repeated by Ryland & Muirhead (1993), none of these authors having had information on the method of budding.

Acknowledgements

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Reproductive strategies of South African corals

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Key words: Corals; reproduction; zooxanthellae; South Africa.

Abstract: South African coral reef communities are the southernmost representatives of this fauna on the African coast. An overview is provided of these limited but unusual reef systems which have a high bio-diversity. A number of facets are under study but coral reproduction has received particular attention to establish whether the corals are self-perpetuating or dependent on recruitment from reefs to the north. Seven corals have been studied, six of which were soft corals as these tend to be preponderant. Reproductive activity has been found in all seven species but detailed information is provided on only three which exemplify the reproductive strategies encountered. *Pocillopora verrucosa* (Ellis & Solander, 1786) is hermaphroditic and has a short breeding season in summer, releasing spawn during one or a few nights. *Sarcophyton glaucum* (Quoy & Gaimard, 1833) is also a broadcast spawner and female colonies invest 16 months in egg development; spawning again appears to be synchronised. Female colonies of *Anthelia glauca* Lamarck, 1816, brood their embryos and release planulae over an extended period. Infection of the developing embryos by zooxanthellae and the presence of a discrete brood chamber have been documented for the first time in this species. The results are discussed in the context of local conditions and reef dynamics, and compared with spawning events elsewhere.

Introduction

Coral-inhabited reefs are limited in distribution in South Africa, occurring mainly in conservation areas in northern KwaZulu-Natal (fig. 1). Schleyer (1995: 131-140) provides a review of these coral reef communities which are becoming increasingly important as venues for eco-tourism. They are the southernmost representatives of this fauna on the African coast and their occurrence at these high latitudes is facilitated by the warm Agulhas Current. They are not true coral reefs but consist of a veneer of coral growth on submerged beach rock formed from fossilized sand dunes. Whereas hermatypic scleractinian corals are a significant component of the systems, soft corals are remarkably abundant. More scleractinian genera are found on the reefs than anticipated by reviewers of coral zoogeography (Achituv & Dubinsky, 1990: 1-9; Veron, 1993: 629-633) and together the hard and soft corals number more than 55 genera and 130 species. Other phyla such as sponges, tunicates and echinoderms are also well-represented.

Considerable research has been undertaken on these reefs over the past four years (Schleyer 1995: 131-140), both in view of their limited distribution and their value for tourism. Recreational diving has increased exponentially in recent years (Schleyer, unpubl. data) and is often in conflict with angling on the reefs. Management of the reefs has thus become necessary to alleviate user-conflict and to ensure

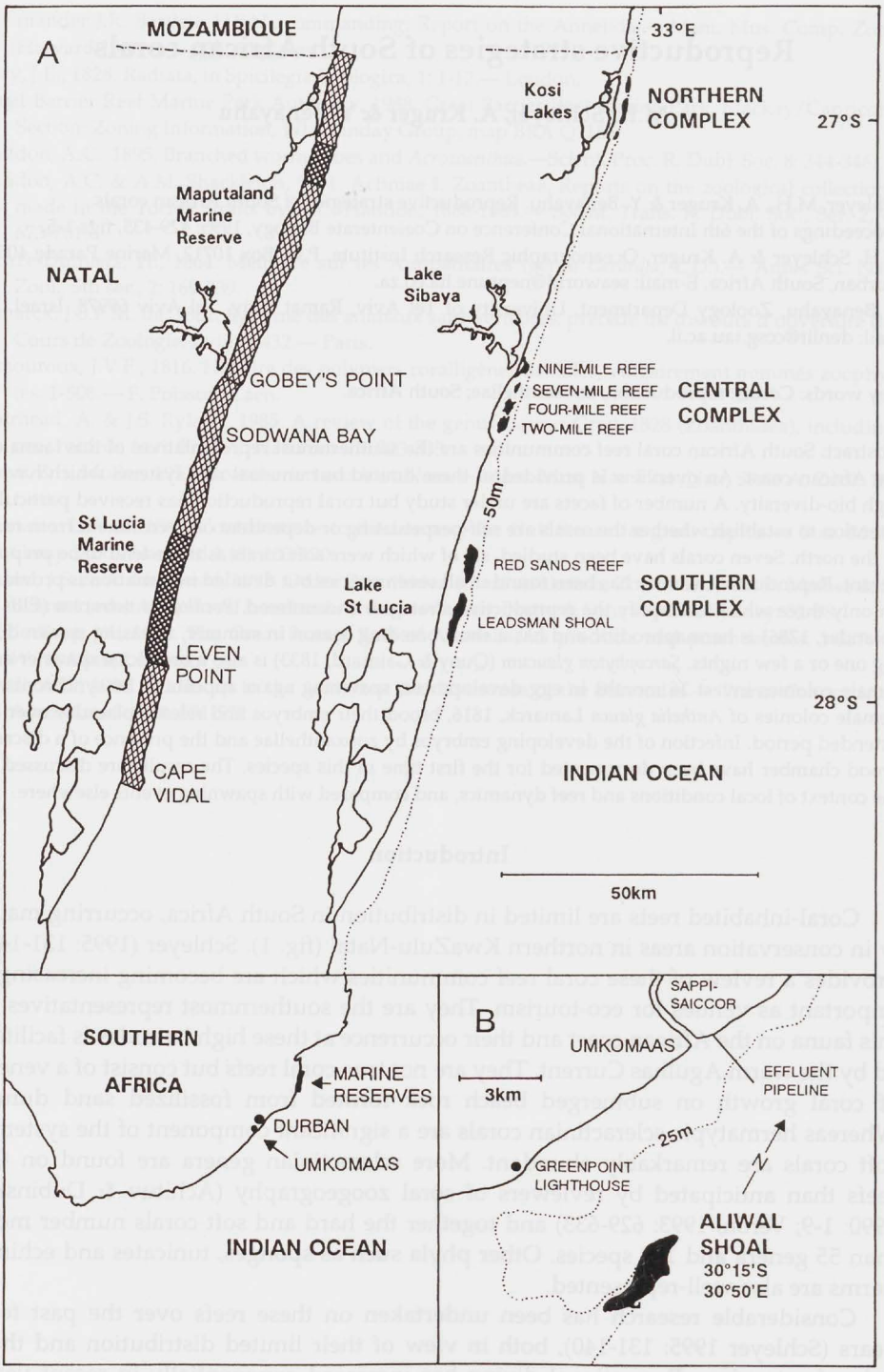

Fig. 1. Location of the major coral-inhabited reefs in KwaZulu-Natal : A) The majority fall within reserve (light shading) or sanctuary areas (dark shading); B) Aliwal Shoal. (From Schleyer, 1995).

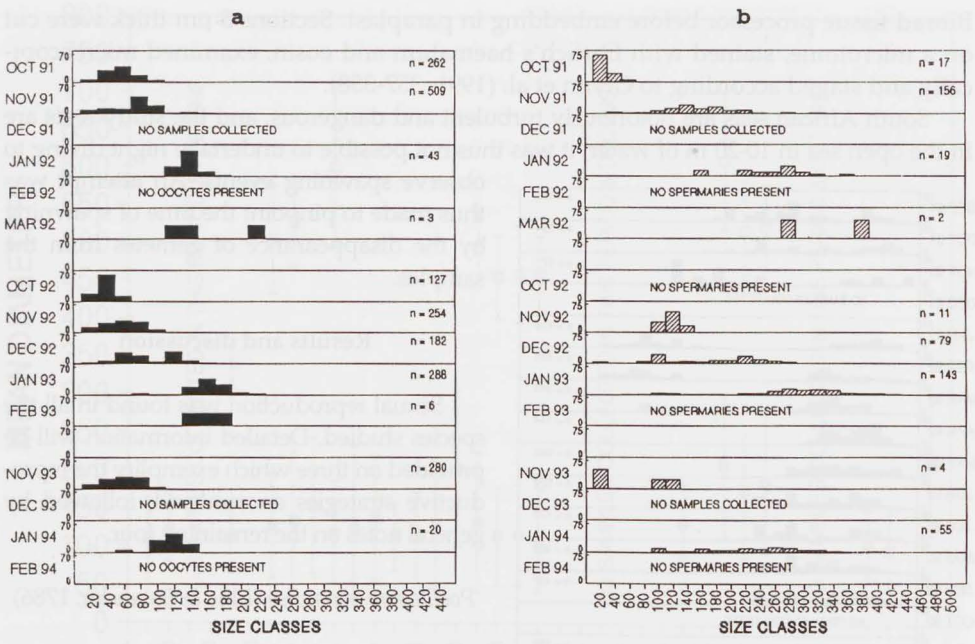

Fig. 2. Monthly percentage size frequencies of *Pocillopora verrucosa* ova (a) and spermaries (b) during the reproductive season. Size classes are in µm.

that they are protected from unsustainable use. As the coral communities are at the limits of their distribution, research on their reproduction was considered essential to establish whether they are self-propagating or dependant on recruitment from reefs to the north.

Seven common species were selected for the study consisting of six soft corals and one hard coral. The subjects of the study were: *Anthelia glauca* Lamarck, 1816; *Lobophytum crassum* von Marenzeller, 1886; *L. depressum* Tixier-Durivault, 1966; *Pocillopora verrucosa* (Ellis & Solander, 1786); *Sarcophyton glaucum* (Quoy & Gaimard, 1833); *Sinularia dura* (Pratt, 1903) and *S. gyrosa* (Klunzinger, 1877).

Materials and methods

Coral samples were collected for the study by SCUBA diving on Nine-mile Reef in the central reef complex of KwaZulu-Natal (fig. 1). At least ten samples of each species were collected during field trips conducted between September 1991 and September 1994, with their greatest frequency in what proved to be the reproductive season. The material was fixed in 4% formalin in seawater, rinsed in fresh water after 24 hours, and then transferred to 70% ethyl alcohol. Subsamples of soft coral tissue were dissected for microscopic measurement of the reproductive products and material, including that of the hard coral *Pocillopora verrucosa*, was decalcified in a formal-nitric acid solution (Mahoney, 1966: 195) for the preparation of histological sections. The decalcified tissues were passed through methanol, ethanol and isopropanol in a

Biorad tissue processor before embedding in paraplast. Sections 5 µm thick were cut on a microtome, stained with Ehrlich's haemalum and eosin, examined microscopically and staged according to Glynn et al. (1991: 357-358).

South African seas are notoriously turbulent and dangerous, and the study reefs are in the open sea in 10-20 m of water; it was thus not possible to undertake night diving to observe spawning events. An attempt was thus made to pinpoint the time of spawning by the disappearance of gametes from the samples.

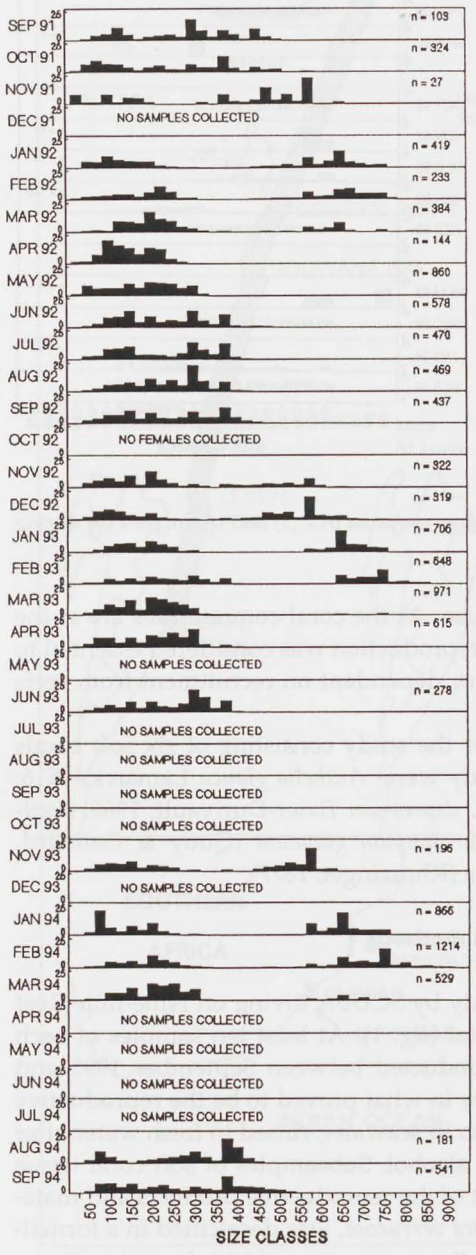

Fig. 3. Monthly percentage size frequencies of *Sarcophyton glaucum* ova. Size classes are in µm.

Results and discussion

Sexual reproduction was found in all the species studied. Detailed information will be provided on three which exemplify the reproductive strategies encountered, followed by general notes on the remaining four.

Pocillopora verrucosa (Ellis & Solander, 1786)

Pocillopora verrucosa proved, typically, to be a simultaneous hermaphrodite (Sier & Olive, 1994: 713-722) with male and female gonads on alternating mesenteries. The species is reproductively active between October and January (fig. 2) and evidence was found of broadcast spawning at or immediately after the new moon in late January, 1993. Whereas Stimson (1978: 173-184) reported that *P. verrucosa* brooded its spawn in the Enewetak Atoll, ours broadcast their spawn, as in the Red Sea (Shlesinger & Loya, 1985: 1333-1335) and Maldive Islands (Sier & Olive, 1994: 713-722).

Sarcophyton glaucum (Quoy & Gaimard, 1833)

Sarcophyton glaucum is a gonochoric broadcast spawner in which the ova are matured over an extended period (Benayahu & Loya, 1986: 32-42). Gametogenesis in South African male and female colonies of *S. glaucum* takes approximately 10 and 18 months respectively, so there are two size classes of ova in female polyps for part of the year (fig. 3). This period of egg

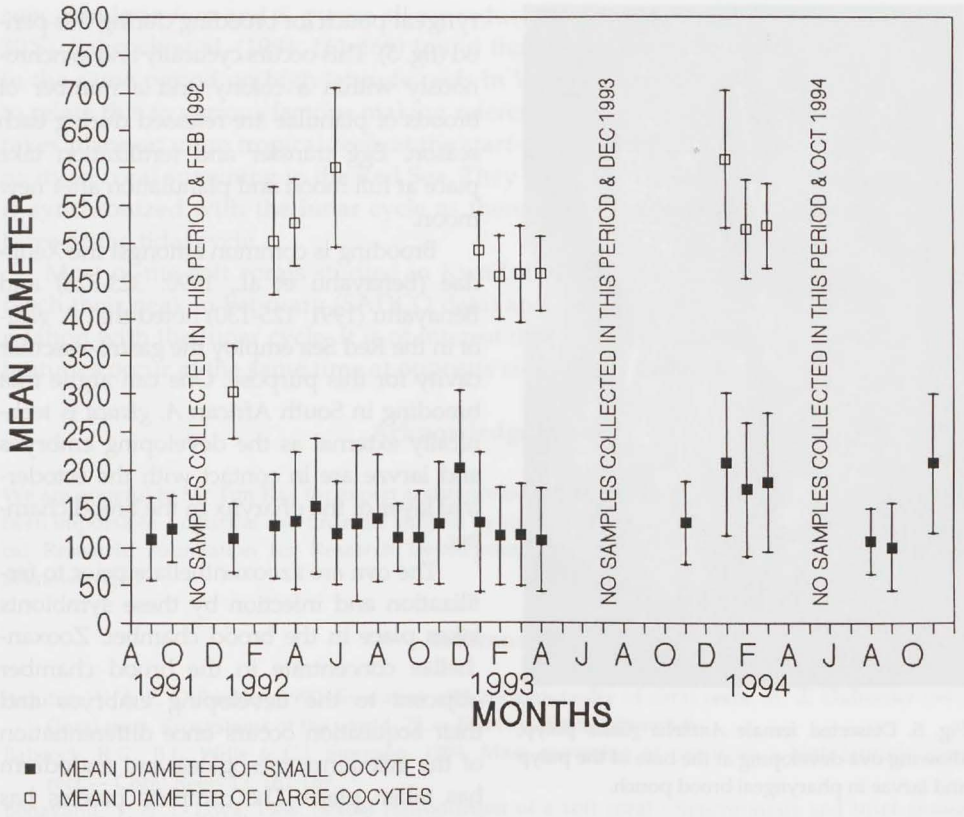

Fig. 4. Monthly mean diameter (μm) and standard deviation of *Anthelia glauca* ova.

maturation is shorter than the 22-23 months it takes in the Red Sea (Benayahu & Loya, 1986: 32-42). Spawning appeared to be synchronized and took place just after the full moon in March during the study. The newer generation of ova are retained for further development in the female polyps.

A feature of the South African population of *S. glaucum* is a 9% incidence of hermaphroditism. In hermaphroditic colonies there are large gametes which are invariably ova of the larger size class and smaller reproductive products which are usually spermaries. This begs the question as to whether a sex change is involved as, after the eggs are shed in the spawning period, the follow-through size class of ova would be absent. It was not possible to tag and sample hermaphroditic colonies from one year to the next to establish whether only spermaries were produced thereafter.

Anthelia glauca Lamarck, 1816

The sexes are separate in *Anthelia glauca* and gametes are found at the base of the polyps throughout the year. Active reproduction takes place between January and June when gametes at different stages of development are found; this is particularly noticeable in female colonies (fig. 4). Mature, fertilized ova are transferred to a pha-

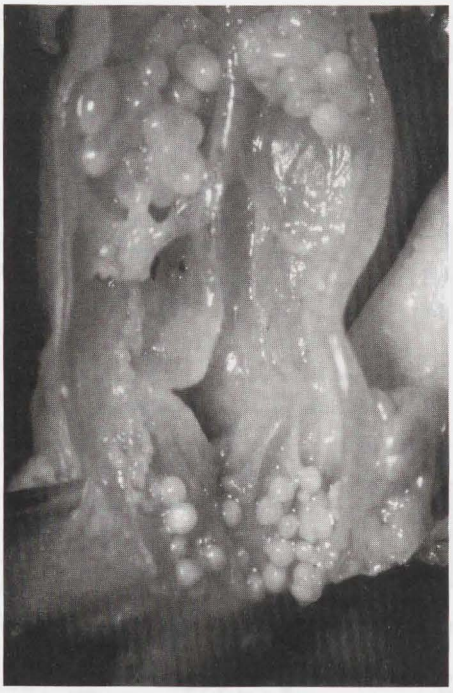

Fig. 5. Dissected female *Anthelia glauca* polyp, showing ova developing at the base of the polyp and larvae in pharyngeal brood pouch.

ryngeal pouch for brooding during this period (fig. 5). This occurs cyclically and synchronously within a colony and a number of broods of planulae are released during each season. Egg transfer and fertilization take place at full moon and planulation after new moon.

Brooding is common amongst the Xeniidae (Benayahu et al., 1990: 323-328) and Benayahu (1991: 125-130) noted that *A. glauca* in the Red Sea employ the gastro-vascular cavity for this purpose. One can argue that brooding in South African *A. glauca* is technically external as the developing embryos and larvae are in contact with the ectodermal layer of the pharynx in the brood chamber.

The ova are azooxanthellate prior to fertilization and infection by these symbionts takes place in the brood chamber. Zooxanthellae concentrate in the brood chamber adjacent to the developing embryos and their acquisition occurs once differentiation of the ectoderm, mesogloea and endoderm has taken place. The complete process has been recorded (Benayahu & Schleyer, in prep.).

The other species

The other species included in our study proved to be gonochoric broadcast spawners. The disappearance of mature gametes from the material suggested that their spawning was synchronized with that of *Sarcophyton glaucum*.

Significance of the results

The primary significance of these findings is that KwaZulu-Natal coral communities at the limits of their distribution in the south-west Indian Ocean are self-propagating. The fact that they are not dependent on recruitment from reefs further north will be of relevance in their management. Larval brooding as well as gonochoric and hermaphroditic spawning were encountered in the study, representing a wide range of reproductive strategies. The southward movement of the Agulhas Current must transport planulae from these reefs beyond the realms of suitability for their settlement. It remains to be established whether the southern coral communities on Aliwal Shoal (fig. 1) are sexually active or are derived from those further north.

Another point of significance is the time at which (synchronized?) spawning of most of the species takes place; *Lobophytum crassum, L. depressum, Sarcophyton glau-*

cum, *Sinularia dura* and *S. gyrosa* all reproduced in late March in our study area (27° 30'S). Babcock et al. (1994: 161-169) found that mass spawning of hard corals occurs in the same period on high latitude reefs in Western Australia (28°-29°S). They tried to relate this to various factors, making reference to the earlier mass spawning which takes place on more tropical reefs at the start of summer and the absence of synchronicity in coral spawning in the Red Sea. They concluded, in their case, that spawning is synchronized with the lunar cycle as there was no correlation with sea temperatures or the tidal cycle.

Most of the soft corals studied in KwaZulu-Natal spawn after sea temperatures reach their peak in February (SADCO data) and there appeared like-wise to be a correlation with the lunar cycle. It is of interest that these coral spawning events at high latitudes occur at the same time at opposite sides of the Indian Ocean.

Acknowledgements

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Medusozoa (Cnidaria: Anthozoa exepted) from the Commander Islands, faunistic composition and biogeography

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Sheiko, O. & S. Stepanjants. Medusozoa (Cnidaria: Anthozoa exepted) from the Commander Islands, faunistic composition and biogeography.
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 437-445, figs 1-6, tabs 1-2.
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Key words: Commander Islands; Medusozoa; hydroids; fauna, biogeography.

Abstract: The medusozoan fauna of the Commander Islands (Komandorsky Ostrova, ca 55°N 167°E) is analysed. Seventy-five species have now been recorded; the geographical distribution of these species indicates that the medusozoan fauna is mainly a cold water fauna. The total number of species so far observed and a comparison with the number observed in adjacent regions justifies the conclusion that the Commander Island medusozoan fauna is still insufficiently known, but that it is closest to that of Alaska and the Aleutian Islands.

Introduction

Literature data on the medusozoan fauna of the Commander Islands (Komandorskye Ostrova, ca 55°N 167°E) are scarce. American publications of the end of the nineteenth and the beginning of the twentieth century give some information on species from this area (Clark, 1877; Nutting, 1899, 1901, etc.). Some species are also mentioned by Jäderholm (1907, four species off Bering Island), Linko (1911, 1912), Kudelin (1914) and later on by Yamada (1959). Naumov (1960) provided evidence for the occurrence of 14 species of hydroids. Scanty information on hydromedusae and scyphomedusae of the Commander Islands is found in papers by Naumov (1960, 1961), Kramp (1961, 1968), and Arai & Brinckmann Voss (1980). Some species of Siphonophora are listed by Stepanjants (1967) and Alvariño (1971).

We examined 198 samples of hydrozoan material from depths between 0 and 500 m (figs 1, 2), collected by scientists from the Laboratory for Benthic Communities of the Institute of Ecology and Nature Treatment of Petropavlovsk-Kamchatskiy (Kamchatka) and the Zoological Institute, Academy of Sciences, St. Petersburg, obtained by SCUBA diving and by research vessels using the dredge "Ocean" and trawls. The material was collected around four islands of the Commander Archipelago: Bering, Medniy, Ariy Kamen and Toporkov (1975, 1985, 1989-1992, 1994).

Results

Both analysis of literature data and study of the available Hydrozoa collection, comprising 41 species, permitted us to compile a list of 75 species of Medusozoa for the Commander Islands, including 61 species of Hydrozoa (33 genera and 14 families), five of Siphonophora (five genera and three families) and nine species of Scyphozoa (seven

genera and six families) (table 1). Amongst these there are no geographically unexpected species.

Thirty-eight percent of the species are Pacific (55% of which high-boreal), 28% are boreal-arctic and amphiboreal (28.5% of which high-boreal), 13% are bipolar (fig. 3). Of the 16% that can be characterized as widely distributed the majority are cold water species, with the exception of *Plumularia setacea* (Linnaeus, 1758), which is a warm water species. All these figures indicate that the Commander Islands medusozoan fauna is mainly a cold water fauna (29.5% high-boreal and 65% boreal, bipolar and widely distributed). Four species are considered east-boreal-arctic: *Sertularia similis* Clark, 1877; *S. cupressoides* Clark, 1877; *Abietinaria turgida* (Clark, 1877), and *Halecium scutum* Clark, 1877. This means that the eastern border zone belongs to the Canadian Arctic Archipelago biogeographically (Calder, 1970) and the western border zone to the East Eurasian seas (Stepanjants, 1989). Other species with this type of distribution can be expected to occur here.

It is interesting to compare the species list of Commander Island Medusozoa with those of nine adjacent regions of the North Pacific: Alaska (120 species), Aleutian Islands (84), East Kamchatka coast (136), Bering Sea (171), Sea of Okhotsk (213), northern part of the Kuril Islands (179), southern part of the Kuril Islands (160), and the northern part of the Sea of Japan (180).

This comparison strongly supports the conclusion that the medusozoan fauna of the Commander Islands is poorer than that of the other regions, indicating primarily that it is still incompletely studied. However, faunistic similarity diagrams (on the level of families and species) indicate that the area investigated is closest to the Aleutian Islands and Alaska (figs 4, 5).

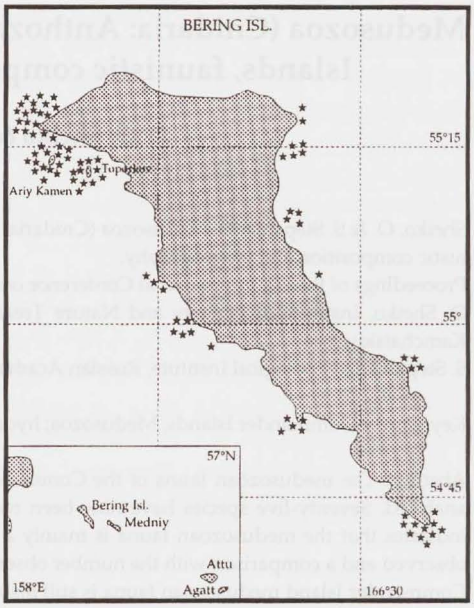


Fig. 1. Map of Bering Island, the largest island of the Commander Archipelago, also showing the islets of Toporkov and Ariy Kamen, situated to the north-west. In the left lower corner the position of the Commander Archipelago is shown between the east coast of Kamchatka and Attu and Agatt, the westernmost of the Aleutian Islands. This and next map are borrowed from "The Commander Islands in 1923" by the famous Russian geographer and Far East investigator V.K. Arsenjev. Stars indicate localities where hydroids were collected.

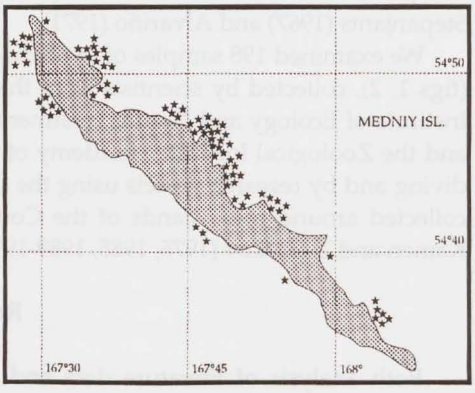


Fig. 2. Map of Medniy Island. Stars indicate localities where hydroids were collected.

Table 1. Medusozoa from the Commander Islands. Index of biogeography: Br = boreal; Ar = arctic; As = Asiatic; BrAr = boreal-arctic; P-Br = Pacific boreal; HBr = High-boreal; AnBr = amphiboreal; B = bipolar; HBrAr = high boreal-arctic; HBrAs = high boreal asiatic; e-HBrAr = east boreal-arctic; W-D = widely distributed; W-D d = widely distributed, deep water; W-D w = widely distributed, warm water; E = endemic; C = cosmopolitan; o = present in our collection.

		Source of information	Biogeographical status
Subphylum Medusozoa			
Class Hydrozoa			
Order Athecata			
Suborder Filifera			
Family Calycopsidae			
1. *Bythotiara depressa* Naumov, 1960		Arai & Brinckmann-Voss, 1980	P-HBr
2. *Heterotiara anonyma* Maas, 1905		id.	W-D
3. *Calycopsis nematophora* Bigelow, 1903		id.	P-HBr
Family Bougainvilliidae			
4. *Rhizorhagium roseum* M. Sars, 1877	o	(present study)	HBrAr
Family Eudendriidae			
5. *Eudendrium vaginatum* Allman, 1863	o	(present study)	HBrAr
6. *Eudendrium* spec.	o	id.	PHBrAs
Suborder Capitata			
Family Tubulariidae			
7. *Tubularia indivisa* Linnaeus, 1758	o	id.	B
Order Thecaphora			
Family Campanulariidae			
8. *Campanularia volubilis* (Linnaeus, 1758)	o	id.	W-D
9. *Orthopyxis compressa* (Clark, 1877)	o	id.	P-Br
10. *Orthopyxis integra* (McGillivray. 1842)	o	id.	B
11. *Tulpa crenata* (Allman, 1876)	o	id.	BrAr
12. *Obelia longissima* (Pallas, 1766)	o	id.	B
13. *Rhizocaulus verticillatus* (Linnaeus, 1758)	o	id.	BrAr
Family Lafoeidae			
14. *Lafoea grandis* Hincks, 1874	o	id.	W-D
15. *Lafoea dumosa* (Fleming, 1820)	o	id.	C
16. *Grammaria abietina* (M. Sars, 1851)	o	id.	B
17. *Grammaria* spec.	o	id.	E As
18. *Filellum serpens* (Hassall, 1848)	o	id.	B
19. *Cryptolarella* cf. *flabellum* (Allman, 1888)	o	id.	?
Family Campanulinidae			
20. *Modeeria plicatile* (M. Sars, 1863)	o	Linko, 1912	BrAr
21. *Calycella syringa* (Linnaeus, 1767)	o	(present study)	B
Family Laodiceidae			
22. *Ptychogena lactea* A. Agassiz, 1865	o	Arai & Brinckmann-Voss, 1980	BrAr
Family Sertulariidae			
23. *Sertularella gigantea* Mereschkowsky, 1878	o	(present study)	BrAr
24. *Sertularella albida* Kirchenpauer, 1884	o	id.	P-HBr
25. *Sertularella rugosa* (Linnaeus, 1758)	o	Kussakin, 1978	BrAr
26. *Sertularella complexa* Nutting, 1904	o	(present study)	P-HBrAs
27. *Sertularella flabella* (Nutting, 1904)	o	Naumov, 1960	P-HBr
28. *Sertularella reticulata* Kirchenpauer, 1884	o	Naumov, 1960	?
29. *Sertularella tenella* (Alder, 1857)	o	(present study)	BrAr
30. *Symplectoscyphus tricuspidatus* (Alder, 1856)	o	id.	B

31. *Symplectoscyphus pinnatus* Clark, 1877	o	id.	AmBr
32. *Sertularia similis* Clark, 1877	o	id.	e-HBrAr
33. *Sertularia cupressoides* Clark, 1877	o	id.	e-HBrAr
34. *Abietinaria abietina* (Linnaeus, 1758)	o	id.	B
35. *Abietinaria variabilis* (Clark, 1877)	o	id.	P-HBr
36. *Abietinaria filicula* (Ellis & Solander, 1786)	o	id.	AmBr
37. *Abietinaria costata* (Nutting, 1901)	o	id.	P-Br
38. *Abietinaria labrata* (Murray, 1860)	o	id.	P-Br
39. *Abietinaria gigantea* (Clark, 1877)	o	Naumov, 1960	P-HBr
40. *Abietinaria gracilis* Nutting, 1904	o	id.	P-Br
41. *Abietinaria turgida* (Clark, 1877)	o	(present study)	e-HBrAr
42. *Abietinaria derbeki* Kudelin, 1914	o	id.	P-HBr
43. *Thuiaria? carica* Levinsen, 1913	o	id.	BrAr
44. *Thuiaria thuja* (Linnaeus, 1758)		id.	BrAr
45. *Thuiaria obsoleta* (Nutting, 1901) (= *T. hartlaubi*)		Naumov, 1960	BrAr
46. *Thuiaria cylindrica* Clark, 1877		id.	HBrAr
Family Haleciidae			
47. *Halecium beringi* Naumov, 1960		(present study)	P-HBr
48. *Halecium scutum* Clark, 1877	o	id.	e-HBrAr
49. *Halecium corrugatum* Nutting, 1899	o	id.	BrAr
50. *Halecium curvicaule* Lorenz, 1886	o	id.	HBrAr
51. *Halecium densum* Calkins, 1899	o	id.	P-HBr
52. *Halecium washingtoni* Nutting, 1901	o	id.	P-W-D
Family Plumulariidae			
53. *Plumularia setacea* (Linnaeus, 1758)	o	id.	W-D w
54. *Nuditheca tetrandra* Naumov, 1960	o	Naumov, 1960	P-BrAs
55. *Schizotricha? cf. divergens?* Naumov, 1960	o	id.	P-BrAs
56. *Plumularia? microtheca* Naumov, 1960	o	id.	P-HBrAs
Order Trachylina			
Family Rhopalonematidae			
57. *Crossota rufobrunnea* Kramp, 1913	o	Arai & Binckmann-Voss, 1980	AmBr
58. *Aglantha digitale* (O.F. Müller, 1766)	o	id.	BrAr
Family Aeginidae			
59. *Aegina rosea* Eschscholtz, 1829	o	id.	W-D
60. *Aeginopsis laurentii* Brandt, 1835	o	Kramp, 1968	HBrAr
Family Cuninidae			
61. *Solmissus incisa* (Fewles, 1886)	o	id.	W-D
Class Siphonophora			
Order Siphonanthae			
Suborder Calycophorae			
Family Hippopodiidae			
62. *Vogtia serrata* (Moser, 1925)		Alvariño, 1971	W-D
Family Prayidae			
63. *Rosacea plicata* Quoy & Gaimard, 1827		id.	W-D
Family Diphyidae			
64. *Lensia conoidea* Keferstein & Ehlers, 1861 (= ?*L. c. pacifica* Stepanjants, 1967)		id.	P-Br
65. *Muggiaea bargmannae* Totton, 1954		Stepanjants, 1967	B
66. *Dimophyes arctica* (Chun, 1897)		id.	B
Class Scyphozoa			
Order Coronatae			
Family Collaspidae			
67. *Atolla wyvillei* Haeckel, 1872		Naumov, 1961	W-D d

Family Periphyllidae		
68. *Periphylla periphylla* (Péron & Lesueur, 1809)	id.	W-D d
Order Semaeostomeae		
Family Pelagiidae		
69. *Chrysaora helvola* Brandt, 1838	id.	P-Br
70. *Chrysaora melanaster* Brandt, 1838	id.	P-Br
Family Cyaneidae		
71. *Cyanea capillata* (Linnaeus, 1758)	id.	W-D
Family Ulmaridae		
72. *Phacellophora camtschatica* Brandt, 1838	id.	P-Br
73. *Aurelia aurita* Linnaeus, 1758	id.	W-D
74. *Aurelia limbata* Brandt, 1838	id.	P-Br
Order Stauromedusae		
Family Eleutherocarpidae		
75. *Haliclystus stejnegeri* Kishinouye, 1899	Kramp, 1961	P-Br

The analysis of hydroid taxocenoses of the tidal and the high-subtidal zones, represented in our collections by 29 species, shows dominance of Sertulariidae (45%), Campanulariidae (20%), Haleciidae (10%) and Lafoeidae (10%).

There are nine species that can be considered to have a bipolar type of distribution (species with antitropical distribution). Representatives of the bipolar family Candelabridae (= Myriothelidae) may be expected to occur here too. The similarity in hydroid composition of the taxocenoses of Commander Islands and the subantarctic islands supports the view of bionomic bipolarity, i.e. the similarity of unrelated species, taxocenoses and biocenoses occurring under similar ecological conditions (Ushakov, 1958; Andriashev, 1987). In the Kerguelen Island region, for instance, the dominance of Sertulariidae is 26%, of Campanulariidae 12% and of Haleciidae 10%.

Comments on some species

Eudendrium annulatum Norman, 1864, is here regarded as a junior synonym of *Eudendrium vaginatum* Allman, 1863. The pseudohydrothecae that are said to be absent in *E. annulatum* but characteristic for *E. vaginatum*, are present in about 20% of the colonies from this area.

A single colony of *Cryptolarella flabellum* (Allman, 1888) represents the third record for the North Pacific. The type locality of this species is in the Caribbean region, which makes the identification of the present specimen slightly doubtful (see for this question Vervoort, 1972: 47-49, fig. 13a, b, where the species and its gonothecae are redescribed).

Grammaria spec. is of interest because it may represent a new species.

Halecium beringi Naumov, 1960, is here considered a valid species, specifically different from *Halecium scutum* Clark, 1877, with which it was synonymized by Antsulevich (1987), a conclusion we reject. In *H. beringi* colony height is less than the maximal colony width and colonies are branched in various planes, whereas in *H. scutum* colonies are longer and branched mainly in one plane. Moreover, the distance from diaphragm to hydrothecal rim is longer in *H. beringi* than it is in *H. scutum* (fig. 6). Some measurements of both species are given in table 2.

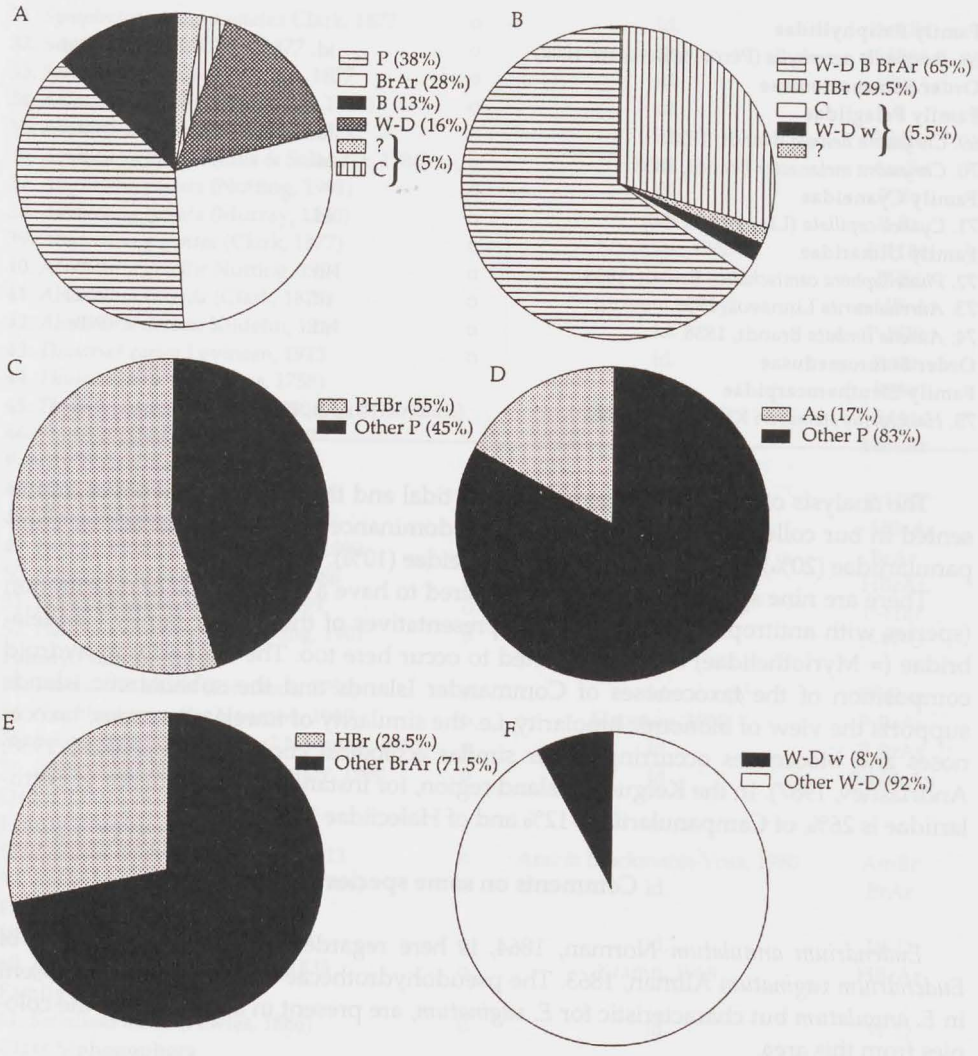
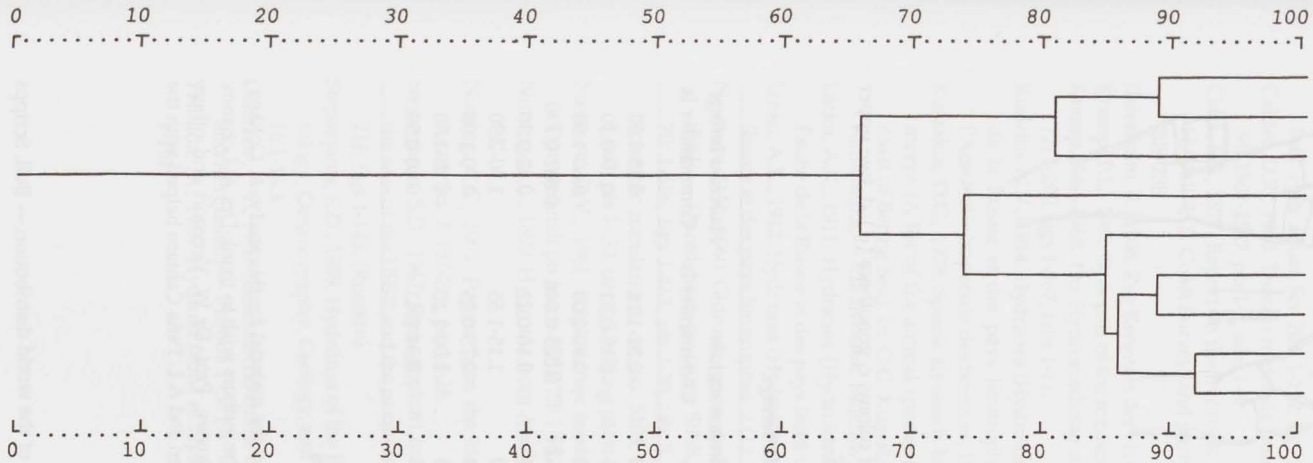


Fig. 3. The faunistic composition of Medusozoa of the Commander Archipelago. The biogeographic abbreviations are the same as in table 1.

Diagrams A and B demonstrate the total species (75) composition: A. Pacific, boreal-arctic, bipolar, widely distributed, and cosmopolitian species; B. High boreal coldwater, boreal-arctic, widely distributed, bipolar cold water, and relatively warm water species.

Diagrams C and D demonstrate the species composition of the Pacific species: C. High boreal and other Pacific species; D. Asiatic and other Pacific species.

Diagrams E and F demonstrate the species composition of the boreal-arctic species (E) and widely distributed species (F).



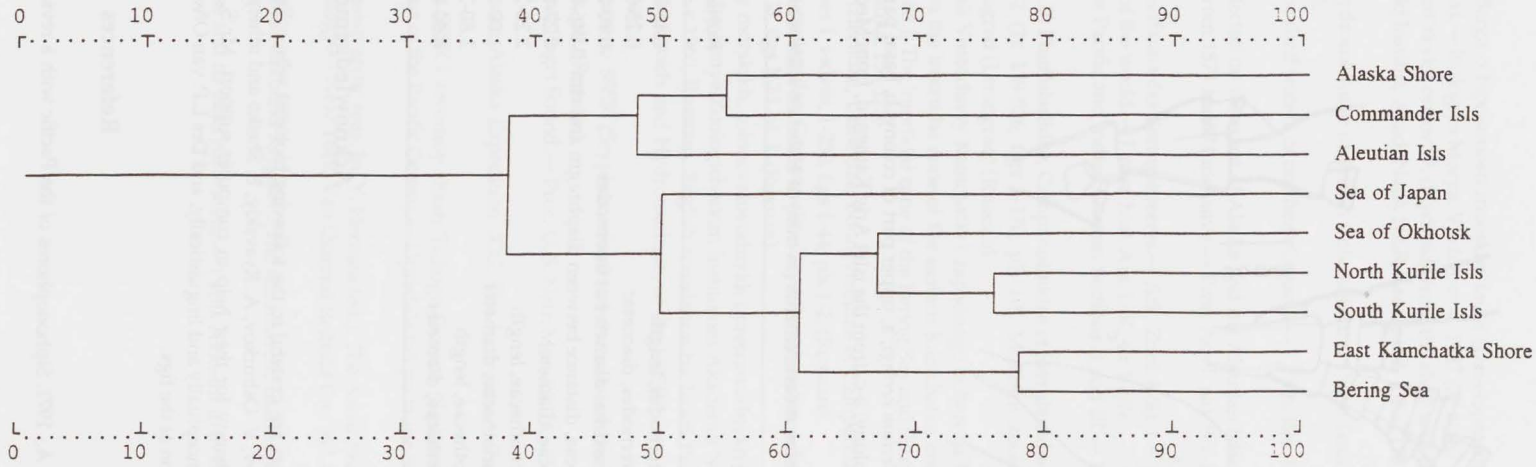


Fig. 4. Similarity of the medusozoan fauna of nine North Pacific areas (index of similarity by Chekanovsky-Soerensen), family distribution.
Fig. 5. The same for the species distribution.

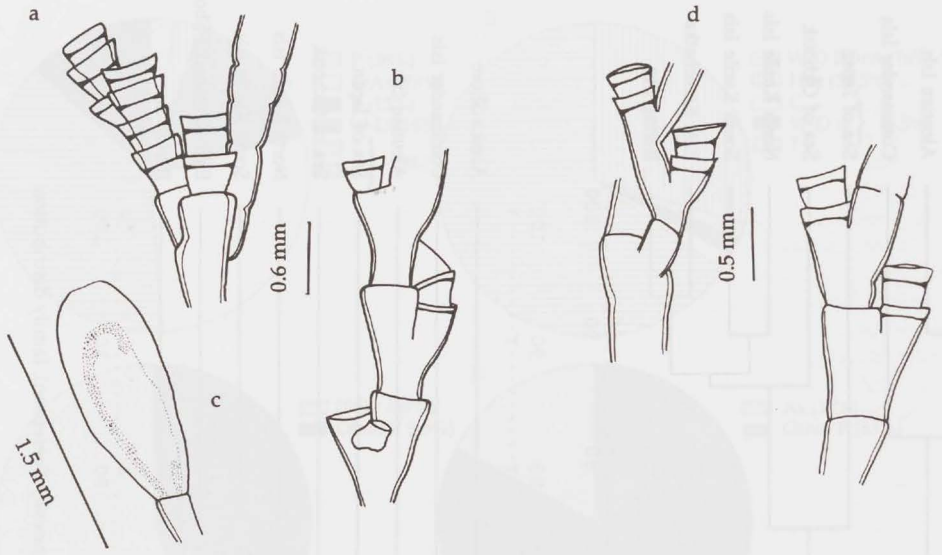


Fig. 6. *Halecium beringi*; a, upper part of colony; b, basal part of colony; c, gonotheca (♂); d, two upper parts of colony; a-c, from the islet Ariy Kamen; d, from Medniy.

Table 2. Some measurements (in mm) of *Halecium scutum* and *H. beringi*.

Parameter	*Halecium scutum*		*Halecium beringi*
	Kurile Is	Commander Is	Commander Is
Branch internodes, length	0.46-1.80	0.31-1.14	0.25-1.00
Branch internodes, diameter	0.25-0.45	0.15-0.31	0.19-0.30
Height : maximal diameter of internodes	0.90-2.50	0.90-2.00	0.60-1.60
Hydrothecae, distance between diaphragm and rim	0.046-0.062	0.025-0.046	0.062-0.130
Hydrothecae, diameter	0.27-0.34	0.16-0.22	0.22-0.30
Female gonothecae, length	2.20-2.60	1.15-1.55	1.30-2.50
Female gonothecae, diameter	1.00-1.20	0.75-0.85	0.70-1.00
Male gonothecae, length	1.80-2.25	1.00-1.10	1.25-1.50
Male gonothecae, diameter	0.50-0.65	0.34-0.43	0.40-0.50

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Effects of weightlessness on budding and ephyra development in *Aurelia aurita* (Linnaeus, 1758) (Scyphozoa: Semaeostomeae)

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Key words: *Aurelia aurita;* jellyfish; weightlessness; microgravity; metamorphosis; budding; statoliths; jellyfish thyroxine; pulsing; swimming.

Abstract: Polyps and ephyrae of *Aurelia aurita* (Linnaeus, 1758) were flown recently on NASA shuttle mission IML-2 (International Microgravity Laboratory -2) three years after polyps and ephyrae were flown on the SLS-1 (Spacelab Life Sciences -1) mission for 9 days at 28°C. The IML-2 experiment was flown for 14 days at 22°C after the polyps were induced to strobilate with iodine (Spangenberg, 1967) in artificial sea water (ASW) 24 h pre-flight. Two groups were centrifuged at 1-g in space for most of the flight duration. Ephyrae from the IML-2 experiment were subjected to the *Aurelia* Metamorphosis Test System (AMTS) post-flight as the ephyrae from the SLS-1 mission had been. The number of ephyrae and their structures (arms, rhopalia, statoliths) were counted as were the number of their pulses per minute. Swimming ability was also noted. Polyps gave rise to ephyrae in space which were able to pulse and swim as they did previously during the SLS-1 mission. More ephyrae with abnormal arm numbers were found in the micro-g (microgravity) group than in controls. In addition, fewer ephyrae that developed in micro-g swam post-flight than 1 g controls. Mean statolith numbers per rhopalia of ephyrae from polyps induced to strobilate 24 h pre-flight were not significantly different from controls in both the IML-2 and SLS-1 experiments. Statoliths were found, however, in higher numbers in space-maintained (IML-2) and space-developed (SLS-1) ephryae in low sulfate ASW than in controls. The group of 6 polyps with immature buds flown during the IML-2 mission in ASW produced more buds with more tentacles than their ground controls.

Introduction

The advent of the "Space Age" provided the opportunity for living organisms to leave the 1g (gravity) Earth environment and to enter an environment with as little as 10^{-5} g. While many organisms, especially humans, have been in this weightless environment for varying periods of time, very little research has been done on the effects of weightlessness on developing organisms in micro-g.

In 1991, the first coelenterate organisms were sent into space by Spangenberg et al. (1994a, 1994b). Polyps and ephryae of the jellyfish *Aurelia aurita* (Linnaeus, 1758)

were launched for a nine day exposure to weightlessness during the SLS-1 mission. One purpose of the experiment was to determine whether polyps could metamorphose (through strobilation) into ephyrae and, if so, whether they would be capable of pulsing and swimming in space. Ephyrae which had developed on Earth and those that developed in space were subjected to the *Aurelia* Metamorphosis Test System (AMTS) (Spangenberg, 1984) post-flight as were the jellyfish from the IML-2 experiment.

Results from the SLS-1 experiment revealed that ephyrae can develop and subsequently pulse and swim in space (Spangenberg et al., 1994a). Ephyrae which developed in space were morphologically very similar to those that developed on Earth. There were no significant differences between the mean numbers of arms between the two groups. The mean number of statoliths formed per rhopalium in the Earth and space-formed ephyrae was not statistically different; however, those ephyrae induced to form in space (L(Launch) + 8 h) made more (statistically significant) statoliths per rhopalium (Spangenberg et al., 1994b). Pulsing abnormalities were found in more space-developed ephyrae post-flight than in controls.

In this paper, the development of ephyrae flown on the IML-2 mission will be compared with that of ground-based controls and with results from the SLS-1 mission. In addition, new data concerning statoliths in ephyrae which developed in low sulfate ASW during the SLS-1 mission will be presented.

Methods

Polyps and ephyrae of the Norfolk VA strain of *Aurelia aurita* were used for the IML-2 experiment. The organisms were maintained in ASW in the laboratory at 19°C and fed newly hatched *Artemia*, according to the methods of Spangenberg (1964). Polyps chosen for this experiment were expected to yield two ephyrae each, based on their size. These polyps were derived through asexual cloning of a polyp that had previously given rise to ephyrae with eight arms. When induced to strobilate, polyps from these cultures continued to give rise to a high number of ephyrae with eight arms over a period of two years. Sub-clones from these animals were developed for superior pulsing and swimming ability. Twenty-four hours pre-flight, four groups of six polyps/group were rinsed 10 times and placed in 4 ml of 10^{-5} M iodine in ASW. The organisms and solutions were transferred into cuvettes (designed and produced by Dr W. Briegleb, DARA, FRG) which allowed videotaping of the organisms while in the NIZEMI (a centrifuging video-microscope). The cuvettes with the jellyfish were maintained at 22°C pre-flight and in-flight in type 1 containers in the Biorack. Two groups of jellyfish were maintained at 1 g in-flight on the Biorack centrifuge and two groups were maintained at micro-g. None of the organisms was fed during the mission. Four ground-based controls were kept at 22°C in the cuvettes in type 1 containers. In addition, five groups of six polyps were placed in 4 ml in auto-analyzer tubes and one was added to each Type I container with two cuvettes. Four of these groups of polyps were placed in 10^{-5} M iodine in ASW and one group was placed in ASW. Polyps in the ASW had a tiny bud anlage on their bodies which was at the same stage of development in each animal. Ground-based controls were prepared simultaneously.

One group of twelve ephyrae was prepared L-29 h in cuvettes at 22°C in four ml

of low-sulfate (0.7 mM) ASW with no air bubbles. These flight ephyrae had developed pre-flight in low-sulfate medium. Ephyrae from this group and their ground controls were examined microscopically pre-flight and no statoliths were found. Post-flight, their statolith numbers were counted. During the SLS-1 experiment, two groups of ephyrae (and ground controls) which developed from polyps strobilating in micro-g in low-sulfate (0.7 mM) ASW with 10^{-5} M iodine, were examined post-flight and their statoliths were counted. These groups of 25 polyps in 40 ml solution were in tissue culture flasks with no air bubbles. They were maintained at 28°C during the 9 day flight and were retrieved within 3 h post-flight.

Ephyrae which developed in space during the IML-2 mission and one group of ephyrae in low sulfate ASW sent from Earth into space (and their Earth controls) were examined using the AMTS after 5.5 h post-flight. The number of pulses per minute per ephyra was recorded during a simple drop test wherein an ephrya was gently released into ASW in a 15 ml test-tube. The swimming ability of the ephyra was also noted. The ephyra was then placed in a wet film and examined microscopically in order to count the numbers of arms, rhopalia, and statoliths per four rhopalia per ephyra. The number of statoliths in two adjacent rhopalia and those directly opposite on the ephyra were also counted. In addition, five days post-flight the number of independent buds in the space-flown groups and controls was counted and their number and their tentacle numbers were also counted six days later. These organisms were kept at 22°C during this period of time and were not fed.

Results

Ephyra development.— Counts of the number of ephyrae and their arms in the three groups of ephyrae which developed during the experiment in 0 g, 1 g in space, and 1 g on Earth revealed that the mean number of ephyrae formed per polyp ranged from 2.0 to 2.16. Arm numbers ranged between 4-10 with 8 arms the predominant number. Arm numbers for the micro-g ephyrae were 7.95 ± 0.21 (mean ± SD); for 1g in space, 8.0 ± 0.86 and earth controls were 7.71 ± 0.72. Statistical analysis of the numbers of ephryae with arm numbers other than eight as compared with those with eight arms are given in table 1. These results revealed that ephyrae which had developed in space at micro-g had significantly more arm abnormality than those that developed in space at 1 g and those that developed on Earth. Indeed, more ephyrae that developed in space at 1 g had 8 arms than those of the other groups.

Table 1. Comparison of numbers of ephyrae with arm numbers other than eight and ephyrae with eight arms. Fisher's Exact Test; $p = 0.030$.

Environment	Number of ephyrae	Other than 8 arms
Micro-g	32	31.25 %
1 g in Space	34	5.88 %
1 g on Earth	85	20.00 %

Further statistical examination of the arm numbers was done comparing the numbers of animals with more than or less than 8 arms in the three groups of ephy-

rae. We found a statistically significant result in the number of ephyrae with greater than 8 arms among the micro-g developed ephyrae (40 %) as compared with the controls (0 %) (table 2). On the other hand, the 1 g controls had more ephyrae with arms less than 8 than did the ephyrae which had developed in micro-g.

Table 2. Comparison of the numbers of ephyrae with more than eight arms with ephyrae with less than 8 arms. Fisher's Exact Test; $p = 0.026$.

Environment	Number of ephyrae without 8 arms	Arms < 8	Arms > 8
Micro-g	10	60 %	40 %
1 g in space	2	100 %	0 %
1 g on Earth	17	100 %	0 %

Rhopalia and their statoliths.— The number of rhopalia was essentially the same as the number of arms since one rhopalium usually forms per arm. The micro-g group, therefore, which had a significantly higher number of arms per ephyra also had a significantly higher number of rhopalia. In addition, a detailed regression analysis was done using the General Linear Models Procedure to determine whether the relationship between the statolith numbers of rhopalia on adjacent arms is closer than those on rhopalia directly across from them. It was found that there was a close relation between the numbers of statoliths of all rhopalia whether they were adjacent to each other or across from each other.

There were no statistically significant differences (Tukey's Studentized Range Test) between the three groups of ephyrae tested with regard to statolith numbers. The mean number of statoliths for the ephyrae which developed in space at micro-g was 19.9 statoliths ± 12.4; for ephyrae from 1 g in space it was 16.28 ± 10.38 and for the ground control ephyrae, 15.68 ± 9.75 statoliths. An unexpected finding, however, was the formation of statoliths in a group of 12 ephyrae sent into space without statoliths. Although the baseline number of statoliths in ephyrae from this group was zero statoliths pre-flight, ephyrae which had been maintained in space for 14 days in ASW deficient in sulfate returned to Earth with a mean of 4.62 statoliths/rhopalium. A group of 12 ground controls had formed only 1.5 statoliths/rhopalium. This data when exposed to the t-test revealed a statistical significance of $p = 0.0002$. The means for arm and rhopalia numbers per ephyra ranged between 7.7 (Earth controls) and 8.0 (1 g in space controls) and no statistically significant difference was found. During the SLS-1 experiment, polyps were flown in iodinated low sulfate ASW and, post-flight, their ephyrae revealed significantly higher numbers of statoliths than the ground controls. Statistical analysis of these statolith numbers using the General Linear Models Procedure and the Duncan's Multiple Range Test revealed a significant difference ($p = 0.0001$) level between the statolith numbers of ephyrae induced L-24 h (7.2/rho), ephyrae induced L-48h (21.0/rho) and ground controls (1.0 and 1.8/rho).

Budding.— Another form of asexual reproduction in polyps is budding. New buds appear on the body of a polyp and, after detaching, grow 16 tentacles (prevalent number). One group of polyps which was sent into space with one tiny bud Anlage and it's ground control were examined four days post-flight The flown jellyfish polyps had given rise to twelve free buds and the ground controls had only

seven buds. These animals and their tentacles were counted again eleven days post-flight when the space-exposed polyps had released another free bud. The numbers of tentacles per polyp (an indication of developmental maturity) were counted. The micro-g group had 8.3 ± 1.3 (mean ± SD) and the ground controls had 6.6 ± 1.6 tentacles. This data was subjected to the t-test procedure and the results were statistically significant to the p = 0.02 level. The space-flown polyps gave rise to more buds which were significantly more mature as determined by tentacle number than the control buds.

Swimming behaviour.— Swimming ability was determined by observing ephyrae's ability to swim for one minute in ASW in a test-tube on Day 1 post-flight. The results were statistically analyzed by the Chi-square test comparing nominal/ordinal variables by location and the Fisher's Exact Test (2 Tail) (table 3).

Table 3. Swimming ability of ephyrae on Day 1 Post-flight. Fisher's Exact Test; p = 0.004.

Environment	Number of ephyrae	% Swim
Micro-g	30	56.67
1 g in space	23	95.65
1 g on Earth	74	74.32

Significantly more ephyrae which developed in space in micro-g did not swim upon return to Earth as compared with the 1 g controls. It is noteworthy that more of the 1 g in space controls swam than the ground based controls. Although the swimming behaviour of ephyrae that developed in space in micro-g was significantly different from that of ephyrae in 1 g, there were no statistically significant differences in the pulsing ability of the animals. Ephyrae from space (micro-g) averaged 57.9 ± 50.4 pulses per minute whereas the 1 g controls averaged 68 ± 50.5 pulses/minute (space) and 57.9 ± 49.7 pulses/minute (Earth).

Discussion

Ephyra development.— The results of the IML-2 experiment confirmed our findings during the SLS-1 experiment that ephyrae can form in micro-g following induction with iodine and they can pulse and swim (Spangenberg et al., 1994a). Since the jellyfish use iodine to synthesize a hormone, jellyfish thyroxine (Jf-T4), (Spangenberg, 1971) in order to strobilate, it is assumed that the jellyfish synthesized this hormone in space. Ephyrae were induced to metamorphose also in space at 1 g on the centrifuge. The IML-2 jellyfish which were in space five days longer than the SLS-1 animals developed at a slower rate due to the lower temperature of 22°C.

The most significant results of the IML-2 experiment are: (1) The discovery that ephyrae which developed in space in micro-g had statistically significantly more arm abnormality (especially regarding more than eight arms) than ephyrae of the control groups. This is important because the organisms were cloned to reduce arm abnormality pre-flight and this abnormality was not present in ephyrae developing in space at 1 g. The counter-measure of 1 g provided to the jellyfish by centrifugation

may have prevented the arm abnormality from occurring. Arm formation during strobilation involves pattern formation and the differentiation of cells to form new structures (arms, rhopalia, statoliths) not found in polyps. Microgravity could possibly interfere with pattern formation or with the genetic programming for arm formation which may involve the activity of the Jf-T4 hormone. (2) The statolith numbers from the IML-2 experiment are not significantly different between the three groups studied. This result is consistent with the findings from the group of SLS-1 organisms which were induced to strobilate on Earth 24 h before launch. Ephyrae which were induced 8 h after launch, however, had statistically significantly higher numbers of statoliths (Spangenberg et al., 1994b). Ephyrae from Earth sent into space with statoliths lost more statoliths in-flight than did the ground controls, indicating that demineralization occurred to a greater degree in the space maintained ephyrae (Spangenberg et al., 1994b). In addition, ephyrae sent into space in low sulfate ASW from a group of ephyrae without statoliths formed statoliths while in space. These ephyrae had nearly three times as many statoliths as the ground controls. Additionally, polyps sent into space in low sulfate ASW gave rise to ephyrae which formed significantly more statoliths per rhopalium than ground controls. Statoliths form intracellularly in lithocytes during strobilation in the rhopalia of the arms. These statolith results suggest that calcium turnover in the ephyrae and/or their statolith-forming lithocytes may be altered in the space environment, especially in animals in low sulfate ASW. Alterations of calcium metabolism, resulting in total body calcium loss and skeletal changes in rats and humans in space have been reported (Nicogossian, Huntoon & Pool, 1994). There are no known available data as to calcium loss from otoconia or reduced otoconia numbers in these flown mammals. (3) Swimming ability of ephyrae tested on the first day post-flight revealed that statistically significantly fewer ephyrae, which had developed in micro-g in space, swam upon return to Earth. Most (95.6%) of those ephyrae which developed while at 1 g in space, however, tended to swim upon return to Earth even though they had a period of two days at micro-g at the end of the mission. Throughout their developmental period, however, they were compensated by their exposure to 1-g. Post-flight, more ephyrae which had developed in micro-g in space during the SLS-1 mission than ground controls had abnormal pulsing activity, including uncoordinated pulsing, spasms, incomplete pulses, and after-twitches. (Spangenberg et al., 1994a). The cause of the abnormal pulsing and reduction of the number of swimmers among the flight organisms is unknown but it seems that some of the jellyfish are especially sensitive to the space environment during development. Further studies of the rhopalia and the neuromuscular system of these organisms along with 1g in space controls, are needed.

Polyp budding.— The effects of the space environment on polyp budding was examined for the first time. We found that more buds had formed in space and they were more advanced developmentally than ground controls when tentacle numbers were counted 11 days post-flight. Budding represents the formation of a new organism of the same form as the "parent" polyp whereas strobilation leads to the formation of organisms of a different form than the "parent" polyp. Hormonal (JF-T4) intervention apparently is required for the new gene expression necessary for ephyra formation. Budding, however, occurs continuously in polyps which are well fed and does not seem to require the Jf-T4 hormone.

Thyroid hormone.— Very little research has been done thus far on the effects of micro-g on metamorphosis, especially that influenced by thyroid hormone(s). Frog embryos developed into tadpoles in space and these tadpoles, upon return to Earth, metamorphosed (a thyroid controlled process) and matured normally (Souza et al., 1995). Thyroxine hormone levels in humans were measured after a Sky-lab flight and were found to be increased (Nicogossian et al., 1994).

Space environment.— While the thyroxine-influenced metamorphosis of jellyfish produces ephyrae in space, the results from the IML-2 and SLS-1 experiments demonstrate that certain developing ephyrae are more sensitive than others to a microgravity environment. Statistically significant numbers of abnormalities in arm numbers, pulsing, and swimming ability were found in some ephyrae, although not all ephyrae had these abnormalities. Similarly, some ephyrae sent into space lost more statoliths over a nine day period than others. The biological diversity seen in jellyfish and other organisms on Earth regarding various environmental factors such as temperature, light, and salinity apparently also occurs in organisms exposed to the microgravity environment of outer space.

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The problem of bipolarity, with emphasis on the Medusozoa (Cnidaria: Anthozoa excepted)

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Abstract: The concept of bipolarity in general and possible explanations of this phenomenon are briefly discussed. Some examples relating to bipolar distribution of Medusozoa (Cnidaria with the exception of Anthozoa) are discussed and a table is presented of 38 species and some 30 genera of Medusozoa that according to the opinion of the authors in their pattern of distribution show this type of phenomenon. Generally bipolar distribution can now better be judged owing to better species diagnoses and the presence of a data base listing the geographical distribution of a great number (692) of Medusozoa in 9 regions of the North Pacific, 12 of the Polar basin and 21 in the Southern Ocean.

Introduction

The authors joined forces in order to summarize their information on bipolarity of the Medusozoa fauna: Arctic, North Pacific, Antarctic (S. Stepanjants); Antarctic and Mediterranean (A. Svoboda). Information on North Atlantic, tropical and Antarctic hydroids was supplied by W. Vervoort. This paper only deals with geographic/ecological phenomena of this group of animals.

The phenomenon of bipolarity

Bipolarity should be taken literally as interrupted distribution of identical or closely related species (or higher taxa) of flora and fauna in polar, temperate, and subtropical regions of both hemispheres and their absence in the tropics. Advocates of bipolarity proceed from "The Origin of species" (Darwin, 1859: chapter XI) where is shown that European plants of the same species, or their subspecies, occur in both hemispheres on plains of temperate regions and on mountain tops of the tropics, but are absent on the tropical plains (Bergh, 1947: 128; Briggs, 1987: 237). Over the last century this problem has been repeatedly discussed, and its opponents (Thompson, 1897-1898: 347-349; Dollo, 1904: 199; Stiasny, 1934: 35-53) brought forward arguments of unreliability in identification or of dissimilarity of the faunae of both hemispheres. However, there are far more supporters of bipolarity (except for those already named: Ross, 1847: 208; Dana, 1854: 36; Pfeffer, 1891: 17; Murray, 1896: 494; Derjugin, 1915: 871; Ekman, 1953: 261; Andriashev, 1964: 335; Beklemishev, 1969: 213; Vinogradova, 1977: 297; Dunbar, 1979: 116, et al.).

Different conceptions concerning the origin of bipolarity are known at present:

1. "Original local creation" of identical species of both hemispheres in similar environments (Dana, 1854: 36).

2. Independent evolution of species living in both hemispheres outside the tropics from warm water species (Hesse, 1924: 293).

3. "Relict theory" (Théel, 1886; Pfeffer, 1891; Murray, 1896) proceeding from the idea of a uniform marine fauna during the Tertiary epoch, when the climate of the earth was warmer and the temperature more uniform. As a result of cooling in polar zones the once homogeneous fauna became extinct or adapted to cold water conditions existing in both hemispheres. Adapted species remained as relics in temperate or polar faunae; in tropical waters the further evolution of the fauna proceeded. Some present day faunists supported the Relict theory. Briggs analysed many new conceptions: "the island integration hypothesis", "the hypothesis of vicariance ", and "the theory of hologenesis", concluding however that "the relict theory appears to provide a mechanism whereby antitropical distributions may be brought about" (1987: 246). Previously Derjugin (1915) derived bipolar distribution from cosmopolitism and came close to accepting the relict theory. His examples on hydroids are highly convincing: *Filellum serpens* (Hassall, 1848), *Lafoea dumosa* (Fleming, 1820) in their distribution are in between cosmopolitism and bipolarity (see below) and demonstrate bipolarity "in statu nascendi" (Derjugin, 1915: 873).

4. The migration theory (Ortmann, 1897) was advanced as an objection to the relict theory. Ortmann basically rejected bipolarity. But in spite of this he recorded closely related *Crangon* species in temperate waters of both hemispheres and he discussed the possibility of present day migrations along the West African coast and along American shores with cold currents or in deep waters (Ortmann, 1897: 571). As will be shown below, this view about the secondary origin of bipolarity by means of present day migrations is quite founded. It is demonstrated by epibiotic species on drifting objects and on macroalgae.

5. Berg (1947: 137) advanced his conception of bipolarity. In the Pleistocene Glaciation Epoch not only arctic-temperate zones were touched by cooling, but also the tropics. In this period several cold water species are presumed to have penetrated into the southern hemisphere across the equator. After the glaciation period the temperature in the tropics increased again and cold water species either were lost there or retired to the North or the South.

6. The assumption about the deep water origin of closely related arctic and antarctic species was evolved by Ross (1847: 208), Ortmann (1897: 580); Andriashev (1987: 62); Vinogradov (1968: 166) and others.

7. The origin of bipolar distribution has been hypothesised by means of an Atlantic mountain range, during the Pleistocene c. 2000 m above sea level. Along this mountain range the exchange between both hemispheres of the terrestrial fauna and flora could have taken place during the pleistocene period (Malaise, 1945: 34).

There is no escape from the fact that the concept of bipolarity also concerns the temperate-subtropical fauna, though in the view of some investigators it primarily concerns the polar arctic and antarctic faunas to which it was initially restricted. First of all the term "bipolarity" induces to speak about the polar fauna; subsequently we find records of bipolar arctic and antarctic species of moss, lichens, Foraminifera

(*Globigerina pachyderma* Ehrenberg, cf. Berg, 1947: 135, 153). In the original scheme of latitudinal-zonal nomenclature Semenov (1982: 193) considers bipolarity as "distribution in cold waters of Arctic and Antarctic". More radical interpretations define it as "the presence in the Arctic and the Antarctic of apparently identical species without their presence in the intervening temperate and tropical regions" (Dunbar, 1979: 116). For good reasons many taxonomists and faunists considered bipolar distribution of the groups they studied as antitropical distribution of polar species (Petrushevskaya, 1967: 180; Kussakin, 1967: 363; Stepanjants, 1967: 95; 1979: 153; 1989: 406).

A new aspect of the concept of bipolarity is the similarity of biota's of both poles as independent entities and taking into account unrelated forms, taxocenoses and biocenoses. This phenomenon was named "bionomic bipolarity" (Andriashev, 1987: 65). A typical example of bionomic bipolarity is the cryopelagic fauna.

Records of bipolar distribution of bottom living and pelagic Medusozoa are known from many taxonomic and faunistic works. However, the characteristics of the areas of bipolar distribution are not always punctual and identification of bipolar species often does not inspire confidence. *Campanularia verticillata* (Linnaeus, 1758) is often given as classical example of a species with bipolar distribution but actually two species are involved: *Rhizocaulus verticillatus* (Campanulariidae) with arctic-boreal distribution in the northern hemisphere and *Stegella grandis* (Hickson & Gravely, 1907) (Campanulinidae) with panantarctic distribution.

Many taxonomists of high qualification now study Medusozoan material from different regions (from Arctic to Antarctic) which assures species identification as exact as possible. We have analysed more than 100 of their publications to prepare the species list which we have used in this work (table 1).

Main material for our work includes a data base with 692 species of 242 genera and 79 families of Hydrozoa, Siphonophora, Cubozoa and Scyphozoa. The data base includes occurrences of these species in 9 regions of the North Pacific, 12 regions of the Polar Basin and 21 regions of the Southern Ocean.

We now dispose of quite exhaustive information on the species composition of those regions. Moreover, we analysed the species distribution in other regions of the World Ocean (based on more than 30 publications). We now have the possibility to include in this analysis new information on tropical faunae (Gibbons & Ryland, 1989: 377; Ryland & Gibbons, 1991: 525; Vervoort & Vasseur, 1977: 98; Rees & Vervoort, 1987: 209; Ramil & Vervoort, 1992: 262; Vervoort, 1993a: 298; 1993b: 537). All this information allows us to give biogeographical characteristics for every species (table 1). As a result we presume to be allowed to judge to which degree the phenomenon of bipolarity occurs in Medusozoa and explore its occurrence with methods based on the peculiarities observed in this group of animals.

Table 1 presents evidences that there are no bipolar species and genera in Cubozoa and Scyphozoa. Thirty-eight species and 30 genera of Hydrozoa and Siphonophora can be treated as bipolar. This can be explained by the "plasticity" of this group and the wider conception of "bipolarity" we presented above. The majority of bipolar species (28) is found in temperate, subtropical zones and in Arctic and Antarctic waters. It appears that there are no true autochthonous arctic or antarctic species with the exception of three: *Botrynema brucei* Browne, 1908, *Paragotoea elegans* Margulis, 1989, and *Yakovia polinae* Margulis, 1989. The remaining have arctic-boreal,

Table 1. The bipolar Medusozoa (species and genera). A = Atlantic; Am = American; Ar = Arctic; As = Asiatic; B = bipolar; Ba = bipolar Atlantic; Bp = bipolar Pacific; Bd = bipolar deepwater; Bl = Black Sea; Bor = boreal; EAr = east Arctic; Eu = European; GalIsl = Galapagos Islands; GlAn = glacial Antarctic; HAr = high Arctic; Mc = Macquarie Id; Nat = natal zone; NZ = New Zealand; P = Pacific; Pat = Patagonian Shelf; SAf = South Africa; SAm = South America; SAust = South Australia; SG = South Georgia; Sub = subtropical; SubAnIsl = subantarctic islands; Tr = tropical; WAr = west Arctic; (*5) = number of bipolar species; if there is no such symbol, the genus is monotypical, "?" = doubtful. Opinions on the classification of higher taxa have not always been considered.

Taxa	General distribution	Distribution N. hemisphere	Distribution S. hemisphere
Subclassis Athecatae/Anthomedusae			
I. Gen. *Rhizogeton* (*5)	B	Ar-Bor A P	SAf
1. *R. nudum*	B?	Bor A	SAf?
II. Gen. *Calycopsis* (*11?)	B?	Bor A P	GlAn SAf
III. Gen. *Bythotiara* (*3)	B	Bor-Sub A P	GlAn
IV. Gen. *Rhizorhagium* (*3)	B	Ar-Bor A P	GlAn
V. Gen. *Monobrachium* (*4?)	B	Ar-Bor A P	GlAn SubAnIsl
2. *Eudendrium rameum*	B	Ar-Bor-Sub A P	Pat SubAnIsl
3. *Tubularia indivisa*	B	Ar-Bor-Sub A P	SubAnIsl SG
VI. Gen. *Paragotoea*(*2)	B	HAr Bor-Sub A	GlAn
4. *P. elegans*	Bd	HAr	GlAn
VII. Gen. *Yakovia*	B	HAr	GlAn
5. *Y. polinae*	Bd	HAr	GlAn
6. *Sarsia tubulosa*	B	Ar-Bor-Sub A P Bl	GlAn SubAnIsl Pat
VIII. Gen. *Monocoryne* (*3)	B	Ar-Bor A P	GlAn SAf
IX. Gen. *Candelabrum* (*13)	B	Ar-Bor A P	GlAn SubAnIsl SAf
X. Gen. *Margelopsis* (*4)	B?	Bor-Sub A P Ind?	GlAn SubAnIsl
XI. Gen. *Rosalinda* (*4)	B?	Bor-Sub P	SAust
Subclassis Thecatae/Leptomedusae			
7. *Orthopyxis integra*	B	Ar-Bor-Sub A P	Pat SAust
8. *Obelia longissima*	B	Ar-Bor-Sub A P Bl	GlAnIsl SubAnIsl Pat NZ
9. *O. geniculata*	B	WAr-Sub A P	Mac SubAnIsl Pat NZ SAust
XII. Gen. *Tulpa* (*3)	B	Ar-Bor-Sub A P	SubAnIsl Pat NZ
10. *Filellum serpens*	B?	Ar-Bor-Sub A P GalIsl?	GlAn Pat SAf TrAf?
XIII. Gen. *Grammaria* (*5?)	B	Ar-Bor-Sub A P	SubAnIsl Pat SG
11. *G. abietina*	B	Ar-Bor-Sub A P	SubAnIsl Pat SG
12. *Acryptolaria conferta*	B?	WAr-Bor-Sub A P	Pat NZ SAf Tr
XIV. Gen. *Zygophylax* (53?)	B?	AR-Bor-Sub A P Tr P ?	SubAnIsl Pat Tr P ?
XV. Gen. *Lafoeina* (*3)	B?	Ar-Bor-Sub A P	GlAn SubAnIsl
XVI. Gen. *Calycella* (*3)	B?	Ar-Bor P Red Sea?	Pat SAf
13. *C. syringa*	B	Ar-Bor A P	Pat
XVII. Gen. *Modeeria* (*3?)	B	Ar-Bor-Sub A P	SubAnIsl Pat SAf NZ SAust
14. *M. rotunda*	B	WAr-Bor-Sub A P	SubAnIsl NZ SAf Pat
XVIII. Gen. *Staurophora*	B	AR-Bor A P	SubAnIsl Pat
15. *S. mertensi*	B	Ar-Bor A P	SubAnIsl Pat
XIX. Gen. *Ptychogena* (*7)	B	Ar-Bor-Sub A P	GlAn SubAnIsl
16. *Halopsis ocellata*	B	WAr-Bor A	Pat
XX. Gen. *Parascyphus* (*2)	B?	WAr-Bor A	Pat SubAnIsl NZ SAf SAust
17. *P. simplex*	B?	Bor A ?	NZ SAust SAf
18. *Sertularella gayi*	B?	Bor-Sub A P Tr?	Pat NZ

19. *S. reticulata*	Bp?	Bor P	SAust?
20. *Symplectoscyphus tricuspidatus*	B	Ar-Bor A P	Pat
XXI. Gen. *Staurotheca* (*6?)	B	Bor P	GlAn SubAnIsl
21. *Abietinaria abietina*	B	Ar-Bor-Sub A P	Pat SAf?
XXII. Gen. *Papilionella* (*4)	B	Bor P	Pat NZ
22. *Thuiaria thuja*	B	WAr-Bor A P	Pat
23. *Halecium tenellum*	B?	Ar-Bor-Sub A P	GlAn NZ Pat SAf SAust
XXIII. Gen. *Kirchenpaueria* (*6?)	B?	Ar-Bor-Sub A P	SAf NZ Tr?
24. *K. pinnata*	Ba	Bor-Sub A	SAf
25. *K. bonnevieae*	B	Sub A	SAf NZ
26. *Ventromma halecioides*	B?	Sub A Bl	SAf?
27. *Plumularia filicaulis*	B?	Bor-Sub P	SAf? Pat?
XXIV. Gen. *Oswaldella* (*6?)	B?	Bor P ?	GlAn SubAnIsl
XXV. Gen. *Schizotricha* (*10?)	B	Ar-Bor-Sub A P	GlAn SubAnIsl SAf Pat
28. *Halopteris catharina*	Ba?	Bor-Sub	Pat
29. *Nemertesia antennina*	Ba?	Bor-Sub A P	SAf Tr?
Subclassis Trachymedusae			
XXVI. Gen. *Botrynema* (*2)	B	Ar-Bor-Sub A P	GlAn
30. *B. brucei*	Bd	Bor P	EBor A GlAn
XXVII. Gen. *Ptychogastria* (*2?)	B	Ar-Bor A P Sub A	GlAn SubAnIsl
Subclassis Limnomedusae			
XXVIII. Gen. *Craspedacusta* (*3)	B	Bor-Sub As Eu Am	Aust SAm
31. *C. sowerbyi*	B	Bor-Sub As Eu Am	Aust SAm
Subclassis Siphonophorae			
Ordo Physophorida			
XXIX. Gen. *Marrus* (*3)	B	Ar-Bor A P	GlAn SubAnIsl SAf NZ
32. *M. antarcticus*	Bd	Bor P	GlAn SubAnIsl SAf NZ
Ordo Calycophorida			
33. *Clausophyes galeata*	Bd	Bor P	GlAn SAust
34. *Lensia achilles*	Bd	Bor A P	GlAn SAf
35. *L. asymmetrica*	Bd	Bor P	GlAn SAf
36. *Muggiaea bargmannae*	Bp	Bor P	GlAn SubAnIsl SG
37. *M. havock*	Bd	Bor P	An SubAnIsl SG SAf
XXX. Gen. *Dimophyes*	B?	Ar-Bor-Sub A P Tr?	GlAn SubAnIsl Nat Tr?
38. *D. arctica*	B?	Ar-Bor-Sub A P Tr?	GlAn SubAnIsl Nat Tr?

arctic-subtropical, or antarctic-natal distribution. Another important feature: of the 38 bipolar species 27 have a bottom dwelling stage, 14 are true epibionts. The other 13 may also be epibiotic. From the 16 species with free medusae, at least 7 species inhabit coastal plankton and their polyps are also epibionts, in any event inhabit drifting objects, ships hulls and macroalgae, and by their presumed ability to tolerate the high temperatures of tropical seawater may migrate. With respect to those species the Migration theory by Ortmann (see above) is quite appropriate. Such species, as e.g. *Obelia geniculata* (Linnaeus, 1758), are represented in the northern hemisphere by dense populations, but in the southern hemisphere occur mainly isolated, often on drifting macroalgae. Migrations are most probably in the direction from north to south.

The eight characteristic pelagic bipolar species include three species of hydromedusae and five species of Siphonophora (see table 1). The polyps of these three

species of hydromedusae are unknown. For *Paragotoea elegans* and *Yakovia polinae* they may be of the same type as other Tubulariidae or Corymorphidae. These species have been recorded from depths between 50 and 2000 m. In polar waters they are known to occur closer to the surface: 50-200 m. Their distribution pattern probably results from contemporary migration (Vinogradov, 1968), including migration of larvae (Mileikovsky, 1977: 105). It seems likely that these species will be detected at bathyal depths of the tropics.

Three species: *Tubularia indivisa* Linnaeus, 1758; *Eudendrium rameum* (Pallas, 1766) and *Grammaria abietina* (M. Sars, 1851) have no free medusae. Their robust colonies often occupy rocky bottoms and form rich, dense settlements in the sub- tidal zone of Arctic and temperate waters of the northern hemisphere. Less rich but viable populations are known to occur near subantarctic islands and on the Patagonian shelf. *Tubularia indivisa* is represented in the Southern Ocean by the subspecies *antarctica* Hartlaub, 1905. It is probably possible to see such species as the relics of an ancient fauna of northern-boreal origin.

Craspedacusta sowerbyi Lankester, 1880 is represented by polyps and medusae in fresh water of the temperate zone of both hemispheres. In the tropics (India, Africa) there is the closely related genus *Limnocnida*. The conception of distribution of terrestrial species in the pleistocene across the Atlantic range may explain bipolarity of *C. sowerbyi*. The presence of the genus *Kirklandia* in Jurassic and Carboniferous formation of Germany and North America confirms the ancient age of this group.

In spite of difficulties in the identification of species of the genus *Filellum* when the coppiniae are absent, it can be stated that only two species are sufficiently well known to state their geographical distribution, viz. *Filellum serpens* (Hassall, 1848), known from cold boreal and warmer subtropical regions, and the subtropical-tropical *Filellum serratum* (Clarke, 1879). For a long time *F. serpens* was accepted by taxonomists as a panoceanic species. It was named as bipolar for the first time by Derjugin (1915: 863). A discussion of the distribution of this species was given later (Stepanjants, 1980: 117). *F. serpens* is known from cold and temperate waters of both hemispheres (9-300 m to 3500 m), was found in the Mediterranean and in tropical waters off West Africa. So formally it is not an antitropical species. But if we bear in mind Darwin's reasoning (see above), it is necessary in this case it use ecological data: the cold water species *F. serpens* may penetrate into deep waters of the tropical regions with the cold water masses along the West African coast.

Similarly the distribution of *Dimophyes arctica* (Chun, 1897) could be assessed. This species was first found in the Arctic and later on in different parts of the World Ocean, in the Caribbean Sea and the tropical zone: consequently it was considered to be cosmopolitan. Investigations in later years testify that the distribution of this species is limited to cold water (Stepanjants, 1975: 96). Moreover, new statistical methods allow us to conclude that *D. arctica* reproduces in water of polar origin, but that the tropics represent a sterile eviction area (Lobanov, Stepanjants & Dianov, in press). The same may hold true for *Muggiaea bargmannae* Totton, 1954, the Pacific bipolar siphonophore. Penetration in the tropics of the above named species involves transportation in specific water masses, which is equivalent to Darwin's and Berg's opinions about bipolarity as a phenomenon caused by climatic conditions. It is logical, therefore, to agree with Derjugin's opinion that cosmopolitism is the predecessor of bipolarity.

Amongst the thirty bipolar genera *Staurophora, Yakovia* and *Dimophyes* alone are monotypic; *Paragotoea* has two bipolar species; *Margelopsis, Monobrachium, Papilionella, Tulpa, Craspedacusta* and others have two to four closely related species from the northern and southern hemispheres, but not a single one in tropical seas (table 1).

Monocoryne and *Candelabrum* constitute the bipolar family Candelabridae. *Monocoryne* has three species: *M. gigantea* (Bonnevie, 1898) (Arctic, temperate North Pacific and Atlantic); *M. minor* Millard,1966 (South Africa) and *M.* spec. (Antarctic). *Candelabrum* includes at least thirteen species: *C. phrygium* (Fabricius, 1780) (Arctic, boreal waters of the North Pacific and Atlantic); *C. cocksii* (Vigurs, 1849) (boreal waters of the eastern Atlantic); *C. giganteum* (Bonnevie, 1898), *C. minutum* (Bonnevie, 1898), *C. mitra* (Bonnevie, 1898) and *C. verrucosum* (Bonnevie, 1898) (northern Atlantic); *C. australis* Briggs, 1928, and *C. harrisoni* Briggs, 1928 (New South Wales); *C. meridianum* Briggs,1938 (Macquarie Id, Marion Id); *C. austrogeorgiae* Jaederholm, 1904; *C. penola* Manton, 1940 (subantarctic Islands, Antarctic); *C. capensis* Manton, 1940 and *C. tentaculata* Millard, 1966 (South Africa). There is no representative of this family in the tropics. All of these species are very close both morphologically and ecologically: they prefer cold water (-1°C to 7°C), and most likely have a deep water nature. In spite of the absence of paleontological data, this species group could be considered to represent relics of a deep water tertiary fauna. Future representatives of this family are likely to be found in deep waters of the tropics.

The bipolar family Kirchenpaueriidae amongst others includes the two genera *Kirchenpaueria* and *Oswaldella*, which like the bipolar genus *Schizotricha* (Plumulariidae) occur in cold water. These three genera, with the possible exception of *Oswaldella*, are distributed in cold, temperate and subtropical waters of both hemispheres but presumably originate from warm water Plumulariidae inhabiting mainly subtropical and tropical zones. The northern *Plumularia microtheca* Naumov, 1960 (North Pacific), *P. fragilis* Hamann, 1882 (Barents Sea) and *P. halecioides* Alder, 1859 (Black Sea) actually belong in Kirchenpaueriidae, not in Plumulariidae, but their present status, in absence of supplementary material, remains uncertain.

There are not many bipolar species in the family Sertulariidae (table 1). *Thuiaria* (including *Selaginopsis*) is represented in the northern hemisphere by about 40 species in the North Pacific, 10 species in the Arctic and c. 5 species in the North Atlantic. No *Thuiaria* species occurs in the tropics. There are some species in the Southern Ocean that are quite close to the northern species of *Thuiaria* but differ in the distribution of the number of rows of hydrothecae on stem and branches of the colony. These antarctic species were placed in *Thuiaria* by Stepanjants (1979: 92). However, besides the difference in the number of rows of hydrothecae referred to above, there are also differences in the shape of the gonothecae. This allows us to remove those southern species from the genus *Thuiaria* Fleming, 1820, and place these in the genus *Staurotheca*. The five *Staurotheca* species so far described are considered endemics of the southern hemisphere and several of the species now added to the genus are likely to be such. *Sertularia staurotheca* Naumov, 1960, is known from the North Pacific and may be affiliated to the genus *Staurotheca* by the crosswise pairs of hydrothecae. There are, however, no data on the gonothecae. If those resemble the gonothecae of southern species of *Staurotheca* and if other morphological details also match, the genus could be supposed to be bipolar.

Zygophylax is convenient for our analysis as it was recently reviewed (Rees & Vervoort, 1987: 51). To date more than 50 species are known, which inhabit mainly subtropical zones of both Atlantic and Pacific, usually at depths exceeding 100 m depth. One Arctic species and two from the subantarctic are known. Twelve species inhabit the tropics, but at 200-1600 m depth. Only five species are known from tropical shallow water localities. We are inclined to refer to deep-water origin of the largely antitropical *Zygophylax* fauna and migration of its species to the north and south may have taken place along the deep, cold water of the Americas, to give independent cold water polar shelf faunae of both hemispheres. This migration may have taken place in the preglacial period since Cambrian *Archaeocryptolaria* and *Archaeolafoea* are known from Tasmania, Australia and North America.

Representatives of Hydractiniidae epibiotic on the ophiurid family Ophiolepididae occur at Arctic depths (*Hydractinia ingolfi* Kramp, 1932) and in the Antarctic (*Hydractinia vallini* Jaederholm, 1926), they allows us to speculate on bionomic (ecological) bipolarity (Svoboda, Stepanjants & Smirnov, in press). It is possible to demonstrate bionomic bipolarity by comparing the hydrozoan faunae of the Commander and Aleutian Islands (123 species) and Kerguelen Island (69 species). There are six species in common: *Tubularia indivisa, Sarsia tubulosa, Lafoea dumosa, Grammaria abietina, Muggiaea bargmannae* and *Dimophyes arctica*. In both regions the same families are dominant: Sertulariidae (26% and 20%), Haleciidae (5.6% and 7.7%), Campanulariidae (5.6% and 11.5%). In Sertulariidae the composition of genera on the northern hemisphere is: *Abietinaria* (12 species), *Sertularella* (8) and *Thuiaria* (7), and on the southern hemisphere: *Symplectoscyphus* (9 species). Cold water Kirchenpaueriidae and Plumulariidae (*Schizotricha*) are represented at the North Pacific Islands by two species [*Plumularia (Kirchenpaueria?) microtheca* Naumov, 1960 and *Schizotricha (= Kirchenpaueria?) divergens* Naumov, 1960], and at Kerguelen by *Oswaldella bifurca* (Hartlaub,1904) and *Schizotricha unifurcata* Allman, 1883.

Conclusions

1. Many bipolar species, genera and families of Hydrozoa and Siphonophora are known. There are no bipolar Scyphozoa and Cubozoa.

2. The term "bipolar" = "antitropical", "bitemperate" for these groups means distribution from Arctic to Antarctic with interruption in the tropics.

3. Certain species and genera occur in cold, temperate and subtropical zones as well as in the tropics. In the last case they are known at depths with cold water of polar origin. We also consider these taxons as being bipolar.

4. Bipolar species distribution of Hydrozoa is largely a result of present day migration from the northern to the southern hemisphere.

5. Only some bipolar species are relics of an old temporate fauna.

6. Some bipolar genera may be considered relics of a cold deep water fauna: *Monocoryne, Candelabrum* and *Zygophylax*; or originate from warm water families: Kirchenpaueriidae from Plumulariidae. There are also some closely related genera in the northern and southern parts of the oceans: *Thuiaria* in the North Pacific and *Selaginopsis* and *Staurotheca* in antarctic waters.

7. Several examples of bionomic bipolarity, on the level of taxa or taxocenoses, are known.

8. All opinions on the origin of bipolarity advocated in the course of time are suitable for the explanation of bipolarity in Medusozoa. There is evidently no universal way to explain the antitropical fauna distribution.

9. Bipolarity is not a unique phenomenon in biogeography. It is most likely the result of different historical or modern ways of the formation of faunae that are similar in their external manifestation.

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Hydromedusae (Cnidaria: Hydrozoa) of Bahía de la Ascensión, Caribbean coast of Mexico: a seasonal survey

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Suárez-Morales, E., M.O. Zamponi & R. Gasca. Hydromedusae (Cnidaria: Hydrozoa) of Bahía de la Ascensión, Caribbean coast of Mexico: a seasonal survey.
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Key words: Hydromedusae; zooplankton; seasonal fluctuations; embayment; Caribbean; Mexico.

Abstract: The composition, distribution and abundance of hydromedusae collected at Bahía de la Ascensión, on the east coast of the Yucatan Peninsula, Mexico, were analysed during three climatic periods of a year cycle (August 1990-July 1991). Highest mean hydromedusan abundance was observed during the rainy period (198 ind./1000 m^3), while lowest values occurred during the 'nortes' season (111 ind./1000 m^3). Twenty-two species and four genera were identified, *Helgicirrha schulzei* Hartlaub, 1909, *Phialidium discoidum* (Mayer, 1900) and *Sarsia gracilis* Browne, 1902, being the most abundant. The former two represented more than 90% of the total number. Spatial distribution of total densities throughout the year seemed to be related to different climatic regimes. From cluster analysis (Bray-Curtis Index), and from the ecological affinities of the species, two main faunistic assemblages could be identified during the three seasons: of resident fauna and of neritic affinity. Distribution of faunistic assemblages in the area was seasonally variable, mainly related to hydrological conditions and the wind regimes.

Introduction

Knowledge of the planktonic fauna in inshore and estuarine systems of the Caribbean region is still limited (Rodríguez, 1975: 32; Suárez & Gasca, 1990: 141). Bahía de la Ascensión is a shallow water embayment located on the central portion of the east coast of the Yucatan Peninsula, Caribbean coast of Mexico. The local zooplankton has been studied only recently (Suárez, 1990: 215; Suárez et al., 1990: 137; 1994: 1; Vásquez-Yeomans et al., 1992: 287; Gasca & Suárez, 1994: 116). However, most of these works are qualitative species inventories and new faunistic records. The hydromedusan fauna of this system has been studied on the same basis by Zamponi et al. (1990: 99; local records of six species), and by Zamponi & Suárez (1991: 42; description of a new genus and species *Tetraotoporpa siankaanensis*).

Hydromedusae have been considered the most important group of Ascensión gelatinous zooplankton in terms of biomass (Gasca & Suárez, 1994: 122), which suggests their trophic relevance. However, there has been no quantitative seasonal information on the community of hydromedusae in this coastal system. In this paper, data on the composition, distribution and seasonal abundance of the local hydromedusan community are analysed for the 1990-1991 cycle.

With an area of ca. 740 km^2, Bahía de la Ascensión is the second largest bay of the Mexican Caribbean coast. Its mean depth is ca. 3.2 m, and it has been described as a typical coastal lagoon with seasonal freshwater input by land effluents and subterra-

nean water discharges (Kjerve, 1987: 232). Lankford (1977: 209) classified it as an "I,F"- type lagoon, a low-energy karstic damped depression. Temperature is highest in August (32°C), and lowest in December (21°C). Salinity has a maximum in July (35.8‰), and a minimum during December (25.6‰). Mean annual salinity is 32‰.

Three different climatic periods (dry, rainy and 'nortes') occur within a typical year cycle. The dry period (March-June) shows a dominant eastern-southeastern wind regime with low precipitation rates. In the rainy period (July-October) eastern-southeastern winds are still dominant and precipitation is higher. The 'nortes' ('northerlies') season (November-February) is characterized by strong northern winds with moderate rains and occasional storms (Merino & Otero, 1991: 34).

Methods

Four-monthly samplings corresponding to each of the three climatic periods surveyed (dry: March-June, 1991; rainy: July, 1991 and August-October, 1990; 'nortes': November 1990-February, 1991) were analysed. Zooplankton was collected by horizontal hauls with a square-mouth (0.45 m per side) standard net (0.5 mm mesh-size). This gear allowed collection of small, medium-sized and large medusae. A digital flow meter was used to estimate the volume of water filtered.

The bay was surveyed at 13 sampling stations (fig. 1). Zooplankton samples were fixed and preserved in a buffered 4% formaldehyde solution (Smith & Richards, 1979: 7). Hydromedusae were sorted and then processed for identification. Density data for each period (corresponding to a four-month data set) were integrated to obtain three seasonal data sets. Shannon-Wiener's diversity was estimated (the index units are bits/individual, which express the uncertainty degree of a prediction about the identity of a given individual), and used along with the Index of Importance Value (IIV) and the Bray-Curtis Index, to analyse on a quantitative basis the local community structure of hydromedusae in each season.

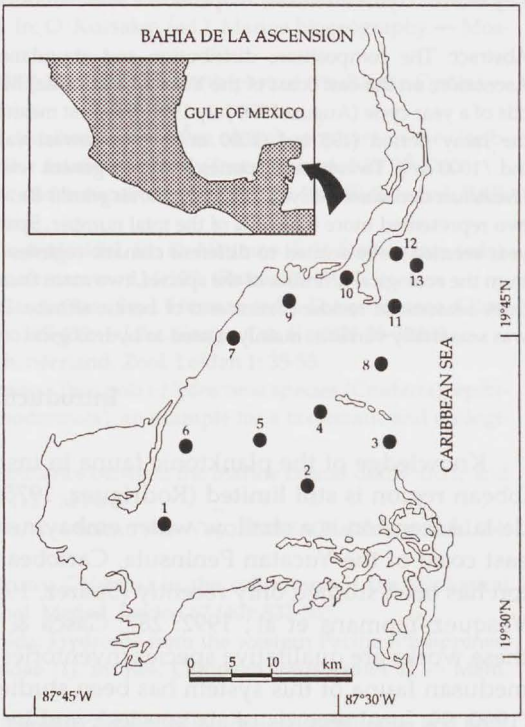


Fig. 1. Survey area with zooplankton sampling stations in Bahía de la Ascensión, Mexico.

Results

During the rainy period, temperature averaged 31.5°C and salinity 32.5‰. Dur-

ing the dry period, temperature averaged 30.2°C and salinity 33.4‰. The 'nortes' period showed the lowest average temperature of the year (26.6°C); average salinity was the lowest of the year (27.75‰). Absolute lowest salinities (13-17‰) were found at several stations of the 'nortes' period.

The distribution of hydromedusan densities showed monthly variations (fig. 2). Highest total mean densities were found during the rainy period (198.3 ind./1000 m^3), followed by the dry (118 ind./1000 m^3) and the 'nortes' (111 ind./1000 m^3) periods. In the dry and rainy seasons, highest densities were found mainly near the innermost portions of the bay; in the dry period, 68.6% of total hydromedusae numbers were distributed at stations on the innermost portions of the bay (sta. 1, 5-7, and 9). During the rainy period, this value was near 44%, while in the 'nortes' period, it was only 19.2%.

Eleven species and seven genera were collected in the surveyed area (table 1). Only *Helgicirrha schulzei* Hartlaub, 1909, *Phialidium discoidum* (Mayer, 1900) and *Sarsia gracilis* Browne, 1902, were found during the three seasons. *Helgicirrha schulzei* and *P. discoidum* were the predominant hydromedusae in the bay. During the 'nortes', they constituted ca. 92% of the total hydromedusan catch, 97% in the rainy season, and 93.7% in the dry period. The other species formed a minor group (table 1).

Helgicirrha schulzei was most abundant during the rainy period, with a mean density of 167 ind./1000 m^3; it was less abundant during the dry (110 ind./1000 m^3) and 'nortes' (41 ind./1000 m^3) periods. It occurred in 70, 79 and 50% of the sampling sta-

Table 1. Mean density (ind./1000 m^3) and relative abundance (%) of hydromedusa species during each of the three seasons (dry, rainy, and 'nortes') in Bahía de la Ascensión.
* = New records for Bahía de la Ascensión.

	Dry season		Rainy season		'Nortes'	
	Density	%	Density	%	Density	%
Helgicirrha schulzei Hartlaub, 1909	1432	93.10	2183	84.6	545	37.7
Phialidium discoidum (Mayer, 1900)	10	0.65	314	12.2	788	54.6
Sarsia gracilis Browne, 1902	29	1.88	12	0.46	17	1.17
Sarsia spp.			1	0.03	12	1.43
**Phialucium* spp.	49	3.18	8	0.31	26	1.8
**Ectopleura* spec.					20	1.4
**Euphysa* spp.	1	0.07	8	0.32	11	0.76
**Eucodonium* spec.			1	0.03	7	0.48
**Solmundella bitentaculata* (Quoy & Gaimard, 1833)					3	0.20
**Eirene lactea* Mayer, 1900					2	0.14
**Eutima mira* McCrady, 1857					2	0.14
Eutima spec.	1	0.07			1	0.07
**Liriope tetraphylla* (Chamisso & Eysenhardt, 1821)			35	1.35	2	0.14
**Eucheilota paradoxica* Mayer, 1900					1	0.07
**Hybocodon forbesi* Mayer, 1894					1	0.07
**Hybocodon* spec.					1	0.07
**Obelia* spp.	12	0.78			1	0.07
**Olindias tenuis* (Fewkes, 1882)	2	0.13	16	0.62		
**Cytaeis tetrastyla* Eschscholtz, 1829			1	0.03		

Fig. 2. Monthly densities (ind./1000 m^3) of hydromedusae at sampling stations during the survey period.

tions during the rainy, dry and 'nortes' periods, respectively. The estimated Index of Importance Value (IIV) for this species was highest during the rainy (IIV = 117.97) and dry (IIV= 149.6) seasons, and lower during the 'nortes' (IIV = 61.29).

Phialidium discoidum was dominant during the 'nortes', with a mean density of 61 ind./1000 m^3. In the rainy and dry seasons densities were lower (24.1 and 0.8 ind./ 1000 m^3, respectively). This species occurred in 11% of the sampling stations of the rainy season, in 4% of the dry, and in 42% of the 'nortes'. Its Index of Importance Value was highest during 'nortes'(IIV = 76.17), with low values in the rainy (IIV = 26.04) and dry (IIV = 9.34) periods.

Neritic species were recorded mainly along the oceanic front of the bay (sta. 3,8,10-13) during the three periods, and were most abundant during 'nortes', with a mean density of 7.15 ind./1000 m^3 (6.4% of hydromedusae total number); during the other periods, neritic species were less representative (dry: 5.15 ind./1000 m^3, 4.35%; rainy: 5.38 ind./1000 m^3, 2.7%).

From the dendrograms of the Bray-Curtis index (figs 3a-c), a zone pattern was outlined for each season (figs 3d-f). During the dry period (D), two groups were defined: the first one (D1) included stations along the oceanic front. This neritic assemblage included stations with very low densities of *Helgicirrha schulzei* (only 8% of the individuals recorded for the species in this season), the occurrence of seven other species (mainly *Phialucium* spec., *Obelia* spec., and *Sarsia gracilis*), and with the highest diversity (>1.0 bits/ind.). The second assemblage (D2) was located at the middle and inner portions of the bay, with resident *H. schulzei* widely dominant (92% of the individuals recorded for the species) and neritic species absent. Diversity was low (>1.0 bits/ind.). The rainy period (R) also showed two groups. The first one (R1), of neritic affinity, was restricted to the northern portion of the bay, with low densities of *H. schulzei* (only 4% of the individuals recorded for the species), the occurrence of eight more species, mainly *Liriope tetraphylla* (Chamisso & Eysenhardt, 1821) and *Phialidium discoidum*, and the highest diversity values (>2.0 bits/ind.). The second group (R2), of resident fauna, showed high densities of *H. schulzei* (96% of the individuals recorded for the species) and low densities of *S. gracilis* and *Olindias tenuis* (Fewkes, 1882). Three groups were defined during the 'nortes' season: in assemblage N1 hydromedusae were absent or with isolated individuals of *Sarsia gracilis*, *S.* spec., and *Eutima* spec. The second group (N2) was located on the southern and central portions of the bay, with low diversity and co-occurring resident species *H. schulzei* and *P. discoidum*. The third group (N3) was dominated by *P. discoidum*, with ten more species (mainly neritic) and high diversity values (>2.0 bits/ind.).

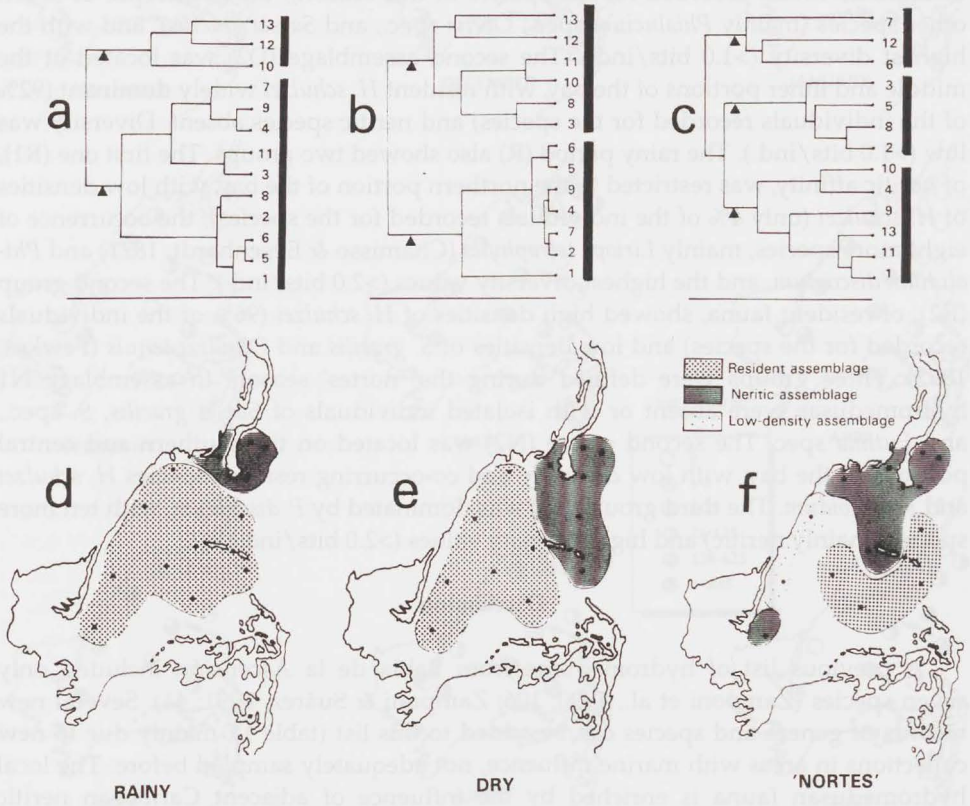
Discussion

A previous list of hydromedusae from Bahía de la Ascensión included only seven species (Zamponi et al., 1990: 106; Zamponi & Suárez, 1991: 41). Several new records of genera and species can be added to this list (table 1), mainly due to new collections in areas with marine influence, not adequately sampled before. The local hydromedusan fauna is enriched by the influence of adjacent Caribbean neritic waters, which are rich in species (Segura-Puertas, 1992: 353).

As in most tropical estuaries (Suárez, 1994: 248), local hydrological conditions

influence the dynamics of zooplankton. The local freshwater input is seasonal and the salinity gradient is weak most of the year; land drainage influence is most relevant during the 'nortes' periods. The degree of marine influence in the bay is related to the strength of a coastal countercurrent moving southwards along the Mexican Caribbean coast (Merino, 1986: 35) and entering to the system by its northern portion. These environmental conditions are determinant when considering the faunistic features of the system. In Bahía de la Ascensión, a number of marine plankton faunistic derivates penetrate year-round into the system via its northern and/or central portion (Gasca & Suárez, 1992: 118; Suárez, 1990: 217; Suárez et al., 1994: 8). Ecological affinities of the hydromedusae occurring in the bay produced a binary general grouping of the resident euryhaline species (*Helgicirrha schulzei*, *Phialidium discoidum*, and probably *Sarsia gracilis*) and the neritic species (*Sarsia* spp., *Phialucium* spp., *Liriope tetraphylla*, and *Euphysa* spp., among others). During the survey period, neritic hydromedusae were present only around the mouth area of the bay. So, the local distribution of the neritic species can roughly indicate the degree of marine influence.

Salinity gradients have been described as a key factor determining the horizontal distribution of zooplankton in bays and inlet waters (Kimmerer et al., 1985). Although the salinity gradient is low in Bahía de la Ascensión, salinity was probably



Figs. 3a-f. Dendrograms from station clustering by Bray-Curtis Index (a-c), and distribution of resulting assemblages during rainy, dry and 'nortes' seasons (d-f). For station numbers, see fig. 1.

associated with the local distribution of hydromedusae, contributing to the limits of the neritic and resident faunistic assemblages in the three seasons. Wind patterns could also affect the seasonal distribution of the highest total densities of hydromedusae. During the dry and rainy periods, winds from the southeast or from the east move the surface layers toward the innermost zone of the bay (sta. 1, 5-7 and 9), where hydromedusae seemed to be accumulated. During the 'nortes' period, in which northern winds are dominant, this pattern is reversed, with highest densities in the central and southern portions of the bay (sta. 2-4 and 8), and with very low densities or absence of hydromedusae at the innermost portions of the bay. This local effect of 'dispersion' by the 'nortes' winds, which is opposite to the 'accumulative' effect of southeastern winds, has been described also for zooplankton biomass (Suárez & Gasca, 1994: 116) and copepods (Suárez et al., 1994: 9).

Station clustering (Bray-Curtis Index) showed a seasonal zonation pattern. During the rainy and dry seasons two groups appeared: a neritic assemblage, with a limited distribution during the rainy period, and with a broader distribution in the dry season. During the rainy season, freshwater input along the innermost portions of the bay was very abundant, restricting the neritic assemblage to a small area and favouring the distribution of the resident assemblage (fig. 4a). In the dry season, a reduced freshwater input allowed a greater influence of neritic derivates which occurred along the mouth zone and reached the middle portions of the system (fig. 4b). The 'nortes' pattern showed both neritic and resident assemblages, plus another one with hydromedusae scarce or absent. The distribution of the neritic facies seemed to be related to both salinity gradients and the effect of northern winds, producing a southward drift of both assemblages.

Among other environmental variables, food condition has been considered as a causal factor of zooplankton distribution (Paffenhöfer, 1983: 15). Greatest densities of hydromedusae were observed mainly at near-shore sampling stations, where crustacean zooplankton showed its highest densities and biomass (Gasca & Suárez, 1994: 116; Suárez et al., 1994: 9). Local herbivorous populations, mainly of decapod larvae and copepods, seem to be the main food source for hydromedusae and should be accounted for as a factor influencing the local distribution of these cnidarians.

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Combined light and temperature effects on *Hydra* periodic behaviour

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Key words: *Hydra vulgaris*; bioelectric activity; photosensitivity; spectral sensitivity; temperature effects.

Abstract: Neither photosensitive organs, nor scattered photosensitive cells have been found in *Hydra*, although quantifiable photoresponses are shown by the animal. Its behaviour is characterized by a continuous alternance of a body shortening phase and a body elongating phase, to which bioelectric Contraction Pulses (CPs), organized in a Contraction Pulse Train (CPT), and a series of Rhythmic Pulses (RPs) are linked respectively and arise at different phases of a Big Slow Wave (BSW). Present results have ascertained that the durations of the behavioural period (inter-CPT period), the inter-CP period related to the shortening phase, as well as the inter-RP period related to the elongating phase, all vary inversely with the temperature variation in the range 5-29°C; contrastingly, the maximum BSW excursion varies directly with the temperature. At 17°C the inter-CPT period duration is inversely proportional to the background illumination wavelength variation in the range 450-700 nm and directly proportional in the range 400-450 nm. By applying to the same animal the variations of both temperature (in the range 9-31°C) and background illumination wavelength (either 450 or 550 nm, or complete darkness for comparison), while the inter-CP period, the inter-RP period and the number of CPs of a CPT are affected by temperature, the inter-CPT period can be influenced by both temperature and wavelength. The combination of the effects of the variation of the two factors simultaneously applied is such that the difference among the values of the inter-CPT period at different wavelengths is higher at lower temperatures.

Introduction

The behaviour of the cnidarian *Hydra* is characterized by a continuous alternation between a phase of body shortening and a phase of body elongation. Bioelectric "Contraction Pulses" (CPs), organized in a "Contraction Pulse Train" (CPT), and a series of "Rhythmic Pulses" (RPs) are linked respectively to shortening and elongation (Passano & McCullough, 1962; 1963; 1964; 1965), and arise at different phases of a "Big Slow Wave" (BSW) (Taddei-Ferretti et al., 1976). This behaviour, which involves neuromuscular pacemakers, is exhibited even under absolutely constant environmental conditions. Its meaning is still a matter of debate, although an osmoregulation function has been proposed (Benos & Prusch, 1973). The behaviour can be modulated by light and well quantifiable photoresponses are performed by the animal (Borner & Tardent, 1971; Ellis, 1970; Feldman & Lenhoff, 1960; Haase-Eichler, 1931; Haug, 1933; Passano & McCullough, 1962; 1963; 1964; 1965; Rushforth, 1971; Rushforth et al., 1963; Singer et al., 1963; Taddei-Ferretti & Cordella, 1976; Taddei-Ferretti et al., 1976; 1987; Tardent & Frei, 1969; Tardent et al., 1976; Trembley, 1744; Wilson, 1891). However, so far neither photoreceptor organs, nor scattered photosensitive cells as those of other Cnidaria (Bouillon & Nielsen, 1974), have been found in *Hydra*.

Light pulse stimuli shift the phase of the contractional sequence. The amount and direction of the shift depend on the application phase of the light pulse, on its polarity (Taddei-Ferretti & Cordella, 1976) and its wavelength (Taddei-Ferretti et al., 1988). Combined phase shifting effects of two light stimuli of different wavelength applied at the same time or in close succession have been observed (Taddei-Ferretti & Cotugno, 1991). The effect of a light pulse of a given wavelength depends also on the wavelength of the background illumination (Taddei-Ferretti et al., 1992b). The system responsible for the behaviour expression is affected by intrinsic noise, as can be inferred both by the slight variability of the behavioural period in undisturbed conditions, and by the skipping phenomenon observed during either the period entrainment or the shortening-phase suppression, both obtained by means of repetitive light pulse stimulation of suitable frequency (Taddei-Ferretti et al., 1992a).

Since preliminary observations have already shown that the length of the contraction period under undisturbed conditions can be influenced by different factors, such as the wavelength of the background illumination (Taddei-Ferretti et al., 1992b) or the temperature (Taddei-Ferretti & Musio, 1994), it seemed interesting to us to investigate in more detail the combined action of steady coloured light and of temperature on the various parameters of the behavioural expression.

Material and methods

A Swiss strain (and a few specimens of an African strain) of *Hydra vulgaris* Pallas, 1766, (syn. *H. attenuata* Pallas, 1766) as well as a few specimens of *H. magnipapillata* Itô, 1947, were used. The animals were cultured and tested in a solution described by Ham et al. (1956) at 18°C, under a circadian 600 lx light-darkness cycle, fed twice a week with *Artemia salina* nauplii, washed 2 h after the meal and used for experiments the day after the last feeding. Only animals without buds were used.

The bioelectric potentials were picked up using a polyethylene suction Ag/AgCl electrode (into which the base and part of the stalk of an animal were lightly sucked), an Ag/AgCl indifferent electrode and a PAR 113 preamplifier and displayed on a Tektronix 5031 oscilloscope and on a YEW 3047 pen recorder. The jar used for recording was a 250 ml pyrex beaker. Recordings were performed in alternating current or, according to experimental needs, in direct current. Unwanted vibrations during the recordings were excluded by means of an antivibration mount consisting of a heavy marble plate on three tennis balls.

Before the beginning of an experiment, each individual was kept in total darkness for 1 h and the period length of the contraction-extension cycle was recorded with the same method above described. When the temporal distribution of the CPTs was so spread that the evaluation of the intervals between the trains was meaningless (i.e., less than four CPTs were performed in darkness at 17°C during one hour), the animal was not tested further; if the evaluation was meaningful, the animal was used in the experiment. All four types of bioelectric events correlated to the periodic behaviour can be a useful monitor of the body behaviour itself. The first CP of a CPT has been arbitrarily chosen by us as a recognizable point of the behavioural pattern appearing repetitively.

The light source was a Philips 6824, 12 V, 100 W lamp. The wavelength was varied by means of Balzers interference filters, 5 cm diameter, 5 nm band, maximum

transmittance at 400, 450, 500, 550, 600, 650, 700 nm; an output of 7×10^{-5} $J.s^{-1}.cm^{-2}$, measured with a digital photometer Tektronix J16 with radiometric head J6502, was provided in all cases by using suitable neutral Balzers filters. The light from the illuminating sources passed only thorough a tube which was blackened on the inside and supported the Balzers filters which modified the output beam.

The temperature of the medium used during the biopotential recording was monitored by means of a YSI 43TD tele-thermometer equipped with a YSI 427 probe. The range of the tested temperatures was 5-31°C. The temperature changes were tested in one run for each animal, either in ascending, or in descending sequence. These changes were discontinuous and each animal was kept at a particular temperature for either 30 min, or 1 h (respectively for figs 1 and 3), in order to exceed the period of temporal hyperactivity that may occur in the animal as a response to a sudden change of the temperature.

A statistical evaluation was performed for some of the results and the mean of the data ± their standard deviation was calculated.

Results

1. The animals were tested in darkness and at different steady conditions of temperature in the range 5-29°C. The durations of the inter-CP period (related to the shortening phase), the inter-RP period (related to the elongation phase), and the behavioural period (inter-CPT period), all vary inversely with the temperature (fig. 1A, B, D). The maximum BSW excursion, however, varies directly with the temperature (fig. 1C).

2. The animals were tested at 17°C and at different steady conditions of background illumination wavelength in the range 400-700 nm. The duration of the inter-CPT period varies inversely to the background illumination wavelength in the range 450-700 nm, and directly in the range 400-450 nm (fig. 2A); the values in darkness and in white light are reported for comparison (fig. 2B); no clear correlation has been observed between background illumination wavelength and the value of either the inter-CP period, the inter-RP period, or the CP number of a CPT.

3. Experiments were then performed by testing each animal at different steady conditions both of temperature (in the range 9-31°C) and of background illumination wavelength (either 450 or 550 nm, or darkness for comparison). The inter-CP period and the inter-RP period are affected by temperature in the ways described in Results 1, while the number of CPs of a CPT follows the same trend (fig. 3A-C). The inter-CPT period is influenced by both temperature and wavelength (fig. 3D). The combination of the effects of the variation of the two factors simultaneously applied is such that the curves expressing the temperature dependence of the inter-CPT period at different wavelengths are not parallel one each other, but the spread among the curves is maximal for the low temperature condition.

Discussion

Temperature effects on *Hydra* reproduction (Dregol'skaya & Korotneva, 1980), feeding behaviour (Hirakawa & Kijima, 1980), budding (Park & Ortmeyer, 1972), and

development (Shostak et al., 1978), as well as on excitable neural membranes either directly, or through enzyme systems responsible of the energy levels for membrane potentials in models other than *Hydra* (e.g., Ayrapetyan, 1969; Carpenter, 1967; Watchel & Wilson, 1973) have been reported. Both light and temperature may control the free running of a rhythmic behaviour (Bünning, 1960; Engelmann et al., 1974; Ikeda & Tamioka, 1993; Winfree, 1980, pp. 379-382, 408-410; Zimmerman et al., 1968), either through an energy supply that modifies the rhythm frequency, or through the production of a signal shifting the system phase.

The spectral sensitivity recorded during the inter-CPT period in steady backgroud illumination conditions is the same as observed when applying different experimental protocols, such as: latency until the next CPT when steps of monochromatic light are applied at the beginning of the body shortening phase (Singer et al., 1963); efficiency of a sudden light onset for the interruption of a CPT in progress (Passano & McCullough, 1964); amount of body elongation during the administration of various series of alternating periods (2 min.) of two different wavelength illumination (Tardent et al., 1976); amount and direction of the phase shift of the contractional sequence with light pulses of different wavelength (Taddei Ferretti et al., 1988). The spectral sensitivity is also the same as that related to the *Hydra* phototropism (Wilson, 1891) and to the delay of the first post-stimulus RP after a light pulse applied during an RP series (Passano & McCullough, 1965).

In addition to the separate effects of temperature and of light wavelength on the behavioural expression, present results have shown an influence of temperature on

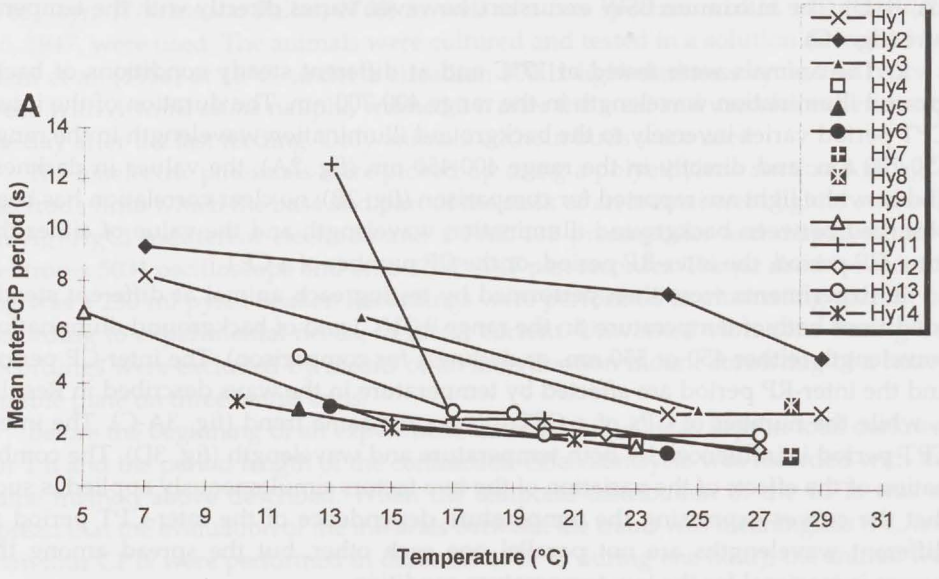
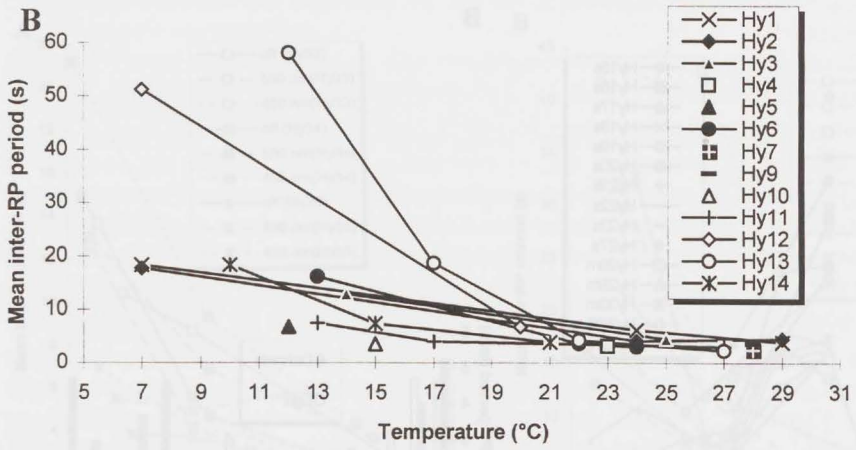
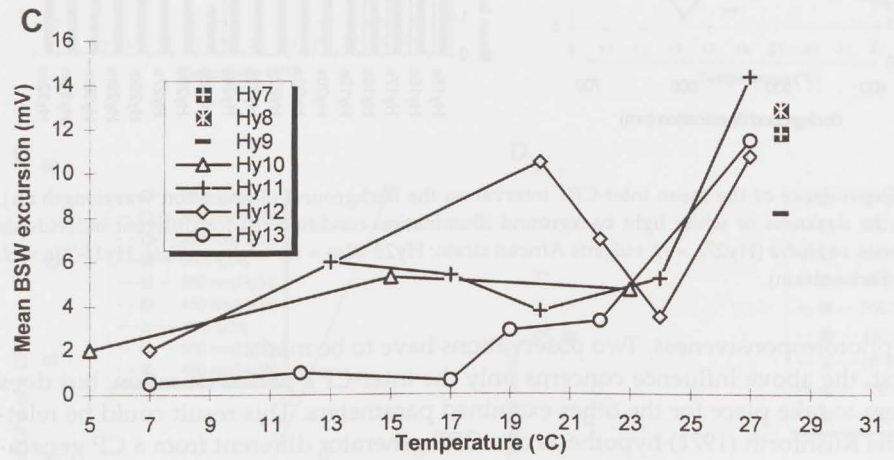
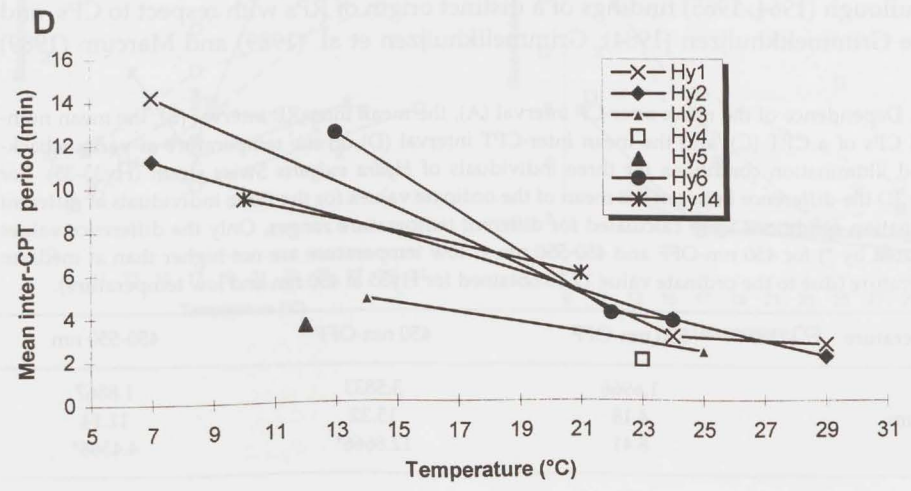


Fig. 1. Dependence of the mean inter-CP interval (A), the mean inter-RP interval (B), the mean BSW excursion (C), and the mean inter-CPT interval (D) on the temperature for different individuals of *Hydra vulgaris* (Hy1-14 Swiss strain). For figure 1C the mean of the ordinate values (m) and their standard deviations were calculated for different temperature ranges (t) and number of observations (n): t = 5-7, m = 1.5 ± 0.71, n = 3; t = 12-13, m = 3.5 ± 2.5, n = 2; t = 15-17, m = 3.8 ± 2.2, n = 3; t = 19-20, m = 5.7 ± 3.4, n = 3; t = 22-24, m = 4.8 ± 1.3, n = 5; t = 27-28, m = 11.6 ± 1.9, n = 6.


B
Mean inter-RP period (s)
Temperature (°C)
Hy1
Hy2
Hy3
Hy4
Hy5
Hy6
Hy7
Hy9
Hy10
Hy11
Hy12
Hy13
Hy14


C
Mean BSW excursion (mV)
Temperature (°C)
Hy7
Hy8
Hy9
Hy10
Hy11
Hy12
Hy13


D
Mean inter-CPT period (min)
Temperature (°C)
Hy1
Hy2
Hy3
Hy4
Hy5
Hy6
Hy14

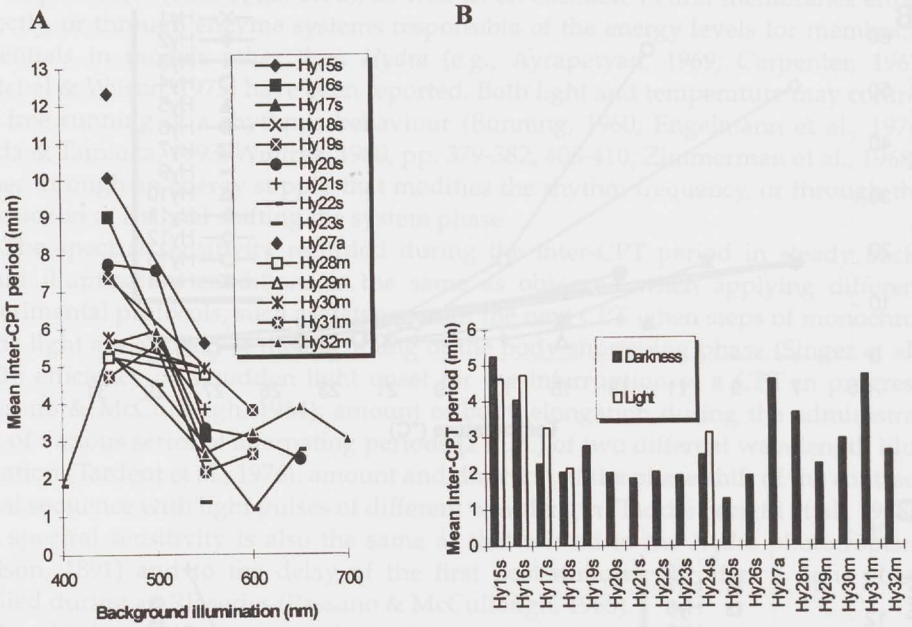


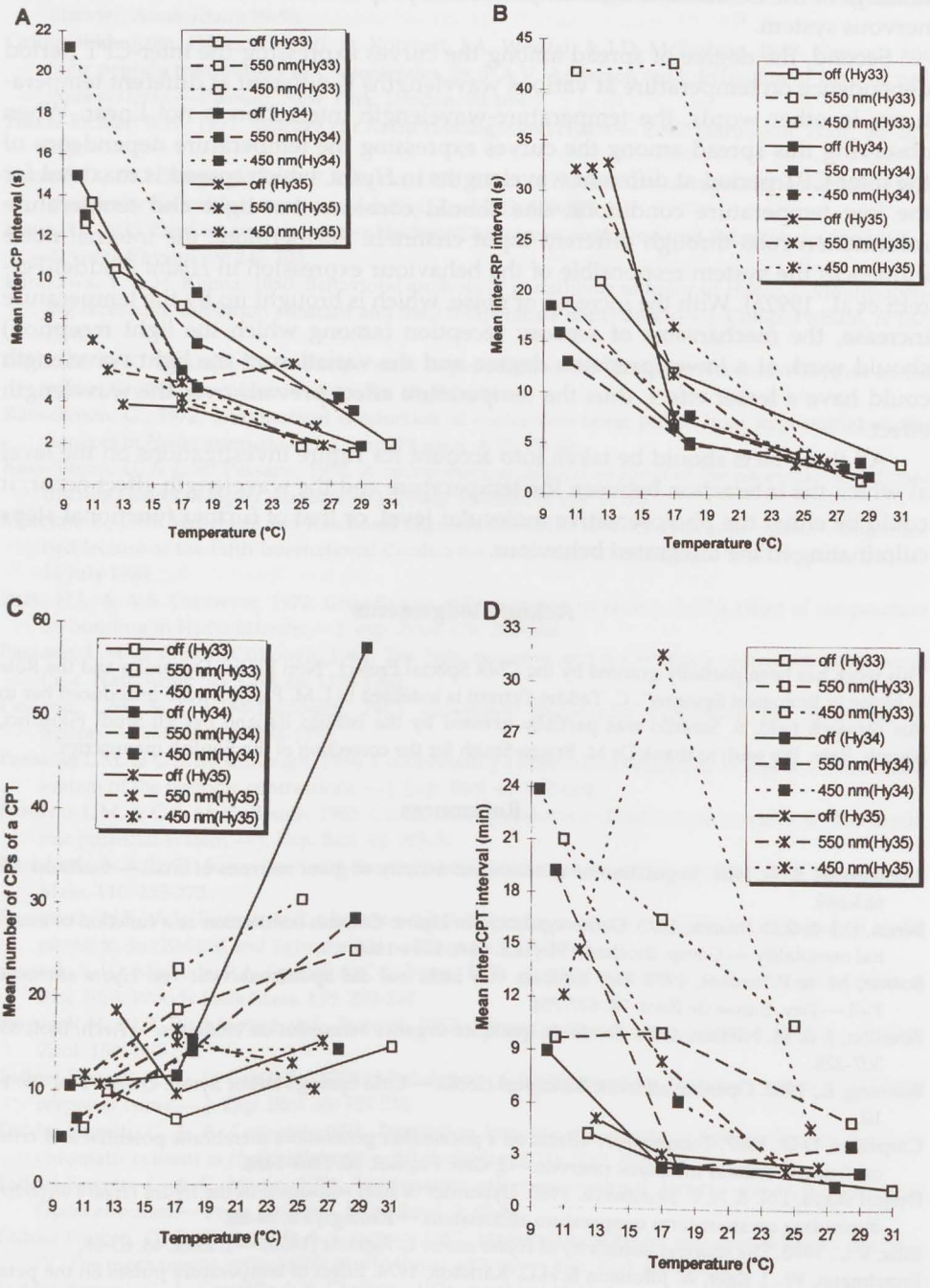
Fig. 2. Dependence of the mean inter-CPT interval on the background illumination wavelength (A), and on the darkness or white light background illumination condition (B) for different individuals and species of *Hydra* (Hy27a = *H. vulgaris* African strain; Hy28-32m = *H. magnipapillata*; Hy15-23s = *H. vulgaris* Swiss strain).

Hydra photoresponsiveness. Two observations have to be made:

First, the above influence concerns only the inter-CPT period duration, but does not seem to take place for the other examined parameters. This result could be related to the Rushforth (1971) hypothesis of a CPT generator different from a CP generator, as well as to the Kass-Simon (1972), Kass-Simon & Passano (1978) and Passano & McCullough (1964; 1965) findings of a distinct origin of RPs with respect to CPs, and to the Grimmelikhuijzen (1984), Grimmelikhuijzen et al. (1989) and Marcum (1989)

Fig. 3. Dependence of the mean inter-CP interval (A), the mean inter-RP interval (B), the mean number of CPs of a CPT (C), and the mean inter-CPT interval (D) on the temperature at various background illumination conditions for three individuals of *Hydra vulgaris* Swiss strain (Hy33-35). For figure 3D the difference between the mean of the ordinate values for the three individuals at different illumination conditions were calculated for different temperature ranges. Only the difference values (indicated by *) for 450 nm-OFF and 450-550 nm at low temperature are not higher than at medium temperature (due to the ordinate value 12.13 obtained for Hy35 at 450 nm and low temperature).

Temperature	550 nm-OFF	450 nm-OFF	450-550 nm
high	1.6966	3.5833	1.8867
medium-	4.18	15.32	11.14
low	8.41	12.8666*	4.4566*


A
Mean inter-CP interval (s)
Temperature (°C)
B
Mean inter-RP interval (s)
Temperature (°C)
C
Mean number of CPs of a CPT
Temperature (°C)
D
Mean inter-CPT interval (min)
Temperature (°C)
off (Hy33)
550 nm(Hy33)
450 nm(Hy33)
off (Hy34)
550 nm(Hy34)
450 nm(Hy34)
off (Hy35)
550 nm(Hy35)
450 nm(Hy35)

findings of the existence of different functional properties of various subsets of *Hydra* nervous system.

Second, the degree of spread among the curves expressing the inter-CPT period dependence on temperature at various wavelengths is different at different temperatures; in other words, the temperature-wavelength interaction is not linear. When observing this spread among the curves expressing the temperature dependence of the inter-CPT period at different wavelengths in *Hydra*, which spread is maximal for the low temperature conditions, one should consider that light and temperature information runs through different input channels. Furthermore, the internal noise influences the system responsible of the behaviour expression in *Hydra* (Taddei-Ferretti et al., 1992a). With the increase of noise, which is brought up by the temperature increase, the mechanisms of sensory reception (among which the light reception) should work at a lower precision degree and the variation of the light wavelength could have a lesser effect; thus the temperature effect prevails over the wavelength effect.

All these facts should be taken into account for future investigations on the level at which the interaction between the temperature and the wavelength effect occur; it could be either the photosensitive molecular level, or that of further functional steps culminating in the integrated behaviour.

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Distribution of *Aurelia aurita* (Linnaeus, 1758) in Tokyo Bay; observations with echosounder and plankton net

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Key words : Acoustic measurement; *Aurelia aurita*; aggregation; vertical distribution; horizontal distribution; coastal front region.

Abstract: A scientific echo sounder and plankton net were employed to investigate the distribution of *Aurelia aurita* (Linnaeus, 1758) in Tokyo Bay during summer 1990 and 1991. Echo traces of *Aurelia* aggregations were similar at 50 kHz and 200 kHz. They were spotted and had unclear edges; many of them were elliptical or round, and light at their center. The observed distribution of aggregations agreed well with results from net catches.
Aurelia aggregations were distributed from the ship's bottom to a few meters above the sea floor. The width of these, as crossed by the ship, varied from 22-790 m (most frequently from 22-140 m) in 1990 and 14-617 m (20-100 m) in 1991. Large aggregations occurred off the mouth of the Tamagawa River and near the narrow Uraga Strait.
Density of *Aurelia* in the aggregations, estimated from the volume scattering strength (Sv) at 200kHz, was 0.004-5.65 inds m^{-3} (mean = 0.3, SD = 0.90, n = 43) in 1990 and 0.06-3.72 inds m^{-3} (mean = 1.1, SD = 1.12, n = 20) in 1991. Densities determined from Sv and from net catches were positively correlated ($r^2 = 0.46$, $P<0.05$, n = 7).
Aurelia aggregations were most frequent within a salinity range of 28-33‰ during both years. Several aggregations were observed to move to the surface early in the morning. They also drifted with tidal currents. At the innermost part of the bay, they apparently aggregated near the estuarine frontal region, especially at the edge of the low salinity water mass.

Introduction

The formation of jellyfish aggregations has been discussed in several recent papers. Some studies have emphasized passive aggregation caused by Langmuir circulations (Hamner & Schneider, 1986: 173-175; Larson, 1992: 234), or wind, current and tidal movement (Zavodnik, 1987: 266-268). Others have focused on active swimming behaviour of jellyfish, such as diurnal migration (Hamner & Hauri, 1981: 415-420), or sun-compass migration (Hamner et al., 1994: 351-353).

Most observations have been done from above the surface visually. Underwater aggregations are mostly undetectable, especially in highly eutrophic coastal regions or at night. Acoustical detection of jellyfish, if feasible, offers at least two advantages; 1) underwater aggregation, invisible from the surface, could be detected, 2) the vertical section of the aggregations could be measured. Shimomura (1959: 100) reported that dense aggregations of *Stomolophus meleagris* L. Agassiz, 1862, in the Sea of Japan were recorded by echogram.

During summer in Tokyo Bay, *Aurelia aurita* (Linnaeus, 1758) forms dense aggregations. Such aggregations sometimes flow into the water intake systems of power plants and damage the cooling systems (Kuwabara et al., 1969: 156). The inner part of the bay is so highly eutrophic that diversity of megaplankton and nekton is low (Murano, 1980: 768; Furota, 1985: 375-376, 382-383). However, conditions of low diversity in the water column were favorable for acoustic sampling.

In this study, we used both a research echosounder and plankton nets. Vertical sections of *Aurelia* aggregations were made and the horizontal distribution of aggregations were studied in relation to coastal hydrodynamic features.

Material and methods

Sampling was carried out in Tokyo Bay on the RV 'Tansei Maru' on 13-16 July 1990, and on 2-3 September 1991. Stations were located to encompass the entire bay in 1990, and were located along a transect line from the mouth of Arakawa River to the mouth of the bay in 1991. Overnight sampling was carried out at Station T both years (fig. 1).

Acoustic scattering strength was measured and recorded during the cruise and samplings by research echosounder (FURUNO FQ50) at 50 kHz and 200 kHz. The transducer was located at the bottom of the ship (ca 4.2 m deep). Echograms from both frequencies were recorded on paper. Volume scattering strength (Sv) generated by echo integrator was recorded as text file and was analyzed later. Sv was integrated vertically at 5 m depth intervals and horizontally at 1 minute intervals or at 0.1 mile intervals. Jellyfish aggregations were determined empirically from echograms according to the type of echo trace (see following section). Sv in a medusa aggregation was standardized using mean Sv immediately before and after passing over the aggregation, and was averaged for the aggregation itself. As jellyfish exist not only in aggregations, this method yielded a conservative estimate.

During daytime at every Station, horizontal hauls were carried out at a depth of 5-10 m using an ORI net of 1.6 m mouth diameter and 1 mm (in 1990) or 2 mm (in 1991) mesh size. The volume of filtered water was calculated from the revolution of a flow meter attached at the mouth of the net.

From 18:00 JST (Japan Standard Time, + 9:00) on 13 July through 5:00 JST on 14 July 1990, and from 15:00 JST on 2 September through 12:00 JST on 3 September 1991, Station T was sampled intensively. A Norpac Twin net (mesh size 0.10 mm and 0.33 mm in 1990, 0.10 mm in 1991) was towed vertically from about a meter above the bottom to the surface. The volume of filtered water was calculated as above. In 1990 another type of net (80 cm diameter, 2 mm mesh) was occasionally towed vertically to confirm jellyfish aggregations under water. No flow meter was installed on these, and 100% filtering efficiency was assumed.

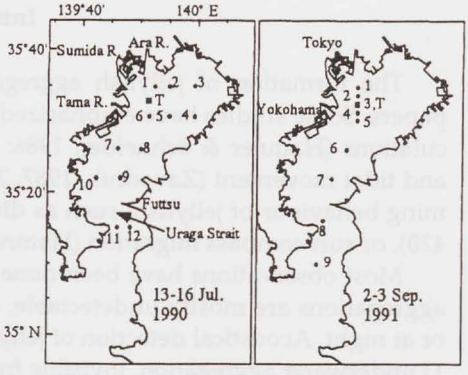

Fig. 1. Location of sampling stations in Tokyo Bay.

Collected jellyfishes were counted and diameters were measured on a pair of acrylic plates which sandwiched a sheet of graph paper. Vertical profiles of water conductivity and temperature were measured by CTD at every Station and at 3 hours intervals at Station T. Salinity was calculated from conductivity.

Results

Determination of *Aurelia* aggregations from the echograms

Echograms made when medusae were abundant, from the results of net catches, are shown in fig. 2a-e. The sampling method and number of jellyfish collected, are shown in table 1.

These echo traces had unique characteristics; 1) they were fuzzy and looked like a cluster of dots, and 2) they were similar at both frequencies. Such characteristics were consistent with the echo traces of *Stomolophus meleagris* (see Shimomura, 1959: 100).

Echo traces of fish have dark, uniform intensity and clear edges (fig. 2f-g). Typically, fishes have a solid body with an air bladder and they scatter sound strongly. In contrast, jellyfishes have a soft body without a bladder, scatter sound weakly. Echo traces of sound scattering layers were also fuzzy and showed dot-like features. However, the traces were much weaker at 200 kHz than at 50 kHz (fig. 2g).

From the accordance between echograms and net catches, and from the unique characteristics of echo traces, we concluded that jellyfish aggregations could be detected from the echo traces. We used the above characteristics to distinguish echo traces of jellyfishes from those of other organisms.

Two large jellyfish species, other than *Aurelia aurita*, were present during these cruises, the scyphomedusa *Chrysaora melanaster* Brandt, 1838, and the ctenophore *Bolinopsis mikado* (Moser, 1907). However, these species were much less abundant than *A. aurita* (<1/100, Toyokawa, unpublished data). We therefore assume that all of the jellyfish aggregations recorded during the two cruises were primarily *A. aurita*.

Vertical sections of *Aurelia* aggregations

In addition to the above features, vertical sections of *Aurelia* aggregations showed

Table 1. Density of jellyfishes estimated from the results of net samplings, when typical echo traces of jellyfish occurred.

Echo trace	Gear / hauling method	Number of jellyfishes caught	Estimated density of jellyfishes (inds/m^3)
Figure 2(a)	Norpac 0.33mm / vertical	*Aurelia* : 5	*Aurelia* : 2.3
Figure 2(b)	ø 80cm, 2mm / vertical 4 replicates	*Aurelia* : 17	*Aurelia* : 2.3
Figure 2(c)	ø 80cm, 2mm / vertical	*Aurelia* : 68	*Aurelia* : 9.0
Figure 2(d)	ø 80cm, 2mm / vertical	*Aurelia* : 101	*Aurelia* : 13.4
		Chrysaora : 1	*Chrysaora* : 0.1
		Bolinopsis : 7	*Bolinopsis* : 0.9
Figure 2(e)	ORI 2mm / horizontal	*Aurelia* : 678	*Aurelia* : 0.9
		Chrysaora : 1	*Chrysaora* : 0.001

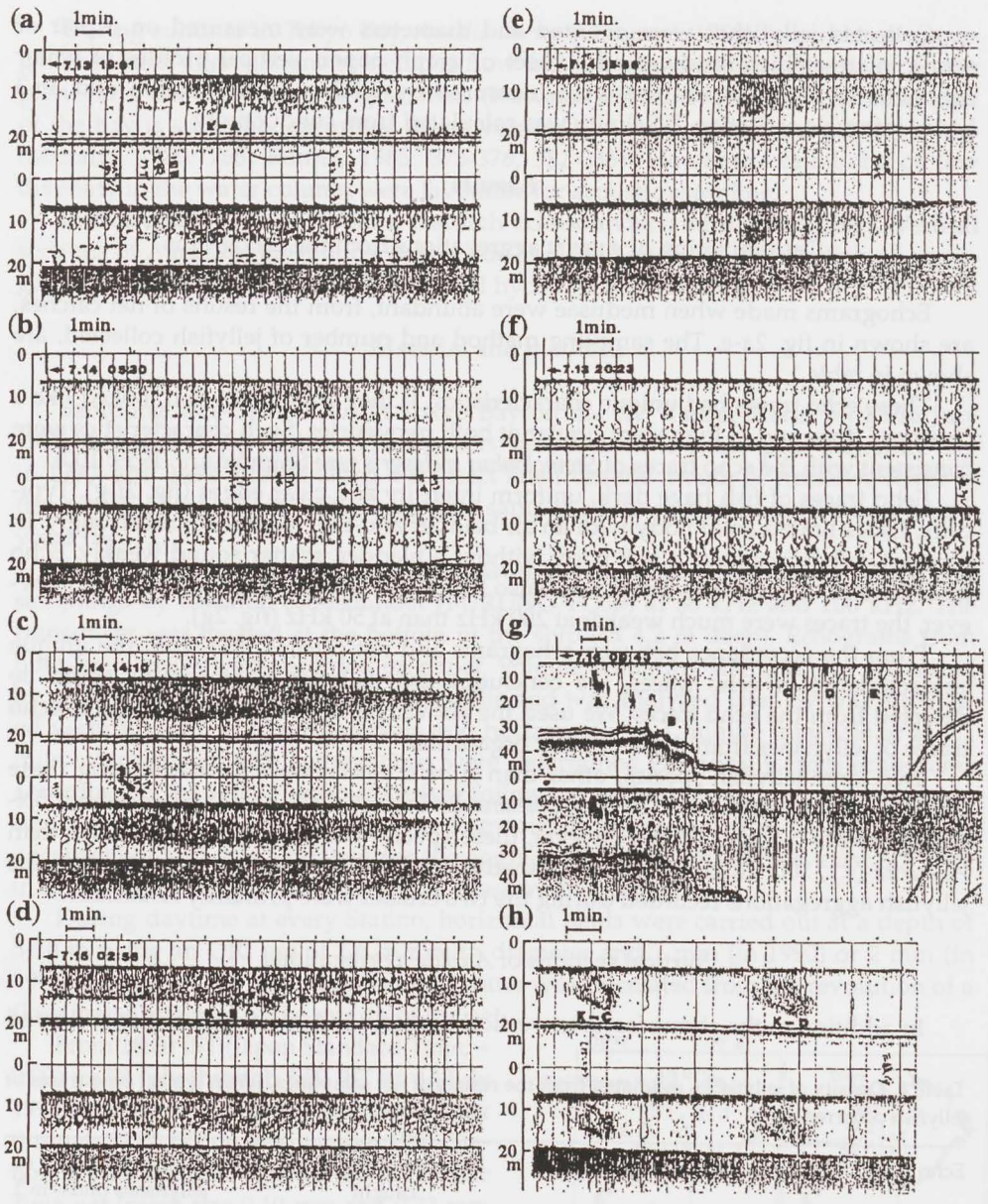

Fig. 2. Echo traces of (a)-(e), (h) *Aurelia* aggregations, (f) small fish at night, and (g) fish schools. Upper traces are at 200 kHz, lower traces at 50 kHz; (a)-(e) were confirmed from the results of net samplings.

the following characteristics. Many of their vertical sections were elliptical or round, and were hollow or light at their centers (fig. 2h). Such ring-like shapes were observed in 20 aggregations out of 45 in 1990, and 11 out of 20 in 1991.

In many cases, the aggregations were not visible or only several individuals were seen from the deck about 4 m above the surface. However, there were two aggrega-

tions which were visible from the deck. One was at dawn at Station T in 1990, and the other was at Station 9 during the same cruise. In most of the aggregations, the upper boundary of the trace was unclear, because of noise at the ship's bottom, and was thought to be 6 m or shallower. However, in 1990, in the innermost part of the bay, where salinity of the surface water was low, the upper boundaries of several aggregations were deeper than 8 m (fig. 5a). Their lower boundaries were restricted to a few meters above the sea floor in most cases (figs 2, 5, 6). The mean depth of the lower boundary of the aggregations was 15.9 m (SD = 2.39, n = 45) in 1990, and 19.9 m (SD = 4.47, n = 20) in 1991. The mean distance from the seafloor to the lower boundary was 4.8 m (SD = 3.31, n = 45) in 1990, and 6.3 m (SD = 2.52, n = 20) in 1991. The variance to mean ratio was little different, whether measured from the surface or the bottom.

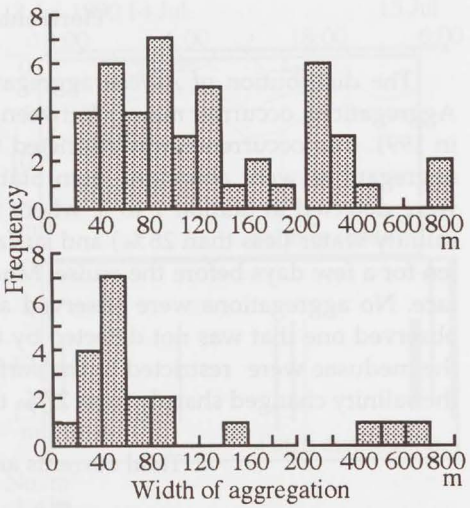

Fig. 3. Frequency distribution of the width of *Aurelia* aggregations passed over by the ship (or passing under the drifting ship).

The width of the aggregations was 22-790 m in 1990, and 14-617 m in 1991. The most frequent range was 22-140 m in 1990, and 20-100 m in 1991 (fig. 3). Large aggregations, the widths of which were more than 400 m, occurred near the mouth of the Tamagawa River and near the mouth of the bay in 1990. In 1991 such large aggregations all occurred slightly southward of the mouth of the Tamagawa River. Increased inflow of freshwater from rivers may have carried the aggregations towards the mouth of the bay (fig. 5b).

The mean diameter of all collected *Aurelia aurita* was 18.2 cm in 1990, and 15.2 cm in 1991. The mean diameter did not differ much among stations. We used the average target strength (-64.4 dB) measured at 200 kHz with a medusa of *Aurelia* whose diameter was 17.0 cm (Saito et al., 1990: 114). The density of *Aurelia* aggregations estimated from the average Sv at 200 kHz, and estimates from net catches, were significantly correlated, but the correlation coefficient was low (r^2 = 0.46, P<0.05, n = 7). In six cases out of seven, estimates from Sv were lower than that from net sampling (fig. 4). The estimated density from Sv was 0.004-5.65 inds. m^{-3} (mean = 0.3, SD = 0.90, n = 43) in 1990 and 0.06-3.72 inds m^{-3} (mean = 1.1, SD = 1.12, n = 20) in 1991.

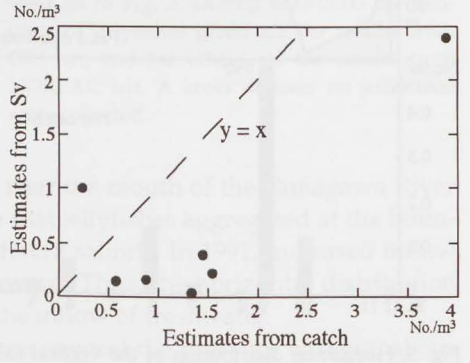

Fig. 4. Relation between the estimated density of *Aurelia* aggregations from net catches and estimates from volume scattering strength (Sv). Dotted line shows where both are equal.

Horizontal distribution

The distribution of *Aurelia* aggregations followed closely the salinity contours. Aggregations occurred mainly between salinity range 30-33‰ in 1990, and 28-33‰ in 1991. The occurrence was restricted to the inner part of the bay (fig. 5). In 1991, aggregations were abundant from Station 3 to Station 7. Almost no aggregations were detected at Station 1 to 3, where the upper 5 m layer was covered with low-salinity water (less than 28‰) and surface salinity was less than 19‰. Rain had fallen for a few days before the cruise. Much wood and waste was observed at the surface. No aggregations were observed at the surface except at Station 7, where we observed one that was not detected by the echosounder. This was probably because the medusae were restricted to the surface layer (shallower than 6 m). At Station 7, the salinity changed sharply from 27‰ to 32‰ within the upper 5 m layer.

Tidal currents and vertical migration

No aggregation occurred at Station T in 1991. In 1990, eight aggregations were

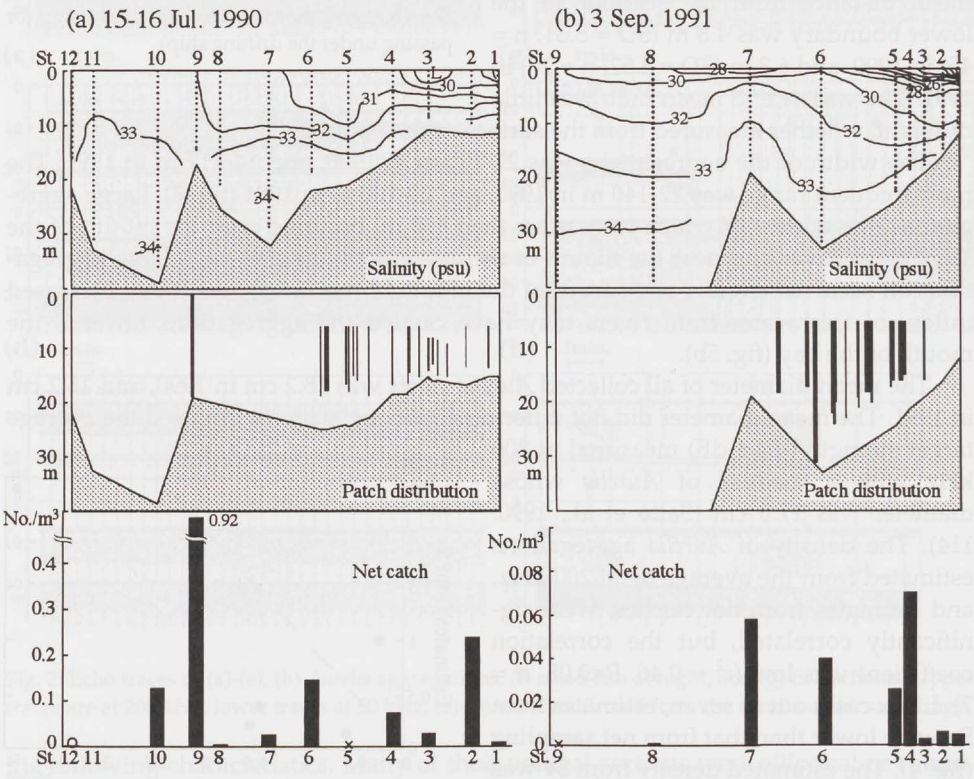

Fig. 5. Horizontal distribution of the vertical salinity profile (top), *Aurelia* aggregations (middle) and density of *Aurelia* estimated from net catches (bottom) during the cruises on 15 July 1990 and on 3 September 1991. In the figures of patch distribution, the width of the bars represent the width of aggregations where the ship passed over them, and the vertical ranges of the bars represent the depth ranges at which the aggregations occurred. In the figures of net catches, a cross denotes no jellyfishes were collected. There were no collection data at Station 5 because of sampling failure.

observed. All of them appeared at ebb tide, when the surface salinity was low (fig. 6), and almost no jellyfish occurred during flood tide. More aggregations occurred when the surface salinity was at normal levels than when the surface salinity was sharply reduced to less than 20‰ (at 6:00 JST on 14 July). This pattern suggests that *Aurelia* aggregations tend to be formed at the edge or just outside the estuarine front-region.

Two aggregations were observed by eye from the deck at dawn on 15 July. Four aggregations were observed from above the surface in 1990. All of these occurred between 4:00 and 7:00 JST. This observation coincides with Yasuda (1973: 1148), who reported that *Aurelia aurita* migrates to the surface at sunrise and sunset.

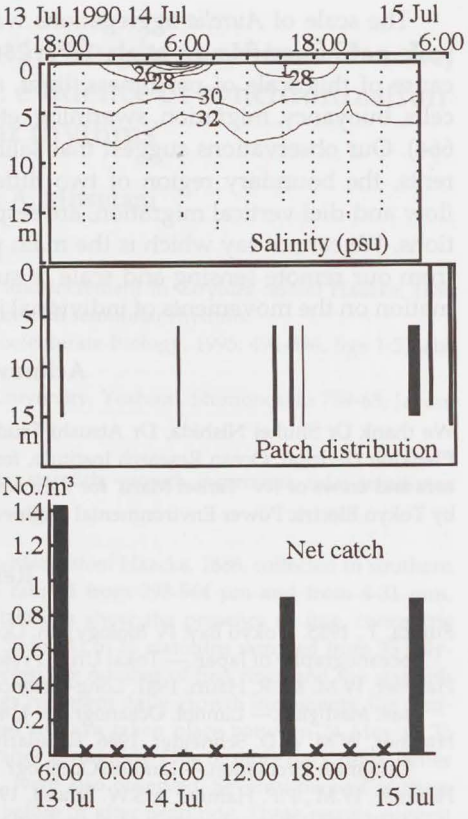

Fig. 6. Temporal distribution of the vertical salinity profile (top), *Aurelia* aggregations (middle) and density of *Aurelia* estimated from net catches (bottom), at station T during 18:00 on 13 July through 6:00 on 15 July 1990. The bars in the figure of patch distribution are the same as in Fig. 5. During 6:00-15:00 the densities of jellyfishes given are the results from ORI net, and the others are the results from NORPAC net. A cross denotes no jellyfishes were collected.

Discussion

The vertical distribution of *Aurelia* aggregations was apparently influenced by the salinity of the water, with 28-33 being their favoured salinity range. Though *Aurelia aurita* can change its density and adapt to that of the ambient seawater, this process takes 3-4 hours (Oshima et al., 1967: 168). These authors also reported that when gravity of the water was lowered as much as 0.008, all of the jellyfishes sank to the bottom. When surface salinity was less than 20‰, the density would be 0.004 lower than at 30‰, at 20°C. It is possible that jellyfishes occurring at a salinity of 28-33‰ would be restricted in their swimming range when surface salinity was lowered by inflow of freshwater.

Large scale aggregations tended to occur near the mouth of the Tamagawa River or near the narrow Uraga Strait. It is possible that jellyfishes aggregated at the boundary region between two watermasses of different salinity. In 1991, increased inflow of freshwater pushed the aggregations southward. Thus, the horizontal distribution of *Aurelia* aggregations is also influenced by the inflow of freshwater.

The ring-like shapes of *Aurelia* aggregations suggest the presence of regional circular flows. However, there was no additional evidence supporting the existence of Langmuir circulations during our study. Simultaneous measurement of flow will be necessary in future studies.

The scale of *Aurelia* aggregations was 20-800 m. This size is in the range of fine-scale patchiness (Haury et al., 1978: 286). Various factors can be expected to be the cause of this scale of patchiness: light, salinity, fronts, tidal current shear, convective cells, buoyancy, migration, swarming, etc. (Haury et al., 1978: 292; Mackas et al., 1985: 664). Our observations suggest that salinity gradients, inflow of freshwater, tidal currents, the boundary region of two different water masses, regional vertical circular flow and diel vertical migration, are responsible for the generation of *Aurelia* aggregations. We cannot say which is the main process generating aggregations in Tokyo Bay from our remote sensing and scale of survey. A smaller scale survey, and more information on the movements of individual jellyfish, are needed in future studies.

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Statolith formation and increment in *Carybdea rastoni* Haacke, 1886 (Scyphozoa: Cubomedusae): evidence of synchronization with semilunar rhythms

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Ueno, S., C. Imai & A. Mitsutani. Statolith formation and increment in *Carybdea rastoni* Haacke, 1886 (Scyphozoa: Cubomedusae): evidence of synchronization with semilunar rhythms.
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 491-496, figs 1-3, tabs 1-2.
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Key words: Cubomedusae; *Carybdea rastoni*; life cycle; statolith; growth increment; tide; semilunar rhythm.

Abstract: Statoliths were taken from specimens of *Carybdea rastoni* Haacke, 1886, collected in southern Japan. The statolith lengths and jellyfish bell widths ranged from 298-564 µm and from 4-31 mm, respectively, in 145 specimens examined. Polished statoliths show the presence of fine, concentric growth rings. The number of these rings ranged from 59 to 141 in 35 statoliths sampled from 35 jellyfishes. There is a significant positive relationship between the number of fine rings and the statolith length ($p < 0.001$, $r = 0.73$). Assuming that the fine rings represent daily growth increments, the commencement of statolith formation in 1994 was inferred to have taken place between 24 May to 23 August, in 86% of the cases within two days before or after spring tide. One or more dark rings, better developed and more conspicuous than the fine rings, were also observed. Seventy percent of these dark rings coincided with a period within three days before or after neap tide. These results suggest that statolith formation is appreciably correlated with semilunar rhythms.

Introduction

Annual rings have been used in the biological study of many kinds of plants and animals, e.g. gymnosperms, angiosperms, molluscs, vertebrates and so on. In cnidarians, annual growth rings are found in the skeleton of gorgonian and antipatharian corals (Grigg, 1974, Grigg, 1977; Grange & Singleton, 1988). Daily growth rings are recognized in fish otoliths and squid statoliths (Pannella, 1971; Natsukari & Komine, 1992). Cubomedusae also have fairly large statoliths in the well-developed statocyst which is composed of calcium sulfate (Spangenberg & Beck, 1968; Chapman, 1985). Recently in jellyfish, Ueno et al. (1995) found for the first time fine growth rings in the statoliths of the cubomedusa *Carybdea rastoni* Haacke, 1886, which were ground with polishing paper. The authors found a significant positive relationship between the number of fine rings and statolith length and suggested that the number of fine rings might correspond with the number of days lapsing between the liberation of young medusae and the sampling date. The present paper examines the relationship between the formation of fine rings of *C. rastoni* and semilunar rhythm.

Material and methods

One hundred and forty-five specimens of *Carybdea rastoni* were collected using

plankton nets and buckets during the period from August to November in 1993 and 1994 at two small fishing ports, Kawatana and Waita, located on the western tip of Honshu, Japan (fig. 1). Statoliths were taken from the statocysts of living jellyfish within a few hours after collection and mounted on glass slides with needle and forceps. The jellyfish bell width and statolith length were measured with a stereoscopic and a standard light microscope, respectively. The statoliths were embedded in epoxy resin after drying in the laboratory for one day. After a further one day for the coagulation of the resin, they were ground using polishing papers with fine alumina grains (minimum grain size 0.3 μm in diameter). Thirty-five polished statoliths were used for observations of the fine rings using a standard light microscope.

Results

The statolith lengths ranged from 298 to 564 μm in the 145 specimens. The mean coefficient of variation of statolith length within an individual jellyfish was 1.3% with a maximum value of 10.0%. The bell widths ranged from 4 to 31 mm (mean ± SD = 17.1 ± 4.4 mm). A significant positive relationship was found between statolith

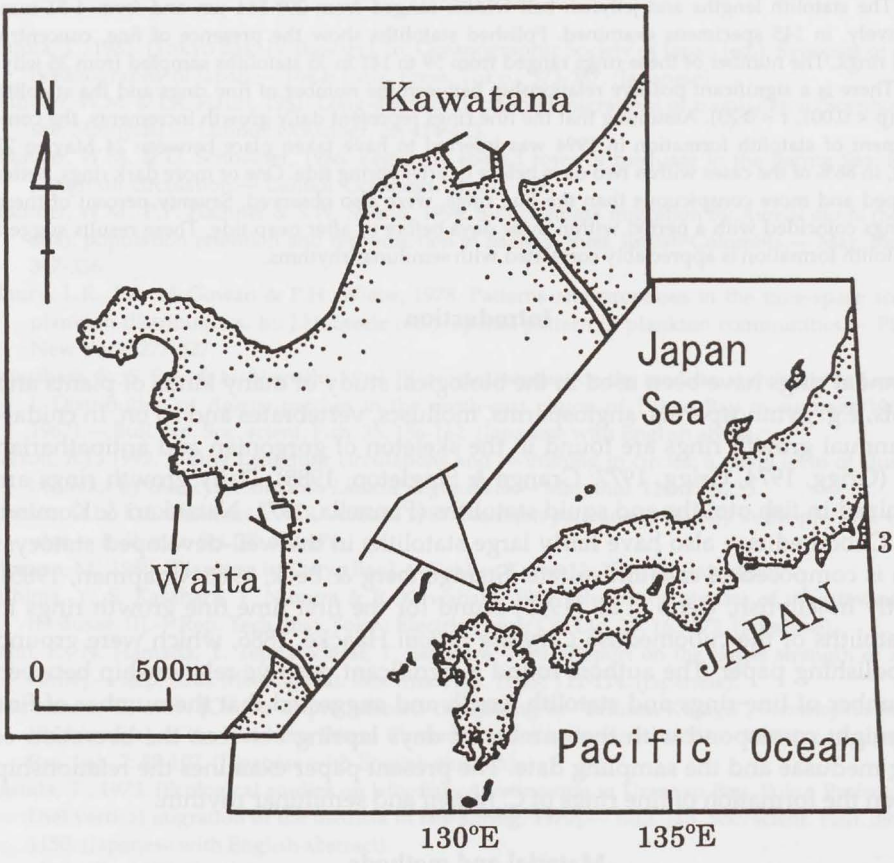

Fig. 1. Sampling sites of specimens of *Carybdea rastoni* used for the present study.

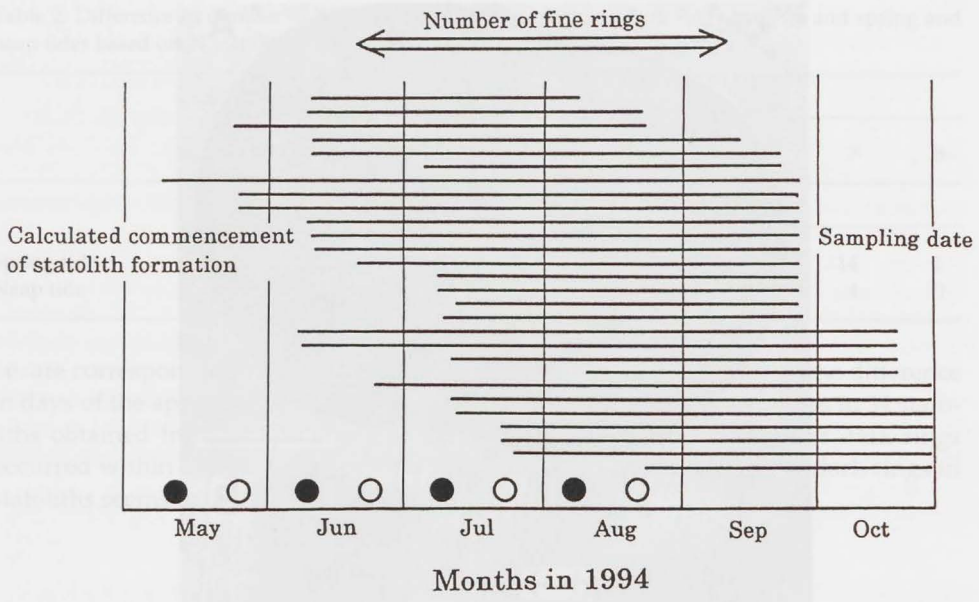

Fig. 2. Dates of commencement of statolith formation (left end of horizontal lines) in 28 specimens of *Carybdea rastoni* calculated from the sampling date and the number of fine rings, assuming that one fine ring is formed per day. ○ = full moon, and ● = new moon.

length and bell width ($p < 0.001$, $r = 0.81$, $n = 145$). The number of fine rings ranged from 59 to 141 among 35 statoliths sampled from 35 jellyfishes (mean ± SD = 102 ± 7.7). We also ascertained a significant positive relationship between the number of fine rings and statolith length ($p < 0.001$, $r = 0.73$, $n = 35$). These relationships indicate that the ring number increases with the statolith length increment and suggest that the fine rings are growth rings.

Assuming that the fine rings are daily increments of the statolith, the commencement of statolith formation was calculated using the sampling date and the number of fine rings. The calculated date of commencement of statolith formation in 1994 was obtained over nearly four months from 8 May to 23 August (fig. 2). The calculated commencements seem to be distributed particularly near full and new moons when spring tides took place. Table 1 shows the frequency of the difference in days

Table 1. Difference in number of days between the calculated commencement of statolith formation and spring and neap tides, based on 28 statoliths (cf. fig. 2).

	Difference in number of days							
	0	1	2	3	4	5	6	7
	Frequency of occurrence of calculated commencement							
Spring tide	7	12	5	1	2	1	0	0
Neap tide	0	0	0	2	0	7	10	9

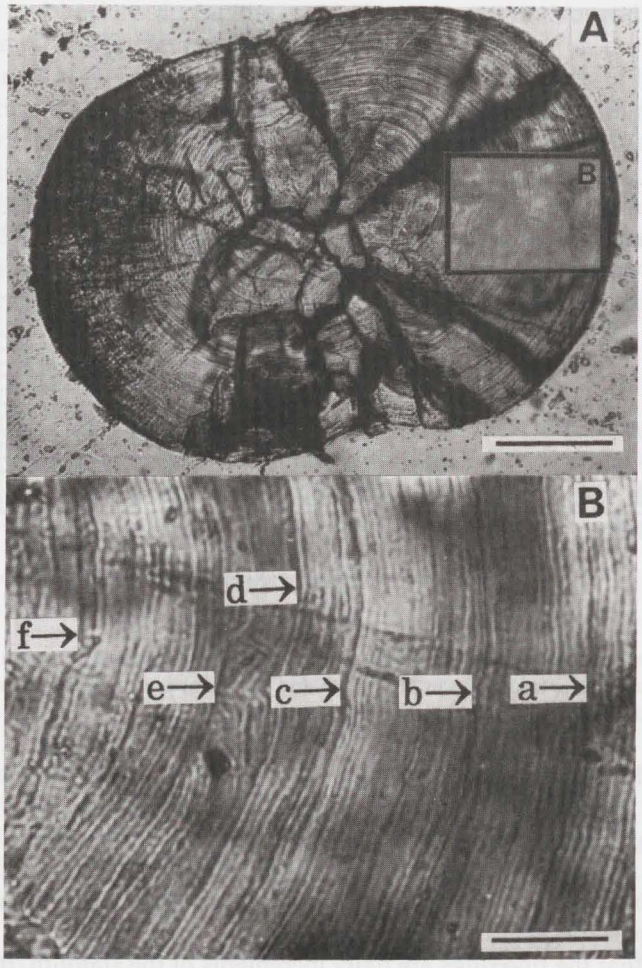

Fig. 3. Dark rings on a polished statolith of *Carybdea rastoni* sampled on 31 October, 1994.
A. Statolith with some large cracks produced by the grinding process. Bar = 100 μm. B. Magnification of outlined rectangle in fig. 3A; a, b, c, d, e and f indicate dark lines which are estimated to have been formed on 17 Oct., 6 Oct., 22 Sep., 14 Sep., 7 Sep. and 21 Aug., respectively. Bar = 25 μm.

between the calculated commencement dates of statolith formation and the dates of spring and neap tides. Ca. 86% of the calculated commencements were within two days before or after spring tide, and on the other hand only ca. 7% were found within four days before or after neap tide. This suggests that the initiation of statolith formation is correlated with spring tide.

In addition to the fine rings we also noted the occasional presence of heavier, more conspicuous rings in the polished statoliths. The number of these dark rings per statolith was variable, but dark rings appeared at a certain interval (fig. 3). Assuming that the fine rings are daily increments, the dates of dark ring formation correspond to 12 October, 30, 14 and 7 September, 29 and 11 August. These dates, except for 7 September, are distributed within 3 days around the first and last quarters of the moon,

Table 2. Difference in number of days between the occurrence of dark rings (n = 82) and spring and neap tides based on 34 statoliths.

	Difference in number of days								
	0	1	2	3	4	5	6	7	8
	Frequency of occurrence of dark rings								
Spring tide	4	4	8	7	11	16	17	14	1
Neap tide	12	14	20	11	8	8	5	4	0

i.e. are corresponding with neap tides. Table 2 presents the frequency of the difference in days of the appearance of dark rings in relation to spring and neap tides in 34 statoliths obtained from 34 specimens of *Carybdea rastoni*. Seventy percent of dark rings occurred within 3 days before or after neap tide. Thus, the formation of dark rings in statoliths seems to correlate with neap tides.

Discussion

The results of this study suggest that the formation of statoliths and fine rings are appreciably correlated with semilunar rhythms. This study also indirectly supports evidence that daily growth increments occur as fine rings on the polished statolith of *Carybdea rastoni*, as suggested by Ueno et al. (1995).

According to Werner et al. (1971), the rhopalia of the cubomedusa *Tripedalia cystophora* Conant, 1897, are formed during the last phase of metamorphosis, taking 4-5 days from the adult polyp to the medusa. This is in accordance with the present calculated commencement of statolith formation in *Carybdea rastoni*, which corresponds essentially to the day of medusa liberation.

Medusa liberation in *C. rastoni* would take place particularly near and around spring tides and dark rings might be formed around neap tides. Among Cnidaria semilunar synchronous spawnings are well-known from many coral species (Babcock et al., 1986; Wyers et al., 1991). But, so far there are no reports on the existence of a semilunar synchronism in medusa liberation. Since semilunar rhythms may be an important factor affecting the life cycle of *C. rastoni* inhabiting in coastal waters, the possible relationship between medusa liberation, dark ring formation and semilunar rhythm is a very attractive subject for ecological study of medusae.

Acknowledgements

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Preliminary assessment of the phylogeny of Pennatulacea (Anthozoa: Octocorallia), with a reevaluation of Ediacaran frond-like fossils, and a synopsis of the history of evolutionary thought regarding the sea pens

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Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 497-509, figs 1-3, tab. 1.
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Key words: Pennatulacea; sea pens; phylogeny; evolution; history of evolutionary thought; Ediacaran fauna.

Abstract: A preliminary assessment of the phylogeny of sea pens is presented, as well as a synopsis of the history of the literature pertaining to the evolution and phylogeny of the Pennatulacea, and a reassessment of the Ediacaran frond-like fossils in light of phylogenetic and fossil evidence. Examination of recently acquired material of broad geographic scope, in addition to older collections and the literature, has allowed for a phylogenetic reassessment of the group using cladistic analyses.
The published history of evolutionary thought regarding the pennatulaceans spans the period 1870-1916. It was not until 1994 that the subject was resurrected.
Distributional and phylogenetic data support the hypothesis that the sea pens first differentiated in tropical shallow-water and subsequently dispersed to and diversified in temperate and polar regions, and to all ocean depths, as well as the shallow-water tropics. Primitive, mostly tropical shallow-water taxa are represented by *Cavernularia* and *Veretillum*, while variously derived deeper water taxa of widespread distribution include *Funiculina, Chunella, Umbellula, Pennatula, Gyrophyllum, Distichoptilum*, and *Kophobelemnon*. *Pteroeides* is an example of a derived taxon represented mostly in tropical shallow-water.
The veretillid sea pens are postulated to represent the most primitive or basal group of Pennatulacea, and at the same time they represent a monophyletic clade distinguished from other octocorals by a distinctive set of apomorphies.
The origin of the Pennatulacea is sought in alcyoniid-like ancestors. At least superficial similarities are found between veretillids such as *Lituaria* and soft coral taxa such as *Verseveldtia* and *Bathyalcyon*. If strong evidence is found for alcyoniid ancestors of the pennatulaceans, then the Alcyonacea *sensu lato* must necessarily be considered paraphyletic.
Morphological and phylogenetic evidence does not support the premise that frond-like fossil taxa of the Ediacaran and Burgess Shale faunas such as *Charniodiscus, Vaizitsinia, Khatyspytia*, and *Thaumaptilon* represent pennatulacean octocorals. The lateral branches of the frond-like fossils and the polyp leaves of leafy sea pens not only appear to be non-homologous but not even functionally convergent.

Introduction

Octocoral systematics at present is often an equivocal and disputatious field (Weinberg, 1976). Our knowledge concerning this group of Anthozoa is far from adequate (Bayer, 1981). Substantial confusion has arisen in the literature because of a general lack of knowledge concerning variability; some octocoral species can be

exceedingly variable and many authors have described separate taxa based on intraspecific variants. Comprehensive collecting in many different geographic localities and detailed comparison of material is essential to adequately assess the degree of phenotypic variation in many taxa due to genetic, geographical, or ecological differences. In addition, the state of the literature for many taxa is inadequate. Many descriptions are very poor in their lack of detail, illustrations, or designation of type or voucher material. Entire groups, such as the gorgonian families Melithaeidae and Ellisellidae, need comprehensive revision before positive identifications of many taxa are possible.

The Pennatulacea or sea pens, are a very distinct and highly specialized group of benthic and sessile coelenterates, found throughout the world's seas at all depths (Hickson, 1909; Bayer, 1956; Williams, 1990). They are morphologically very diverse and exhibit trends toward bilateral symmetry, the development of lateral processes as polyp leaves or ridges, localization and concentration of polyps, and reduction in size and number of sclerites. The group as a whole was last monographed by Kükenthal (1915), who provided only brief, sparsely-illustrated descriptions. Hickson (1916) and Williams (1990a) provided regional faunas in which many taxa were included. Williams (1989) reviewed the genus *Cavernularia*, provided a synopsis of the living genera of sea pens (Williams, 1995a), and revised the genus *Sarcoptilus* (Williams, 1995b) .

Of the 436 nominal species names appearing in the literature, approximately one half are at present considered valid (Williams, 1995a). Thirty-two genera in fifteen families are currently recognized. The greatest diversity of taxa is found in the Indo-Pacific. Several species exhibit near-cosmopolitan distributions. The sparse fossil record extends back to the Cretaceous and Tertiary Periods; it is represented by *Virgularia*, *Graphularia*, and *Pteroeides*, with likely alcyonacean sclerites from the Silurian (Bengtson, 1981) and controversial and problematic taxa from the Ediacaran fauna (Bayer, 1956). Unlike other octocorals, sea pens are characterized by having the bulk of the body composed of a single large primary polyp, the oozooid. Lateral budding of the oozooid body wall gives rise to two or three kinds of secondary polyps (always autozooids and siphonozooids, and rarely mesozooids). The oozooid has a basal fleshy peduncle that acts to anchor the animal in soft substrata such as sand, mud, or abyssal ooze deposits, by peristaltic contractions and hydrostatic pressure. The secondary polyps are arranged on the upper portion of the animal, the rachis, which projects above the surface of the sea bottom.

Historical review

A few authors have attempted to deal with the subject of the evolution and phylogeny of veretillids and other sea pens, these works being antiquated to say the least (1870-1916)! The only modern phylogenetic analyses are those by Williams (1994 & 1995c). Kölliker (1870 [1872]: 449) was the first to address the phylogenetic development of sea pens, where he considered *Umbellula* along with *Protoptilum* to be primitive offshoots of the pennatulacean prototype and the veretillids as highly specialized forms derived from kophobelemnoid ancestors. Koch (1878) countered the second part of Kölliker's premise, viewing the veretillids as transitional forms between the Alcyonacea and the Pennatulacea: "Ich betrachte sie (Pennatuliden) als Verwand-

te der Alcyoniden, zu denen einige Veretilliden ganz gute Uebergangsformen bilden".

Kölliker (1880: 39) believed the deep sea pennatulaceans to be primitive forms and the probable relicts of an ancient extinct fauna: "The simpler forms of the Pennatulida, especially those with sessile polyps, inhabit great depths ..... These simpler forms are probably also the oldest, and may be regarded as the last remnants of an extinct primary creation. The Protoptilidae and the Umbellulidae are the principal representatives of these old forms". Kölliker's argument should be viewed from the perspective of the mid-nineteenth century school of thought that considered the virtually unknown abyss as the home of the primordial ooze, the "Bathybius" of English science and the "Urschleim" of German science. Although, contrary to our modern interpretations, Kölliker's views can be considered discerning in light of the status of knowledge at the time; he viewed all forms with sessile polyps as primitive and those with polyps emanating from accessory appendages as derived. Our modern interpretation views this character state as only one in a suite of characters that are important in determining relationships and in differentiating primitive versus derived forms. Some of the forms with sessile polyps such as *Chunella* and *Umbellula* are now considered highly derived (fig. 1; see also Williams, 1994). In addition, the shallow water veretillids (currently regarded as the most primitive group), were not well known during Kölliker's time. Even with this limitation, however, Koch was perceptive enough to recognize their relevance to the evolution of the sea pens as a whole.

Marshall (1887: 148) disputed Kölliker's 1880 claim (as well as the first part of his 1872 premise) by acknowledging the striking diversity of deep sea forms together with the markedly derived nature of *Umbellula*: "The most noteworthy point is the great abundance and variety of specimens dredged ..... from a depth of 555 fathoms ..... These results ..... do not lend any material support to Kölliker's conclusion, that 'the simpler forms of Pennatulida, especially those with sessile polyps, inhabit great depths'. Kölliker ranks among primitive forms of Pennatulida the *Umbellulidae*, which are an essentially deep water family ..... and cites this distribution in evidence of the view that the lower forms of Pennatulida are, as a rule, deep water forms. *Umbellula* appears to me, however, to be not a primitive form but a highly modified one ..... In all these respects *Umbellula* is far less primitive than *Funiculina*, which is essentially a shallow water form, attaining its maximum of development at about 30 fathoms depth".

Bourne (1900: 33) considered bilateralism and the presence of polyp leaves as derived characters: "The higher members of the Pennatulacea have a distinct bilateral symmetry, due to the zooids being borne like the barbs of a feather on two sides of the rachis only, leaving a sterile band on the two remaining sides". However, he considered the radial symmetry of the Veretillidae and the strongly bilateral symmetry of the Funiculinidae to be separate derivatives of a *Protocaulon*-like archetype: "The existing families of the Pennatulaceae appear to have diverged from an ancestral form resembling *Protocaulon molle*". *Protocaulon molle* Kölliker, 1880, exemplifies bilateral symmetry, and was subsequently considered as an unidentifiable species of the genus *Virgularia* by Kükenthal (1915: 71). *Protoptilum* and *Funiculina* were considered to be members of closely related families by Kükenthal & Broch (1911: 456), and the

present study supports this premise. In addition, Bourne viewed the Umbellulidae and the various taxa with polyp leaves as two separately derived lineages of the Funiculinidae, while he considered the Veretillidae to be a terminal offshoot of *Protocaulon*.

Kükenthal & Broch (1911: 556) in their concluding chapter entitled, "Die Stammesgeschichte der Seefedern", gave a detailed account of pennatulacean phylogeny. They supported Marshall's contention that *Umbellula* is a derived rather than primitive taxon and corroborated Koch's position that the veretillids are the most primitive surviving pennatulaceans. The acceptance of the veretillid taxa as primitive sea pens was reiterated by Kükenthal (1912: 563) and Niedermeyer (1913: 263). Kükenthal (1915) produced the first comprehensive taxonomic synthesis of the Pennatulacea, and placed the Veretillidae first at the beginnining of his systematic arrangement for the order.

Hickson (1916: 25) gave a detailed assessement of the evolution of the Pennatulacea. He recognized the dilemma of Bourne's view in the great morphological disparity between an alcyonacean ancestor and Bourne's primitive sea pens: "Bourne ..... was of opinion that 'the existing families of Pennatulacea appear to have diverged from an ancestral form resembling *Protocaulon molle*', but the difficulty of this view is that it leaves such a great gap in structure between the Pennatulacea and the other Alcyonaria". Although Hickson viewed the Veretillidae as the most primitive existing family, he disagreed with the contention of Kükenthal's (1912) that *Lituaria* is the most primitive genus simply because of the presence of tuberculated sclerites: "Kükenthal ..... regards the genus *Lituaria* as the most primitive of all the Pennatulacea and places it at the root of his family tree. With that conclusion I am not entirely in agreement". He considered the presence of an axis and the occasional hint of bilateral symmetry as ample evidence to regard *Lituaria* as a somewhat specialized veretillid, and he also recognized the superficial similarity between the alcyonacean *Verseveldtia trochiforme* (Hickson, 1900) (see Williams, 1990b) and various veretillids, and chose it to illustrate a form similar to the prototype of the Pennatulacea: "..... we have an Alcyonacean which shows certain features of resemblance to a Veretillid. It is dimorphic, the colony shows a marked division into regions corresponding to the rachis and stalk, it is radially symmetrical and it has a very narrow base of attachment. These points of resemblance may be due to convergence but the species gives us a representation of what the hypothetical ancestor may have been like before it became detached and took to an independent existence".

Lastly, Williams (1994, 1995c) published the first cladogram of pennatulacean phylogeny, in which the veretillids represent the least derived clade and *Umbellula* is depicted as one of several highly derived clades. *Funiculina* is portrayed as being more derived than the veretillids but less derived than *Umbellula* and *Pteroeides*.

Phylogenetic assessment

Williams (1994; 1995c) provided the first modern phylogenetic analyses regarding the pennatulacaean octocorals. Very few other cladistic works have appeared that pertain to "lower invertebrate" groups. These include Weerdt (1985), Hooper (1987; 1990) for the demospongian genera *Acarnus* and *Rhabderemia* (Porifera), Hoeksema (1989; 1991) for fungiid scleractinians, Cairns (1984a; 1984b; 1987) for stylasterine and scleractinian

corals, Gerhart (1983) for gorgonian octocorals, and Schmidt (1972; 1974) for the anthozoan coelenterates as a whole.

Williams (1994) provided several reasons why cladistic analyses for lower metazoans such as the Pennatulacea are often seemingly intractable, explaining the consequent paucity of cladistic work in the literature regarding these taxa. These are (1) the scarcity of useful characters and the difficulty of character analysis; (2) a poorly represented and problematic fossil record, which makes identification and comparison with extinct groups difficult or impossible (Conway-Morris, 1991); (3) difficulty of obtaining fresh material of many taxa for study (especially for application of molecular and genetic techniques); (4) a high frequency of homoplasy (parallelisms, convergences, and reversals); and (5) unusually high degrees of intraspecific variability. Solé-Cava & Thorpe (1991: 69) have shown that mean heterozygosity values for sponges and coelenterates are very high compared to other groups of organisms such as vertebrates, arthropods, and plants.

Table 1. Matrix of twenty-two characters used to generate the cladogram in fig. 1. Polarity is expressed as follows: 0 = absent (ancestral state); 1 = present (derived state).

	OUTGROUP	VERETILLIDAE	ECHINOPTILIDAE	SCLEROBELEMNON	FUNICULINA	CHUNELLA	UMBELLULA	VIRGULARIA	PENNATULA	PTEROEIDES
primary polyp	0	1	1	1	1	1	1	1	1	1
peduncle	0	1	1	1	1	1	1	1	1	1
radially arranged polyps	0	1	1	0	0	0	0	0	0	0
smooth sclerites	0	1	0	1	0	0	0	0	0	1
retractile polyps	1	1	0	1	0	0	0	1	0	1
three-flanged sclerites	0	0	1	0	1	0	0	0	1	0
bifurcated polyps calyces	0	0	1	0	0	0	0	0	0	0
naked dorsal tract	0	0	0	1	1	1	1	1	1	1
bilateral symmetry	0	0	0	1	1	1	1	1	1	1
multiserial polyps	0	0	0	1	0	0	0	0	0	0
rachis elongation	0	0	0	0	1	1	1	1	1	1
biserial polyps	0	0	0	0	1	0	0	0	0	0
congested polyps	0	0	0	0	0	1	1	1	1	1
whorled polyps	0	0	0	0	0	1	1	0	0	0
reduced terminal polyp	0	0	0	0	0	1	0	0	0	0
terminal polyp cluster	0	0	0	0	0	0	1	0	0	0
polyp leaves	0	0	0	0	0	0	0	1	1	1
reduced leaf sclerites	0	0	0	0	0	0	0	1	0	0
polyp zonation	0	0	0	0	0	0	0	0	1	1
trimorphic polyps	0	0	0	0	0	0	0	0	1	0
zooid pads	0	0	0	0	0	0	0	0	0	1
supporting rays	0	0	0	0	0	0	0	0	0	1

If we accept Hickson's premise that the veretillids are intermediate between the alcyonaceans and the other pennatulaceans, then a detailed study of the veretillids is essential to shed light on the phylogenetic relationships of the pennatulaceans in general as well as on the nature of the pennatulacean ancestor and sister group relationships. Studying a more derived clade such as the pteroeidids, which may be easier to define as a monophyletic group, would not help to define the sea pen ancestor or elucidate broader aspects of pennatulacean phylogeny.

Along with the echinoptilids, the veretillids are here considered the least derived of the extant sea pens and exhibit the greatest diversity in the relatively shallow waters of the Indo-Pacific, while a great variety of more derived forms is present world-wide with unrestricted bathymetric range. The veretillid taxa represent a particularly important group for study since they occupy a basal position in the Pennatulacea, and are therefore ideally suited to help elucidate the nature of the sea pen ancestor and the phylogenetic affinities of the group in general. Highly derived forms such as *Virgularia* and *Pteroeides* are also present in tropical shallow water and are sympatric with the more primitive taxa, while other highly derived forms such as *Chunella* and *Umbellula* are restricted to deep water. It has therefore been postulated by Williams (1994: 734) that the sea pens as a group initially differentiated in the

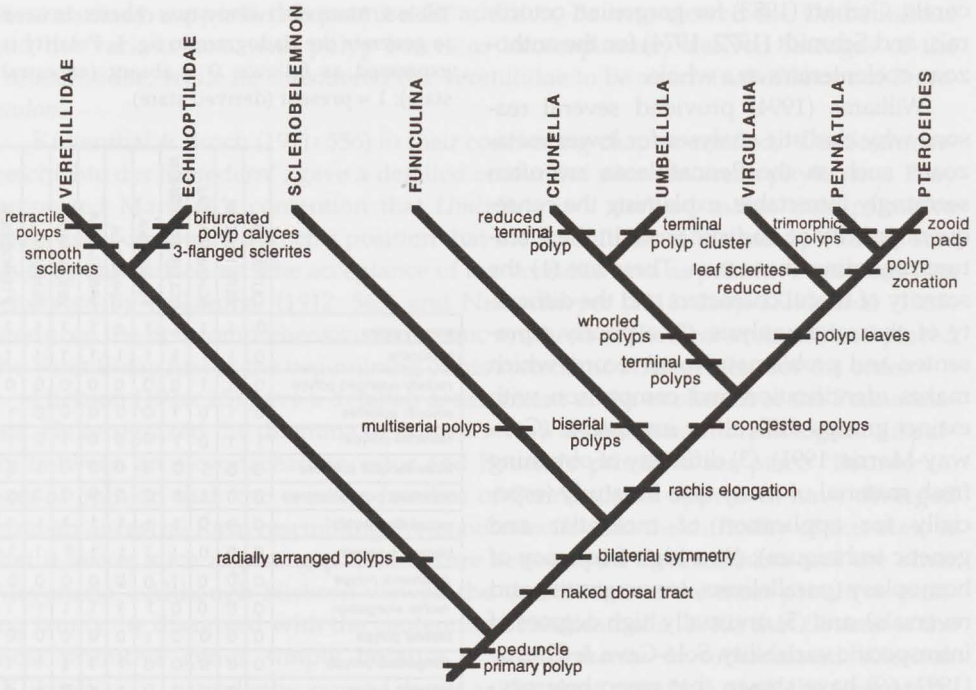

Fig. 1. Preliminary cladogram for pennatulacean exemplars, illustrating the monophyly of the Pennatulacea and the basal position of the veretillid taxa. Synapomorphies correspond to those in table 1.

shallow waters of tropical oceans and have subsequently diversified and dispersed to all depths of the temperate and polar regions, as well as the tropics (figs 1-2).

The veretillid genera *Cavernularia*, *Cavernulina*, *Lituaria*, and *Veretillum* are restricted to shallow water (0-300 meters in depth, but mostly in less than 100 meters), and are distributed from southern Europe and western Africa, throughout much of the Indo-Pacific, to parts of the Panamic Province (Mexico to Ecuador). The greatest species diversity occurs in the western Pacific triangle formed by the Philippines, Indonesia, and New Guinea (Williams, 1994: 732). Of the 61 nominal species names appearing in the literature, at least 35 are presumably valid (table 1). Williams (1995a: 103) expressed doubt concerning the validity of the genus *Cavernulina* and its differentiation from *Cavernularia*. At present, the distinctions between the various species of veretillids rely mainly on the differences in size and shape of the sclerites and development of the axis. The axis varies from being well-developed as in some species of *Lituaria* to rudimentary or absent altogether in some species of *Cavernularia*. New taxa are anticipated to be discovered and described as new collecting and examination of previously collected material is undertaken.

The veretillids are a group of pennatulacean octocorals that are very unlike other sea pens. They are thought to represent a monophyletic group, which occupies a basal position in the phylogeny of the Pennatulacea. Because of this, they no doubt have symplesiomorphic characters which do not serve to segregate them as a monophyletic clade. However, preliminary investigation has revealed several apomorph-

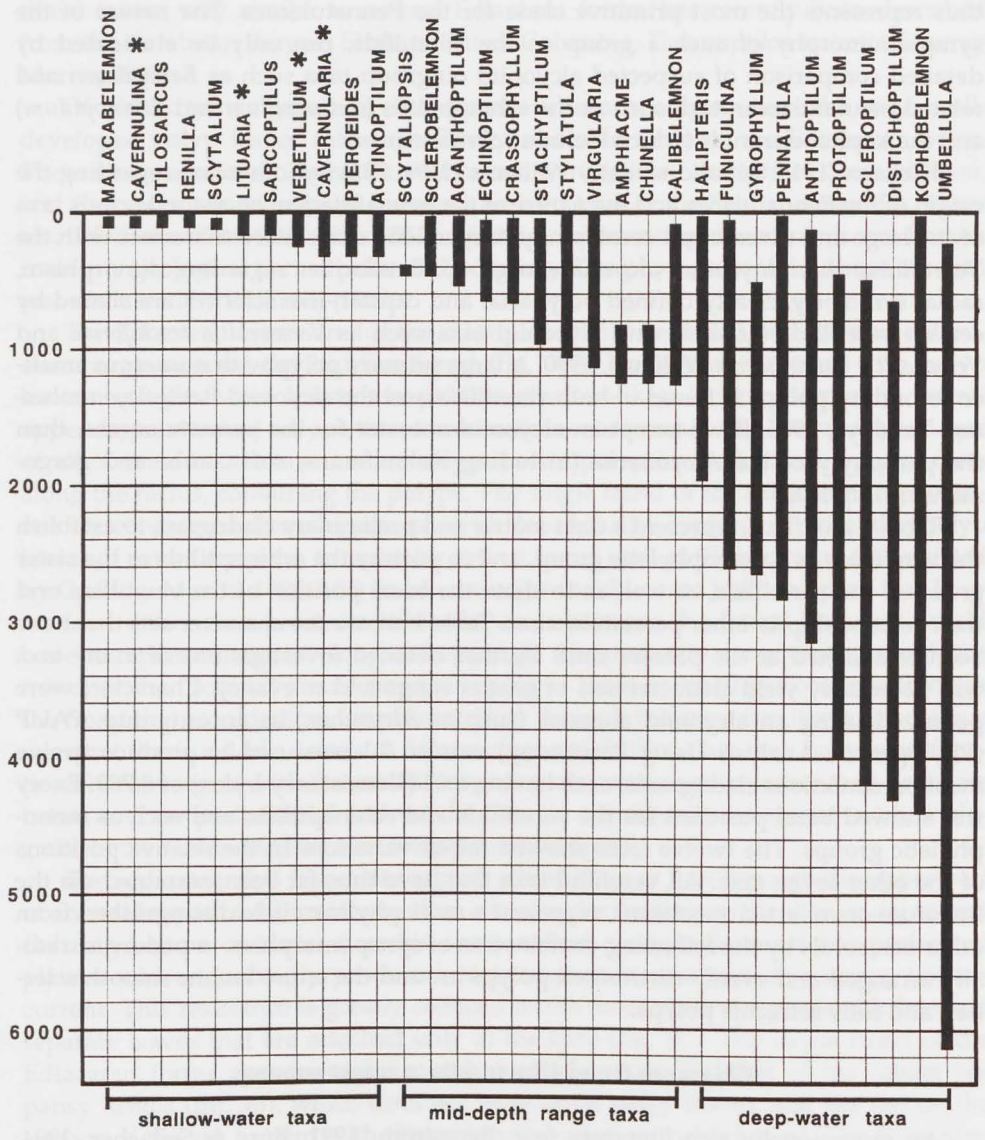

Fig. 2. Bathymetric distribution of Pennatulacea; recorded depth ranges in meters for all valid living genera (* = veretillid taxa).

ies to define the veretillids as a distinguishable and unique clade of pennatulaceans - a peduncle, a primary polyp, radially arranged and evenly distributed polyps around the entire rachis, smooth sclerites, and fully retractile polyps.

The sister group to the Veretillidae is hypothesized to be the Echinoptilidae (fig. 1), the species of which share radial symmetry with the veretillids, but have three-flanged sclerites and permanent polyp calyces unlike the veretillids, which have smooth sclerites and completely retractile polyps. The clade containing both families

thus represents the most primitive clade for the Pennatulacaea. The nature of the symplesiomorphy of such a group as the veretillids, can only be elucidated by detailed comparison of suspected alcyoniid outgroup taxa such as *Bathyalcyon* and related pennatulacean taxa such as the echinoptilids (*Actinoptilum* and *Echinoptilum*) and the kophobelemnids (which include *Sclerobelemnon*).

Hickson (1916: 26) and recently Williams (1994: 733) hypothesized regarding the origin of the Pennatulacea and the nature of the pennatulacean ancestor. Various taxa of the large and diverse soft coral family Alcyoniidae share more characters with the Veretillidae than any other alcyonacean group. Similarities regarding dimorphism, radial symmetry, clearly defined polyparia, and capstan-like sclerites are shared by certain veretillids (*Lituaria*) and alyconiid taxa such as *Verseveldtia trochiforme* and *Verseveldtia bucciniforme* Williams, 1990. A large primary polyp with numerous smaller secondary polyps is found in both veretillids and the alcyoniid *Bathyalcyon robustum* Versluys, 1906. If we accept an alcyoniid ancestor for the pennatulaceans, then the paraphyly of the Alcyonacea (including stoloniferans, soft corals, and gorgonians) is implicit.

Table 1 and fig. 1 represent a data matrix and preliminary cladogram to establish the veretillids as a monophyletic group, and to portray the echinoptilids as the sister group of the veretillids, as well as to show the basal position of the veretillids and their relationship to other pennatulaceans. Table 1 shows the character sets that have been established at the present time. Further detailed investigations of many taxa will potentially yield character sets of greater range and relevancy. Characters were polarized using an alcyoniid ancestor (such as *Alcyonium*) as an outgroup. PAUP (Phylogenetic Analysis Using Parsimony) version 3.1 was used to produce twelve most parsimonious cladograms, each having a CI (Consistency Index) of 0.783. Every tree showed basal positions for the veretillids and echinoptilids, and each as monophyletic groups. The twelve trees showed minor variations in the relative positions of the other seven taxa. All veretillid taxa that have thus far been examined via the literature or collected specimens, represent a monophyletic clade distinguished from other octocorals by the following combination of synapomorphies - a peduncle, radially arranged and evenly distributed polyps around the entire rachis, smooth sclerites, and fully retractile polyps.

Ediacaran frond-like fossils, a reassessment

An exceptionally rich literature (e.g. Bergström, 1991; Buss & Seilacher, 1994; Conway Morris, 1991; Fortey, Briggs, and Wills, 1996; Glaessner, 1979 & 1984; Glaessner & Wade, 1966; Retallack, 1994; and Seilacher, 1989), has arisen to describe and/or assess the taxonomic status of various pennatulacean-like Precambrian fossils from the Ediacaran fauna, first described from Australia, but subsequently found in Namibia, Russia, and England. These fossils have been defined in a variety of ways: as a phylum of extinct metazoans (Vendobionta), as a separate non-metazoan kingdom (Vendobionta), as lichens, and as sea pens. Some of the fossils are generally either frond-like forms with an elongate or a bulbous holdfast (including the genera *Charnia, Charniodiscus, Glaessnerina, Vaizitsinia,* and *Khatyspytia),* or are bag-like foliate forms with similarly bulbous holdfasts such as *Pteridinium* and *Rangea.* An addi-

tional taxon, very similar to several Ediacaran forms, has recently been described from the Cambrian Burgess Shale of British Columbia, *Thaumaptilon walcotti* Conway Morris, 1993.

Of the thirty-two genera of extant pennatulaceans, only six have the large, well-developed polyp leaves that superficially resemble those of the Ediacaran forms. These six taxa, namely *Pennatula, Ptilosarcus, Gyrophyllum, Sarcoptilus, Crassophyllum,* and *Pteroeides,* are considered the most derived taxa of the Pennatulacea (fig. 1; see also Williams, 1994: 733; 1995c; 326). Material representing these taxa is not known from any geological period prior to the Tertiary. The phylogeny of the above six taxa was discussed by Williams (1995c).

The Ediacaran forms are for the most part foliate (leaf-like) and have a continuous margin around the leaf-like frond (fig. 3D). The extant sea pens with polyp leaves, on the other hand, are pinnate (feather-like), having numerous lateral appendages attached only at their bases to the central rachis. The lateral appendages of all of these extant taxa are actually separate polyp leaves in two opposite rows along the rachis, containing the polyps. The single frond of the Ediacaran forms cannot be considered homologous or even functionally convergent with the polyp leaves of extant sea pens. In order for extinct forms such as *Thaumaptilon* (as depicted by Conway Morris, 1993: 602) to be convergent with extant leafy sea pens that have an erect rather than prostrate habit, the areas between the lateral branches would have to represent slits or elongated openings that perforate completely through the frond, or the "zooids" would have to represent holes or small perforations through the frond that would allow for water to flow through the organism like a sieve. If we interpret the frond-like fossils as "sieve organisms", then they may have been functionally convergent with the extant leafy sea pens, with an erect rather than prostrate habit, and the perforated frond would then be considered analogous, not homologous, to the rachis and leaves of leafy pennatulaceans.

The separate polyp leaves of sea pens allow water to flow through the upper part of animal in quite a different way, thus allowing for feeding on planktonic organisms utilizing bottom currents. A rachis composed of a single flattened non-perforated plate would undoubtedly produce a high amount of resistance in a strong bottom current. This resistance is greatly reduced in the feather-like sea pens with free and separate leaves that are attached only at the base (fig. 3C). The single frond of the Ediacaran forms appears to be morphologically more like that of the foliate sea pansy *Renilla* (fig. 3B), which does not have serial polyp leaves, and lies flat on the surface of the sea bottom with the peduncle buried in the sediment. The Ediacaran forms may have had a similar life habit, or could have lived totally immersed in the sediment as postulated by Buss & Seilacher (1994: 3).

As previously stated, the least derived extant taxa, including the most primitive sea pens, the veretillids, are not feather-like but rather vermiform or clavate to cylindrical in shape, and lateral appendages resembling polyp leaves are absent altogether (fig. 3A). None of these taxa are represented in the fossil record. *Graphularia* and *Virgularia,* sea pens with relatively weakly-developed polyp leaves, are the only other pennatulacean genera represented by fossils, and these are not recorded from strata earlier than the Jurassic.

Based on the paleontological and phylogenetic evidence, as well as that of com-

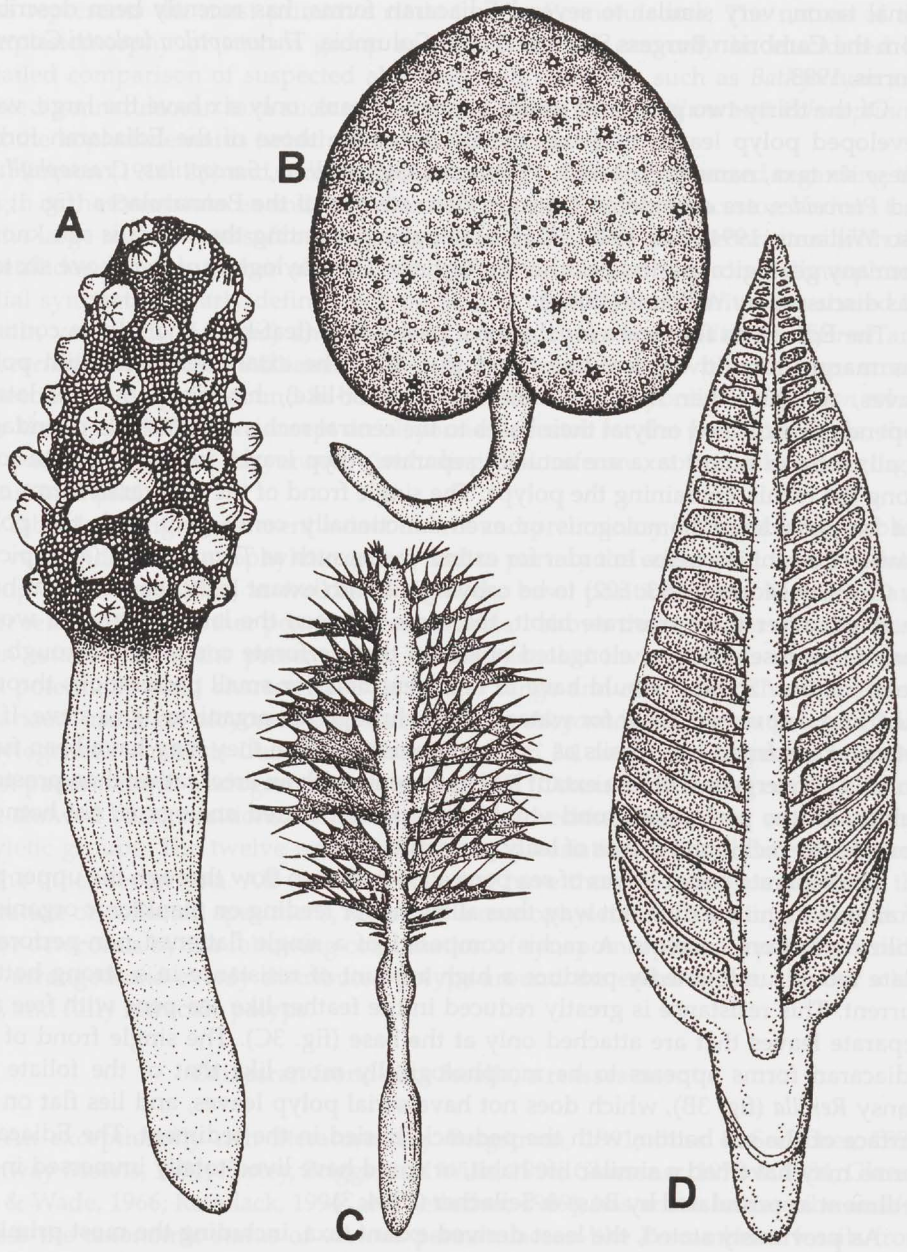

Fig. 3. Morphological comparisons. A. A veretillid sea pen *Cavernularia elegans* (Herklots, 1858). B. A sea pansy *Renilla amethystina* Verrill, 1864. C. A pteroeidid sea pen *Pteroeides spinosum* (Ellis, 1764). D. Reconstruction of the Burgess Shale frond-like fossil *Thaumaptilon walcotti* Conway Morris, 1993 (redrawn from Conway Morris, 1993: 609).

parative morphology, it is here hypothesized that the Ediacaran/Burgess Shale "sea pens" such as *Thaumaptilon* are not fossilized pennatulacaean octocorals, but rather represent another, unrelated but superficially similar lineage without living representatives, a lineage that was probably extinct by the end of the Cambrian. At present, there is no evidence to suggest that the pennatulaceans were present prior to the Mesozoic.

In my view, the problematic nature of interpreting the nature of the Ediacaran fossils is in part due to the specialized state of science in which workers in different fields concerned with similar issues are unaware of each other's work and findings. It would be more productive if neontologists (i.e. systematists) and Precambrian paleontologists worked together to decipher the affinities of the organisms in the Ediacaran and Burgess Shale faunas.

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Behaviour and settlement of actinula larvae of *Tubularia mesembryanthemum* Allman, 1871 (Hydrozoa: Tubulariidae)

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Yamashita, K., S. Kawaii, M. Nakai & N. Fusetani. Behaviour and settlement of actinula larvae of *Tubularia mesembryanthemum* Allman, 1871 (Hydrozoa: Tubulariidae).
Proceedings of the 6th International Conference on Coelenterate Biology, 1995: 511-516, fig. 1.
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Key words: *Tubularia mesembryanthemum*; actinula larvae; settlement; nematocyst-printing; external/internal Ca^{2+}.

Abstract: Behaviour of the actinula larva of the hydroid *Tubularia mesembryanthemum* Allman, 1871, was observed during settlement and metamorphosis using a range of microscopic techniques and time-lapse video recordings. Factors affecting larval behaviour and settlement were examined, and intracellular [Ca^{2+}] dynamics during nematocyst discharge were visualized by fura-2/AM. Actinula larvae demonstrated two forms of attachment to substrata: temporary attachment by discharge of atrichous isorhizae from the tentacle tips and permanent settlement by cement secretion from the columnar gland cells of the basal protrusion. During settlement, nematocyst-printing behaviour was executed by rolling the tentacle tips on the substrata. Temporary attachment was induced by 20-100 mM external [K^+] and inhibited by ≤0.9 mM [Ca^{2+}], while settlement behaviour was induced by 30-50 mM [K^+] but inhibited by ≤ 2.3 mM [Ca^{2+}]. Larvae became increasingly sensitive to inducers with time. Atrichous isorhizae were discharged from the tentacle tips following the addition of K^+; a simultaneous surge of $[Ca^{2+}]_i$ was observed in the tentacle tips. Dynamic changes in nematocyst composition of the aboral tentacles were observed throughout larval and post-larval development. These results are discussed in relation to functional changes of the tentacles during the transition from the larva to the juvenile polyp.

Introduction

Tubularia mesembryanthemum Allman, 1871, is a common marine colonial hydroid, whose relatively large polyp size render it attractive as a potential experimental animal. Regeneration (Barth, 1940: 405; Tardent & Eyman, 1958: 280), development of the gonophore (Brauer, 1894: 551; Berrill, 1952: 583; Nagao, 1965: 9), growth in culture (Mackie, 1966: 397), field ecology (Hughes, 1983: 467), physiology (Josephson & Mackie, 1965: 293; Neufeld et. al., 1978: 347; Mithel & Cose, 1986: 295), and taxonomy (Tardent, 1980: 17; Petersen, 1990: 101) of adult *Tubularia* spp. have already been described. The life cycle of *Tubularia* species contains a morphologically unique dispersive phase, the actinula larva. Unlike the adult phase, only preliminary studies of actinula behaviour have been performed (Pyefinch & Downing, 1949: 21; Berrill, 1952: 583; Hawes, 1958: 147), and little is known about factors affecting the settlement of actinulae. Along the Japanese coast, dense colonies of *T. mesembryanthemum* can be observed throughout the year, with the exception of August, and their attachment to artificial substrata used in aquaculture is a serious problem for fisheries.

Detailed observations were made on the behaviour and morphological changes of actinula larvae during settlement and metamorphosis by employing a range of micro-

scopic and video techniques. We also examined factors influencing larval attachment and settlement (contact-collision, microbial films, external/internal ion environment). Here we briefly describe our results concerning larval behaviour, morphological transformation, external/internal ion environment, and nematocyst dynamics.

Materials and methods

Biological material

T. mesembryanthemum colonies were collected mainly from fishing nets in Sagami bay (35°20'N, 139°30'W) and Kagoshima bay (35°70'N, 139°30'W), and transferred to the laboratory in cooled, insulated containers. Colonies were maintained and cultured in roughly filtered running seawater and were fed on *Artemia* nauplii reared on a diet of the *Isochrysis* spec. (Haptophyceae). Larvae were obtained by placing branches of female colonies bearing many actinulae in beakers containing fresh filtered seawater (FSW). Larvae sank to the bottom of beakers and were collected and rinsed in FSW. Larval age was defined as the period following liberation from the maternal gonophore. Settlement assays were performed at 19°C ± 1°C.

Larval behaviour and morphology

Larval behaviour was observed on both clean and microbially filmed glass (suspended in roughly-filtered seawater for 1 day - 3 weeks) under either a stereoscopic microscope or an inverted microscope coupled with time-lapse video. Larval and post-larval dimensions were recorded until stolon elongation occurred. In order to examine histological changes, larvae were fixed in 10% neutral formalin, dehydrated, embedded in resin (Technobit®), sectioned (6 µm thickness), and stained with hematoxylin-eosin.

Effects of K^+ and Ca^{2+}, and $[Ca^{2+}]_i$ dynamics during nematocyst discharge

Van 't Hoff artificial seawater (ASW) was used as a control, while $[K^+]$ and $[Ca^{2+}]$ were varied under almost isotonic conditions as follows; 10(control), 20, 30, 40, 50, 60, 80, and 100 mM of $[K^+]$ in ASW, and 0.23, 0.92, 2.3, 4.6, 9.2 (control) mM of $[Ca^{2+}]$ in ASW. The final volume of test solutions was 40 ml. For settlement assays, 12-16 larvae (< 8 h old) were placed in a deep glass petri-dish containing test solution. Larvae were exposed to high $[K^+]$ ASW for 24h, and low $[Ca^{2+}]$ ASW for 48 h. Following treatment with low $[Ca^{2+}]$, experimental solutions were exchanged for ASW in order to check that larvae were not adversely affected by the treatment.

A specially devised imaging-system was assembled for the visualization of $[Ca^{2+}]_i$ using larvae loaded with the fluorescent calcium indicator fura-2/AM (see Kawaii et al., in prep.). The tentacle tips were immobilized by gentle suction through a micropipette and nematocyst discharge was induced by addition of 200 mM KCl solution.

Nematocyst composition

Nematocyst composition was examined in the following developmental stages:

embryo, preactinula, 2-4 h old larva, 24 h old larva, nematocyst-printing larva, settled post-larva, and juvenile polyp. Each larva was placed in circa 200 mM Mg^{2+} in ASW and gently covered with a cover-slip. The type and number of nematocysts in tentacles were then recorded under a Nomarski interference microscope.

Results

Larval behaviour and morphology

Newly released actinulae had 4-12 (mean = 8) aboral tentacles and 4-7 oral tentacles (fig. 1A). Larvae 2-8 h after liberation demonstrated the following characteristics: aboral tentacle length, 600-1100 µm; oral tentacle length, 15-60 µm; body length, 230-340 µm; body width, 200-320 µm. Within 12 h, the larvae initiated repeated contraction and expansion of the body, and the aboral tentacles became more sticky due to increased tip adherency, eventually resulting in temporary attachment following surface contact. Numerous atrichous isorhizae (AI) were observed at the aboral tentacle tips at this stage. Adhesion was achieved by the tubes of the discharged AIs. Approximately 1 day after liberation, the oral tentacles elongated (50-130 µm), and body length increased (240-470 µm) and the aboral pole gradually began to extend downwards in the region which will later became the location of permanent attachment (= basal protrusion). Larvae then positioned themselves for settlement by rolling the previously rigid tentacle tips so that all of the cnidocils came into contact with the substratum, as did the basal protrusion (fig. 1B, C). Contact of the tentacle tips with the substrata resulted in extensive nematocyst discharge around the place of settlement (fig. 1F). This nematocyst discharge was distinct from that used for locomotion by actinulae prior to this phase and by other cnidarians, and was termed "nematocyst-printing". On clean glass surfaces in still water, larvae usually started and stopped this nematocyst-printing behaviour repeatedly and erratically before settlement occurred.

The basal protrusion was composed of long columnar gland cells filled with strongly eosinophilic secretory granules. Settlement of larvae involved pressing the basal protrusion against the glass surface (orientation), followed by release of the cement substance. This process was accompanied by nematocyst-printing and body-swaying (fig. 1D). Subsequently, stem and stolon differentiation occurred (fig. 1E), during which phase the cushion-like tissue of the aboral endoderm degenerated. Three days after liberation, juvenile polyps showed the following characteristics: aboral tentacle length, 700-1300 µm; oral tentacle length, 60-140 µm; body length, 600-970 µm; body width, 140-250 µm.

Effects of K^+ and Ca^{2+} on larval settlement, and $[Ca^{2+}]_i$ dynamics during nematocyst discharge

Temporary attachment by nematocyst discharge from the tentacle tips was induced by 20-100 mM $[K^+]$ and 2.3 mM $[Ca^{2+}]$, but was inhibited by $\leq$ 0.9 mM $[Ca^{2+}]$. Nematocyst-printing behaviour and settlement were induced by 30-50 mM $[K^+]$, but was inhibited by $\leq$ 2.3 mM $[Ca^{2+}]$. Larvae whose attachment and settlement had been

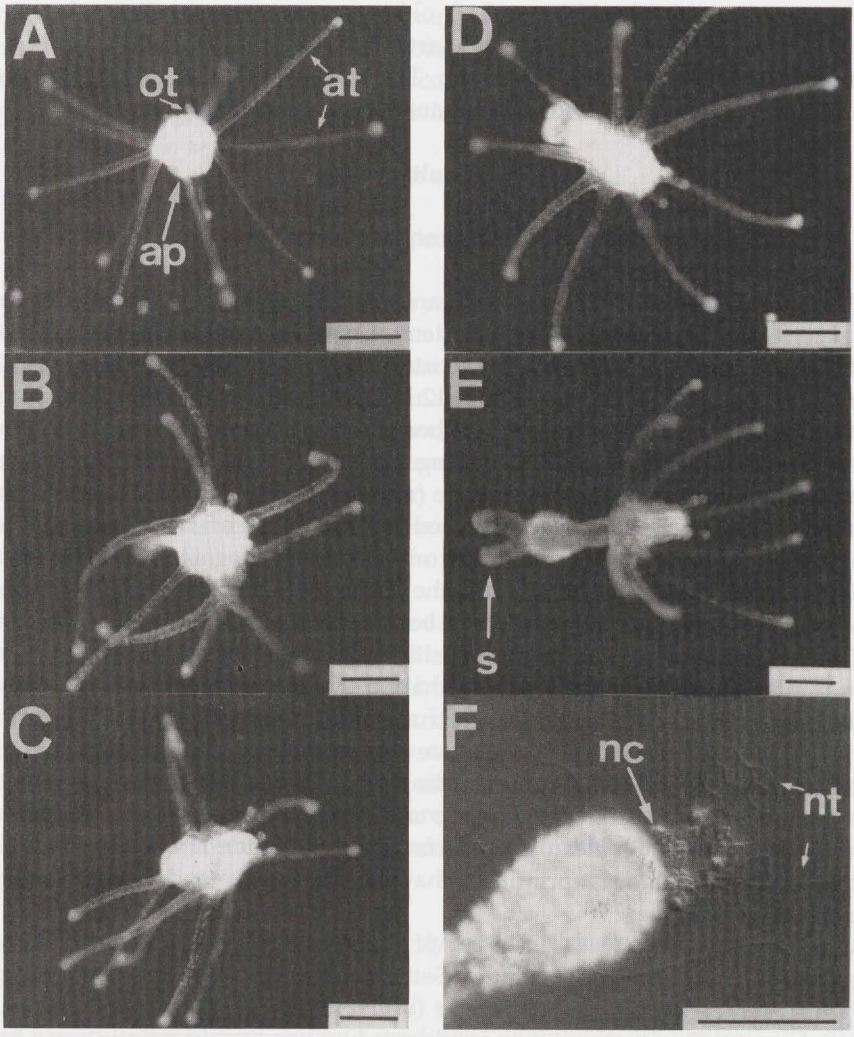

Fig. 1. Nematocyst-printing behaviour during settlement of the actinula larva.
A. Larva 2h after liberation from the maternal gonophore.
B. and C. Nematocyst-printing by 1 day-old larva.
D. Post-larva permanently attached by the aboral pole.
E. Juvenile polyp 1 day after settlement, demonstrating stolon elongation.
F. Numerous nematocysts were discharged from the tentacle tip resulting in printing around the place of settlement.
Scale bars = 300 µm (A-E) and 500 µm (F); ap = aboral pole (= basal protrusion), at = aboral tentacle, nc = nematocyst capsule, nt = nematocyst tubes, ot = oral tentacle, s = stolon.

inhibited by exposure to ≤ 2.3 mM $[Ca^{2+}]$ demonstrated rapid settlement behaviour following transfer to ASW.

Immediately following the direct addition of 200 mM KCl, sudden discharge of many atrichous isorhizae from the tentacle tips occurred. At this stage, a simultaneous transitory surge of $[Ca^{2+}]_i$ occurred in the tentacle tip.

Nematocyst composition

No nematocysts were observed in embryonic tentacles. In the preactinular tentacle, only several atrichous isorhizae (AI) were observed, but these were not deployed (see Campbell, 1988: 15) at the epidermal surface of the tentacle tip. In 2-4 h old larval tentacles, four types of nematocysts were observed: atrichous isorhizae (AI), stenotele (S), desmoneme-like (D), and microbasic mastigophore-like (MM). Many AI, used for temporary attachment, were deployed at the tentacle tips at this stage. The number of AI in the tentacles then gradually decreased, and other types of nematocysts (S, D, MM), utilized in prey capture or possibly defense, increased rapidly in number. During pre-settlement and post-settlement stages, S and D nematocytes migrated from the ectoderm of the body wall to the tentacle tips, thereby resulting in dynamic change in the tentacular nematocyst composition. Newly-settled individuals (prior to stem and stolon elongation) retained small amounts of AI which were not found in the tentacles of juvenile polyps following stem and stolon elongation, although several holotrichous isorhizae-like nematocysts were observed in the tentacles of the matured adult polyps.

Discussion

Detailed observations of actinula larvae revealed a complex series of behavioural and morphological changes previously unreported. The coordination of tentacular function and the basal protrusion suggested a physiologically complex set of interactions controlling actinular settlement behaviour. Nematocyst composition of the tentacles changed dynamically from AI dominance to S and D dominance before and following nematocyst-printing behaviour, suggesting that nematocyst-printing behaviour is essential for non-reversible changes in aboral tentacle function, that is, from attachment to feeding and defense.

Actinulae proved very sensitive to changes in external $[K^+]$ and $[Ca^{2+}]$, and nematocyst-printing behaviour was clearly regulated by these cations. These results suggest that signal transduction systems play an important role in actinula settlement and metamorphosis as in other hydrozoan planulae (Müller, 1985: 216; Leiz & Müller, 1987: 82; Berking, 1988: 1; Freeman & Ridgeway, 1990: 63).

Temporal attachment by actinulae was achieved by AI nematocyst discharge, and external Ca^{2+} proved essential for this process. The transitory surge of intracellular $[Ca^{2+}]_i$ during AI nematocyst discharge further suggested that an influx of external Ca^{2+} was involved in this process, similar to that already suggested for other coelenterate species (Salleo et. al., 1994: 201; Watson & Hessinger, 1994: 473).

The relationships between actinula behaviour and environmental factors will be subject to further investigation in order to further clarify mechanisms of actinula settlement and metamorphosis.

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Taxonomic index

(Captions of figures and tables not taken into account; † = fossil taxon)

General index

(Mainly based on entries selected by the authors)

Explanatory additions in square brackets; slashes separate related entries; characters or syllables in parentheses combine verbal forms, singular and plural forms of nouns, adjectives and/or adverbs, or variable spelling of the same entry; words in parentheses combine single and compound entries.

PROCEEDINGS OF
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The present volume contains the results of recent research on Coelenterata world-wide presented at the 6th International Conference on Coelenterate Biology held in 1995 at conference centre the 'Leeuwenhorst', Noordwijkerhout, The Netherlands. It includes important review papers by leading scientists as well as new results of research on various topics. Many contributions can be classified among the best research available on the subject. Being an important source of current research, this book is recommended to coelenterate specialists who have not been able to attend the conference, and for libraries of research institutes, natural history museums, universities and colleges.