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Narcotisation, fixation and preservation experiments with marine zooplankton

R.P. Heyman

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I. INTRODUCTION

In the years 1980-1983 the Institute of Taxonomic Zoology (University of Amsterdam) carries out four expeditions in the North Atlantic Ocean, within its general programme of variation and speciation research Project 101A (see also the IInd Report Oceanographic Research I.T.Z. 10-5-1979).

As a preparation for this research a 'test cruise' was carried out with the oceanographic vessel H.M.S.'Tydeman' in the area of the Bay of Biscay, in the period 26-2-1979 to 10-3-1979. Among other things, this journey aimed at testing methods for narcotising, fixing and preserving marine plankton. These experiments were necessary in order to test some preparation methods (e.g. histology, karyology) which had hitherto not been used for plankton, and in order to find the most efficient way of storing great quantities of material on board.

Up to now the material caught during expeditions has been mainly fixed and/or preserved in solutions of alcohol or formalin. Although especially the formalin solution is very suitable for fixing and preserving 'museum' material, it can become necessary also to use other materials. The fact is that certain ways of processing plankton animals are very difficult and sometimes altogether impossible with specimens preserved in formalin or in alcohol. Besides, it appears to be desirable to find better preservation methods for delicate plankton organisms as e.g. Chaetognatha, Ctenophora and Thaliacea.

It is a well-known fact that each animal group reacts somewhat differently to the chemicals applied. It is not feasible to take along special narcotisation, fixation and preservation media for each animal group one expects to catch. Moreover, treatments aboard should be simple, standardised and preferably not dangerous in order to avoid serious mistakes and accidents.

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The squash technique is applied in karyological research on chromosomes during cell-division stages, enabling one to study both their numbers and their morphology. In taxonomy, karyology is especially helpful when the criterion of interbreeding cannot be tested, as is the case with most marine zooplankton. Furthermore, the number of chromosomes of most marine zooplankton taxa is unknown.

When working with animal tissue, the acetic-orcein squash technique is employed, as described by La Cour(1941) for fresh material. This causes problems when treating marine zooplankton. The squash technique cannot be employed very easily aboard ship because of its complicated procedure which takes up a lot of time, and because of the impossibility of adequate use of a microscope aboard ship. The logical consequence is that, before squashing can be carried out in a laboratory, the organisms undergo some extra treatments aboard in order to fix and preserve them for a longer or shorter period. Fixation and preservation with formalin is not possible as this blocks those groups within the nuclein acids necessary for the dyes to attach themselves to, which renders staining impossible (Burck,1973, p.39).

Unesco(1976) published a summary of the 1972 symposium on the 'Fixation and Preservation of Marine Zooplankton' which served as a basis for the present research.

The aim of the present study was to test

- narcotisation
- fixation and preservation of material suitable for histological treatment
- specific treatment as a preparation for squashing
- combinations of various narcotisation, fixation and preservation methods suitable for long-term storage
- special treatment of some selected specimens
- special fixation and preservation solutions for calcareous organisms.

For these reasons the material caught during the cruise has been divided into three parts.

First representative subsamples were made to test narcotisations, fixations and preservations.

Secondly, some selected specimens were taken from the remainder to undergo special treatment or squashing.

Thirdly, calcareous organisms were put into a calcium fixative

II. METHODS

As was already stated in the introduction, the composition of the media to be used had to meet basic requirements. The treatments should not be too com-

plicated or dangerous and their procedures should be standardised. On the other hand they should be effective for most groups of planktonic organisms. The treatments tested follow below.

II 1. Narcotisation

Narcotisation serves as a preliminary treatment, before fixation, to prevent the organisms from warping, retracting their protuberances or contracting. Narcotised organisms are considered to be more suitable for future use as museum-material.

Narcotisation is also very helpful when photographically recording organisms. At the same time, however, their colour, form and size should be preserved. The narcotisation has to act rapidly, within a few minutes to one hour, and the concentration should be such that the organisms do not die during the narcotisation period.

From Unesco(1976) it appears that narcotisations for zooplankton are but fragmentarily known.

Experience led to the decision to test the following liquids:

a) Acetone-chloroform
   formula: 1% in filtered sea-water(hitherto not used for plankton)

b) Magnesium-chloride (after Pantin,1948)
   formula: 7,5 g $MgCl_2 6H_2O$
   92,5 ml distilled water } isotonic with seawater
   100 ml filtered sea-water
   Narcosis is recorded to set in after 10-20 min.

c) Nembutal with M.S.222 (both tradenames) (after Joosse & Lever,1959; Lever et al.,1964)
   formula: Nembutal is a short-working barbiturate $C_{11}H_{17}N_2O_3Na$
   M.S.222 is a tetracain metosulphate
   There are two ways of employing these reagents. One of these was not tested because of its complicated procedure, the other method has undergone some changes due to the circumstances.
   The original procedure for fresh-water pulmonates is:
   Place the organism for 20-30 minutes in the following solution:
   0.08 g nembutal in 100 ml distilled water;
   afterwards the organism is to remain for another 10 minutes in that same solution with 0.3 g M.S.222 added.
   The procedure has now been changed in order to test a nembutal solution and a M.S.222 solution separately as narcotics. The solutions must be made with sea-water to prevent the organisms from dying immediately.

The formulas are now as follows:

0.08 g nembutal in 100 ml filtered sea-water

0.3 g M.S.222 in 100 ml filtered sea-water

Hitherto these solutions had not been used for marine zooplankton

d) Chloral hydrate

formula: 1 ml for 1½ l filtered sea-water

This liquid had not been used for plankton before.

e) Menthol and ethyl alcohol (after A.F.de Fluiter)

formula: 5 g menthol for 100 ml 100% alcohol

Of this solution 1 ml is to be diluted with 1 l filtered sea-water

This solution had not been used with plankton before.

f) Valerian

procedure: drop into sea-water

This procedure had hitherto not been used for plankton.

g) Menthol + chloral hydrate (after Gray,1953; Van Eeden,1958)

formula: 12 g pulverized menthol; add
13 g chloral hydrate; they will form a fluid

procedure: dip a glass rod in the narcotising fluid; lower it into the container with organisms; the result is a thin film on the surface.

Maximum time of narcotisation: 30 hrs.

This solution has been described for narcotising Coelenterata and Gastropoda. It was tested, although the narcotisation time is too long for shipboard use.

II 2. Fixation of non-calcareous organisms

Fixation is one of the most important elements in the treatment of organisms. It is important to kill the organism in a quick way without seriously impairing its quality, that is to say, avoiding shrinkage, swelling or crooking. Besides, the medium used should penetrate the organism quickly and thoroughly. Other criteria, such as the possibility also to use the fixing agent for preservation and the ultimate results in microscopic slides further determined the choice of fixatives to be tested:

a) Alcohol 70%

Standard fixative and preservative

Time of fixation: a few days.

b) Formalin

Like alcohol this may be used as standard fixing and preserving agent as a 4% solution in distilled water. For very delicate and small plankton organisms it is best to use a solution of 2% for both fixation and preservation

For reasons of comparison also the following solutions have been tested:

4% borax buffered formalin in distilled water;

4% solution in filtered sea-water

2% solution in filtered sea-water

Reported time of fixation: ca 2 days.

c) Bouin (after Romeis,1948: 305)

formula: 5 ml formaldehyde 36%
15 ml picric acid saturated solution
1 ml acetic acid 97%

Picric acid: 30-50 g picric acid in 2 l distilled water of ca 90°C; shake well. According to Romeis the solution has to be prepared freshly, but this turned out not to be necessary.

Time of fixation is between a few hours and a few days. After fixation the organisms should be preserved in alcohol 70%; the alcohol is to be changed a few times.

Disadvantage: Bouin hydrolyses very strongly during fixation.

Maybe Bouin can also be used for preservation; hardly anything was known about this possibility.

This fixation is suitable for general microscopic slides, as the acetic acid fixes the nucleus and as picric acid makes the organisms easy to stain.

d) Carnoy (after Romeis,1948: 226)

formula: 6 ml alcohol 100%
3 ml chloroform
1 ml acetic acid 97%

Reported time of fixation: Carnoy quickly permeates the organisms (ca 2.9 mm per four hours)

After fixation, the organisms should be preserved in alcohol 96%. A disadvantage is that this makes the material shrink and harden.

Maybe Carnoy can also be used for preservation, but nothing is known about this possibility (Burch,1962-'64).

This fixation is suitable for nucleus fixation and especially for squashing (Natarajan et al.,1965).

e) Perenyi (after Romeis,1948: 322)

formula: 40 ml 10% solution of nitrous acid
30 ml alcohol 100%
30 ml 0.5% watery solution of chromic acid

Reported time of fixation: for 3-4 mm thick tissues 3-5 hrs.

After fixation, the organism should be preserved on alcohol 70%.

According to Romeis(1948) this is not a good fixative. Nevertheless it was tried as it had not been used for plankton before.

II 3. Preservation of non-calcareous organisms

After fixation, preservation should make it possible to keep the organisms in good condition for an indefinite time. It is important that enough preservation liquid is used. The rule of thumb is to store 1 volume-unit of organisms in 3 volume-units of liquid.

Besides the standard media alcohol 70% and formalin, also fixatives have been used which might be used as preservatives as well, namely Bouin and Carnoy.

For the smaller planktonic organisms a specific preserving agent, propylene phenoxetol plus propylene glycol (pppg) has been chosen, which cannot be used as a fixative:

formula: 0.5 ml propylene phenoxetol (3-phenoxypropanol) $C_9H_{12}O_2$
4.5 ml propylene glycol (1-2 propane diol)
95 ml distilled water

The combination of these two chemicals has advantages: the flexibility of the organisms remains intact, the organisms do not fall apart and the medium is a powerful inhibitor of mould. Moreover, propylene phenoxetol is readily soluble in combination with propylene glycol, and finally, it does not break down the lipids because it is an anti-oxidant (Steedman,1976,pp.180 and 226).

There was no experience with the results in the long run. For short periods good results had been obtained, especially with small planktonic organisms.

II 4. Fixation and preservation of calcareous organisms

Most fixation and preservation agents are acid, they dissolve away the hard, calcareous parts. Particularly the shells of planktonic molluscs, e.g. Pteropoda and Heteropoda, which consist of aragonite (the most soluble configuration of calcium) dissolve very quickly. However, in media with pH 8.2 or more the shells keep well (Turner,1976). A disadvantage of high pH is that it makes the condition of the soft parts of the organism deteriorate sharply during preservation, and extremely so when pH exceeds 8.4 (Steedman,1976, p.222). So, whenever the soft parts are also important it is necessary to preserve part of the sample with a normal preservative and part with a preservative with high pH. A good compromise for preservation is not known. If one wants to study the hard parts with scanning electron microscopy, one should not use a preservation liquid containing glycerol.

Fixation can be carried out with alcohol 70%, with pH raised to ca 8 with borax or sodium glycerophosphate. The second calcium-fixative to be tested was the following:

0.5 ml propylene phenoxetol
4.5 ml propylene glycol
5 ml formaldehyde 36%
95 ml distilled water
It is necessary to keep the pH at ca 8 by adding borax. The pH has to be checked daily (Universal Indikator pH 0-14 paper), until it does not change any more.

The above solution cannot be improved upon by using sea-water as this ultimately dissolves the calcium.

Besides preservation by way of alcohol with borax-raised pH, with regular pH-check, it was the intention to use the preservative of Owen-Steedman(1958), with pH-check (which the authors did not mention).
formula: 1% propylene phenoxetol
3% alcohol 100%
5-10% glycerol
rest: distilled water
The presence of glycerol makes it unsuitable for scanning electron microscopy. Therefore, if necessary, the formula can be changed on the basis of the one discussed above, of pppg, using borax for raising the pH, which is regularly checked.
formula: 1% propylene phenoxetol
3% alcohol 100%
10-15% propylene glycol
rest: distilled water
The two preceding media, however, could not be used due to a shortage of propylene phenoxetol.

Formalin with raised pH has not been tested because of the bad results obtained when using it for calcareous organisms (pers.communication S.van der Spoel).

II 5. Processing material before squashing

In order to obtain a squash preparation of zooplankton tissue or of an organism, it first has to undergo the following treatments: 1) pre-treatment; 2) fixation; 3) preservation; 4) squashing itself.

- The pre-treatment aims at obtaining dividing tissue with as many fission-stages as possible, showing well-discernible and well-spread-out chromosomes. Colchicin ($C_{22}H_{25}NO_6$) inhibits growth and the forming of microtubuli, and therefore the forming of the spindle, thereby putting the development of the cells to a standstill in a fixed stage of the nucleus-fission (prometaphase).

Due to the high degree of affinity of colchicin with the microtubuli, only a very slight quantity of colchicin is needed. According to E.Raubos (Free University, Amsterdam, pers.communication), the same effect can be obtained with fresh-water pulmonates, by storing the organisms, without any chemicals added, for ca 12 hours in the refrigerator. According to the manual General Histological Techniques(Free University, internal report), the colchicin has to be used for at least 4-5 hours in a 0.002 or 0.005 M. solution.

- Fixation (see II 2.d.): Carnoy; a fixation particularly suitable for squash slides; may also be used for preservation (unknown for how long)

Another pre-squash fixation is Newcomer fluid (Newcomer,1953).

formula: 600 ml isopropyl alcohol
300 ml propionic acid
100 ml petroleum ether
100 ml dioxane
100 ml acetone

This mixture can be made beforehand and kept.

Fixation time: ca 5 hrs; according to Pearse(1968) ca 12-24 hrs. According to Newcomer this fixation method is suitable for squash slides in combination with the Feulgen staining procedure.

- Preservation: according to Inaba's(1963) data, Newcomer fluid can also be used for preservation, for at least 9 years.

- Squashing: before actually squashing the organism its DNA has to be stained. The quickest staining-method, the acetic-orcein one, has been opted for (La Cour,1941).

Procedure:

Dissect out a piece of gonad tissue and place it in a drop of acetic-orcein dye which lies on a thin piece of glass. Staining should take between 20-40 minutes (to be tested). Prevent the drop from drying up by adding more dye, if necessary. During staining the drop should be heated on a heating plate until it takes on the form of a mercury drop. As staining is optimal at that stage, do not heat further. If necessary, snip the tissue into smaller pieces during staining, with dissecting needles. After staining, the tissue should be transferred to a drop of acetic acid 45% for two minutes. Subsequently, transfer to a drop of distilled water and snip the tissue very finely with crossed needles. Cover with a coverslip and press firmly with the thumb. Remove superfluous liquid.

Mounting:
For temporary mounting (a few days) acrylic resin should be run over the sides of the cover in order to prevent drying up.
Final mounting: first ran in 70% alcohol under the cover, afterwards 100% alcohol, and finally euparal which serves to embed the preparation.
N.B. as final mounting of Chaetognatha and Pteropoda tissues tends to be problematic, it is advisable in those cases to reverse the procedure. The tissue is then first very finely snipped, while in a drop of euparal without coverslip, and is squashed and embedded afterwards.
Squashing has been carried out with gonad tissue of Cymbulia peroni peroni and spermatogenetic tissue of various Sagitta species.

III. MATERIAL

The material was cought during the 'test cruise'. All the specific data on the hauls are to be found in the station list (table I). The net used was a Rectangular Midwater Trawl(R.M.T.1): an opening-and-closing-net with an opening of $1m^2$, mesh-size $0.33mm^2$ and a calculated effective opening of ca $0.8m^2$ at a ship's speed of max. 2 knots (Baker et al., 1973).

With the R.M.T.1 successful open hauls were made. The quality of the catches was exceedingly good. All planktonic organisms reached the ship undamaged and alive and it was possible to keep all of them alive in the refrigerator for a few days, if neccesary e.g. for photographing or pre-treatment.

IV. EXPERIMENTAL SET-UP

In table II the scheme is given for the treatment of non-calcareous organisms which, being museum-material, have to be preserved for a long time, and with which it should be possible to test various histological overall stains. Within this programme no stains were actually tested, this will yet have to be carried out.

From table II alone, 441 combinations can be made. It proved useless to test all of them. To test all possibilities of fixation and preservation with narcotisations which are certain to be unsuccessful, has no sense. That is why the narcotisations described were employed with the zooplanktonic organ-

isms from the first haul. This made it possible to assess their value and decide which ones to use for future catches (see Results and table IV).

Furthermore, those combinations known to be illogical from literature or from earlier experience were not carried out.

Control-treatments with alcohol 70% and buffered formalin 4% were always made. Whenever the formula mentions a changing of the preservation liquid, a sample was preserved without changing the preservative.

Table III gives the transfer list for non-calcareous material for long-term preservation and the ultimate scores taken from the quality assessment (p.11, tables IX-X); in table III transfer data of all specimens are represented.

Sub-sample 96b, fixed in 4% buffered formalin and preserved in pppg, was transferred to alcohol 70% seven months after having been caught. Sub-sample 33b, fixed in alcohol 70% and preserved in pppg was also transferred to alcohol 70% seven months after the catch. Half of sub-sample 63, preserved in pppg was transferred to 2% formalin in distilled water eight months after the catch, and was given the number 63I.

These transfers to alcohol 70% and to formalin 2% in distilled water of organisms preserved in pppg took place to assess the effect of standard museum preservatives on the quality of the samples. In practice, such a transfer would probably take place if the samples were given in loan to musea where pppg is not used as a preservative.

In table V the scheme is given for the processing of calcareous organisms which, being museum material, have to be preserved for a long time. The 28 combinations of this table have been reduced in the same way as those of table II. A further reduction was caused by the fact that the calcium-preservative could not be used (see II 4). Table VI gives the transfer list for calcareous material and the ultimate scores taken from the quality assessment (p.11 and table XI).

Table VII consists of the scheme for squash material. In principle all 20 possibilities have been carried out as indicated in table VIII, which gives the transfer list for the squash material.

Squashing was also tried on material which had been fixed for long-term preservation with Carnoy or Newcomer, but not preserved in either of these liquids; the data of this material will be found in table III.

For an assessment of the quality of the variously processed sub-samples the following characteristics were taken into account:

slimy	intransparent
sticky	contracted
softened	fallen apart
hardened	decoloured
crooked	turbid

The first quality assessment took place three months after the catch-date (table IX A & B). For each of the negative characteristics the sample clearly showed, it was given one point. If the characteristic was less clearly present, ½ point was allotted. "Good samples" were considered those with a number of points of max. 3; these were also studied after 8 and 15 months.

The results of each treatment-series were studied. In those cases in which a treatment-series was represented by more than one jar, initially only one jar was selected at random; the remaining jars were only studied (8 months after catch-date) if the first jar scored 3 points or less (table IX C).

Finally, a quality assessment has been carried out of three specific animal groups: Chaetognatha, Crustacea and Pteropoda (table X) and of the calcium-series (table XI).

V. RESULTS

V 1. Narcotisations

The narcotisation results are given in table IV. The narcotisation media which turned out to be the best were chloroform/acetone and M.S.222. They were easy to use and worked well and quickly. They did not give rise to crookedness and there was no visible decolouring. The external gills of Euphausiidae turned milky white after 8 minutes, but this does not matter as these organisms take only ca 4 minutes to become narcotised. These results led to the decision taken aboard to continue with these media. Side-effects of M.S. 222 are the ejection of ink in Cephalopoda and changes in size of the chromatophores in Cephalopoda and Crustacea.

The above media were also used to reduce the activities of the organisms during photographing. However, the narcotisations should also be seen in combination with the fixations and preservations and therefore also sub-samples without narcotisations should be studied as a control-group.

V 2. Quality-assessment of the subsamples

The quality assessment of the subsamples is given in table IX. The samples with a low score are best. The numbers of the jars correspond with those in table III, where only the last total scores are given.

It is useless to discuss all treatments separately, because there is a large number of bad results. Only the samples with a low score (max. 3) have been studied more closely.

The best preserved sample is no 94 which scored 1 point at the first check. At the second check, 8 months after the catch, nothing had changed. Subsample 63, which had been treated similarly, also scored 1 point after 8 months. These subsamples were fixed in 4% buffered formalin, without narcotisation and preserved in pppg. These samples do not show decolouring.

The subsample next in quality is no 96b which had 1½ point at the first and at the second check. It had been narcotised with M.S.222, fixed in 4% buffered formalin and preserved in pppg. There was no subsample which had been treated similarly.

When comparing the quality of the subsamples with scores of 1-3 at the first and at the second check, the following can be concluded: except for one sample, no 22c, the subsamples had not visibly deteriorated between the two checks. No 22c had been fixed with alcohol 70% and preserved in pppg; its quality had seriously deteriorated. The samples which had been treated similarly as no 22c were also in bad shape.

After 15 months the subsamples which had been best at the previous checks were looked at again; their condition had remained unchanged.

When comparing all results one can conclude that the best fixation is 4% buffered formalin and that pppg is the best preservation medium, although there is not much difference between the latter and 2% formalin with sea-water.

However, there is a marked difference in narcotisation results. Preservation with pppg gives better results when M.S.2222 was used for narcotisation than with acetone/chloroform. Exactly the opposite is the case for preservation with 2% formalin in seawater. This clearly shows that the narcotisation applied does influence the preservation. However, from the subsamples one cannot conclude that the narcotisations are important for the ultimate quality, as the best final result was obtained without narcotisation.

The control-subsamples which have been preserved in alcohol 70% or 4% formalin (samples 14,15 and 97) are also good. Their score was between 2-3. As it is a well-known fact that these media are more or less satisfactory, the similarly treated subsamples in alcohol and formalin have not further been included in the quality assessment. However, most planktonic organisms ultimately become very hard and intransparent in alcohol, and very brittle when stored in 2% or 4% formalin, due to the disrupture of the albumen-structure.

A remarkable result was obtained by the use of Perenyi in subsample 75

As a preservation liquid it is not very good, but for fixation purposes it is very appropriate in combination with alcohol 70% for preservation. The alcohol has to be changed after two days, if this does not take place the result is markedly worse.

All further narcotisations and preservations following an alcohol 70% fixation are bad, as alcohol dissolves the lipids and oils. This is also the reason why alcohol cannot very well be used as both fixative and preservative.

Also all samples treated with Carnoy, Newcomer and Bouin show quite rapidly bad results. After 8 months already, both the Carnoy fixed and preserved material and the Carnoy fixed and alcohol 96% preserved material are in bad condition. After 8 months the Carnoy preserved subsamples are almost dried out, notwithstanding the closed plastic lids. Accordingly, Carnoy turns out to dissolve plastic quite rapidly and so do Carnoy vapours, albeit slowly. Consequently, Carnoy is utterly unsuitable for museum preservation.

Besides the quality assessment of the subsamples, also the calcareous organisms, the 'calcium series' were checked twice. These results are given separately in table XI. Although there were few calcareous organisms in these small samples, the only three that were present remained in good condition in the calcium fixative. Notwithstanding the high pH(ca 8), they were comparable with the best subsamples.

V 3. Quality assessment of some specifically treated animal groups (table X)

a. Crustacea. It is difficult to state anything definite with regard to the few specific Crustacea samples. However, a few things seem to stand out clearly. Firstly: formalin fixation and preservation yields better results than alcohol 70%. Formalin 4% preservation is only suitable for big Crustacea. For the small ones a 2% formalin solution with sea-water is much better. It is a pity that pppg could not be tested.

b. Pteropoda. The best results were obtained with formalin 4% fixation combined with formalin 2% in sea-water preservation, or combined with a preservation in the calcium fixative. Once again, alcohol 70% is much less suitable. Additionally, also the pteropods have been checked which had been preserved for squash purposes. There is a marked difference between the Carnoy preservation, in so far as these samples had not dried out 8 months after the catch, and the Newcomer preservation: the Carnoy treated material is in better condition.

c. Chaetognatha. Also here, the best results were obtained with 4% formalin fixation in combination with a 2% formalin in sea-water preservation, and with

2% formalin in sea-water fixation and preservation. Alcohol 70% was not used as it is known (pers.communication Pierrot-Bults) that chaetognaths preserve very badly in alcohol 70%: they become hard and intransparent. The quality of the organisms fixed and preserved in Bouin is good for histology. Also in this group the specimens preserved for squash purposes have been checked. Also here the quality of the Carnoy preserved material, in so far as these samples had not dried out 8 months after the catch, was remarkably different from that of the Newcomer preserved material: in this case the Newcomer material is better, the opposite result from the Pteropoda.

V 4. Squash results

The preparations seemed to be all right, but it was not possible to trace any metaphase nuclei, all the preparations turned out to contain only interphase nuclei with inactive dividing tissue, and consequently inactive gonads. Only one Cymbulia peroni peroni from jar 36a manifested a different division stage. Although it is not clear which stage is concerned, due to a lack of material for comparison and because of the fact that this sample had not been pretreated with colchicin, it seems to be a diakinetic stage of the primary spermatocyte.

To check whether the method worked adequately Chaetognatha were squashed which had been caught in the Central North Sea in August 1978. These Chaetognatha had been fixed and preserved in Newcomer's fluid, without a colchicin pre-treatment. In the tail coelom of these Chaetognatha all fission stages of the spermatogenesis were found together, which shows that the squash- and staining-method is satisfactory.

With the samples 82-84, which had been fixed with Carnoy and preserved in alcohol 96%, the making of a squash preparation did not succeed.

All combinations of narcotisations, fixations and preservations carried out but not discussed here were of such bad quality that they are not recommended.

VI. DISCUSSION

The tests carried out do not pretend to offer something entirely new but they serve to acquire experience with less standardised media than alcohol and formalin. On the basis of the results a more considered choice is proposed for the use of particular chemicals or combinations of chemicals.

For mixed plankton samples narcotisation turned out to be useless: the quality of the samples is not improved and furthermore, narcotisation in combination with fixatives and preservatives may have a negative influence on the quality of some organisms in a mixed sample or on the sample as a whole.

As a way to anaesthesize organisms for purposes of study and photography narcotisation did prove useful.

During the 1980 expedition a M.S.222 stock fluid was made in a saturated solution. It was possible to drop any amount of this stock fluid around an organism with a drop-pipette, until the result was satisfactory, without the risk of the animal dying.

It still remains to be studied whether veronal can be used as slow-working barbiturate and whether barbituric acid can be used as a quick-working medium.

For subsamples to be preserved as museum material alcohol 70%, Bouin, Carnoy and Newcomer do not yield satisfactory results, to be judged from their condition 8 months after the catch. Perenyi gave a good result with alcohol 70% preservation, provided the alcohol was changed. However, the use of Perenyi plus alcohol on a large scale is complicated and expensive and enormous quantities of alcohol cannot be stored aboard ship.

Fixation with 4% formalin has proved a necessary step in the procedure. Preservation can take place in various ways. On the grounds of the quality of the subsamples after 8 months it is advised to use: a 2% formalin solution in sea-water, a 2% formalin solution in distilled water or a 4% buffered solution. As the I.O.S. (Institute of Oceanographic Sciences) acquired the experience that organisms preserved in buffered formalin (e.g. with hexamin) ultimately fall apart, they preserve the organisms in a solution of slightly acid formalin with pH ca 6, in which they seem to remain in good condition (pers.communication Mr.P.M.David)

This information brought about a pH check of the various subsamples of good quality, ca 8 months after the catch. Formalin 2% + sea-water had a pH of ca 6.5. Formalin 2% + distilled water and pppg in distilled water both had a pH of ca 5.5. (This datum will cause pppg solutions to be made with sea-water, without further additions, during future expeditions.)

When using formalin as preservation medium one should take into account the Sorenson(1908) reaction. This reaction causes the pH to decrease with an increasing rate of plankton/formalin. Therefore, the following proportion should be observed: 1 volume-unit of organisms in 3 volume-units of liquid.

For the pppg mixture nothing is yet known about the behaviour of the pH after some time, nor about the various quantities of material and preservative. What is known (Steedman,1976) is that a 1% propylene phenoxetol solution in distilled water, in which fixed material had been preserved, had

a constant pH of between 6 and 6.5 for a number of years. In view of the chemical character of propylene glycol it is not likely that a pH-decreasing reaction will take place with the already fixed material. However, this will have to be verified for subsamples preserved in pppg.

Up to now the results show that the best way is to fix the samples for 48 hrs in 4% borax buffered formalin and subsequently to preserve them in pppg in distilled water.

Preservation with a 2% formalin solution in filtered sea-water yields almost equally good results. For purely practical reasons concerning the work aboard ship this latter treatment is to be preferred due to the simplicity of the process and the small amount of distilled water needed.

A third practical method is to fix with 4% borax buffered formalin and to leave the sample like that until the application of preservatives on shore. A disadvantage is that the organisms are longer subjected to a high concentration of formalin with the concomitant progression of the "tanning" process.

One other possibility which still has to be worked out is the use of the calcium fixative also for non-calcareous organisms. As the results showed, the samples in this fixative were still in very good condition ca 8 months after the catch, notwithstanding the high pH of ca 8.2. Therefore, this calcium fixative (formula on p.7) should be tested in a slightly acid state, for larger samples than those of the test journey. Besides, this solution will be tested with filtered sea-water, with a pH of ca 6: a high pH is of no use for non-calcareous organisms; for calcareous organisms this solution is worthless due to the fact that sea-water dissolves the calcium.

The advantage of the calcium fixative compared with a formalin solution in distilled water is the more rapid penetration in the tissues, because of the combination of formalin with propylene phenoxetol (Baker, 1966). Although also formalin in sea-water penetrates more rapidly than in distilled water, the difference with the calcium fixative is not known (Steedman, 1976).

To this point fixatives and preservatives dissolved in distilled water or in sea-water have been discussed without talking about the osmotic value of these solutions. Needless to say, the ideal is to be able to use a fixative and a preservative which are as isotonic as possible with sea-water. That is why there still has to be carried out research into the osmotic values of the various media.

For the preservation of larger plankters, e.g. large Crustacea, it is probably best to apply 4% formalin. Dr.C.F.E.Roper (personal communication)

advises to fix and preserve Cephalopoda with 10% formalin. Especially these larger organisms will often be narcotised which necessitates a study of the correlation between the narcotisation fluid and the rest of the treatment.

A clear illustration of the fact that specific animal groups react differently to a particular medium is provided by the use of Bouin: only Chaetognatha remain well preserved in Bouin, for at least 8 months.

The transfer, 8 months after the catch, of samples 96b and 33b into alcohol 70% and of sample 63I into 2% formalin/distilled water showed the best result for sample 63I (one month after the transfer). Seven months after the transfer sample 63I was still in the best condition. Samples 96b and 33b had not further deteriorated but showed the characteristics typical of any alcohol sample: hardening, intransparency and a tendency to become white. Besides, these samples had formed clots at places.

One should bear in mind that the results of the treatments described above cannot be fully objective, as the quantities worked with were not 100% qualitatively measurable. To further assess the usefulness of media like pppg as preservatives for museum material, long-term experience will be indispensable, especially as to the preservation time and the possibility to carry out haemaluin–eosin and other overall stains. The same holds for the use of Perenyi in combination with alcohol and for the specific use of Bouin with Chaetognatha.

The squashing did not yield any direct karyological results. The question which arose was: what is the matter with the organisms caught during the test-cruise in which only sexually inactive tissue was found?

For Cymbulia the reason was thought to be sexual inactivity as they had hardly any developed gonad tissue, which could be due to the fact that the sexual reproduction just had not yet set in that early in spring. The Chaetognatha, however, showed several stages of sexual development, to judge from the developmental condition of the tail coelom, according to the coding system of Pierrot-Bults(1975).

Chaetognatha, caught in August 1978 in the Central North Sea and fixed and preserved without pre-treatment, in Newcomer, displayed the entire spermatogenesis immediately after having been squashed. This datum leads to the strong assumption that the Chaetognatha of the test-cruise were indeed sexually inactive, but that this rest-phase can occur during various sexual stages. It is, of course, also possible that the coding-system of the sexual stages is not directly coupled with the real sexual activity.

Nevertheless, the squash results were such that can be stated that it is possible to squash Carnoy and Newcomer fixed and preserved material.

However, it should be borne in mind that squashing of the Carnoy and Newcomer fixed/preserved material should take place within roughly three months as the quality sharply deteriorates, as mentioned before.

During future cruises in other seasons it will be necessary to fix and preserve specimens from animal groups to be squashed separately in Carnoy and Newcomer for reasons of comparison.

The above-discussed progress of the squash tests has not led to a conclusion as to the influence of colchicin on cell-fission and as to the concentrations to be used. Therefore, the various colchicin concentrations for the pre-treatment will have to be tested anew. The colchicin has to be prepared freshly with sea-water. During the pre-treatment the organisms must remain alive, in the refrigerator at ca 4°C. As the colchicin kills the micro-organisms in sea-water, the sea-water with colchicin goes bad, which excludes the possibility of making a stock fluid. Furthermore, it seems advisable to try, during the squashing procedure, to macerate the tissue in 1M HCl of 60°C during 5 min. or more (Dyer,1979). Although, according to Dyer, this is only necessary for plant material, it may loosen the animal tissue which had become hard through fixation and preservation, thus facilitating the actual squashing.

It is not useful to just put the organisms in the refrigerator as a pre-treatment, as many of the organisms caught normally also live in a low temperature comparable with that of the refrigerator.

I should very much like to thank Mr.A.F.de Fluiter for his great part in the making of the various mixtures, for his good and well-organised way of packing the chemicals, his help in deciding which media to apply and his help in making the squash preparations.

I wish to thank Els van der Zee for her work on the manuscript. I am very much indebted to the project-leader Prof.Dr.S.van der Spoel for the chance he offered me to participate in the cruise, in the framework of my studies.

SUMMARY

In this report narcotisation, fixation and preservation experiments with marine zooplankton are described.

Narcotisation turns out to be useless for mixed plankton samples. M.S. 222 works well as narcotisation medium for organisms to be photographed. Fixation with 4% formalin proved to be a necessary treatment. Afterwards the best preservation method is to use a propylene phenoxetol plus propylene glycol solution in distilled water or a 2% formalin solution in filtered sea-water. Further study is necessary of the use of sea-water as a solution

medium, of the pH changes, the osmotic value of the solutions, the long-term use and the subsequent processability of the organisms for histological purposes.

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101A

Handleiding voor het behandelen van organismen tijdens een expeditie.

Na de vangst gaan de organismen in koelboxen gevuld met vers gekoeld zeewater (± 4°C).
Hierna wordt een verdeling en sortering gemaakt van het materiaal:

I: de te fotograferen organismen
II: Vissen
III: Cephalopoda (inktvissen)
IV: tere planktonten, zoals kwallen, salpen en tunicaten
V: stevige planktonten
VI: Chaetognatha (pijlwormen)
VII: kalkhoudende organismen
VIII: squash materiaal.

De verwerking vindt hoofdzakelijk plaats volgens de nu volgende puntsgewijze beschrijvingen.

ad I: Organismen die gefotografeerd dienen te worden, gaan na voorzien te zijn van een potnummer apart in de koelkast op vers gefilterd zeewater. Na het fotograferen worden de dieren volgens één van de onderstaande beschrijvingen verwerkt.

ad II: De vissen die in de collectie van het museum gaan worden gefixeerd op 4% gebufferde formaldehyde met zeewater gedurende minimaal 48 uur. Daarna worden zij overgebracht op alcohol 70%. De vissen waaraan de otolieten en wervels bestudeerd moeten worden gaan direct op alcohol 70%. In dit geval weinig vissen op veel alcohol zetten (bijv. 1:5).

ad III: Grote inktvissen dienen volgens Dr.C.F.E.Roper (Smithsonian Institution) gefixeerd en gepreserveerd te worden op 10% gebufferde formol + aqua dest. Kleinere en tere inktvissen fixeren en preserveren op gebufferde 4% formol + aqua dest.

ad IV: Tere planktonten 48 uur fixeren op gebufferde 2% formaldehyde + zeewater, daarna preserveren op pppg.

ad V: Stevige planktonten 48 uur fixeren op gebufferde 4% formaldehyde + zeewater, daarna 50% van het materiaal preserveren op gebufferde 4% formaldehyde + aqua dest en 50% preserveren op pppg.

ad VI: Chaetognatha behandelen als IV.

ad VII: Kalkhoudende organismen. 50% van het materiaal fixeren en preserveren op alcohol 70% waarvan de pH op ± 8,2 wordt gehouden met Borax. De eerste dagen regelmatig controleren (met pH indicator papier). De pH op 8,2 houden totdat deze niet meer verloopt. De andere 50% van het kalkhoudende materiaal fixeren en preserveren op kalkfixatief. In dit geval, 1 deel organismen op 5 delen kalkfixatief. Ook hier de pH op 8,2 houden. Nooit zeewater gebruiken daar dit de kalk doet oplossen!

ad VIII: Het materiaal voor squash doeleinden wordt eerst levend voorbehandeld. Hiertoe wordt 50% van het materiaal gebracht op een verse 0,002 mol colchicine oplossing met vers zeewater (A). De andere 50% van het materiaal wordt op een verse 0,005 mol colchicine oplossing met vers zeewater gebracht (B). Het materiaal moet minimaal 5 uur op deze oplossingen blijven bij een temperatuur van ± 4°C;
Al het uitwendig en inwendig contact met deze colchicine oplossingen moet vermeden worden. Colchicine is zeer giftig. Draag handschoenen en werk in zuurkast.
Na deze voorbehandeling 50% van A en 50% van B fixeren en preserveren op Carnoy in glazen potjes, de andere 50% van A en B op Newcomer's fluid brengen. Ook hierbij handschoenen gebruiken.

./.

Vergeet nooit:

A) Een potnummer.
B) Doe dit nummer in de pot en op de deksel.
C) Vul de monsterkaartjes duidelijk in met potlood.
D) Noteer de loop van de verwerking in de doorvoerschema's van de monsters plus de tijdstippen van de verschillende behandelingen.
E) Zorg dat de nog door te voeren monsters gescheiden blijven van de niet meer door te voeren monsters.
F) Als er iets speciaals met een monster gebeurt, noteer dit dan in het doorvoerschema.
G) Zet de potten altijd zeevast met dichte deksel.
H) Als de monsters uiteindelijk opgeslagen kunnen worden, verstrek dan alle gegevens betreffende het monster aan de centrale administratie.
I) Potten die opgeslagen worden, dienen dicht gesealed te worden met paraplast of paraffine.
J) Laat altijd voor de volgende ploeg een duidelijke lijst achter waar opstaat wat er nog gebeuren moet en wanneer.

Table I. Station list

position - the position at the start of each haul is given

at surface: temp./sal. - the temperature (in °C) and the salinity (in ‰) at the start of the haul at the sea surface are given

gear - the abbreviations used are: RMT1 = Rectangular Midwater Trawl 1; Ring = open Ringnet

stat.	haul	date 1979	position N:	position W:	depth in m	time start	time end	at depth start	at depth end	at surface temp.	at surface sal.	speed in kn.	angle wire	gear
1	1	28.2	50°07.0	2°47.0	0-30	18.40	20.44	18.45	20.42	7.91	35.32	2.6		RMT1
2	2	2.3	47°14.3	6°50.6	0-1000	19.35	22.20	20.15	20.15	11.26	35.65	0.0	30°	RMT1
2	3	2.3	47°13.4	6°43.6	0-50	23.00	00.20	23.15	00.10	11.26	35.65	0.0	32°	RMT1
2	4	3.3	47°07.7	6°39.5	0-500	02.10	04.40	02.35	03.39	11.25	35.65	0.0	30°	RMT1
3	5	3.3	47°06.1	6°45.1	0-300	21.14	23.20	21.50	22.50	11.28	35.67	0.5	30°	Ring
3	6	3.3	47°06.3	6°36.2	0-300	23.35	01.38	00.05	01.07	11.28	35.67	0.0	30°	RMT1
3	7	4.3	47°08.6	6°30.7	0-300	01.49	03.50	02.21	03.22	11.26	35.66	0.5	30°	Ring
3	8	4.3	47°05.3	6°27.3	0-50	03.58	04.50	04.06	04.36	11.28	35.66	0.5	25°	RMT1
4	9	4.3	47°03.4	6°19.5	0-1000	13.20	17.57	15.15	15.29	11.37	35.65	1.4	30°	RMT1
5	10	5.3	47°21.4	6°18.6	0-45	23.59	01.15	00.10	01.08	11.31	35.64	1.5	30°	RMT1

Table II. Scheme for long-term preservation of non-calcareous organisms in taxonomic collections.

narcotisation	fixation	preservation
acetone-chloroform	Bouin	Bouin
magnesium chloride	Carnoy	Carnoy
Nembutal	buffered formalin 4% + distilled water	buffered formalin 4% + distilled water
M.S.222	formalin 4% + sea water	formalin 4% + sea water
chloral hydrate	formalin 2% + sea water	buffered formalin 2% + distilled water
menthol + ethylalcohol	Perenyi	formalin 2% + sea water
valerian	alcohol 70%	propylene phenoxetol + propylene glycol
		alcohol 70%
		alcohol 96%

Table III. Transfer list for non-calcareous material for long-term preservation.

haul	jar	organism	narcotisation	fixation	fix.time in hrs	preservation	changings	remarks[1]
1	7	subsample	none	Newcomer	-	-	-	7
1	8	,,	,,	Bouin	-	-	-	3½
1	9	,,	,,	Carnoy	-	-	-	-
1	10	,,	,,	,,	-	-	-	5
1	11	,,	,,	alcohol 70%	-	-	-	-
1	12	,,	,,	,,	18	alcohol 70%	after 48 hrs	
1	13	photographed organisms	,,	buffered formalin 4%	min.48	formalin 2% + sea-water	-	-
1	14	subsample	,,	,,	-	-	-	2½
1	15	,,	,,	,,	min.48	formalin 2% + sea-water	-	2½
1	16	Crustacea	,,	alcohol 70%	,,	alcohol 70%	-	4
2	17	Ctenophora + other soft organisms	,,	formalin 2% + sea-water	,,	pppg[2]	-	-
2	18	Ostracoda	acetone/chloroform	buffered formalin 4%	,,	formalin 2% + sea-water	-	-
2	19	subsample	none	calcium fixat.	-	-	-	pH does not change
2	20	,,	,,	alcohol 70%	min.48	alcohol 70%		7
2	21	,,	M.S.222	buffered formalin 4%	-	-	-	3
2	22a	,,	acetone/chloroform	alcohol 70%	min.48	alcohol 70%	-	3
2	22b	,,	,,	,,	-	-	-	4½
2	22c	,,	,,	,,	min.48	pppg	-	5
2	23	Chaetognatha	,,	buffered formalin 4%	,,	formalin 2% + sea-water	-	1½
2	24	Crustacea	acetone/chloroform	,,	-	-	-	½
2	25	,,	,,	alcohol 70%	min.48	alcohol 70%	after 48 hrs	2
2	26a	subsample	,,	buffered formalin 4%	,,	formalin 2% + sea-water	-	2
2	26b	,,	,,	,,	,,	formalin 2% + dist.water	-	2½

[1] numbers represent total scores from quality assessments on p.11 and in table IX and, for specific animal groups, table X
[2] pppg = propylene phenoxetol plus propylene glycol

table III continued

haul	jar	organism	narcotisation	fixation	fix.time in hrs	preservation	changings	remarks
2	26c	subsample	acetone/chloroform	buffered formalin 4%	min.48	pppg	-	3
2	27	Fish	none	alcohol 70%	min.48	alcohol 70%	-	-
2	28	,,	,,	,,	,,	,,	-	-
2	29	Cymbulia	acetone/chloroform	,,	,,	,,	-	$3\frac{1}{2}$
3	30	Medusa	M.S.222	diluted Bouin	-	-	-	Bouin:dist.water=1
3	31	Pteropoda	none	Carnoy	-	-	-	$2\frac{1}{2}$
3	32	,,	,,	Newcomer	-	-	-	4
3	33a	subsample	M.S.222	alcohol 70%	min.48	alcohol 70%	-	6
3	33b	,,	,,	,,	,,	pppg	-	4; transferred to alcohol 70% seven months after catch
3	34a	,,	,,	Bouin	-	-	-	4
3	34b	,,	,,	,,	min.48	alcohol 70%	-	-
3	35a	,,	,,	Carnoy	-	-	-	4
3	35b	,,	,,	,,	min.48	alcohol 96%	-	5
3	36a	,,	,,	Newcomer	-	-	-	7; used for squash
3	36b	,,	,,	,,	min.48	alcohol 70%	-	7; ,,
3	37	,,	,,	Perenyi	,,	,,	-	$4\frac{1}{2}$; ,,
3	38	,,	acetone/chloroform	Bouin	-	-	-	$4\frac{1}{2}$
3	39	,,	,,	Carnoy	-	-	-	,,
3	40	,,	none	alcohol 70%	min.48	alcohol 70%	-	-
3	49	,,	acetone/chloroform	buffered formalin 4%	-	-	-	$2\frac{1}{2}$
3	50	Tomopteris	M.S.222	,,	min.48	formalin 2% + sea-water	-	-
3	51	subsample	acetone/chloroform	,,	,,	,,	-	2
3	52	photographed organisms	M.S.222	alcohol 70%	,,	alcohol 70%	-	-
4	53	Chaetognatha	none	Bouin	12	,,	-	-
4	54	,,	,,	,,	-	-	-	$2\frac{1}{2}$
4	55	Medusa	,,	formalin 2% + sea-water	-	-	-	-
4	56	subsample	acetone/chloroform	Bouin	28	alcohol 70%	after 48 hrs	$4\frac{1}{2}$
4	57	,,	,,	Carnoy	,,	,,	-	$6\frac{1}{2}$
4	58a	,,	none	Bouin	-	-	-	-

table III continued

haul	jar	organism	narcotisation	fixation	fix.time in hrs	preservation	changings	remarks
4	58b	subsample	none	Bouin	min.48	alcohol 70%	-	4
4	59a	,,	,,	Carnoy	-	-	-	-
4	59b	,,	,,	,,	min.48	alcohol 96%	-	6½
4	60a	,,	,,	Newcomer	-	-	-	-
4	60b	,,	,,	,,	min.48	alcohol 70%	-	5
4	61	,,	,,	buffered formalin 4%	-	-	-	2
4	62	,,	,,	,,	min.48	formalin 2% + dist. water	-	-
4	63	,,	,,	,,	,,	pppg	-	1
4	63I	,,	,,	,,	,,	,,	-	transferred to formalin 2% + dist. water 8 months after catch
6	72	Fish	M.S.222	alcohol 70%	,,	alcohol 70%	-	-
6	73	subsample	none	Perenyi	-	-	-	3½
6	74	,,	acetone/chloroform	Newcomer	-	-	-	used for squash
6	75	,,	,,	Perenyi	7	alcohol 70%	after 48hrs	2½
6	76a	,,	none	Carnoy		-	-	6
6	76b	,,	,,	,,	1	alcohol 96%	-	5½
6	77a	,,	,,	,,	-	-	-	5
6	77b	,,	,,	,,	3	alcohol 96%	-	5
6	78a	,,	,,	,,	-	-	-	5½
6	78b	,,	,,	,,	5	alcohol 96%	-	6
6	79a	,,	M.S.222	,,	1	,,	-	5½
6	79b	,,	,,	,,	,,	,,	after 48hrs	4½
6	80a	,,	,,	,,	3	,,	-	6
6	80b	,,	,,	,,	,,	,,	after 48hrs	4½
6	81a	,,	,,	,,	5	,,	-	6
6	81b	,,	,,	,,	,,	,,	after 48hrs	4½
6	82a	,,	acetone/chloroform	,,	1	,,	,,	accidentally changed; used for squash
6	82b	,,	,,	,,	,,	,,	,,	used for squash
6	83a	,,	,,	,,	3	,,	-	,,
6	83b	,,	,,	,,	,,	,,	after 48hrs	,,

table III continued

haul	jar	organism	narcotisation	fixation	fix.time in hrs	preservation	changings	remarks
6	84a	subsample	acetone/chloroform	Carnoy	5	alcohol 96%	-	used for squash
6	84b	,,	,,	,,	,,	,,	after 48hrs	,,
6	85	,,	none	buffered formalin 4%	min.48	formalin 2% + sea-water	-	$2\frac{1}{2}$
7	86	Cymbulia	,,	alcohol 70%	,,	alcohol 70%	-	-
7	87	Medusa	,,	buffered formalin 4%	,,	pppg	-	-
6	88	,,	,,	formalin 2% + sea-water	,,	,,	-	-
6	89	Fish	M.S.222	alcohol 70%	,,	alcohol 70%	after 48hrs	
6	90	Medusa	,,	formalin 2% + sea-water	,,	pppg	-	-
6	91	Crustacea	,,	buffered formalin 4%	,,	formalin 2% + sea-water	-	1
8	92	subsample	none	,,	-	-	-	$2\frac{1}{2}$
8	93	,,	,,	,,	min.48	alcohol 70%	-	3
8	94	,,	,,	,,	,,	pppg	-	1
6	95	phytoplankton	,,	formalin 2% + sea-water	-	-	-	-
9	96a	subsample	M.S.222[1]	buffered formalin 4%	min.48	formalin 2% + sea-water	-	3
9	96b	,,	,,	,,	,,	pppg	-	$1\frac{1}{2}$; transferred to alcohol 70% seven months after catch
9	97	,,	,,	alcohol 70%	-	-	-	$3\frac{1}{2}$
9	100	,,	none	,,	-	-	-	6
9	101	,,	,,	formalin 4% + sea-water	-	-	-	-
9	102	,,	,,	buffered formalin 4%	-	-	-	2
9	103	,,	,,	Newcomer	-	-	-	-
9	104	,,	,,	Carnoy	-	-	-	-

[1] in the following three cases the M.S.222 solution was: 2 grs in 360 cc sea-water

table III continued

haul	jar	organism	narcotisation	fixation	fix.time in hrs	preservation	changings	remarks
9	105	subsample	none	Carnoy	-	-	-	-
9	106	Fish	,,	alcohol 70%	min.48	alcohol 70%	after 48hrs	-
9	107	,,	,,	buffered formalin 4%	-	-	-	-
9	108	Ostracoda	M.S.222	,,	-	-	-	-
9	109	Pteropoda	,,	alcohol 70%	min.48	alcohol 70%	after 48hrs	-
9	118	Decapoda	valerian(4 hrs)	buffered formalin 4%	-	-	-	valerian was not effective
9	119	Chaetognatha	M.S.222	,,	min.48	formalin 2% + sea-water	-	2
4	120	Ctenophora	none	formalin 4% + sea-water	,,	pppg	-	before fixation 48 hours in fridge
4	121	Decapoda	,,	,,	-	-	-	,,
9	122	Medusa	,,	,,	min.48	pppg	-	-
9	123	Cephalopoda	M.S.222	,,	-	-	-	-
9	124	Cymbulia	,,	alcohol 70%	min.48	alcohol 70%	-	3
9	125	Pteropoda	,,	calcium fixat.	-	-	-	2
9	126	Crustacea	,,	buffered formalin 4%	-	-	-	$2\frac{1}{2}$
10	129	subsample	none	alcohol 70%	min.48	pppg	-	$3\frac{1}{2}$
10	130	,,	,,	,,	,,	alcohol 70%	-	-
10	131	,,	,,	formalin 4% + sea-water	,,	formalin 2% + sea-water	-	-
10	132	Pteropoda	M.S.222	alcohol 70%	,,	alcohol 70%	-	-
9	133	,,	,,	buffered formalin 4%	,,	formalin 2% + sea-water	-	-
9	136	Chaetognatha	none	formalin 2% + sea-water	-	-	-	$1\frac{1}{2}$
10	137	Ctenophora	,,	,,	-	-	-	-
10	138	Cymbulia	M.S.222	buffered formalin 4%	min.48	formalin 2% + sea-water	-	2
10	139	,,	,,	calcium fixat.	-	-	-	2
10	140	Phronima	,,	alcohol 70%	min.48	alcohol 70%	-	-

Table IV. Narcotisations (all the organisms are from haul no 1)

organism	narcotisation	results and remarks
Euphausiidae and Copepoda	$MgCl_2$	Euphausiidae narcotised after ca 15 min. Copepoda after ca 5 min. The organisms turn white after ca ½h. narcotisation. Narcotisation time proportionate to body size.
Euphausiidae	chloral hydrate	after 5-10 min. narcotised. The colour of the organisms changes.
Euphausiidae	chloroform + acetone	narcotised after 1-1½ min. without perceptible crookedness.
Euphausiidae	alcohol + menthol	works very slowly. After narcotisation the organisms become very crooked. Organisms larger than 2cm do not react to the narcotisation.
Euphausiidae	Nembutal	narcotised after ca 10 min. Pleiopods contract against one another.
Euphausiidae	M.S.222	good narcosis after ca 4 min. Gills become milky white after ca 10 min.
Euphausiidae and Decapoda	valerian	the concentration of 1-25 drops/20 ml did not affect these organisms at all.

Table V. Scheme for long-term preservation of calcareous organisms in taxonomic collections.

narcotisation	fixation	preservation
acetone-chloroform magnesium-chloride nembutal M.S.222 chloral hydrate menthol + ethylalcohol valerian	alcohol 70%[1] calcium fixative[1]	alcohol 70%[1] calcium preservative[1]

[1] pH should be kept ≥ 8.2

Table VI. Calcium series (calcareous organisms, all from haul no 9).

jar	narcotisation	narc.time	fixative/preservative	results (cf. p.11 and table XI)
110	-	-	alcohol 70%	4½
111	M.S.222	20 min.	alcohol 70%	4
112	acetone/ chloroform	20 min.	alcohol 70%	5½
113	menthol + chloral hydrate	30 min.	alcohol 70%	5½
114	-	-	calcium fix.	2
115	M.S.222	20 min.	calcium fix.	2
116	acetone/ chloroform	20 min.	calcium fix.	2
117	menthol + chloral hydrate	30 min.	calcium fix.	2½

Table VII. Preparation scheme for squash technique.

pre-treatment	fixation/ preservation	stain
colchicine 0.002 colchicine 0.005 refrigerator	Newcomer Carnoy	acetic-orcein

Table VIII. Squash series.

haul	jar	organism	pre-treatment	duration pretreatm.	fixation/ preserv.	results (cf.table X)
2	41	Chaetognatha	colchicine 0.002	5 hrs	Newcomer	3
2	42	Chaetognatha	colchicine 0.002	5 hrs	Carnoy	5
2	43	Chaetognatha	colchicine 0.005	5 hrs	Newcomer	3
2	44	Chaetognatha	colchicine 0.005	5 hrs	Carnoy	dried out
2	45	Pteropoda *Cymbulia*	colchicine 0.002	5 hrs	Carnoy	$2\frac{1}{2}$
2	46	Pteropoda *Cymbulia*	colchicine 0.002	5 hrs	Newcomer	3
2	47	Pteropoda *Cymbulia*	colchicine 0.005	5 hrs	Carnoy	$2\frac{1}{2}$
2	48	Pteropoda *Cymbulia*	colchicine 0.005	5 hrs	Newcomer	3
3	64	Pteropoda *Diacria trispinosa*	refrigerator 4°C	15 hrs	Carnoy	[1][2]
3	65	Pteropoda *Diacria trispinosa*	refrigerator 4°C	15 hrs	Newcomer	[1][2]
3	66	Pteropoda	refrigerator 4°C	15 hrs	Carnoy	dried out[1]
3	67	Pteropoda	refrigerator 4°C	15 hrs	Newcomer	4[1]
4	68	Chaetognatha	refrigerator 4°C	15 hrs	Carnoy	dried out[1]
4	69	Chaetognatha	refrigerator 4°C	15 hrs	Newcomer	3[1]
4	70	subsample	refrigerator 4°C	15 hrs	Carnoy	
4	71	subsample	refrigerator 4°C	15 hrs	Newcomer	
6	98	subsample	refrigerator 4°C	12 hrs	Carnoy	
6	99	subsample	refrigerator 4°C	12 hrs	Newcomer	
9	127	subsample	colchicine 0.002	5 hrs	Newcomer	there was no Carnoy left
9	128	subsample	colchicine 0.005	5 hrs	Newcomer	
9	134	subsample	colchicine 0.002	5 hrs	Newcomer	
9	135	subsample	colchicine 0.005	5 hrs	Newcomer	

[1] after 15 hrs in the refrigerator the organisms were still alive
[2] for fixation the shells were cracked

Table IX. Quality assessments of fixation/preservation treatments (see p.11 and table III; jar numbers correspond with those in table III)

A. Results after three months, with the totals after eight months (8) and fifteen months (15) added.

jar no	slimy	sticky	softened	hardened	crooked	intransparent	contracted	fallen apart	decoloured	turbid	total	(8)	(15)	remarks
94									1		1	1	1	
96b					½	½			½		1½			after 6 months into alc.70%
51					½	1			½		2	2	2	
75					½	½			1		2	2½	2½	became somewhat contracted
15					½	½	½		1		2½	2½	2½	
26b					½	½	½		1		2½	2½	2½	
14					½	½	½		1		2½	2½	2½	
22a					1	½	½		½		2½	3	3	became more decoloured
49						1	½		1		2½	2½	2½	
26c					1	½	½		1		3	3	3	
93					½	1		½	1		3	3	3	
22c		½	½		½			½	1		3	5	5	1st check: somewhat lumpy; 2nd check: more fallen apart and crooked; intransparent
21					½	½	½	1	½		3	3	3	
96a					½	1		1	½		3	3	3	
97					½	1		½	1		3	3½	3½	became somewhat contracted

B. After three months only.

73					1	1	½		1		3½			
8					1	1	½		1		3½			everything yellow
129					1		1	1	½		3½			
58b					1	1	½	½	1		4			everything yellow
34a					1	1	1		1		4			everything yellow
33b	1		1		½			½	½	½	4			
35a					1	½	½	½	1	½	4			fluid splits into two layers, plastic dissolves; after 8 months fluid almost evaporated through pl. lid
22b			1		1	½		1	1		4½			
37					1	1	1		1	½	4½			salps look beautiful
38		½			1	1	½	½	1		4½			everything yellow
80b		1			1	1		½	1		4½			
81b		1			1	1		½	1		4½			
56		½			½	1	1	½	1		4½			everything yellow
79b		1			1	1		1	½		4½			
77a					1	1		1	1	1	5			
60b		1			1	1	½	½	1		5			
35b		1			1	1	½	½	1		5			

table IX B continued

jar no	slimy	sticky	softened	hardened	crooked	intransparent	contracted	fallen apart	decoloured	turbid	total	(8)(15) remarks
77b		1			1	1	1		1		5	
10			1		1	1	½	½	1		5	
76b		1			½	1	1	1	1		5½	
79a		1			1	1	1		1	½	5½	
78a					1	1	½	1	1	1	5½	organism stuck to jar
80a					1	1	1	1	1	1	6	
33a		½			1	1	1	½	1	1	6	
100	½	1	1			1	1	½	½	½	6	
78b		1			1	1	1	½	1	½	6	
76a		½			1	1	1	½	1	1	6	
81a	•	½			1	1	1	½	1	1	6	
57		1			1	1	1	½	1	1	6½	
59b		½			1	1	1	1	1	1	6½	
7	1	1			1	1	1	½	1	½	7	lumpy; pigment dissolved
20			1		1	1	1	1	1	1	7	
36b	1	1			1	1	1	1	1		7	
36a	1	1			1	1	1	½	1	½	7	

C. After eight months only.

63									1		1	
26a						½	1		½		2	
61					½	½	½		½		2	
102					½	½	½		½		2	
85					½	½	½		1		2½	
92					½	½	½		1		2½	

Table X. Quality assessments of fixation/preservation treatments (see p, 11) of Chaetognatha, Pteropoda and Crustacea carried out 8 months after treatment (jar numbers correspond with those in tables III and VIII)

	jar no	slimy	sticky	softened	hardened	crooked	intransparent	contracted	fallen apart	decoloured	turbid	total	
Pteropoda	29						1	½	½	1	½	3½	
	31						1	½		1		2½	
	32						1	1	½	1	½	4	
	124						1		½	1	½	3	cf. table III
	125						1			1		2	
	138						1			1		2	
	139						1			1		2	
	45						1	½		1		2½	
	46						1	½	½	1		3	cf. table VIII
	47						1	½		1		2½	
	48						1	½	½	1		3	
	66[1]												
	67						1	1	½	1	½	4	
Chaetognatha	23						½			1		1½	
	54				½	1				1		2½	
	119						1			1		2	cf. table III
	136						½			1		1½	
	41					1	½			1	½	3	
	42		½	½			1	½	1	1	½	5	
	43					1	½			1	½	3	
	44[1]												cf. table VIII
	68[1]												
	69					1	½			1	½	3	
Crustacea	16						1	1		1	1	4	
	24										½	½	
	25							1	½		½	2	cf. table III
	91										1	1	
	126	1							½		1	2½	

[1] dried out

Table XI. Quality assessments of the fixation/preservation treatments (see p.11) of the calcium series, carried out four months (and 2.total: 8 months) after treatment (cf. table VI; the jar numbers correspond with those in table VI).

jar no	slimy	sticky	softened	hardened	crooked	intransparent	contracted	fallen apart	decoloured	turbid	calcium dissolved	total	2.total	remarks
110					1	1	1		1		½	4½	4½	the calcium is brittle
111			½		½	1		½	1	½		4	4	
112			1		½	1		1	1	½	½	5½	5½	
113			1		½	1	½	1	1	½		5½	5½	
114							1		1			2	2	
115[1]						½	1		½		?	2	2	all the Ostracoda had
116							1		1			2	2	opened valves
117					½		1		1			2½	2½	

[1] no calcareous organisms