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<https://doi.org/10.1002/tax.603016>

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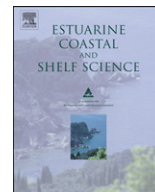
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Contents lists available at SciVerse ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Spatial synchronies in the seasonal occurrence of larvae of oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis/galloprovincialis*) in European coastal waters

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ARTICLE INFO

Article history:

Received 28 May 2011

Accepted 8 May 2012

Available online 28 May 2012

Keywords:

spatial synchrony

synoptic scale

larval occurrence

Crassostrea gigas

Mytilus edulis

Mytilus galloprovincialis

ABSTRACT

Reproductive cycles of marine invertebrates with complex life histories are considered to be synchronized by water temperature and feeding conditions, which vary with season and latitude. This study analyses seasonal variation in the occurrence of oyster (*Crassostrea gigas*) and mussel (*Mytilus edulis/galloprovincialis*) larvae across European coastal waters at a synoptic scale (1000s of km) using standardised methods for sampling and molecular analyses.

We tested a series of hypotheses to explain the observed seasonal patterns of occurrence of bivalve larvae at 12 European stations (located between 37°N and 60°N and 27°W and 18°E). These hypotheses included a model that stated that there was no synchronisation in seasonality of larval presence at all between the locations (null hypothesis), a model that assumed that there was one common seasonality pattern for all stations within Europe, and various models that supposed that the variation in seasonality could be grouped according to specific spatial scales (i.e., latitude, large marine ecosystems and ecoregions), taxonomic groups, or several combinations of these factors.

For oysters, the best models explaining the presence/absence of larvae in European coastal waters were (1) the model that assumed one common seasonal pattern, and (2) the one that, in addition to this common pattern, assumed an enhanced probability of occurrence from south to north. The third best model for oysters, with less empirical support than the first two, stated that oysters reproduced later in the south than in the north. For mussels, the best models explaining the seasonality in occurrence of

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larvae were (1) the model that assumed four underlying trends related to large marine ecosystems, and (2) the one that assumed one common seasonal pattern for larvae occurrence throughout Europe.

Such synchronies in larval occurrences suggest that environmental conditions relevant to bivalve larval survival are more or less similar at large spatial scales from 100s to 1000s of km. To unravel the underlying mechanisms for this synchronisation is of particular interest in the light of changing environmental conditions as the result of global climate change and the possible consequences for marine food webs and ecosystem services.

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1. Introduction

Evolution in marine invertebrates with complex life-cycles, such as bivalves, has resulted in synchronised reproductive cycles within species. The ultimate selective advantage of synchronisation is to produce larvae under those circumstances which maximise their survival (Olive, 1995). Knowledge of temporal patterns of occurrence of larval stages of various species under different ecological settings may help, therefore, to identify environmental factors that enhance species-specific larval survival. This information can only be gained from robust comparison of heterogeneity across broad geographical scales, and is crucial for predicting the consequences for invertebrate populations under environmental changes such as eutrophication and global climate change (Olive, 1995; Harley et al., 2006; Goberville et al., 2010).

Synchronised reproduction has often been linked with water temperature, which varies with season and latitude (Orton, 1920; Giese, 1959). However, seasonal cycles of photoperiod or changes in food availability may also steer the timing of reproduction of marine benthic invertebrates (Starr et al., 1990; Bentley and Pacey, 1992). Species with latitudinally separated populations may have, therefore, predictably different reproductive cycles (Orton, 1920; Thorson, 1946; Deksheniaks et al., 1993; Hofmann et al., 1994; Bhaud, 2000).

If waters are warm and food availability is relatively constant, e.g. in the tropics, bivalves generally display multiple spawning events within one year (Pouvreau et al., 2006). In continuously cold waters where food availability is strongly seasonal, such as polar regions, bivalves and other invertebrates often show one strong spawning peak per year (Thorson, 1946; Kang et al., 2009). In temperate waters where both temperature and food availability are seasonal, the timing of spawning of bivalves is also strongly seasonal. Furthermore, boreal-temperate invertebrates tend to spawn earlier and for a more extended period in the warmer part than in the colder part of their distribution range (Thorson, 1946; Seed, 1976; Bauer, 1992; Defeo and Cardoso, 2002; O'Riordan et al., 2004).

Many of the findings corroborating a latitudinal gradient in environmental conditions and in bivalve reproduction come from large-scale (100s–1000s of km) observations in marine waters along long and uninterrupted north–south orientated coastlines such as the Pacific coast of North America (Bauer, 1992) and the Atlantic coast of South America (Defeo and Cardoso, 2002). More detailed studies along these coastlines, however, often revealed mesoscale (10s–100s of km) variation in reproductive factors which appears to be related to coastal topographic features such as bays and headlands (e.g., Lester et al., 2007). These features structure local hydrodynamics such as residence times (Frantzen, 2007) and upwelling events (e.g., Brink, 1983; Ebert and Russell, 1988; Connolly et al., 2001; Largier, 2003). The subsequent local and mesoscale variation in the timing of bivalve reproduction and in larval distribution may interfere with synoptic-scale relationships between latitude, environmental conditions and the seasonality in the presence of larvae in coastal waters.

In contrast to the Pacific coast of North America and the Atlantic coast of South America, Europe does not have an uninterrupted north–south orientated coastline because it comprises semi (e.g. North Sea) to almost fully (e.g. Baltic Sea, Mediterranean and Black Sea) land-locked seas. This topographical complexity may result in a decoupling of latitude and environmental conditions. Chlorophyll-*a* concentrations tend to be higher in coastal than open seas as the result of nutrient supply by rivers or upwelling events (e.g., Babin et al., 2003). The enclosed seas have different SSTs compared to the more open marine waters of Europe at similar latitudes (Belkin, 2009).

In this paper, we investigated the presence of bivalve larvae at 12 sites in European coastal waters, ranging from 37°N to 60°N (distance \approx 2400 km) and from 27°W to 18°E (distance \approx 4000 km), during one year of observations. Although previous studies have examined mesoscale (100s of km) relationships between latitude, environmental conditions and invertebrate reproduction in European marine waters (e.g., Haury et al., 1978; Brown, 1984; Lewis, 1986; Jenkins et al., 2000; O'Riordan et al., 2004; Cardoso et al., 2007; Burrows et al., 2010), our study was the first which examined the seasonality in bivalve larvae at a synoptic scale (1000s of km) in this part of the world.

We assumed that the presence of larvae in the water is a proxy for the timing of reproduction. We analysed the larval samples for the presence of two bivalve species, i.e. Pacific oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis/galloprovincialis*). We tested how much of the observed seasonal patterns of presence of bivalve larvae at 12 European stations could be explained by various models, including one that stated that there was no synchronisation in seasonality of larval presence at all between the locations (null hypothesis), one that assumed that there was one common seasonality pattern for all stations within Europe, and various models which supposed that the variation in seasonality could be grouped according to specific spatial scales, taxonomic groups, or several combinations of these factors.

2. Materials and methods

2.1. Sampling

Sampling was carried out at 12 coastal sites in Europe. Sites were selected to maximise the range of environmental conditions and latitude in European coastal waters (Table 1). All study sites were permanently submerged, and preferably far from freshwater inputs and other major sources of impacts such as dredging activities and harbours. In addition, the sites were accessible throughout the year to be able to sample at the required frequency and for the full study period. Sites consisted of a single station except for the Azores where two stations were sampled.

To standardize sampling and laboratory procedures, the Royal Netherlands Institute for Sea Research (NIOZ) supplied all materials to filter and store the samples. Sampling started on December 15, 2006, and was aimed to take place once every two weeks throughout the year until December, 2007. Water samples of 10 L

Table 1
Name and positioning of sampling sites, ranked from high to low latitudes, and the name of the Large Marine Ecosystem (www.lme.noaa.gov) and Marine Ecoregion (Spalding et al., 2007) in which the sites are situated. Furthermore, the potential presence of *Mytilus* groups is indicated (Mt = *M. trossulus*, Me = *M. edulis*, MgA = Atlantic *M. galloprovincialis*, MgM = Mediterranean *M. galloprovincialis*) based on literature (Quesada et al., 1995; Śmietanka et al., 2004, 2009).

Name site	Ctry	Latitude	Longitude	Samples		Large marine ecosystem (LME)	Marine ecoregion (ME)	<i>Mytilus</i> groups			
				n	Period			Mt	Me	MgA	MgM
Oslo	NO	59°53'57"N	010°39'03"E	15	Mar07–Dec07	North Sea	North Sea	x	x		
Sylt	DE	55°01'00"N	008°26'24"E	27	Dec06–Dec07	North Sea	North Sea		x		
Helgoland	DE	54°10'45"N	007°53'36"E	27	Dec06–Dec07	North Sea	North Sea		x		
Texel	NL	53°00'19"N	004°47'46"E	27	Dec06–Dec07	North Sea	North Sea		x		
Oostende	BE	51°14'14"N	002°54'05"E	17	Dec06–Dec07	North Sea	North Sea		x		
Roscoff	FR	48°42'30"N	003°57'09"W	25	Jan07–Dec07	Celtic-Biscay Shelf	Celtic Seas		x	x	
Banyuls	FR	42°29'26"N	003°09'38"E	25	Jan07–Dec07	Mediterranean	Western Mediterranean				x
Povoa	PT	41°21'01"N	008°46'21"W	27	Dec06–Dec07	Iberian Coastal	South European Atlantic Shelf			x	
Lecce	IT	40°15'08"N	017°54'23"E	23	Jan07–Dec07	Mediterranean	Ionian Sea				x
Mallorca	ES	39°31'14"N	002°39'12"E	17	Feb07–Dec07	Mediterranean	Western Mediterranean				x
Arrabida	PT	38°39'08"N	009°20'09"W	6	Sep07–Dec07	Iberian Coastal	South European Atlantic Shelf			x	
Faial-North	PT	38°37'23"N	028°38'22"W	21	Mar07–Dec07	(NE Atlantic)	Azores Canaries Madeira				
Faial-South	PT	38°33'34"N	028°46'08"W	21	Mar07–Dec07	(NE Atlantic)	Azores Canaries Madeira				
Algarve	PT	36°58'14"N	007°58'21"W	25	Dec06–Dec07	Iberian Coastal	South European Atlantic Shelf			x	

Abbreviations countries: NO = Norway, DE = Germany, NL = The Netherlands, BE = Belgium, FR = France, PT = Portugal, ES = Spain, IT = Italy.

were taken from the water surface using a bucket, preferably at high tide for tidal stations and around noon for non-tidal stations. One subsample of 2 L was taken from the bucket, while the water was gently stirred to keep the larvae into suspension. This subsample was filtered over a 31.5 µm-mesh nylon filter with a diameter of 47 mm (tailor-made by “Merrem & la Porte BV”, Zaltbommel, The Netherlands) using minor suction of 0.5–1 bar. Material was washed down onto the filter with 0.2 µm-filtered seawater with salinity comparable to that of the samples. The filters were stored separately in pre-labelled cryovials at –80 °C. At the end of the field campaign, the frozen samples were transported by couriers on dry-ice to NIOZ and immediately stored at –80 °C after arrival.

For several stations, we measured chlorophyll-a concentrations and sea surface temperature (SST) at or close to the larvae sampling stations (i.e., Sylt, Texel, Oostende and Lecce). Data on environmental conditions for Roscoff and Banyuls were provided by the French Coastal Environmental Monitoring Service (SOMLIT). For all other stations, monthly water temperatures (SST) were derived from monthly averaged satellite data (<http://oceancolor.gsfc.nasa.gov>) from December 2006 to December 2007.

2.2. Species

In the mid-1960s, the Pacific oyster (*Crassostrea gigas*) was introduced for commercial purposes from the Northwest Pacific to several locations in Europe. Since then it has spread widely and wild populations can now be found along most of Europe's coastlines, from the northern Mediterranean to southern Norway (Miossec et al., 2009). In northern European waters, there has been a transition from triploid sterile oyster populations sustained by hatchery production to recruitment from wild-settlement (Gollasch et al., 2009).

The absence of Pacific oysters in northern European waters may be caused by too low temperatures for survival and reproduction. Pacific oysters cannot survive at temperatures below 3 °C (Kobayashi et al., 1997; Cardoso et al., 2007). While Pacific oysters initiate gametogenesis between 8 and 11 °C, they spawn after a threshold temperature of 18–20 °C has been reached (Mann, 1979; Fabioux et al., 2005). For optimal development, oyster larvae need water temperatures of at least 22 °C (Kobayashi et al., 1997; Shatkin et al., 1997; Rico-Villa et al., 2008). Reproduction appears to be more sensitive to low temperatures than survival. For example, although Pacific oysters can grow such as the Bay of

Morlaix (Roscoff sampling station), they are not able to reproduce in these relatively cold waters (Lejart, 2009).

The southern edge of distribution may also be temperature-limited. The temperature tolerance of Pacific oysters ranges between 3 °C and 35 °C (Cardoso et al., 2007), and summer adult mass mortalities have been documented at hatcheries in France and Ireland since the mid-1980s and mid-1990s, respectively (Cotter et al., 2010; Huvet et al., 2010).

The *Mytilus edulis* complex comprises three nominal species, namely *Mytilus edulis*, *Mytilus galloprovincialis* and *Mytilus trossulus* (Śmietanka et al., 2009). Whilst *M. trossulus* inhabits the Baltic Sea and Loch Etive in Scotland, *M. edulis* occurs from the Barents Sea in the north to the Atlantic coast in France to the south, and *M. galloprovincialis* extends from the Orkney Island in the north to the Mediterranean and Black Sea in the south (Gosling, 1992; Berge et al., 2005; Zbawicka et al., 2010). These mussel species can also interbreed and produce fertile hybrids where their distributions overlap, i.e. the French Atlantic coast up to northern Scotland (Bierne et al., 2003; Śmietanka et al., 2004, 2009).

Whilst *Mytilus edulis* is genetically homogeneous throughout its range, *Mytilus galloprovincialis* is subdivided into a Mediterranean group and an Atlantic group with a break point at the Almeria-Oran oceanographic front in the Mediterranean Sea (Quesada et al., 1995). *Mytilus galloprovincialis* is slowly spreading northwards invading territory once exclusive to *M. edulis* which may be a result of global climate change (Beaumont et al., 2007). Adults of *Mytilus* species are virtually absent in the Azores (Hawkins et al., 2000) and whether larvae of mussels are present is still unknown.

2.3. Molecular analyses

Total DNA was extracted by the UltraClean™ Soil DNA Isolation Kit (MOBIO Laboratories Inc.) using the alternative protocol for maximum yields (version 03252005). The filter was incised by a sterile scissor to increase the surface area before being subjected to the steps in the extraction protocol. The DNA extracts were checked on a 1% agarose gel stained with ethidium bromide and visualized.

Specific primers for *Crassostrea gigas* and *Mytilus edulis/galloprovincialis* were developed to amplify a part of the cytochrome oxidase subunit I (COXI) mitochondrial gene (Table 2). Primers for *C. gigas* had at least one mismatch at the 3' terminal base with *Crassostrea angulata*, *Crassostrea sikamea*, *Crassostrea nippona*, *Crassostrea ariakensis*. Primers for *M. edulis/galloprovincialis*

Table 2
Primer sequences and additional information.

Species	Primer name	Sequence (5'–3')	Length
<i>Mytilus edulis/galloprovincialis</i>	Myt3L	CTCTATTAGTAGGTGGTA	18-mer
	Myt6H	AATACGGCAGTAACTCT	17-mer
<i>Crassostrea gigas</i>	GigasF	TCTCTTATTCGTTGGAGA	18-mer
	GigasR	ACCTTAATAGATCAAGGG	18-mer

matched exactly with most sequences of female individuals of these species, except for some sequences from Nedlands (Australia) and Dichato (Chile). The primers did not match with the male lineage of *M. edulis/galloprovincialis* (two mismatches in the final four bases at the 3' side). For *Mytilus trossulus* (both females and males), there were no mismatches in the final four bases at the 3' side, but three mismatches further on.

Each PCR mixture contained: 2.5 μ L BioTherm™ PCR Buffer (contains 15 mM MgCl₂), 2.5 μ L dNTP's (Bioline, 2.5 mM), 0.25 μ L of each primer (50 μ M), 0.5 μ L BSA (20 mg ml⁻¹), 0.125 μ L BioThermPlus™ Taq (5 units μ l⁻¹), 15.875 μ L PCR H₂O, 3 μ L DNA template. After an initial denaturation step at 94 °C for 120 s, the PCR programmes followed 36 cycles at 94 °C for 30 s, annealing at 52.5 °C (*Crassostrea gigas*) or at 50 °C (*Mytilus edulis/galloprovincialis*) for 30 s, extension at 72 °C for 60 s and a final extension step at 72 °C for 420 s.

Presence of PCR inhibitors in a sample (e.g. from the seawater) may result in a false negative, i.e. a negative amplification result whilst larvae were present in that sample. Our results were therefore checked by means of PCR inhibition tests. For this test we used the protocol as described above, but instead of 3 μ l sample we used 2 μ l of the diluted sample (1:1, 1:5, 1:25 and 1:125) and added 1 μ l of positive control consisting of adult DNA of each target species and visualized the results on gel.

The presence/absence of larvae of the target species was scored from the gels immediately after PCR amplification. Presence was detected if there was at least one D-larva (early veliger larval stage of bivalves) larger than 50 μ m in the 2-l sample. This implied that it was possible to score larvae from mean concentrations of 0.5–2.5 larvae per litre (=500–2.500 larvae per m³) onwards. We scored the visibility of the bands as an index of presence: larvae were assumed to be absent (0) if no band was visible, and present (1) if the bands were weakly to strongly visible. Molecular analyses, including scoring, were performed by only one person (BTR).

Table 3

Hypotheses and statistical models for the presence/absence data of larvae of *Crassostrea gigas* and *Mytilus edulis/galloprovincialis* in coastal European waters at a synoptic scale (LMEs = Large Marine Ecosystems, MEs = Marine Ecoregions).

#	Hypothesis	Statistical model
H0	There is no trend, just noise (local variation dominates geographic variation in larval presence)	$\text{logit}(p_{is}) = \alpha + a_i + \varepsilon_{is}$
H1	All the time series have one (similar) underlying seasonal trend	$\text{logit}(p_{is}) = \alpha + f(\text{DayNr}_{is}) + a_i + \varepsilon_{is}$
H2	There is one underlying latitude effect and one underlying seasonal trend for all the time series	$\text{logit}(p_{is}) = \alpha + f(\text{DayNr}_{is}) + f(\text{Latitude}_{is}) + a_i + \varepsilon_{is}$
H3	The latitude effect changes over time	$\text{logit}(p_{is}) = \alpha + f(\text{DayNr}_{is}, \text{Latitude}_{is}) + a_i + \varepsilon_{is}$
H4	All the time series have four underlying seasonal trends which are related to LMEs	$\text{logit}(p_{is}) = \alpha + f_j(\text{DayNr}_{is}) + a_i + \varepsilon_{is}, j = 1, \dots, 4$, where j refers to LMEs
H5	All the time series have five underlying seasonal trends which are related to MEs	$\text{logit}(p_{is}) = \alpha + f_k(\text{DayNr}_{is}) + a_i + \varepsilon_{is}, k = 1, \dots, 5$, where k refers to MEs
H6	There is one seasonal trend and the latitude effect differs per LME	$\text{logit}(p_{is}) = \alpha + f(\text{DayNr}_{is}) + f_j(\text{Latitude}_{is}) + a_i + \varepsilon_{is}, j = 1, \dots, 4$, where j refers to LMEs
H7	There is one seasonal trend and the latitude effect differs per ME	$\text{logit}(p_{is}) = \alpha + f(\text{DayNr}_{is}) + f_k(\text{Latitude}_{is}) + a_i + \varepsilon_{is}, k = 1, \dots, 5$, where k refers to MEs
H8	There is one seasonal trend and there is a subspecies effect	$\text{logit}(p_{is}) = \alpha + f(\text{DayNr}_{is}) + \text{Subspecies} + a_i + \varepsilon_{is}$
H9	There is one seasonal trend, the latitude effect is the same for all the time series and there is a subspecies effect	$\text{logit}(p_{is}) = \alpha + f(\text{DayNr}_{is}) + f(\text{Latitude}_{is}) + \text{Subspecies} + a_i + \varepsilon_{is}$

2.4. Spatial scales

We tested if the observed seasonal patterns of presence of bivalve larvae at 12 European stations could be best explained:

- by local variation dominating the geographical variation in larval presence (null hypothesis), or
- by one common seasonality pattern for all stations within Europe (Hypothesis 1), or
- by groups of seasonal patterns which are related to specific spatial scales such as latitude, large marine ecosystems, and marine ecoregions (Hypotheses 2–5), or
- by taxonomic groups (Hypothesis 8), or
- by several combinations of these factors (Hypotheses 6, 7 and 9) (Table 3).

European marine waters can be classified into several Large Marine Ecosystems, LMEs (Table 1; Fig. 1). These LMEs are relatively large regions on the order of 200,000 km² or greater, characterized by distinct bathymetry, hydrography, productivity, and trophically dependent populations (see <http://www.lme.noaa.gov>). Based on a more detailed distribution overview of marine species than the one that was originally used for the definition of the LMEs, Spalding et al. (2007) subdivided the coastal and shelf areas of the world into 232 marine ecoregions (MEs). Within European marine waters, however, most MEs more or less overlap with LMEs with exception of the Iceland Shelf and the Norwegian Sea (which are each split into 2 ecoregions) and the Mediterranean (which is subdivided into 7 ecoregions).

2.5. Statistical analyses

Following the protocol in Zuur et al. (2010), an initial data exploration was applied. Hereafter, we modelled the data by means of a generalised additive mixed effects model (GAMM) with a binomial distribution, i.e.:

$$M_{is} = \text{Binomial}(p_{is}, 1)$$

$$\text{logit}(p_{is}) = \alpha + \text{covariates} + a_i + \varepsilon_{is}$$

where M_{is} is the observed presence or absence of *Crassostrea gigas* or *Mytilus edulis/galloprovincialis* at site i at time s , and p_{is} is the probability that larvae of oysters or mussels are present at site i at time s . The random intercept a_i allows for a random variation around the intercept α , and is assumed to be normally distributed with mean 0 and variance σ_{site}^2 . The random intercept ensures that

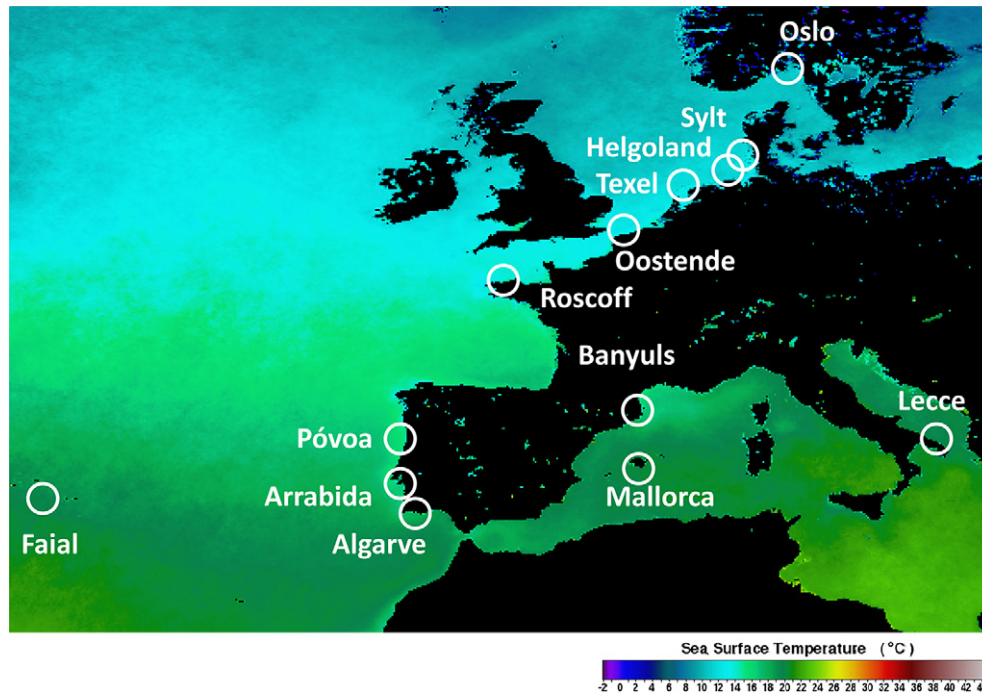


Fig. 1. Locations of sampling sites, projected on annual average of sea surface temperatures in 2007 as measured by the MODIS sensors aboard the Aqua EOS PM satellite (<http://oceancolor.gsfc.nasa.gov>).

observations from the same site are allowed to be correlated. Due to the short length of the time series, we did not apply more advanced correlation structures like auto-regressive correlation (Zuur et al., 2009), but instead only used the symmetric compound correlation as imposed by the random intercept.

Based on the underlying questions (see Section 2.4), a series of hypotheses and models were used for the 'covariates' part (Table 3). The smoothers $f(\cdot)$ were estimated using the R package (R Development Core Team, 2009), gamm4 (Wood, 2009) and cubic regression splines. The covariate subspecies was fitted as a categorical variable. In order to fit all models on the same data, the observations from the Azores (Faial North and South) were removed from the analysis.

To compare models, we followed an Information Theoretic approach (Anderson and Burnham, 2002). We calculated differences Δ_i between the Akaike Information Criterion (AIC) of each model and the minimum AIC. Anderson and Burnham (2002) state that the level of empirical support for model i is:

- substantial if Δ_i is between 0 and 2 (hence, these are models with similar AICs as the optimal model),
- considerably less if Δ_i is between 4 and 7, and
- essentially none if Δ_i is larger than 10.

Akaike weights w_i were calculated following Anderson and Burnham (2002), which place the Δ_i on a more interpretable scale by using:

$$w_i = \frac{e^{-\frac{1}{2} \times \Delta_i}}{\sum_i e^{-\frac{1}{2} \times \Delta_i}}$$

These weights have the convenient ability that they can be interpreted as probabilities that a given model is judged the best model on repeated sampling. If the weight for a particular model has a value of 0.75, for example, this implies that this model has a probability of 75% of being the best model within the series

tested. Note that the AIC values obtained by a GAMM with a binomial distribution should be considered as approximate.

3. Results

3.1. Oysters (*Crassostrea gigas*)

Larvae of *Crassostrea gigas* were observed at 5 of the 12 sites, and occurred more often at Sylt, Texel and Roscoff than at Oostende and Algarve (Fig. 2). The values of the Akaike weights w_i indicated that model H1 has the highest probability (58%) of being the best model within the series tested, followed by model H2 (25%) and model H3 (17%) (Table 3).

The best model explaining the presence/absence of oyster larvae in European coastal waters was the one that assumes one common seasonal trend at all stations (H1 in Table 4). This model suggested that oyster larvae are most likely to occur in summer with peak values around early August (day number experiment \approx 230) (Table 5; Fig. 3).

The other model for which the level of empirical support is substantial (i.e. the value of Δ_i is between 0 and 2, so its AIC is considered to be similar to that of the optimal model H1) was the one that assumes one underlying seasonal trend and one latitudinal effect for all the time series (H2; Table 5; Fig. 4) with higher probability of occurrence in the north than in the south.

The model that assumes that the latitude effect changes over time (H3) had some empirical support as well ($\Delta_i = 2.4$). The results of model H3 suggested that larvae have a higher probability of occurrence at higher latitudes and exhibit a seasonal pattern for day number with earlier occurrences at higher latitudes in the range of those tested (Fig. 5).

3.2. Mussels (*Mytilus edulis/galloprovincialis*)

Mussel larvae were observed at all 12 sites, and were present during most of the year at Povoia, Sylt, Texel, Oslo and Banyuls but

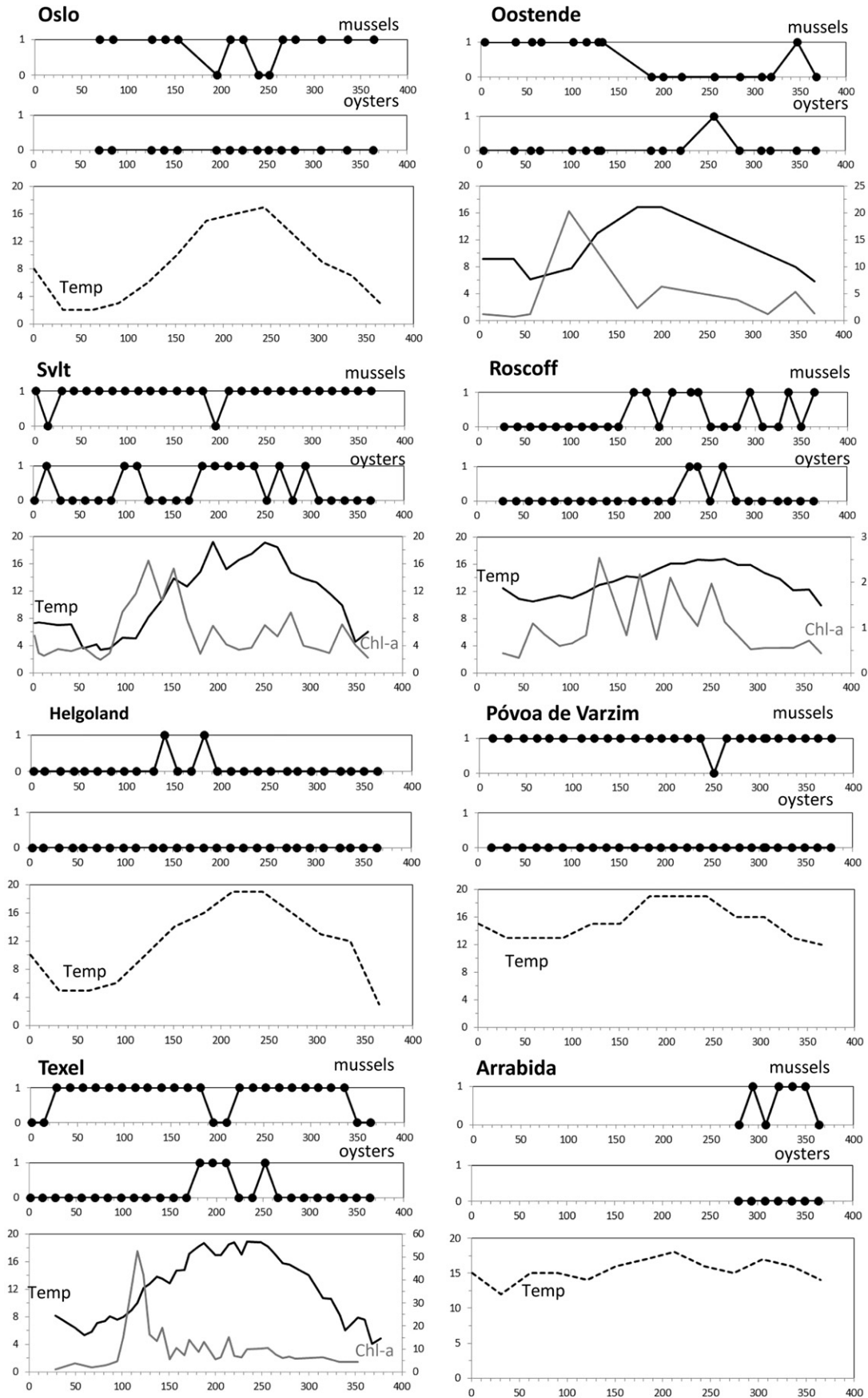


Fig. 2. Occurrence of larvae of Pacific oysters (*Crassostrea gigas*), occurrence of mussels (*Mytilus edulis/galloprovincialis*), water temperature ($^{\circ}\text{C}$; black line; left y-axis) and chlorophyll-a concentrations ($\mu\text{g l}^{-1}$; grey line; right y-axis) at 12 sites within European coastal waters between December 15, 2006 (Day number experiment = 1) and December 18, 2007 (Day number experiment = 368). Presence of larvae in the samples is indicated by 1, their absence by 0. The environmental conditions were determined *in situ* (solid lines) at several stations (i.e., Svlt, Texel, Oostende, Roscoff, Banyuls and Lecce) and derived from satellite images (dotted lines) for the others.

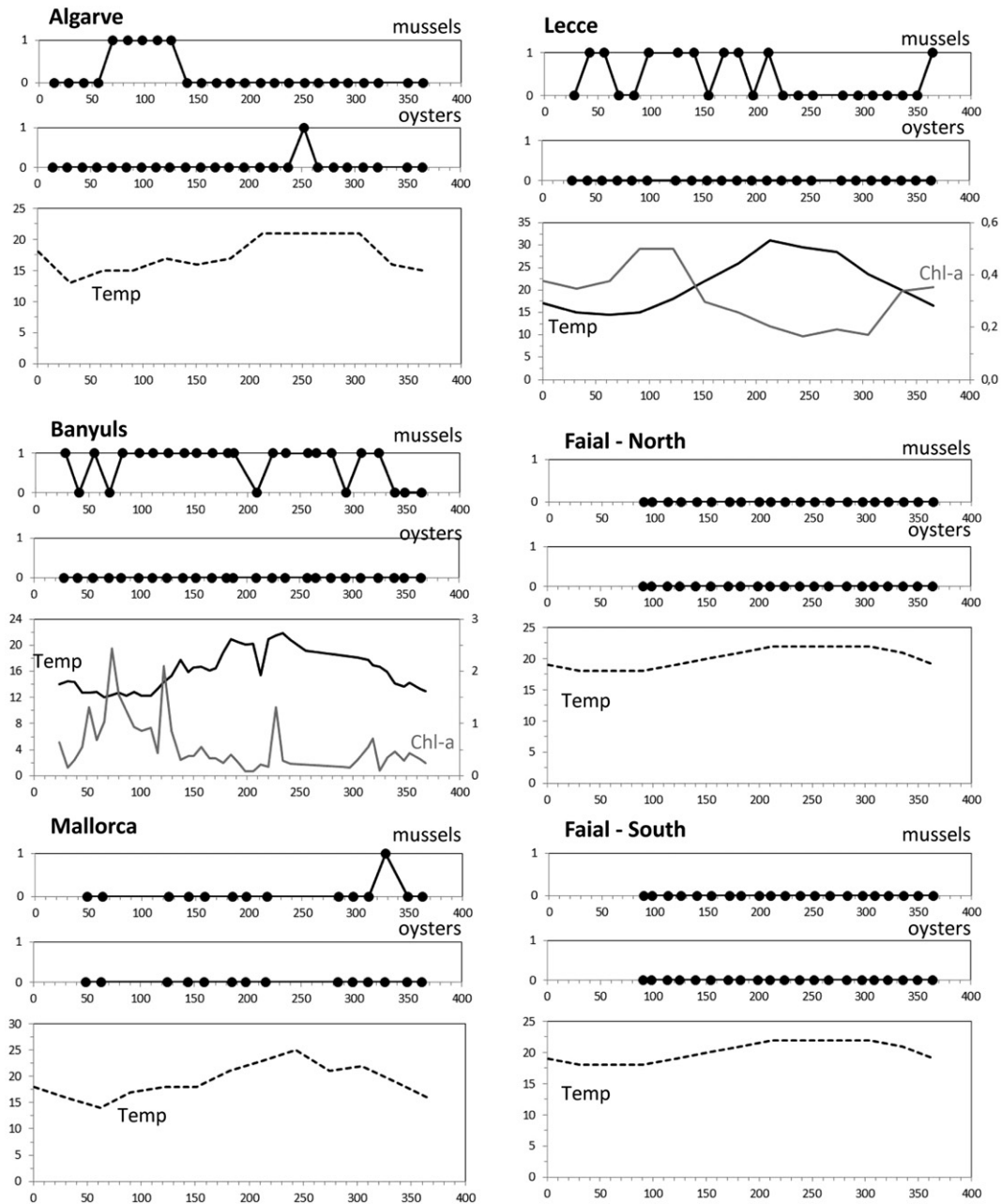


Fig. 2. (continued).

were rarely observed at Helgoland and Mallorca (Fig. 2). The values of the Akaike weights w_i indicate that that model H4 has the highest probability (52%) of being the best model within the series tested, followed by model H5 (25%) (Table 3).

The best model explaining the presence/absence of mussel larvae in European coastal waters was the one that assumed four different seasonal trends, one for each LME (H4 in Table 3). For the North Sea and the Iberian Coast, this model suggested that the highest probability of occurrence of mussel larvae was in late winter (end of February) and around the end of September (Table 6; Fig. 6). The Celtic-Biscay Shelf was characterised by an increase in probability of occurrence of mussel larvae between February and June, whilst the Mediterranean shows a decrease in probability of occurrence of mussel larvae throughout spring and summer (Fig. 6).

The other model for which the level of empirical support is substantial (i.e. the value of Δ_i is between 0 and 2) is the one that assumes that all the time series have one underlying seasonal trend (H1 in Table 3) with peak values around mid-April (day number experiment ≈ 120) (Table 6; Fig. 7).

4. Discussion

4.1. Limitations of our study

At optimal water temperatures and feeding conditions, larvae of oysters and mussels may reach metamorphosis within two weeks (Satuito et al., 1994; Rico-Villa et al., 2009). This implies that only under such maximum growth conditions for larvae, reproductive periods could have been missed for those stations

Table 4

Values and differences of the Akaike Information Criterion (AIC), and the Akaike weight (w_i) for statistical models 0–9 in Table 3. Models for which the level of empirical support for model i is substantial (i.e. the value of Δ_i is between 0 and 2) are underlined, the best fit (for which AIC has the lowest value) is printed in bold.

Model	<i>Crassostrea gigas</i>			<i>Mytilus edulis/galloprovincialis</i>		
	AIC	Δ_i	w_i	AIC	Δ_i	w_i
H0	119.9	16.0	0.000	281.0	6.6	0.019
H1	103.9	0.0	0.576	275.9	1.5	0.246
H2	<u>105.6</u>	<u>1.7</u>	<u>0.246</u>	279.2	4.8	0.047
H3	106.3	2.4	0.173	281.1	6.7	0.018
H4	116.6	12.7	0.001	274.4	0.0	0.519
H5	120.6	16.7	0.000	277.7	3.3	0.100
H6	114.6	10.7	0.003	288.0	13.6	0.001
H7	118.5	14.6	0.000	291.5	17.1	0.000
H8	Not relevant for this species			281.8	7.4	0.013
H9	Not relevant for this species			279.7	5.3	0.037

where we were able to sample as planned, i.e. once every fortnight.

Due to the presence of a detection limit of larvae in our samples, we cannot distinguish between the presence of larvae at low densities (<500 larvae per m^3) and the actual absence across sampling sites and/or sampling periods. We might, therefore, have missed the presence of larvae if their densities were low, e.g. as the result of low reproductive output of bivalve stocks and/or as the result of dilution if the sampling site was not close to the nearest spawning stock.

In addition, our sampling and analyses techniques were not quantitative and did not allow us to discriminate between a few larvae exceeding the threshold or higher larval densities. Interpretation of the results should, therefore, be done within the light of these limitations.

The strength of our methods, however, lies within the standardisation of the sampling techniques throughout Europe and the consistency of the analyses of the samples which were performed in one laboratory by one person. This reduced the possible noise in the data as the result of variation in sampling and analysis techniques between laboratories to a large extent.

4.2. Oysters (*Crassostrea gigas*)

The best model on the occurrence of oyster larvae in European coastal waters (Fig. 3) suggested that the reproduction of oysters

Table 5

Crassostrea gigas. The estimates, estimated degrees of freedom (obtained by cross-validation) and approximate significance for intercepts and smoothers of best statistical models for oyster larvae (see Table 4). Day number is referring to the day number of the experiment, ranging from 1 (December 15, 2006) to 368 (December 18, 2007). To fit models 2 and 3, both covariates were scaled to have the same variation.

Parameter	Estimate	Std Error	T value	Pr(> t)
Model H1. One underlying seasonal trend				
Intercept	-10.550	-5.496	<2e-16	0.001
Smooth terms	edf	Ref.edf	F	p-value
Day number	3.758	3.758	4.264	0.003
Model H2. One underlying seasonal trend + one underlying latitude effect				
Intercept	-5.359	0.791	-6.773	< 0.001
Smooth terms	edf	Ref.edf	F	p-value
Day number (scaled)	3.718	3.718	4.124	0.004
Latitude (scaled)	2.932	2.932	1.872	0.136
Model H3. Latitude effect changes over time				
Intercept	-5.045	0.784	-6.891	< 0.001
Smooth terms	edf	Ref.edf	F	p-value
Day number, latitude (scaled)	6.918	6.918	1.864	0.077

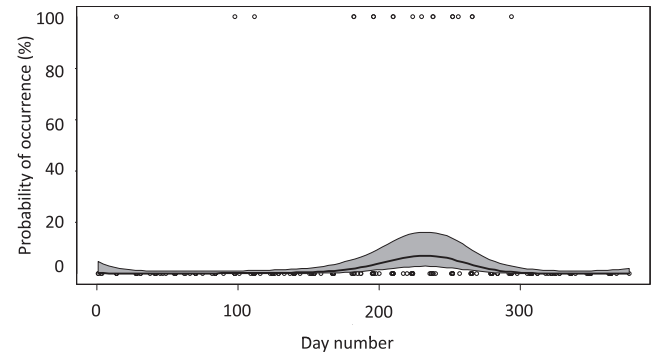


Fig. 3. Estimated smoother obtained by the model in H1 (see Table 4) for the occurrence of Pacific oyster (*Crassostrea gigas*) larvae in European coastal waters between December 15, 2006 (Day number experiment = 1) and December 18, 2007 (Day number experiment = 368). The solid line is the smoother (plus the intercept) and the grey area determines the 95% confidence bands. Confidence bands are calculated by adding the estimated smoother and 95% confidence bands, and then applying the logit transformation. The white dots are the observations on presence (value = 1) and absence (value = 0) of larvae of the Pacific oyster at the sampling sites.

most probably exhibits one common seasonal trend at all stations where larvae were observed, with highest probability of larval occurrence around early August. This is in agreement with previous observations that natural populations of Pacific oysters in north-western Europe generally spawn in summer and autumn

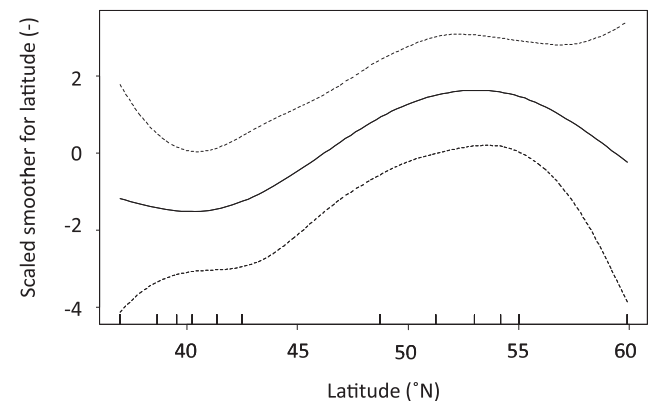
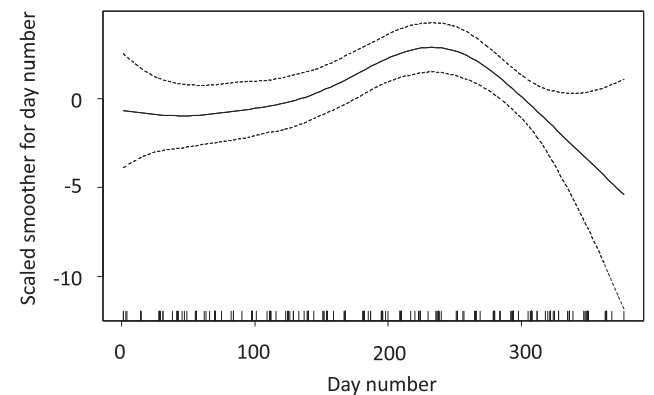


Fig. 4. Fitted values on field observations on probability of occurrence of Pacific oyster (*Crassostrea gigas*) larvae as measured at 12 sampling sites in European coastal waters between 15 December 2006 and 18 December 2007, obtained by Generalized Additive Mixed Modelling (GAMM) with a 1-dimensional smoother (see Model H2 in Table 4). Top panel: The vertical axis shows the fitted values (logistic scale) and the horizontal axis the day number (with values between 1 and 368). Bottom panel: The vertical axis shows the fitted values, and the other axis the latitude (from 45°N to 80°N).

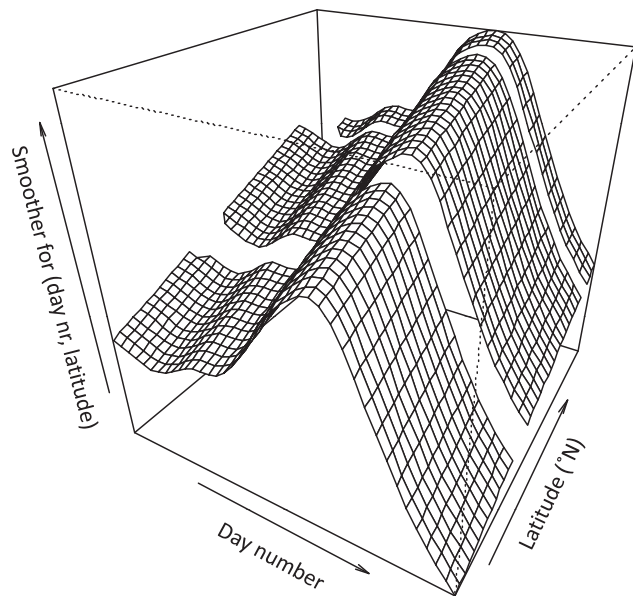


Fig. 5. Fitted values on field observations on probability of occurrence of Pacific oyster (*Crassostrea gigas*) larvae as measured at 12 sampling sites in European coastal waters between 15 December 2006 and 18 December 2007, obtained by Generalized Additive Mixed Modelling (GAMM) with a 2-dimensional smoother (see model H3 in Table 4). The vertical axis shows the fitted values (logistic scale) and the horizontal two axes the day number of the experiment (with values between 1 and 368) and the latitude (H2-LAT; from 45°N to 80°N). To fit the two-dimensional smoother, both covariates were scaled to have the same variation, but this did not affect the AIC.

(Pouvreau et al., 2006; Cardoso et al., 2007; Enríquez-Díaz et al., 2009; Troost et al., 2009; Dutertre et al., 2010).

The second best but comparably good model (Fig. 4) suggested that larvae exhibit one seasonal trend and one underlying latitudinal effect in probability of occurrence, while the third best model (Fig. 5) suggested that oyster larvae also occurred earlier at higher latitudes in the range of those tested. If the timing of reproduction is predominantly triggered by a threshold in temperature, it would be expected that spawning would occur earlier in the south than in the north. Our findings were in agreement with previous observations by Cardoso et al. (2007), however, who showed that spawning events (identified by sudden decreases in gonadal mass) of Pacific oysters occurred earlier in northern than in southern sites along the west European coast (from 46 to 53°N).

The above evidence suggests that the onset of spawning is not only determined by temperature but also by other factors, possibly food availability. Energy flow models of *Crassostrea virginica* indicated, for example, that both temperature and food supply affect the timing of the spawning season and the reproductive output (Deksheniaks et al., 1993; Hofmann et al., 1994). Within NW-Europe, increased food conditions in the north were suggested to advance the timing of spawning of Pacific oysters during the warmest months of the year (Cardoso et al., 2007). Their suggestion on the importance of food availability on the timing of spawning of Pacific oysters were corroborated by our findings that chlorophyll a concentrations were considerably higher at the northern locations than at Roscoff (Fig. 2).

Larvae of *Crassostrea gigas* were detected by molecular techniques in winter and spring at the sampling site near Sylt (Fig. 2) which was confirmed by microscopic analyses of water samples taken at the same time (R. Asmus, unpublished data). These observations suggest that spawning can occur at much lower temperatures (less than 10 °C; Fig. 2) than previously indicated as

threshold values (Mann, 1979; Fabioux et al., 2005; Lejart, 2009). Whether this might be the result of rapid adaption of the invasive Pacific oysters towards a relatively new environment (Prentis et al., 2006) or that Sylt harbours a different subpopulation of Pacific oysters remains to be investigated, e.g. by determining the traits with regard to phenology and larval survival of oysters from various origins and possible subpopulations.

4.3. Mussels (*Mytilus edulis/galloprovincialis*)

The timing of reproduction and the duration of the reproductive cycle in *Mytilus edulis*, *Mytilus galloprovincialis* and their hybrids have been shown to vary both spatially and temporally under local environmental conditions like temperature and food availability (Wilson and Seed, 1974; Bayne, 1975, 1976; Pieters et al., 1980; Sunila, 1981; Kautsky, 1982; Lowe et al., 1982; Sprung, 1983; Villalba, 1995; Pulfrich, 1997; Cáceres-Martínez and Figueras, 1998; Frantzen, 2007; Duinker et al., 2008; Dias et al., 2009; Doherty et al., 2009; Troost et al., 2009).

Based on our results, larval presences of mussels appear not to be determined by small-scale (local) variation in environmental conditions (null hypothesis), but by environmental conditions that operate at larger (100s–1000s of km) spatial scales. The best model (Fig. 6) suggested that, for the North Sea and the Iberian Coast, the probability of occurrence of mussel larvae is high at the end of March with a second peak in end September (North Sea) to the beginning of November (Iberian Coastal). The model results for the Celtic-Biscay and Mediterranean areas were different for the beginning and the end of the year (Fig. 6), which suggested that conditions in the study year and the following year were different. To exclude the possibility of an artefact in the models, this type of study ideally needs to be conducted across more than a single year. The second best but comparably good model (Fig. 7) suggested that on seasonality in occurrence of mussel larvae showed a common seasonal pattern with one peak around mid-April for all sites.

Previous observations on the reproductive cycle of *Mytilus edulis* suggested a shift from one to two periods of reproduction within a year during the past decades, i.e. the observations of a single spawning event in spring were made in the 1950s to the 1980s (Chipperfield, 1953; Pieters et al., 1980; Sunila, 1981; Kautsky, 1982; Sprung, 1983), whilst multiple spawning in both spring and autumn was observed from the 1990s onwards (Villalba, 1995; Pulfrich, 1997; Cáceres-Martínez and Figueras, 1998; Frantzen, 2007; Duinker et al., 2008; Dias et al., 2009; Troost et al., 2009). It is not

Table 6

Mytilus edulis/galloprovincialis. The estimates, estimated degrees of freedom (obtained by cross-validation) and approximate significance for intercepts and smoothers of the best statistical models for mussel larvae (see Table 4). Day number is referring to the day number of the experiment, ranging from 1 (December 15, 2006) to 368 (December 18, 2007) (CBS = Celtic-Biscay Shelf; IBC = Iberian Coastal; MED = Mediterranean; NOS = North Sea).

Parameter	Estimate	Std Error	T value	Pr(> t)
Model H4. Four underlying seasonal trends related to LMEs				
Intercept	0.2776	0.1861	1.491	0.137
Smooth terms	edf	Ref.edf	F	p-value
Day number CBS	1.896	1.896	2.945	0.057
Day number IC	4.607	4.607	3.088	0.012
Day number M	1.698	1.698	2.999	0.060
Day number NS	4.562	4.562	3.205	0.010
Model H1. One underlying seasonal trend				
Intercept	−0.4165	0.6210	−0.671	0.503
Smooth terms	edf	Ref.edf	F	p-value
Day number (scaled)	3.896	3.896	4.354	0.002

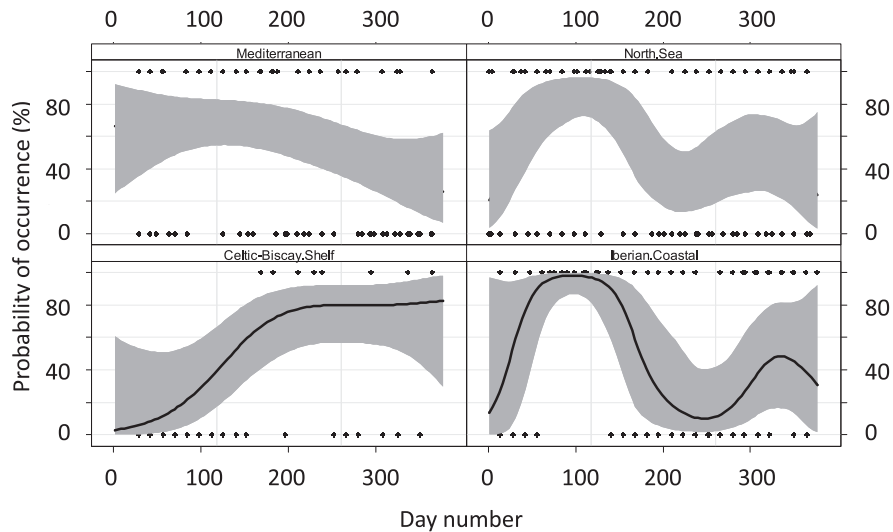


Fig. 6. Estimated smoothers obtained by the model in H4 for the presence/absence of mussel (*Mytilus edulis/galloprovincialis*) larvae in European coastal waters. The solid line is the smoother (plus the intercept) and the grey areas determine the 95% confidence bands. Confidence bands were calculated by adding the estimated smoothers and 95% confidence bands, and then applying the logit transformation.

possible, however, to infer from this information whether the reproductive behaviour of mussels has actually changed during the past decades, because most observations in the older publications were restricted to a limited period of the year.

This possibility of long-term changes in phenology of mussels deserves future investigation, e.g. by studying the phenology of spawning of mussels from various origins under different temperature and food conditions, because climate-induced changes in spawning frequencies have been observed for other marine invertebrates in Europe during the past decades. In south-west Britain, for example, *Patella depressa* (a warm temperate gastropod species) originally showed single spawning events only in the 1940s whilst multiple periods of spawning and gonad redevelopment occurred during the reproductive season throughout the 2000s (Moore et al., 2011).

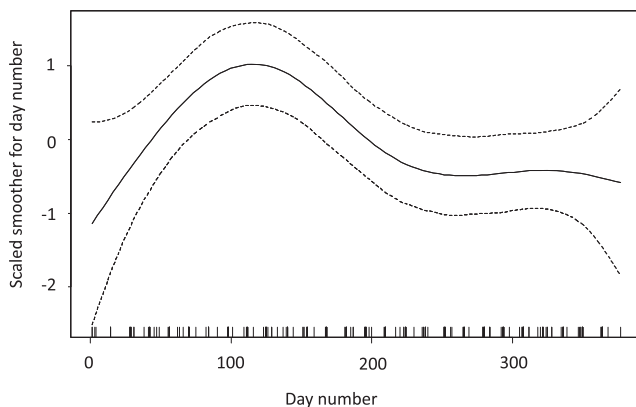


Fig. 7. Fitted values on field observations on probability of occurrence of mussel (*Mytilus edulis/galloprovincialis*) larvae as measured at 12 sampling sites in European coastal waters between 15 December 2006 and 18 December 2007, obtained by Generalized Additive Mixed Modelling (GAMM) with a 1-dimensional smoother (see Model H1 in Table 4). The vertical axis shows the fitted values (logistic scale) and the horizontal axis the day number (with values between 1 and 368).

5. Conclusion

Based on the variety in environmental conditions such as temperature and chlorophyll-a concentrations at our sampling stations (Fig. 2) and potential patchiness in larval distributions, we would have expected that the null hypothesis (local variation) would best explain observed occurrence of oyster and mussel larvae throughout Europe. Both bivalve species, however, exhibited synchronised patterns in occurrence of larvae at mesoscale (100s of km) to synoptic scales (1000s of km). Such synchrony in larval occurrence suggests that environmental conditions relevant to bivalve reproduction and larval survival are more or less similar at these spatial scales.

As the result of our comparative approach, we cannot determine the actual underlying mechanisms for these patterns, nor exclude the possibility that different locations harboured different subpopulations adapted to local conditions. Our results might help, however, to design experiments to test competing hypotheses regarding causal mechanisms such as food availability and temperature at the appropriate ranges and scales (Underwood and Petraitis, 1993; Jenkins et al., 2000). The unravelling of the nature and strength of structuring factors of phenology of bivalve reproduction is of particular interest in the light of changing environmental conditions as the result of global climate change and the possible consequences for marine food webs and ecosystem services (e.g., Philippart et al., 2003; Kirby et al., 2009).

Acknowledgements

The authors thank the coordinators and funders of the EU-Network of Excellence “Marine Biodiversity and Ecosystem Functioning” (MarBEF) for financially supporting this research. We acknowledge the SOMLIT French network for the provision of environmental data. We are grateful to Jan Beukema, Mardik Leopold and four anonymous referees for their valuable comments on earlier drafts of this manuscript. We are particularly grateful to John S. Gray, who initiated the sampling efforts near Oslo in 2006 but passed away in October 2007.

References

- Anderson, D.R., Burnham, K.P., 2002. Avoiding pitfalls when using information-theoretic methods. *Journal of Wildlife Management* 66, 912–918.
- Babin, M., Stramski, D., Ferrari, G.M., Claustre, H., Bricaud, A., Obolensky, G., Hoepffner, N., 2003. Variations in the light absorption coefficients of phytoplankton, nonalgal particles, and dissolved organic matter in coastal waters around Europe. *Journal of Geophysical Research* 108, 3211. doi:10.1029/2001JC000882.
- Bauer, R.T., 1992. Testing generalizations about latitudinal variation in reproduction and recruitment patterns with sicyoniid and caridean shrimp species. *Invertebrate Reproduction and Development* 22, 193–202.
- Bayne, B.L., 1975. Reproduction in bivalve molluscs under environmental stress. In: Vernberg, F.J. (Ed.), *Physiological Ecology of Estuarine Organisms*. University of South Carolina Press, Columbia, pp. 259–277.
- Bayne, B.L., 1976. Aspects of reproduction in bivalve molluscs. In: Wiley, M. (Ed.), *Estuarine Processes. Uses, Stresses and Adaptation to the Estuary*, vol. 1. Academic Press, New York, pp. 432–448.
- Beaumont, A.R., Gjedrem, T., Moran, P., 2007. Blue mussel *Mytilus edulis*, Mediterranean mussel *M. galloprovincialis*. In: Svasand, T., Crosetti, D., Garcia-Vasquez, E., Verspoor, E. (Eds.), *Genetic Impact of Aquaculture Activities in Native Populations*, pp. 62–69. Genimpact Final Scientific Report.
- Belkin, I.M., 2009. Rapid warming of large marine ecosystems. *Progress in Oceanography* 81, 207–213.
- Bentley, M.G., Pacey, A.A., 1992. Physiological and environmental control of reproduction in polychaetes. *Oceanography and Marine Biology – An Annual Review* 30, 443–481.
- Berge, J., Johnsen, G., Nilsen, F., Gulliksen, B., Slagstad, D., 2005. Ocean temperature oscillations enable reappearance of blue mussels *Mytilus edulis* in Svalbard after a 1000 year absence. *Marine Ecology Progress Series* 303, 167–175.
- Bhaid, M.R., 2000. Some examples of the contribution of planktonic larval stages to the biology and ecology of polychaetes. *Bulletin of Marine Science* 67, 345–358.
- Bierne, N., Borsa, P., Daguin, C., Jollivet, D., Viard, F., Bonhomme, F., David, P., 2003. Introgression patterns in the mosaic hybrid zone between *Mytilus edulis* and *M. galloprovincialis*. *Molecular Ecology* 12, 447–461.
- Brink, K.H., 1983. The near-surface dynamics of coastal upwelling. *Progress in Oceanography* 12, 223–257.
- Brown, R.A., 1984. Geographical variations in the reproduction of the horse mussel, *Modiolus modiolus* (Mollusca: Bivalvia). *Journal of the Marine Biological Association of the United Kingdom* 64, 751–770.
- Burrows, M.T., Jenkins, S.R., Robb, L., Harvey, R., 2010. Spatial variation in size and density of adult and post-settlement *Semibalanus balanoides*: effects of oceanographic and local conditions. *Marine Ecology Progress Series* 398, 207–219.
- Cáceres-Martínez, J., Figueras, A., 1998. Distribution and abundance of mussel (*Mytilus galloprovincialis* Lmk) larvae and post-larvae in the Ria de Vigo (NW Spain). *Journal of Experimental Marine Biology and Ecology* 229, 277–287.
- Cardoso, J.F.M.F., Langlet, D., Loff, J.F., Martinez, A.R., Witte, J.J., Santos, P.T., van der Veer, H.W., 2007. Spatial variability in growth and reproduction of the Pacific oyster *Crassostrea gigas* (Thurnberg, 1793) along the west European coast. *Journal of Sea Research* 57, 303–315.
- Chipperfield, P.N.J., 1953. Observations on the breeding and settlement of *Mytilus edulis* (L.) in British waters. *Journal of the Marine Biological Association of the United Kingdom* 32, 449–476.
- Connolly, S.R., Menge, B.A., Roughgarden, J., 2001. A latitudinal gradient in recruitment of intertidal invertebrates in the northeast Pacific Ocean. *Ecology* 82, 1799–1813.
- Cotter, E., Malham, S.K., O'Keeffe, S., Lynch, S.A., Latchford, J.W., King, J.W., Beaumont, A.R., Culloty, S.C., 2010. Summer mortality of the Pacific oyster, *Crassostrea gigas*, in the Irish Sea: the influence of growth, biochemistry and gametogenesis. *Aquaculture* 303, 8–21.
- Dafeo, O., Cardoso, R.S., 2002. Macroecology of population dynamics and life history traits of the mole crab *Emerita brasiliensis* in Atlantic sandy beaches of South America. *Marine Ecology Progress Series* 239, 169–179.
- Deksheniek, M.M., Hofmann, E.E., Powell, E.N., 1993. Environmental effects on the growth and development of eastern oyster, *Crassostrea virginica* (Gmelin, 1791), larvae – a modeling study. *Journal of Shellfish Research* 12, 241–254.
- Dias, P.J., Batista, F.M., Shanks, A.M., Beaumont, A.R., Davies, I.M., Snow, M., 2009. Gametogenic asynchrony of mussels *Mytilus* in a mixed-species area: implications for management. *Aquaculture* 295, 175–182.
- Doherty, S.D., Brophy, D., Gosling, E., 2009. Synchronous reproduction may facilitate introgression in a hybrid mussel (*Mytilus*) population. *Journal of Experimental Marine Biology and Ecology* 378, 1–7.
- Duinker, A., Håland, L., Hovgaard, P., Mortensen, S., 2008. Gonad development and spawning in one and two year old mussels (*Mytilus edulis*) from Western Norway. *Journal of the Marine Biological Association of the United Kingdom* 88, 1465–1473.
- Dutertre, M., Beninger, P.G., Barillé, L., Papin, M., Haure, J., 2010. Rising water temperatures, reproduction and recruitment of an invasive oyster, *Crassostrea gigas*, on the French Atlantic coast. *Marine Environmental Research* 69, 1–9.
- Ebert, T.A., Russell, M.P., 1988. Latitudinal variation in size structure of the west coast purple sea urchin: a correlation between headlands. *Limnology and Oceanography* 33, 286–294.
- Enríquez-Díaz, M., Pouvreau, S., Chávez-Villalba, J., Le Pennec, M., 2009. Gametogenesis, reproductive investment, and spawning behavior of the Pacific giant oyster *Crassostrea gigas*: evidence of an environment-dependent strategy. *Aquaculture International* 17, 491–506.
- Fabioux, C., Huvet, A., Le Souchu, P., Le Pennec, M., Pouvreau, S., 2005. Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. *Aquaculture* 250, 458–470.
- Frantzen, S., 2007. Recruitment of blue mussels, *Mytilus edulis* L., on suspended collectors in Finnmark, North Norway (70–71°N). *Marine Biology Research* 3, 37–48.
- Giese, A.C., 1959. Comparative physiology – annual reproductive cycles of marine invertebrates. *Annual Review of Physiology* 21, 547–576.
- Goberville, E., Beaugrand, G., Sautour, B., Treguer, P., SOMLIT Team, 2010. Climate-driven changes in coastal marine systems of western Europe. *Marine Ecology Progress Series* 408, 129–148.
- Gollasch, S., Haydar, D., Minchin, D., Wolff, W.J., Reise, K., 2009. Introduced aquatic species of the North Sea coasts and adjacent brackish waters. *Biological Invasions in Marine Ecosystems*. Ecological Studies 204, 507–527.
- Gosling, E., 1992. Genetics of *Mytilus*. In: Gosling, E. (Ed.), *The Mussel Mytilus: Ecology, Physiology Genetics and Culture*. Elsevier, Amsterdam, pp. 309–382.
- Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomaneck, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems. *Ecology Letters* 9, 228–241.
- Haurly, L.R., McGowan, J.A., Wiebe, P.H., 1978. Patterns and processes in the time-space scale of plankton distribution. In: Steele, J.H. (Ed.), *Spatial Patterns in Plankton Communities*. Plenum Press, New York, pp. 277–327.
- Hawkins, S.J., Corte-Real, H.B.S.M., Pannacchiulli, F.G., Weber, L.C., Bishop, J.D.D., 2000. Thoughts on the ecology and evolution of the intertidal biota of the Azores and other Atlantic islands. *Hydrobiologia* 440, 3–17.
- Hofmann, E.E., Klinck, J.M., Powell, E.N., Boyles, S., Ellis, M., 1994. Modeling oyster populations II. Adult size and reproductive effort. *Journal of Shellfish Research* 13, 165–182.
- Huvet, A., Normand, J., Fleury, E., Quillien, V., Fabioux, C., Boudry, P., 2010. Reproductive effort of Pacific oysters: a trait associated with susceptibility to summer mortality. *Aquaculture* 304, 95–99.
- Jenkins, S.R., Aberg, P., Cervin, G., Coleman, R.A., Delany, J., Della Santina, P., Hawkins, S.J., LaCroix, E., Myers, A.A., Lindgarth, M., Power, A.-M., Roberts, M.F., Hartnoll, R.G., 2000. Spatial and temporal variation in settlement and recruitment of the intertidal barnacle *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) over a European scale. *Journal of Experimental Marine Biology and Ecology* 243, 209–225.
- Kang, D.H., Ahn, I.Y., Choi, K.S., 2009. The annual reproductive pattern of the Antarctic clam, *Laternula elliptica* from Marian Cove, King George Island. *Polar Biology* 32, 517–528.
- Kautsky, N., 1982. Quantitative studies on gonad cycle, fecundity, reproductive output and recruitment in a Baltic *Mytilus edulis* population. *Marine Biology* 68, 143–160.
- Kirby, R.R., Beaugrand, G., Lindley, J.A., 2009. Synergistic effects of climate and fishing in a marine ecosystem. *Ecosystems* 12, 548–561.
- Kobayashi, M., Hofmann, E.E., Powell, E.N., Klinck, J.M., Kusaka, K., 1997. A population dynamics model for the Japanese oyster, *Crassostrea gigas*. *Aquaculture* 149, 285–321.
- Largier, J., 2003. Considerations in estimating larval dispersal distances from oceanographic data. *Ecological Applications* 13, S71–S89.
- Lejart, M., 2009. Etude du processus invasif de *Crassostrea gigas* en Bretagne: état des lieux, dynamique et conséquences écologiques. PhD thesis, Université de Bretagne Occidentale, Brest.
- Lester, S.E., Gaines, S.D., Kinlan, B.P., 2007. Reproduction on the edge: large-scale patterns of individual performance in a marine invertebrate. *Ecology* 88, 2229–2239.
- Lewis, J.R., 1986. Latitudinal trends in reproduction, recruitment and population characteristics of some rocky littoral molluscs and cirripedes. *Hydrobiologia* 142, 1–13.
- Lowe, D.M., Moore, M.N., Bayne, B.L., 1982. Aspects of gametogenesis in the marine mussel *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* 62, 133–146.
- Mann, R., 1979. Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. *Journal of the Marine Biological Association of the United Kingdom* 59, 95–110.
- Miossec, L., Le Deuff, R.M., Goulletquer, P., 2009. *Crassostrea gigas* (Pacific oyster). ICES Cooperative Research Report 299.
- Moore, P.J., Thompson, R.C., Hawkins, S.J., 2011. Phenological changes in intertidal con-specific gastropods in response to climate warming. *Global Change Biology* 17, 709–719.
- Olive, P.J.W., 1995. Annual breeding cycles in marine invertebrates and environmental temperature: probing the proximate and ultimate causes of reproductive synchrony. *Journal of Thermal Biology* 20, 79–90.
- Orton, J.H., 1920. Sea-temperature, breeding and distribution in marine animals. *Journal of the Marine Biological Association of the United Kingdom* 12, 339–366.
- O'Riordan, R.M., Arenas, F., Arrontes, J., Castro, J.J., Cruz, T., Delany, J., Martínez, B., Fernandez, C., Hawkins, S.J., McGrath, D., Myers, A.A., Oliveros, J., Pannacchiulli, F.G., Power, A.M., Relini, G., Rico, J.M., Silva, T., 2004. Spatial variation in the recruitment of the intertidal barnacles *Chthamalus montagui* Southward and *Chthamalus stellatus* (Poli) (Crustacea: Cirripedia) over a European scale. *Journal of Experimental Marine Biology and Ecology* 304, 243–264.
- Philippart, C.J.M., van Aken, H.M., Beukema, J.J., Bos, O.G., Cadée, G.C., Dekker, R., 2003. Climate-related changes in recruitment of the bivalve *Macoma balthica*. *Limnology and Oceanography* 48, 2171–2185.

- Pieters, H., Kluytmans, J.H., Zandee, D.I., 1980. Tissue composition and reproduction of *Mytilus edulis* in relation to food availability, Netherlands. *Journal of Sea Research* 14, 349–361.
- Pouvreau, S., Bourles, Y., Lefebvre, S., Gangnery, A., Alunno-Bruscia, M., 2006. Application of a dynamic energy budget model to the Pacific oyster, *Crassostrea gigas*, reared under various environmental conditions. *Journal of Sea Research* 56, 156–167.
- Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M., Lowe, A.J., 2006. Adaptive evolution in invasive species. *Trends in Plant Science* 13, 288–294.
- Pulfrich, A., 1997. Seasonal variation in the occurrence of planktic bivalve larvae in the Schleswig-Holstein Wadden Sea. *Helgoland Marine Research* 51, 23–39.
- Quesada, H., Zapata, C., Alvarez, G., 1995. A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. *Marine Ecology Progress Series* 116, 99–115.
- R Development Core Team, 2009. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. URL: <http://www.R-project.org>.
- Rico-Villa, B., Woerther, P., Mingant, C., Lepiver, D., Pouvreau, S., Hamon, M., Robert, R., 2008. A flow-through rearing system for ecophysiological studies of Pacific oyster *Crassostrea gigas* larvae. *Aquaculture* 282, 54–60.
- Rico-Villa, B., Pouvreau, S., Robert, R., 2009. Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*. *Aquaculture* 287, 395–401.
- Satuito, C.G., Natoyama, K., Yamazaki, M., Fusetani, N., 1994. Larval development of the mussel *Mytilus-edulis galloprovincialis* cultured under laboratory conditions. *Fisheries Science* 60, 65–68.
- Seed, R., 1976. Ecology. In: Bayne, B.L. (Ed.), *Marine Mussels: Their Ecology and Physiology*. Cambridge University Press, Cambridge, pp. 13–65.
- Shatkin, G., Shumway, S.E., Hawes, R., 1997. Considerations regarding the possible introduction of the Pacific oyster (*Crassostrea gigas*) to the Gulf of Maine: a review of global experience. *Journal of Shellfish Research* 16, 463–477.
- Śmietanka, B., Zbawicka, M., Wolowicz, M., Wenne, R., 2004. Mitochondrial DNA lineages in the European populations of mussels (*Mytilus* spp.). *Marine Biology* 146, 79–92.
- Śmietanka, B., Burzynski, A., Wenne, R., 2009. Molecular population genetics of male and female mitochondrial genomes in European mussels *Mytilus*. *Marine Biology* 156, 913–925.
- Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdana, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience* 57, 573–583.
- Sprung, M., 1983. Reproduction and fecundity of the mussel *Mytilus edulis* at Helgoland (North Sea). *Helgoland Marine Research* 36, 243–255.
- Starr, M., Himmelman, J.H., Therriault, J.C., 1990. Direct coupling of marine invertebrate spawning with phytoplankton blooms. *Science* 247, 1071–1074.
- Sunila, I., 1981. Reproduction of *Mytilus edulis* L. (Bivalvia) in a brackish water area, the Gulf of Finland. *Annales Zoologici Fennici* 18, 121–128.
- Thorson, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates. *Meddelelser fra Kommissionen for Danmarks Fiskeri- og Havundersøgelser Series Plankton* 4, 1–523.
- Troost, K., Gelderman, E., Kamermans, P., Smaal, A.C., Wolff, W.J., 2009. Effects of an increasing filter feeder stock on larval abundance in the Oosterschelde estuary (SW Netherlands). *Journal of Sea Research* 61, 153–164.
- Underwood, A.J., Petraitis, P.S., 1993. Structure of intertidal assemblages in different locations: how can local processes be compared. In: Ricklefs, R., Schluter, D. (Eds.), *Species Diversity in Ecological Communities*. University of Chicago Press, Chicago, pp. 38–51.
- Villalba, A., 1995. Gametogenic cycle of cultured mussel, *Mytilus galloprovincialis*, in the bays of Galicia (N.W. Spain). *Aquaculture* 130, 269–277.
- Wilson, J.H., Seed, R., 1974. Reproduction in *Mytilus edulis* L. (Mollusca: Bivalvia) in Carlingford Lough, Northern Ireland. *Irish Fisheries Investigations B* 15, 1–30.
- Wood, S., 2009. gamm4: Generalized Additive Mixed Models Using mgcv and lme4. R Package Version 0.0-2. <http://CRAN.R-project.org/package=gamm4>.
- Zbawicka, M., Burzyński, A., Skibinski, D., Wenne, R., 2010. Scottish *Mytilus trossulus* mussels retain ancestral mitochondrial DNA: complete sequences of male and female mtDNA genomes. *Gene* 456, 45–53.
- Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer, Berlin, 574 pp.
- Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoiding common statistical problems. *Methods in Ecology and Evolution* 1, 3–14.