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ORIGINAL ARTICLE

Impact of trematodes on the population structure and shell shape of the estuarine mud snail *Hydrobia ulvae* from a Southern European estuary

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Abstract

The occurrence of trematodes within the gastropod *Hydrobia ulvae* was studied in two areas (a *Zostera noltii* bed and a eutrophic area) in the Mondego Estuary, Portugal. The aim was to assess trematode infections across snail age classes, as well as to investigate any influence of parasitism on the shell shape and size of these gastropods. In the *Z. noltii* bed, infection occurred in snails with shell height of 1.5–6 mm, but a higher prevalence was seen in intermediate size classes (3–5 mm). Infected individuals were never found among the elder gastropods. In the eutrophic area, infection was commonly observed within small individuals (1.6–2.5 mm), as large individuals were rarely found. The warm temperate climate in this coastal system may cause infected specimens to die before they can grow to larger dimensions, in contrast to Northern European populations. The frequency of snails with thin, distorted and corroded shells was higher among infected than non-infected snails. Infected individuals were frequently colonized by epibionts that may have additional detrimental effects and probably promote shell erosion. Infection with trematodes and their influence on snail size needs consideration in the evaluation of parameters related to population dynamics and population structure. If the implications of parasite infection are ignored, inadequate decisions about ecosystem management may be taken, resulting mainly from erroneous assessment of population structure, age composition and ecological longevity. The role of parasites is especially important to consider for systems under environmental stress.

Introduction

Digenean trematodes are common parasites of marine organisms (Lauckner 1980; de Montaudouin *et al.* 2003). They typically have complex indirect life cycles, with obligatory alternation between sexual and asexual reproduction. Several trematode species use gastropods as their first intermediate hosts, where they reproduce asexually, producing rediae or sporocysts that later will give rise to free larvae (cercariae) in large numbers. Redial stages are known to be more damaging to the host than the sporocysts, since they feed directly on the snail's tissues,

whereas sporocysts feed by absorbing nutrients (Probst & Kube 1999; Sorensen & Minchella 2001).

Parasites may potentially harm their hosts, depending on the intensity of infection or the way they interact with their hosts. Trematodes can influence their snail hosts' survival, reproduction, growth and behaviour (Huxham *et al.* 1993; Mouritsen & Jensen 1994; Gorbushin 1997; Probst & Kube 1999; Mouritsen & Poulin 2002; de Montaudouin *et al.* 2003; Fredensborg *et al.* 2005). Infected snails are generally more vulnerable than uninfected ones to adverse environmental factors, such as desiccation, osmotic stress, oxygen deficiency, and tem-

perature fluctuations (Huxham *et al.* 1995; Jensen *et al.* 1996; Shinagawa *et al.* 2001; Marcogliese 2008). Eventually, the combined effect of trematode infections and extreme environmental conditions may cause high mortality rates, with potential implications for the host population (Jensen & Mouritsen 1992; Mouritsen & Jensen 1997; Mouritsen *et al.* 2005).

Trematode rediae and sporocysts normally develop in the digestive gland and gonad complex of the snails (Gorbushin 1997; Probst & Kube 1999). Parasites established in the gonads may lead to castration of the host, by tissue destruction, space competition and reallocation of resources or other chemical mechanisms (Hall *et al.* 2007; Lafferty & Kuris 2009). In these cases, the energy that was destined for gastropod reproduction may be diverted to the production of parasite offspring (Lafferty & Kuris 2009) or to somatic growth of the snail, leading to gigantism (Mouritsen & Jensen 1994; Gorbushin 1997; Probst & Kube 1999).

There is still debate as to whether such enhanced growth is a parasite adaptation to enhance the available space for parasite biomass and increased cercarial production (McCarthy *et al.* 2004), or whether it is an adaptive host response that may improve future reproductive output if the host survives the infection (Minchella 1985). Enhanced growth might also be a passive side-effect of parasitic infection. The observed relationship between snail size and prevalence is often explained by the fact that larger, and hence older, snails have been exposed to potential infection for a longer time. Increased food consumption rate with snail size may also enhance the possibility of ingesting trematode eggs, thus leading to infection (de Montaudouin *et al.* 2003).

Trematodes are known to influence not only the growth rates of their snail hosts, but also host shape (Krist 2000; McCarthy *et al.* 2004; Levri *et al.* 2005; Thieltges *et al.* 2009). Morphological variation in snail individuals is determined by a combination of genetic and environmental factors that influence development (Poulin & Thomas 1999; Krist 2000; Alda *et al.* 2010). A variety of factors such as substratum, diet, food availability, and parasitism may impact shell morphology (Fernandez & Esch 1991; Probst & Kube 1999). Alterations induced by parasites span the intracellular to organism levels (Miura *et al.* 2006). Given the different degrees of impact that rediae and sporocysts inflict on snail hosts (Probst & Kube 1999; Sorensen & Minchella 2001), it is expected that they would also have dissimilar effects on host shell morphology and size.

Hydrobia ulvae is host to a variety of digenean trematodes (Deblock 1980; Huxham *et al.* 1995; Field & Irwin 1999; de Montaudouin *et al.* 2003; Bordalo *et al.* 2011). It is considered a significant link in estuarine food webs

(Cardoso *et al.* 2002, 2005) with great importance for the functioning of ecosystems in which they are present (Lillebø *et al.* 1999; Cardoso *et al.* 2002, 2005). This mud snail is one of the key species in macrobenthic intertidal communities of the Mondego Estuary (Portugal) (Cardoso *et al.* 2002, 2005). In this system, *H. ulvae* recruits four times a year (March, June, July and September) and its life span varies from 16 to 20 months (Cardoso *et al.* 2002, 2005). Trematode species composition within this mud snail population and density patterns of infected individuals have been addressed before (Bordalo *et al.* 2011). In the present study, it was intended to assess how parasites influence the size and morphology of these gastropods and accordingly how they might interfere with analyses of population structure, thus potentially influencing studies of their population dynamics. Consequently, the main goals were: (i) to evaluate whether parasite prevalence increases with size/age, and (ii) to investigate any alterations in host morphology connected with parasitism.

Material and Methods

Study site

The Mondego Estuary is a small coastal system (1072 ha) located in a warm temperate region on the central western coast of Portugal (40°08′ N, 8°50′ W). It comprises a north and a south arm, separated by Murraceira Island (Fig. 1). The north arm is deeper and constitutes the main navigation channel, being frequently dredged. The south arm is shallower and is characterized by large areas of exposed intertidal flats during low tide. Despite being relatively less impacted by human activities, the south arm is particularly susceptible to eutrophication due to its shallow depth, high water residence time, and elevated nutrient loading (Martins *et al.* 2001; Lillebø *et al.* 2005).

Two sampling areas in the south arm were regularly sampled, representing dominant habitats: (i) *Zostera noltii* bed: a mud flat covered with the seagrass *Z. noltii,* situated downstream, and (ii) eutrophic sand flat area: located upstream, in the inner section of the south arm, where *Z. noltii* disappeared decades ago as a result of eutrophication-induced seasonal green algal (*Ulva* sp.) blooms (Bordalo *et al.* 2011).

The nearby Coimbra forecasting station of the Portuguese Institute of Meteorology provided the monthly precipitation and atmospheric temperature data. Mean air temperature patterns vary according to the warm temperate climate of the Mondego region, increasing from spring (mean: 17 °C) to summer (mean: 21 °C) and decreasing in autumn (mean: 14 °C), towards winter (mean: 12 °C). In the summer, maximum air temperatures
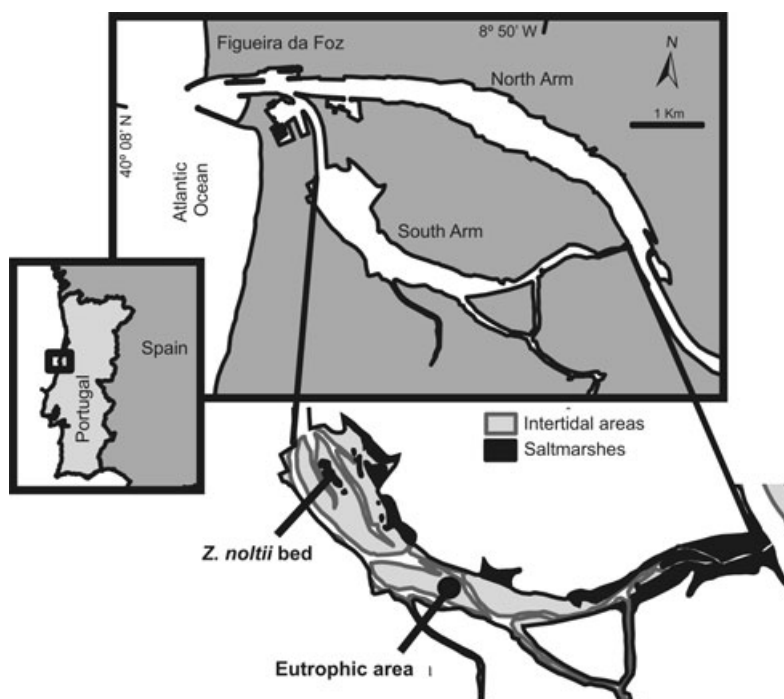
Fig. 1. Location of the sampling stations along the south arm of the Mondego Estuary.

frequently reach values near 40 °C; temperature in intertidal pools can be higher than 30 °C (Bordalo *et al.* 2011).

Sampling programme and laboratory work

Macrobenthos was sampled between January 1993 and September 1995. This period has already featured in several studies (Cardoso *et al.* 2002; Ferreira *et al.* 2004; Jensen *et al.* 2004; Pardal *et al.* 2004; Dolbeth *et al.* 2007), including examinations of *H. ulvae* population dynamics (Lillebø *et al.* 1999; Cardoso *et al.* 2002) and an analysis of trematode diversity in the mud snails (Bordalo *et al.* 2011). Samples were taken in a haphazard manner each month during morning low tides, using six cores of 141 cm^2 at each station. Cores were taken to a depth of 20 cm in both the *Zostera noltii* bed and the eutrophic area. The resulting material was washed on a 500-μm-mesh sieve and preserved in 4% buffered formalin. For every sampling date at each sampling area, temperature and salinity were measured in low tide pools by means of a portable conductivity meter (model WTW v330i) with a standard TetraCon® 325 conductivity cell (Weilheim, Germany).

Hydrobia ulvae population structure was previously determined by recognition of cohorts from successive dates (Cardoso *et al.* 2002). In the present study, *H. ulvae* specimens collected in the sampling cores were sieved using a 1-mm mesh, to remove immature, and thus uninfected, juveniles (Probst & Kube 1999). Adult specimens retained on the sieve mesh were sub-sampled using a Folsom splitter (Wildco®, Yulee, FL, USA) for a total of at least 300 individuals from each monthly sample. Replicates with smaller numbers of individuals were screened completely. *Hydrobia ulvae* individuals were measured for shell width, dissected, and inspected for parasites, which were identified according to Deblock (1980). It was only possible to identify infections with mature cercariae and therefore immature infections were not considered. The shell was removed by crushing it with a pair of tweezers and later cleaned in distilled water. Maximum shell width was measured instead of shell height because in most individuals the apex was missing or damaged. Therefore, shell height (mm) was estimated according to the following equation: shell maximum width = 0.4369 × shell height + 0.2091 (N = 339, r^2 = 0.97) (Lillebø *et al.* 1999). The relative proportion of infected individuals within each cohort was calculated to follow the evolution of their prevalence over time.

The shell of each specimen from the *Z. noltii* bed was described with respect to shape and state, including corrosion marks, epibionts, ballooning of the whorls, thinning of the shell, asymmetrical development of the spire and shell fragility (assessed while crushing the mud snails with tweezers for parasite inspection). It was impossible to give a similarly detailed description of the shells of preserved snails from the eutrophic area, as a large fraction of these shells were found to have

deteriorated after storage in formalin and because of their generally small size.

Data analysis

A Scheirer–Ray–Hare (SRH) test was used as a non-parametric equivalent of a two-way ANOVA with replication (Dytham 2003) to test for differences in shell size of snails infected by trematodes from the most abundant families (Microphallidae, Haploporidae and Heterophyidae) between the two sampling areas (factors: site and trematode family).

Differences between the frequencies of shell shape abnormalities in relation to infection were examined by a chi-square test, including whether they were infected by trematode species whose life cycles include redia or just sporocyst larval stages. Statistical analyses were performed using IBM® SPSS® Statistics 20 (Armonk, NY, USA).

Results

Trematode fauna in relation to *Hydrobia ulvae* size and shape

A total of 14,260 *Hydrobia ulvae* specimens from the *Zostera noltii* bed and 11,014 individuals from the eutrophic area were examined. The population structure differed between the two sampling areas (Fig. 2). In the *Z. noltii* bed, the population was characterized by the presence of a wide range of size classes, whereas in the eutrophic area, mostly juveniles were found (Fig. 2).

Trematode species from five families were found within *H. ulvae* from the Mondego estuary: Microphallidae, Haploporidae, Heterophyidae, Notocotylidae and Echinostomatidae. Species from the first family produced only sporocysts, whereas species from all other families also had a redial stage. Neither double nor multiple infections were found. Only mature cercariae, within rediae or sporocysts, could be identified and therefore infections with immature larval stages were not considered in this analysis.

Digenean trematodes were found in specimens with shell heights of between 1.6 and 7.0 mm. In the *Z. noltii* bed, the proportion of infected individuals peaked in the intermediate size classes (3–5 mm), whereas at the eutrophic area they were more frequently found between 1.6 and 2.5 mm (Fig. 2a,b). Furthermore, the sizes of infected *H. ulvae* in the *Z. noltii* bed were significantly higher than those found in the eutrophic area (SRH test, P < 0.05; Table 1, Fig. 3) in accordance with the different population structures observed in the sampling areas (Fig. 2a,b). On the other hand, the size of infected *H. ulvae* did not differ among trematode families (SRH test,
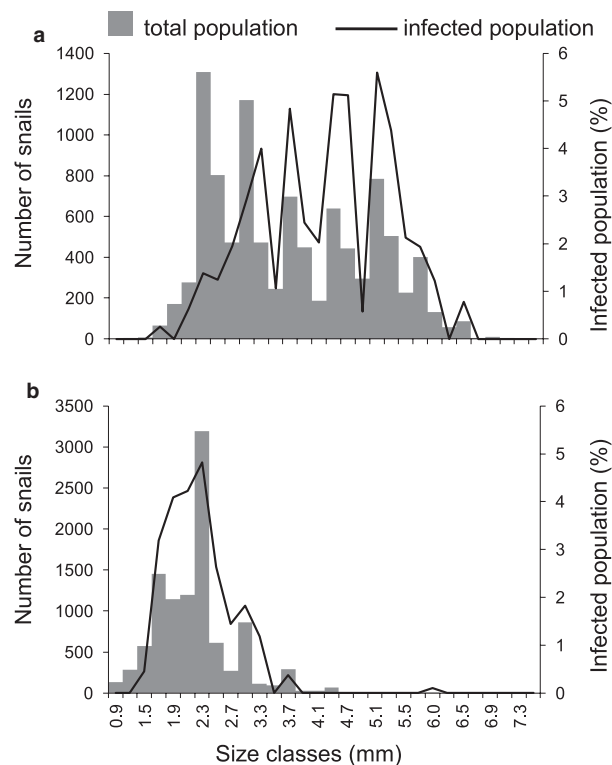
Fig. 2. Size structure of the *Hydrobia ulvae* population (absolute values of inspected individuals) and prevalence of infection (%) throughout size classes at (a) *Zostera noltii* bed and (b) eutrophic area.

Table 1. Results of a Scheirer-Ray-Hare (SRH) test with site (*Zostera noltii* bed or eutrophic area) and trematode family (infected with Microphallidae, Heterophidae or Haploporidade) as fixed factors, using shell size as a dependent variable.

Source of variation	SS	SS/MSotal	df	P-value
Site (factor)	3043.06	7.57	1	0.01
Family (factor)	71.29	0.18	2	0.92
Size * family (interaction)	105.05	0.26	2	0.88

P > 0.05; Table 1, Fig. 3). There was no interaction between the two factors: trematode families and sampling areas (SRH test, P > 0.05; Table 1, Fig. 3).

By tracking the evolution of the cohorts in the *Z. nolti* bed, it was observed that the percentage of infected individuals was relatively constant in median age/size individuals, and usually decreased in the larger size classes (Table 2a, Fig. 2a). In some cohorts (C2, C3, C4, C5, C9, C10, C11, C12) the infection decline occurred during or following the summer period, whereas in C7 and C8 infected individuals were found almost up until the disappearance of the cohorts. In these cases, the cohorts did not achieve their largest sizes during the summer period.
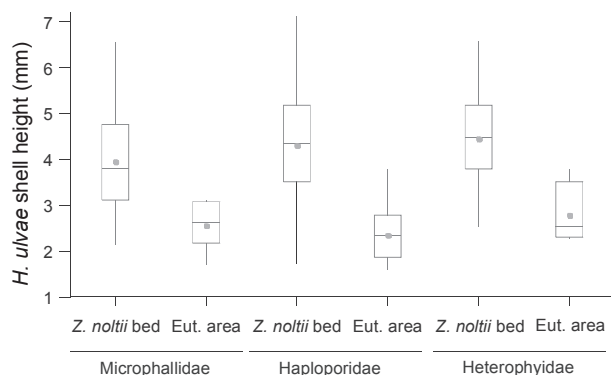
Figure 3. Box plot of infections in *Hydrobia ulvae* individuals by the most abundant trematode families Microphallidae, Haploporidae and Heterophyidae, in two sampling stations in the Mondego Estuary, *Zostera noltii* bed and eutrophic area, showing the shell sizes in which the infection is present. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles, respectively. The grey circles indicate mean values.

Figure 4. (a) Proportion of regular and irregular shells of *Hydrobia ulvae* in non-infected individuals and individuals infected with sporocysts and rediae, in the *Zostera noltii* bed, in the Mondego Estuary, from January 1993 to September 1995. (b) Shells of two *H. ulvae*, one infected with *M. subdolum* (left) and the other not infected (right). Both have the same width (1.2 mm) but the infected mud snail has a shell height of 2.5 mm whereas the non-infected snail measures 2.3 mm.

In the eutrophic area, infection appeared in young individuals that corresponded to the major stock of potential trematode hosts (Fig 2b). In practically all cohorts from this site, infection lasted until the disappearance of snails from this area, which occurred relatively early (Table 2b, Fig 2b) when they reached a shell length of approximately 3 mm (Fig. 2b). No obvious patterns were evident here, as infections in this area were scarce (Table 2b).

The frequency of abnormal shell shapes was significantly higher among infected than uninfected individuals ($\chi^2_1 = 160.845$, P < 0.05; Fig. 4) but there were no significant differences in the frequency of abnormal shells between snails infected with trematode species whose life cycles included rediae or those with just sporocyst larval stages ($\chi^2_1 = 0.813$, P > 0.05; Fig. 4).

Discussion

In several previous studies of snail–trematode systems, an increased prevalence in the largest size classes was observed (Rothschild 1936; Mouritsen & Jensen 1994; Huxham *et al.* 1995; Gorbushin 1997; Field & Irwin 1999; Gorbushin & Levakin 1999; Probst & Kube 1999; de Montaudouin *et al.* 2003) (Table 2). In the present study, there was no evidence of larger individuals having more prevalent infections when compared with the rest of the population. Instead, infections were observed more often within the intermediate size classes. The impact of infection on the snail host is strongly dependent on its life history (Gorbushin 1997). In contrast to similar stud-

ies from systems at northern latitudes (Rothschild 1936; Mouritsen & Jensen 1994; Huxham *et al.* 1995; Gorbushin 1997; Field & Irwin 1999; Gorbushin & Levakin 1999; Probst & Kube 1999), infected snails from the present system may not live long enough to become 'giant'. These individuals commonly inhabit shallow-water pools formed during low tide, where temperatures may reach above 30 °C during summer, much higher than the values found in northern coastal systems. Considering the low thermal tolerance of debilitated infected snails (Jensen *et al.* 1996; Marcogliese 2008), an increased mortality of *Hydrobia ulvae* would be expected in the summer. These individuals might not outlive this season and consequently, the infected fraction of the population may not surpass 6–7 mm within a 16- to 20-month life span of the total population. The larvae living inside the snail inflict serious injuries in the tissues as well as

Table 2. Prevalence of infection within each cohort of *Hydrobia ulvae* (calculated as the proportion of infected individuals in relation to the number of individuals that belong to a given cohort) in the *Zostera noltii* bed and eutrophic area. The shaded areas represent the evolution of the cohort. The first column corresponds to the year of recruitment of the cohort. The second line of the column corresponds to the month of recruitment of the cohort (red – March, blue – June, green – July, yellow – September).

A

Year	Cohort	F93	M93	A93	J93	J93	A93	O93	D93	J94	F94	M94	A94	J94	S94	O94	N94	D94	F95	M95	A95	M95	J95	J95	A95	S95
92	C1			0.38																						
92	C2		0.55	0.38	0.43																					
92	C3	0.49		0.33	0.33		0.02	0.02	0.33																	
92	C4			0.25			0.02	0.02	0.33	0.33		0.40														
93	C5								0.33	0.33		0.40	0.40													
93	C6										0.35	0.40	0.40													
93	C7										0.35		0.37		0.33											
93	C8											0.74		0.40	0.44	0.33	0.30	0.38	0.28	0.17	0.24	0.37				
94	C9													0.40	1.32	0.66	0.61	0.75	0.41	0.25	0.40	0.25				
94	C10														0.44	1.00	0.53	0.57	0.55	0.33	0.24	0.41	0.32	0.24	0.20	0.26
94	C11																0.30	0.75	0.28	0.33	0.24		0.64	0.24	0.20	0.22
94	C12																0.30	0.38					0.64	0.39	0.20	0.45
95	C13																	0.30			0.24	0.49	0.32	0.47	0.20	0.15
95	C14																									0.15

B

Year	Cohort	F93	M93	A93	J93	J93	A93	S93	O93	N93	D93	J94	F94	M94	A94	M94	J94	J94	S94	O94	N94	D94	F95	M95	A94	M95	J95	J95	A95	S95
92	C3	0.24		0.20																										
92	C4	1.67	0.10	0.07	0.07	0.20	0.50																							
93	C5			0.07	0.07	0.20	0.50	0.36	0.23	0.31	0.30																			
93	C6							0.54	0.31	0.11																				
93	C7							0.11								0.40														
93	C8															0.40	0.40													
94	C9																0.40	2.46	0.18	0.18	0.55									
94	C10																			0.83							0.21			
94	C11																				1.64		1.10	0.11		0.42	0.61		0.31	
94	C12																							0.11		0.42	0.42	0.45	0.21	
95	C13																											0.45		0.37
95	C14																													
95	C15																													

pathological changes (Probst & Kube 1999). Moreover, keeping in mind that increased water temperatures usually promote a higher cercarial output (Mouritsen 2002; Poulin 2006; Marcogliese 2008; Studer et al. 2010) and that larger snails produce more of these free-living larval stages (Miura et al. 2006; Poulin 2006), very high temperatures may especially affect larger specimens. Hence, the deleterious effects of parasites may interact with extreme environmental conditions, leading to the death of the host (Jensen & Mouritsen 1992; Meissner & Bick 1999; Mouritsen 2002). Both stress factors influence the life history of these organisms, and both should be considered when analysing population structure. In this study, these effects were observed at low prevalence values (<6%). In highly infected snail populations (Table 3), parasites may be responsible for an even more noticeable variation in shell size, which might generate mistaken interpretations of the population structure, with strong repercussions in its further analysis.

The two sites studied represent distinct habitats that provide different environmental conditions for the mud snails, with implications for their population structures, which in turn have an influence on infection. Trematodes are found preferentially within adult individuals, as adult snails have mature gonads and a larger size, providing more soft tissue for parasite development and more energy resources (Probst & Kube 1999; Graham 2003; Miura et al. 2006). The Zostera noltii bed yielded an abundant and well age-structured mud snail population (Lillebø et al. 1999; Cardoso et al. 2005) and therefore considerable availability of potential hosts in this area. Infection in the Z. noltii bed was observed in almost all of the recognized cohorts, but was most obvious in the middle size/age classes. The largest/oldest size classes were represented mainly by uninfected specimens, probably because infected individuals died sooner than uninfected individuals, for the reasons mentioned above. On the other hand, in the eutrophic area, where the mud snail population was dominated by juveniles, potential hosts were in short supply, so trematodes have no alternative but to infect small individuals in this location.

Shell shape modifications are frequently observed following trematode infection (Krist 2000; McCarthy et al. 2004; Hay et al. 2005). Infected mud snails often had thinner shells, with some distortions and with epibionts on the surface. According to Mouritsen & Bay (2000), trematode-infected individuals are more likely to become fouled by epibionts, probably because the parasites interfere with the anti-fouling mechanisms developed against epibiosis, mainly the production of toxic secondary metabolites. It may also be possible that the epibionts weaken the immune system of the snail, so that consequent infection is more likely (Mouritsen & Bay 2000). Parasitism may influence host morphology by altering rates of growth in one dimension but not in another (Levri et al. 2005), resulting in asymmetric shapes deviating from the 'normal' phenotype. Parasites may obtain profit from alterations in host shell shape (McCarthy et al. 2004), as the ballooning of some whorls may result in an increase in the total volume inside the shell, giving more room for the parasite and thus enhancing its cercarial production. Nonetheless, there is competition for space within the shell between host and parasite tissues (Sorensen & Minchella 2001). Alterations in the shape are visible if the parasites have a long period of time to grow within the snail (Krist 2000). For that reason, some infected individuals had a normal shell shape, in the case of early infections, when the effects of parasitism were still not evident. In addition, the parasite may not interfere with shell shape if it is not affecting growth rate, or not inflicting its maximum effect on the host (Krist 2000). A small proportion (6%) of non-infected mud snails also had an irregular shell shape, possibly due to a natural deficiency in the snail's defence mechanism not connected with parasitism. The thinner shells that frequently occurred in the infected individuals could be explained by the high energetic

Table 3. Prevalence data from studies of digenean trematodes in *Hydrobia ulvae* populations from other European localities. The presence or absence of a relationship between size and infection is indicated.

Author	Study site	Mean prevalence (%)	Max prevalence (%)	Min prevalence	Relationship with size
Fish & Fish (1974)	Dovey Estuary	2.8	3.8	1.4	n.a.
Huxham et al. (1995)	Ythan Estuary, Scotland	–	16.8	1.5	> 6.1 mm all infected
Sola (1996)	Bidasoa Estuary, Spain	6.5	–		n.a.
Field & Irwin (1999)	Belfast Lough, N. Ireland	12.6	25.5	–	Presence
	Ythan Estuary, Scotland	13.5	24.8	–	
de Montaudouin et al. (2003)	Arguin,Arcachon Bay, France	–	16.0	0.0	> 5.1 mm all infected
	La Canellete,Arcachon Bay, France	–	6.0	0.5	Absence
	Lette Douce,Arcachon Bay, France	–	5.1	1.3	Absence

n.a., not applicable; indicates that such a relationship was not mentioned.

demand of the parasites that diverts important resources away from shell maintenance. This is probably due to the fact that the construction of shell material is energetically costly (Brusca & Brusca 2002). Accordingly, these snails are more likely to be crushed than others, being indeed more vulnerable than the non-infected snails.

Infected *H. ulvae* from the Mondego Estuary displayed a similar morphology irrespective of trematode type, with no size or shape differences being found. It was expected that redial and sporocyst infections might have different outcomes for the snail host, but instead a generalized response to infection was found. According to Sorensen & Minchella (2001) trematode species with redia larvae were expected to induce gigantism in their snail host more than those with sporocysts alone. Rediae are known to impose more physical damage on the host tissues than sporocysts because they feed directly on the host. Considering that rediae have a higher impact on the host snail, differences were expected to emerge regarding the degree of shell shape modification. In the present study, the results showed that trematode species induced changes in shell morphology of the snail host. However, no significant difference was found between species asexually reproducing by redial and sporocyst larval stages.

As the shell is a gastropod's main defense, alterations in shell shape or size may alter the effectiveness of the shell in protecting against predators (Krist 2000) or in competing with other individuals. As a result, alterations in shell morphology are likely to have fitness consequences for the snail host. Parasites may also play an important role in the phenotypic variation within the population, this role being increasingly important with higher prevalence. The influence of parasites on the population structure of the snail host can have implications in studies of population dynamics. Erroneous interpretation of the size frequency of the individuals of a given population will compromise understanding of age structure, individual growth rates, ecological longevity (by the recognition of cohorts, through size frequency analysis) and secondary production. Such errors may consequently influence the overall ecosystem investigation. Digenean trematodes arise as a cryptic factor that can interfere with the management of ecosystems, especially those that are under environmental stress, when their presence, quantity and implications are ignored.

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ORIGINAL ARTICLE

Relationships between mechanical disturbance and biomass of the invasive amphipod *Gammarus tigrinus* within a charophyte-dominated macrophyte community

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Abstract

Disturbance may alter the resistance of communities to non-indigenous species as it frees space and removes competitively superior species. In a factorial field experiment we studied how different types of mechanical disturbance affected the biomass level of the non-indigenous amphipod *Gammarus tigrinus* in a brackish water charophyte community. Mechanical disturbance affected the biomass of *G. tigrinus* with a time lag between disturbance and response of the gammarid species. In general, all types of disturbance reduced gammarid biomasses. The effect persisted until the end of the experiment regardless of the recovery of macrophyte communities in terms of species number and biomass of benthic invertebrate and plant species. Thus, a possible cause of reduced biomass of the gammarid amphipods relates to the decreased biomass of *Chara aspera* and its structural changes. This indicates that the dominance of *G. tigrinus* in a low saline system has less to do with strong species interactions (e.g. competitive displacement) than with habitat-level processes (e.g. changes in habitat structural characteristics and food supply).

Introduction

Non-native species are ranked among the greatest threats to global biodiversity (Bax *et al.* 2003), with coastal marine systems being the most invaded systems on the planet (Carlton 1996). Earlier studies have shown that successful exotics may render previously stable systems unbalanced and unpredictable (Carlton 1996; Ruiz *et al.* 1997) and severely affect the structure and functioning of invaded ecosystems (Ricciardi & Cohen 2007; Orav-Kotta *et al.* 2009). Despite the importance of coastal invasions, marine non-native species are much less well studied than terrestrial and freshwater counterparts (Grosholz 2002).

Dating back as far as 1958, Elton realized that species-rich communities should be more stable and less susceptible to non-native species compared to species-poor communities (Elton 1958). To date most of the smaller-scale experimental work has supported this biotic resistance hypothesis (e.g. Stachowicz *et al.* 1999, 2002) and

the observed inverse relationship is explained by more efficient utilization of available resources by highly diverse communities (Levine & D'Antonio 1999) and/or the sampling effect, i.e., the occurrence of suppressive species increases with diversity (Wardle 2001). Competition with an ecologically similar native species is an obvious example of such biotic resistance (Kotta *et al.* 2001; Kotta & Ólafsson 2003).

Disturbance may alter the resistance of communities to non-native species as it frees space and removes competitively superior species, thus relaxing competitive interactions among non-native and resident species (Shea & Chesson 2002). Nevertheless, non-native species often seem to have idiosyncratic and context-specific characters, urging experimental studies on how disturbance affects the population dynamics of these species. Even more, the role of disturbance in the success of non-native species has initiated a long debate because non-native species could be considered the drivers or consequences of

biological changes (Didham *et al.* 2005 and references therein).

Due to its short geological history, the Baltic Sea biota has a low number of species (e.g. Segerstråle 1957). Despite high numbers of non-native species, the coastal biota shows no loss of biodiversity and, even more, non-native species actually expand ecosystem functioning (Kotta *et al.* 2006; Reise *et al.* 2006). In recent years the range of non-native amphipods has increased tremendously in the Baltic Sea. Crustacean invaders seem to expand their distribution at the expense of the resident species of the area, suggesting strong competitive interactions among resident and non-native species (Jazdzewski *et al.* 2002; Szaniawska *et al.* 2003; Orav-Kotta *et al.* 2009).

Although the exotic *Gammarus tigrinus* Sexton was occasionally found in the southern Baltic Sea in the mid-1970s, it was not until the early 2000s that the species expanded its distribution throughout virtually the entire Baltic (Herkül *et al.* 2009). A recent climate change may have facilitated this rapid range expansion, as elevated winter storms are expected to increase the amount of drifting macroalgae, an efficient transport vector of amphipod crustaceans in the water column (Biber 2007). More importantly, extensive erosion and alteration of depositional coasts has been observed in the Baltic Sea in recent years (Orviku *et al.* 2003). The lack of evidence for sea level rise during this period suggests that such erosion is largely due to the recent increased storminess in the Eastern Baltic Sea. Severe storms efficiently erode sediment and destroy benthic macrophyte communities, thereby facilitating the large-scale spread of the non-native species through relaxation of competitive interactions. Besides these episodic extreme disturbances, benthic communities are impoverished by increasing intensities of smaller mechanical stressors, mainly of human origin, such as trampling by feet, boating and dredging.

Earlier studies have shown that *G. tigrinus* tolerates broad environmental conditions (Pinkster *et al.* 1977; Wijnhoven *et al.* 2003; Devin & Beisel 2007) and has low habitat selectivity (Bousfield 1973; Daunys & Zettler 2006); therefore, it may potentially inhabit very different types of coastal habitat. However, in the coastal reach of the Northern Baltic Sea, the species is becoming a dominant mesoherbivore in the sheltered charophyte communities (Herkül *et al.* 2009). Being extremely fragile, such charophyte habitats are potentially very sensitive to any type of mechanical disturbances.

In an *in situ* experiment, we studied how different types of mechanical disturbance affected the biomass level of *G. tigrinus* in a brackish water charophyte community. We predict that a moderate mechanical disturbance leav-ing a significant proportion of the charophyte canopy intact may increase the biomass of the non-native amphipod as the majority of native herbivores are expected to be sensitive to such stress. Consequently, the competitive interactions among non-native and resident species are expected to be weakened (e.g. Herkül *et al.* 2006). *Gammarus tigrinus* is an opportunistic species and is therefore expected to be less severely affected by mechanical disturbance than the resident species. We also predict that due to the phytophilous nature of *G. tigrinus*, the disturbances associated with mechanical removal of vegetation or sediment will significantly reduce the biomass of the non-native species.

Study Area

Rame Bay is a shallow and semi-enclosed bay in the Northeastern Baltic Sea (58.5749° N, 58.5671° E; surface area 4 km^2). The maximum depth of the area is 1.5 m but most of the bay is shallower than 1 m. The bottom is composed of fine sand and a thick layer of finely fractioned silt. Salinity varies between 3 and 5 and is highly dependent on rainfall. Being sheltered, Rame Bay provides excellent habitat for luxurious charophyte populations together with aquatic phanerogams. The most widespread species is *Chara aspera* Willd., which dominated the entire sheltered part of the bay (Torn & Martin 2003). The non-native amphipod *Gammarus tigrinus* only recently invaded Rame Bay and 2 years after establishment (i.e., at the beginning of this study) it made up the majority of gammarid abundance and biomass in the bay.

Material and Methods

Experimental procedures

The experiment was carried out from June 2007 to July 2008. Experimental plots (1.5 × 1.5 m) were established within a dense *Chara aspera* community at 1 m depth. Mechanical disturbance was applied once in June 2007 and involved the following treatment levels: (i) control (i.e., undisturbed plots), (ii) cutting the tips of plants, (iii) removal of plants, (iv) mixing of the sediment surface layer, (v) removal of the surface sediment layer. We used a full random design. For the second treatment, the upper 5 cm of the vegetation was cut by a diver. For the third treatment, plants were removed gently by hand. These two treatments mimic the foraging of herbivorous birds. For the fourth treatment, sediment was mixed together with vegetation to approximately 0.1 m depth. Mixing of the sediment surface layer was used to imitate the influence of motorboat anchoring, running of scooters and/or trampling. And finally, for the fifth treatment,

the sediment layer was removed down to about 0.1 m depth. Removal of the sediment layer represents disturbances due to dredging and/or ice scrape that also result in the removal of vegetative layer. The plots were sampled in July, August, September and October 2007, and July 2008. For each treatment level and sampling occasion three replicate biomass samples were collected, totaling 75 samples. A SCUBA diver collected samples by gently removing the biota within the algal canopy and sediment epifauna using a 20 × 20 cm frame. Samples were stored at −20 °C. In the laboratory all macrophytes and benthic invertebrates were determined to the species level. The dry weight of species was obtained after drying the individuals at 60 °C for 2 weeks (Torn *et al.* 2010).

Statistical analyses

Repeated measures ANOVA (StatSoft Inc 2007) was used to compare the effect of different types of disturbance (levels: tips cut, plants removed, sediment mixed, sediment removed and control) on species number and biomass among different months (from July 2007 to July 2008). The Mauchly sphericity test was used to check the assumption of equality of variance. We used the following multivariate tests to seek the statistical significance of different types of experimental disturbance on vegetation biomass: Wilks' lambda, Pillai–Bartlett trace and Hotelling–Lawley trace tests. These tests were used as they do not make the strict, often unrealistic, assumptions about the structure of the covariance matrix. As all these tests resulted in similar significance levels, only the output of the Wilks' lambda test (as the most commonly used) was reported. A post-hoc Fisher LSD test was used to analyse which treatment levels were statistically different from each other.

Multivariate data analyses on plant and invertebrate communities were performed by the statistical program PRIMER version 6.1.5 (Clarke & Gorley 2006). Similarities between each pair of samples were calculated using a zero-adjusted Bray–Curtis coefficient. This coefficient is known to outperform most other similarity measures and enables samples containing no organisms at all to be included (Clarke *et al.* 2006). Non-metric multidimensional scaling analysis (MDS) was used to visualize the dissimilarities between treatments and times.

Results

The studied benthic invertebrate communities were characterized by a few taxa. The plots contained mainly the amphipods *Gammarus tigrinus*, *Gammarus salinus* Spooner, *Gammarus zaddachi* Sexton, *Gammarus oceanicus* Segerstråle, *Gammarus locusta* (Linnaeus), the isopod *Asellus aquaticus* (Linnaeus), the snails *Bithynia tentaculata*

(Linnaeus), *Hydrobia ulvae* (Pennant), *Lymnaea peregra* (Müller), *Theodoxus fluviatilis* (Linnaeus), the cockle *Cerastoderma glaucum* (Poiret) and larvae of Chironomidae, Coleoptera, Odonata and Trichoptera. *Gammarus tigrinus* was by far the most dominant herbivore species and constituted 98% of gammarid biomass, with moderate biomasses from June to September and high biomasses in October.

Mechanical disturbance affected the biomass of *G. tigrinus* with a 4-month time lag between disturbance and response of the gammarid species (Fig. 1, Table 1). Regardless of type, the disturbed communities had significantly lower gammarid biomasses than control communities in October 2007 (repeated measures ANOVA, post-hoc Fisher LSD test: control *versus* disturbed plots P < 0.001) and in July 2008 (repeated measures ANOVA, post-hoc Fisher LSD test: control *versus* disturbed plots P = 0.023–0.025, except for control *versus* tips cut treatment P = 0.071).

Mechanical disturbance had no effect on the species number of benthic invertebrates and the biomasses of any other invertebrate species including juvenile gammarids (repeated measures ANOVA, separate disturbance effect and disturbance × time interaction P > 0.05) (Fig. 2).

The studied macrophyte communities contained mainly the charophyte *Chara aspera*, the chlorophytes *Cladophora glomerata* (L.) Kütz. and *Ulva intestinalis* L. and the higher plants *Najas marina* L. and *Potamogeton*
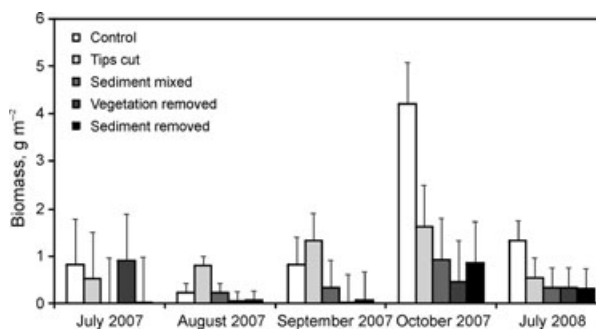
Fig. 1. Seasonal variation in average (±95%CI) biomass of *Gammarus tigrinus* for the studied disturbance treatments in 2007–2008.

Table 1. Repeated measures ANOVA multivariate test of significance for the effect of disturbance on biomasses of *Gammarus tigrinus* in 2007–2008.

Effect	SS	Df	MS	F	P
Intercept	35.70	1	35.70	121.35	<0.001
Disturbance	16.44	4	4.11	13.98	<0.001
Error	2.94	10	0.29		
TIME	16.92	4	4.23	15.40	<0.001
TIME × disturbance	20.06	16	1.25	4.56	<0.001
Error	10.99	40	0.27		
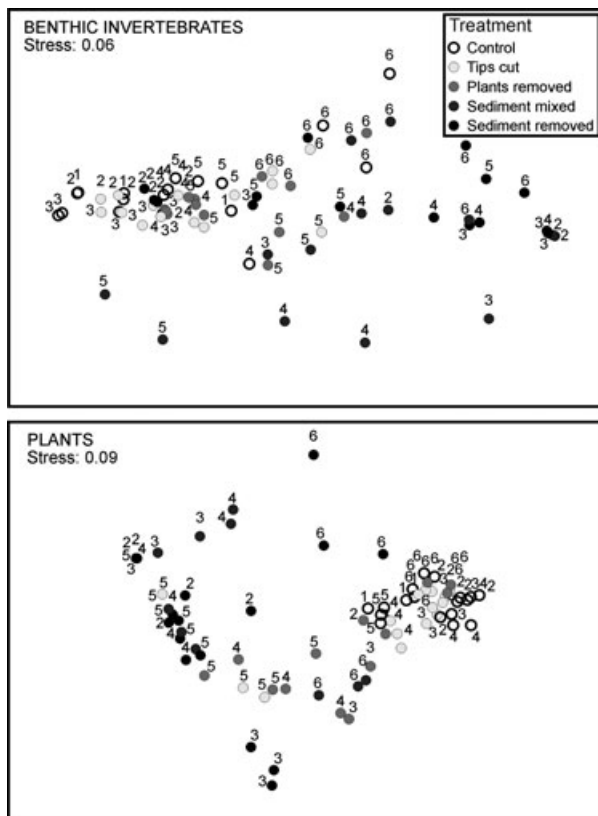
Fig. 2. Multidimensional scaling analysis (MDS) ordination of benthic invertebrate and macrophyte biomasses. Increasing distances between points denote larger dissimilarities among treatments and times. The time code is as follows: 1: June 2007, 2: July 2007, 3: August 2007, 4: September 2007, 5: October 2007, 6: July 2008.

pectinatus L. *Chara aspera* was by far the most dominant macrophyte species and constituted 96% of total plant biomass, with high biomasses from June to September and moderate biomasses in October.

Mechanical disturbance affected the biomass of *C. aspera* instantaneously and the effects persisted until the end of the experiment (Fig. 3, Table 2). Similarly to *G. tigrinus*, the disturbed communities had significantly lower biomasses compared with control communities. The disturbances associated with mechanical removal of vegetation or sediment had the largest impact on the charophyte community throughout the experiment (repeated measures ANOVA, *post-hoc* Fisher LSD test: control *versus* heavily disturbed plots P < 0.001). Disturbances that only partly damaged the plants had minor effects in 2007 and the communities had recovered by July 2008.

Mechanical disturbance also reduced the species number of macrophytes (Fig. 4, Table 3). In general, the disturbances associated with mechanical removal of vegetation or sediment had significantly lower species number compared with other treatments in 2007
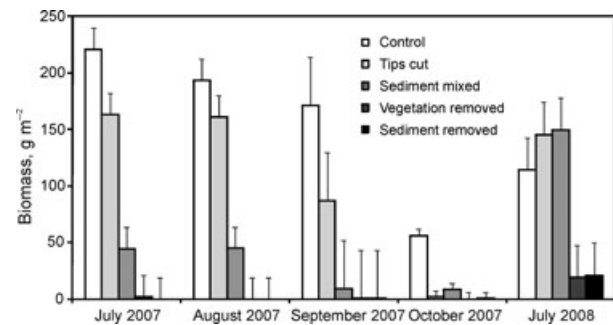
Fig. 3. Seasonal variation in average (±95%CI) biomass of *Chara aspera* for the studied disturbance treatments in 2007–2008.

Table 2. Repeated measures ANOVA multivariate test of significance for the effect of disturbance on biomasses of *Chara aspera* in 2007–2008.

Effect	SS	Df	MS	F	P
Intercept	311,731	1	311,731	2081	<0.001
Disturbance	258,034	4	64,509	431	<0.001
Error	1498	10	150		
TIME	61,465	4	15,366	32	<0.001
TIME × Disturbance	89,820	16	5614	12	<0.001
Error	19,113	40	478		
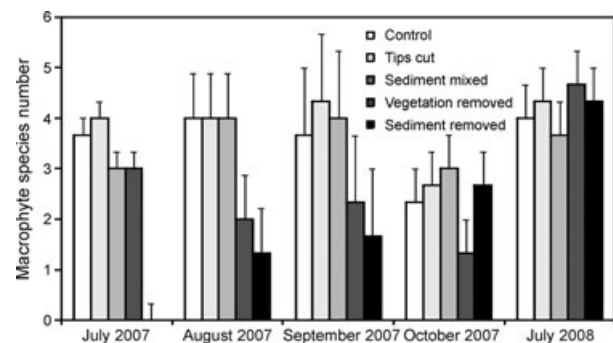
Fig. 4. Seasonal variation in average (±95%CI) species number of macrophytes for the studied disturbance treatments in 2007–2008.

Table 3. Repeated measures ANOVA multivariate test of significance for the effect of disturbance on the species number of macrophytes in 2007–2008.

Effect	SS	Df	MS	F	P
Intercept	730.080	1	730.080	1479.892	<0.001
Disturbance	35.387	4	8.847	17.932	<0.001
Error	4.933	10	0.493		
TIME	27.653	4	6.913	16.862	<0.001
TIME × Disturbance	37.547	16	2.347	5.724	<0.001
Error	16.400	40	0.410		

(repeated measures ANOVA, *post-hoc* Fisher LSD test: control *versus* heavily disturbed plots P < 0.05). Macrophyte species number within the heavily disturbed plots had recovered by July 2008. Mechanical disturbance did not affect the biomasses of other macrophyte species (repeated measures ANOVA, separate disturbance effect and disturbance × time interaction P > 0.05; Fig. 2).

Discussion

We predicted that a moderate mechanical disturbance that leaves the charophyte community almost intact but at the same time significantly impoverishes native invertebrate communities, may increase the biomass of the non-native amphipod because the competitive interactions are relaxed. However, our experiment did not support this prediction, as the biomass of *Gammarus tigrinus* was significantly reduced under all of the different types of mechanical disturbance, whereas the native species were not affected. The result suggests that the dominance of *G. tigrinus* in our system has less to do with strong species interactions, such as competitive displacement, than with habitat-level processes, such as changes in habitat structural characteristics and food supply (e.g. Kotta *et al.* 2000, 2004, 2008b). Moreover, the experiment demonstrated that *G. tigrinus* constituted most of the benthic invertebrate biomass. This would indicate that it is unlikely that *G. tigrinus* is influenced by competition from the native fauna, as the native fauna is simply too sparse to have significant competitive interactions with *G. tigrinus*.

Although there is solid evidence that disturbance enhances the densities of non-native species both in terrestrial and aquatic ecosystems, the results are not fully consistent. The effects of disturbance are known to be scale-specific, with strong positive effects often associated at small spatial scales but not at larger scales (Thompson *et al.* 2001; Gross *et al.* 2005). At small spatial scales the disturbance opens space in the community, which is then quickly used by the opportunistic non-native and native species and consequently animal densities at the community level are increased. On the other hand, disturbance does not enhance the likelihood of a species to become dominant, as high disturbance probably wipes out any species including the opportunistic species. Such patterns can be explained by purely statistical processes using neutral models with no species-specific interactions involved (Herben 2009). However, the neutral model does not predict that the non-native species should prevail in the absence of disturbance.

The Northeastern Baltic Sea is a physically limited environment. Strong natural disturbances due to low salinity, temperature extremes and ice scrape have resulted in impoverished benthic communities, with many native and alien species inhabiting the area at the edge of their tolerance limits (Kotta *et al.* 2008a). In such environments, competitive interactions are not expected to play a large role; rather, habitat characteristics should be responsible for shifts in the benthic communities. In our experiment the strong link between the biomass of *Chara aspera* and *G. tigrinus* suggests a strong affinity of non-native gammarids to pristine charophyte habitats. Nevertheless, none of the disturbance treatments had any significant effect on the biomass of native species and thus the expectation that the majority of native herbivores are sensitive to environmental stresses does not hold true. We may also argue that the resistance of native species is an inevitable consequence of natural selection, as only the fittest can cope with the permanent environmental stresses presented by the Baltic Sea environment (Herkül *et al.* 2006).

Macrophytes provide important habitat and food resources for a variety of associated mobile animals. This is consistent with our study (and our second hypothesis) in which dense charophyte communities supported high biomasses of the non-native gammarid species. It is generally believed that mobile herbivores respond more strongly to the amount of available resources than the diversity of plants providing it (Parker *et al.* 2001; Christie *et al.* 2009), suggesting that mesoherbivores often have a broad diet and selectivity is rare (Cruz-Rivera & Hay 2001). The results of our experiment also hint that the gammarid amphipods are generalist foragers, as high densities of *G. tigrinus* did not match with macrophyte species number but rather to the biomass of *C. aspera*. It is plausible that besides food value, dense charophyte communities support high structural complexity and thus provide better value as a refuge from predators (Orav-Kotta & Kotta 2004; Kinzler & Mayer 2006; Christie *et al.* 2009).

Most aquatic macrophytes are seasonal, providing habitats of limited duration (Pihl *et al.* 1996; Kiirikki & Lehvo 1997), whereas species of gammarid amphipods have a lifespan of approximately up to 3 years (e.g. Wilhelm & Schindler 1996). Gammarids may therefore show a preference for macrophytes with higher longevity, such as charophytes, over ephemeral algae. However, due to the poor nutritional value of charophytes, grazers often just live within their bushes and feed on the epiphytes attached to the host plant (Coops & Doef 1996; Kotta *et al.* 2004). The low levels of herbivory are also related to the unpalatability or resistance of the algae. According to Hunter (1976), fresh *Chara* are heavily calcified, which may greatly reduce their appeal to herbivores. In the course of decomposition the cell walls of the algae become less resistant to herbivory and the concentration of nutrients increases in the decomposing material as a result of increased microbial activity (Hunter 1976; Buchsbaum

et al. 1991). As a consequence, the charophytes may occasionally become more attractive to benthic invertebrates, especially in the late autumn months, when gammarids are known to consume a significant number of charophytes (Van den Berg 1999; Noordhuis *et al.* 2002; Kotta *et al.* 2004). By October, the density of filamentous algae had notably declined in the study area. The abundant population of *G. tigrinus* that had relied on these algae was forced to switch to an alternative diet. Compared with other macroalgae in the area, the decomposing charophytes seemed to be the most rewarding food for gammarid amphipods. Thus, high aggregation of *G. tigrinus* within the *Chara* stands in October may be explained by high mobility of *G. tigrinus*, ensuring high dispersal out of macrophyte beds and quick utilization of rewarding habitats (e.g. Jørgensen & Christie 2003; Salovius *et al.* 2005). Mechanical disturbance resulted in lower gammarid biomasses but such stress affected only the qualities of adult habitats, whereas effects on juveniles were not found. The statistical differences in the gammarid biomasses between disturbed and undisturbed plots in October may be explained partly as a statistical sampling effect, as it corresponds with the peak biomass of *G. tigrinus*.

However, *G. tigrinus* retained elevated biomasses within undisturbed charophyte communities in July 2008, a year after the disturbance. Although the communities of *C. aspera* have recovered from moderate disturbances (i.e., levels: tips cut, sediment mixed), *G. tigrinus* had a systematically lower biomass in disturbed communities than in control treatment and the biomasses did not vary among disturbance levels. Thus, a high biomass of charophytes does not necessarily ensure that it will be colonized by a high number of *G. tigrinus*. Instead, the availability of resources and/or structural properties of charophytes may better explain the recovery of gammarid amphipods in the disturbed charophyte habitat. Repeated measures ANOVA analysis showed that mechanical disturbance had no effect on the algal resource availability, as assessed by a ratio of the biomass of macroalgae to the biomass of *G. tigrinus* (repeated measures ANOVA, separate disturbance effect and disturbance × time interaction P > 0.05). In the light of this evidence, the lack of recovery of gammarid amphipods a year after the disturbance hints that the three-dimensional structure of charophyte canopy rather than absolute or relative algal quantity determines the patterns of *G. tigrinus* in the studied habitat.

Conclusions

To conclude, mechanical disturbance affected the biomass of *Gammarus tigrinus* but with a significant time lag, possibly reflecting the seasonal dynamics of the gammarid amphipods and macroalgae. In general, the disturbed communities had significantly lower gammarid biomasses than control communities throughout the experiment. A possible cause of reduced biomass of the gammarid amphipods relates to the decreased biomass of *C. aspera* and its structural changes but not to the diversity of macrophyte communities and the biomasses of macrophyte and benthic invertebrate species. Thus, the dominance of *G. tigrinus* in our system has less to do with strong species interactions, such as competitive displacement, than with habitat-level processes, such as changes in habitat structural characteristics and food supply.

Acknowledgements

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Comparisons of individual and community photosynthetic production indicate light limitation in the shallow water macroalgal communities of the Northern Baltic Sea

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Abstract

Primary production is of special importance in ecology, since it fuels other trophic levels with energy and biomass. In aquatic ecosystems, almost all primary production is performed by algae. The primary production of aquatic macrophytes has been mainly quantified using detached phyto-elements (fronds and algal thalli) or whole plants, whereas the community level is usually ignored. In a field experiment we evaluated macroalgal photosynthesis at individual and community levels. We used natural communities dominated by the ephemeral green alga *Cladophora glomerata* (L.) Kützing and the perennial brown alga *Fucus vesiculosus* L. *Fucus vesiculosus* had temporally stable and *C. glomerata* highly variable photosynthetic production. On a biomass basis, net and gross photosynthetic production was always higher at the individual level than at the community level. The ratio of individual photosynthetic production to community photosynthetic production increased with light intensities. The observed relationships give clear evidence that in shallow water, where light is supposedly plentiful, macroalgae are nevertheless strongly light-limited. Although photosynthetic production estimates at individual levels are traditionally extrapolated to the community level, such estimates largely overestimate reality and give a false impression of the potential photosynthetic production of macroalgae.

Introduction

Primary production in the sea is the major process that supplies energy and matter to marine organisms. In coastal ecosystems, macroalgae constitute the most productive habitats and virtually all primary production is performed by them (Field *et al.* 1998; Mann 2000). Primary production is limited by abiotic and biotic factors (Field *et al.* 1998; Hauxwell *et al.* 2003), with abiotic constraints being the most important in natural ecosystems (Hill *et al.* 1995).

The availability of light is known to play a crucial role in regulating primary production of macroalgae (e.g. Pregnall & Rudy 1985; Field *et al.* 1998; Hauxwell *et al.* 1998; Duffy & Hay 2000; Binzer *et al.* 2006). The hyperbolic tangent function has been traditionally used to describe the relationship between light and photosynthetic production

(Jassby & Platt 1976) as it can easily represent the transition from one state (light limitation) to another (light saturation). Light limitation may arise from the amount of irradiance arriving at the sea surface but more likely from self-shading. Thus, in addition to the light environment above the algal canopy, macroalgal density and structure determine overall community photosynthetic production. Therefore, it is not only the total light availability but also the distribution of light photons between different parts of the algal thalli that largely determine the community photosynthetic production (Binzer & Sand-Jensen 2002). Often the light is unevenly distributed and most of the photons are absorbed in the upper layers of photosynthetic tissue. Even though algal photosynthesis will be saturated locally with increasing irradiance, community photosynthesis will not become saturated because the lower photosynthetic tissue in the community will have an unused photosynthetic

potential (Binzer & Middelboe 2005). In general, light availability is inversely related to algal densities, is highest in tips and decreases with distance from the tip along the thallus. Shaded algal parts are subject to less seasonal variation in irradiance (Stengel & Dring 1998; Binzer & Sand-Jensen 2002) and, due to photoacclimation, their photosynthetic performance may differ from of that of the upper thallus (Middelboe & Binzer 2004; Binzer & Middelboe 2005; Binzer et al. 2006).

The shape of irradiance–production curves may vary among macroalgae. Measurements of individual photosynthetic production have shown that thin, sheet-like and filamentous algae are capable of fast growth, which is coupled to high photosynthetic rates per unit biomass (Johansson & Snoeijs 2002). On the other hand, perennial bush-like macroalgae grow slowly and have low photosynthetic rates per unit biomass (King & Schramm 1976; Wallentinus 1984; Middelboe & Binzer 2004; Sand-Jensen et al. 2007). Moreover, the relationship and variability between irradiance and photosynthetic production may change with seasonal variations in thallus photosynthesis (King & Schramm 1976; Wallentinus 1978; Stengel & Dring 1998; Middelboe et al. 2006).

However, measurements of community photosynthetic production have shown a much more stable photosynthetic production than predicted from their individual photosynthetic production (Middelboe et al. 2006). Thus, macroalgae seem not to realize their individual potential at community level due to impoverishment in the light environment within the algal canopy. This allows us to conclude that a three-dimensional structure of communities and high species-richness can make community photosynthetic production more stable and predictable because different parts of the algal canopy and different species complement each other in utilizing all of the available light (Middelboe & Binzer 2004; Middelboe et al. 2006).

In a factorial field experiment, we evaluated the photosynthetic production of different species at individual and community levels in the Northern Baltic Sea in May, June and August 2008. The experiment involved shallow-water communities dominated either by the ephemeral green alga *Cladophora glomerata* (L.) Kützing or the perennial brown alga *Fucus vesiculosus* L. Our hypotheses were as follows: (i) per unit biomass photosynthetic production is higher at an individual level than at community level; (ii) elevated algal biomasses increase light limitation, as shown by larger differences in individual and community level photosynthetic production; (iii) due to higher structural and species diversity, the *F. vesiculosus* community has a more stable community photosynthetic production than the *C. glomerata* community. To date, most of the studies on macroalgal photosynthesis have been conducted under fully marine conditions (e.g. Middelboe & Binzer 2004; Binzer & Middelboe 2005; Middelboe et al. 2006). The current study reports the photosynthetic production of macroalgae at a reduced salinity.

Study Area

The study was conducted in the shallow semi-enclosed Kõiguste Bay, Gulf of Riga, Northern Baltic Sea (58°22.10′N, 22°58.69′E). In general, the bottom relief of the area is quite flat, gently sloping towards deeper areas. The prevailing substrate types of the bay are sandy clay mixed with pebbles, gravel or boulders, at between 1 and 4 m. The Gulf of Riga receives fresh water from a huge drainage area and therefore Kõiguste Bay has a reduced and stable salinity of 4.0–6.5 psu and elevated nutrient levels (Kotta et al. 2008). The benthic vegetation is well developed (Kersen et al. 2011) and an extensive proliferation of ephemeral macroalgae has been reported from the area in the recent years (Lauringson & Kotta 2006).

Material and Methods

In situ experiments were performed adjacent to Kõiguste Marine Biological Laboratory in May, June and August 2008. During the course of the experiment the macroalgal community had 50–70% algal coverage and consisted mainly of *Cladophora glomerata*, *Pilayella littoralis* and *Fucus vesiculosus*.

Stones with growing macroalgae were collected from a shallow (1 m) area and were placed in experimental mesocosms, with replicates in separate chambers. Experimental treatments consisted of communities dominated either by *F. vesiculosus* or *C. glomerata*. Both macroalgae had small amounts of epiphytes, mainly *P. littoralis*, *Ceramium tenuicorne* and *Ulva intestinalis*.

Algal photosynthetic production was estimated each month at individual and community levels over 4 days. On each day, three replicates were made for each combination of treatments. The individual-level photosynthetic production was measured in transparent and dark incubation bottles. About 0.5 g (dry weight) of algal material was incubated in 600-ml transparent and dark glass bottles, filled with the unfiltered seawater and incubated horizontally on special trays at 0.5 m depth. Bottles without algae served as the controls. The community-level production was measured in transparent and dark chambers holding 29 l of water. The surface area of the chamber was 0.08 m². Macroalgal communities were placed in the chamber at coverage relevant to field conditions. The chambers were deployed on the sea floor at 1 m depth, i.e. close to the site of individual-level photosynthetic production estimates.

Oxygen concentration in the incubation bottles and chambers was measured using a calibrated Optode type oxygen sensor (Aanderaa Instruments) connected to a data logger (Compact-Optode data recorder by Alec Electronics). Changes in dissolved oxygen per g dry weight of algal tissue were used as a proxy of photosynthetic production and respiration. Rates of change in the light (includes both primary production and community respiration) indicated community net photosynthesis and rates of change in the dark represented community respiration. Community gross photosynthesis was a sum of community net photosynthesis and respiration. Production experiments lasted 40 min and dark respiration experiments 20 min, respectively. During deployment, the irradiance above the canopy was measured every second using a calibrated spherical quantum sensor connected to a data logger (ultra-miniature logger for light intensity by Alec Electronics).

After the experiment, all algae were stored in a deep-freezer at −20°C and subsequent sorting, counting and determination of algal species were performed in the laboratory using a stereomicroscope. All species were determined to species level. The dry weight (dw) of species was obtained after drying the individuals at 60°C for 2 weeks.

Repeated measures ANOVA was used to compare total macroalgal biomass, community species number and light intensities among macrophyte communities (levels: *Cladophora glomerata*, *Fucus vesiculosus* community) and months (levels: May, June, August). Repeated measures ANCOVA was used to compare the effect of organizational level (levels: individual, community) and macroalgal community (levels: *Cladophora glomerata*, *Fucus vesiculosus* community) on the photosynthetic production of macroalgal communities among months (levels: May, June, August). In the ANCOVA models, light and temperature were included into analysis as time-varying covariates. *Post-hoc* Bonferroni tests were used to analyse which treatment levels were statistically different from each other. Multiple regression analysis was used to learn more about the relationship between light, total macroalgal biomass and the ratio of individual net photosynthetic production to community net photosynthetic production.

Results

On a sample basis the *Fucus vesiculosus* community had on average about fivefold higher biomasses (Fig. 1, repeated measures ANOVA, *post-hoc* Bonferroni test $P < 0.001$) and higher number of macrophyte species compared to the *Cladophora glomerata* communities (Fig. 2, repeated measures ANOVA, *post-hoc* Bonferroni test $P < 0.001$). However, due to more equal spread of biomasses among species, the Shannon diversity values were
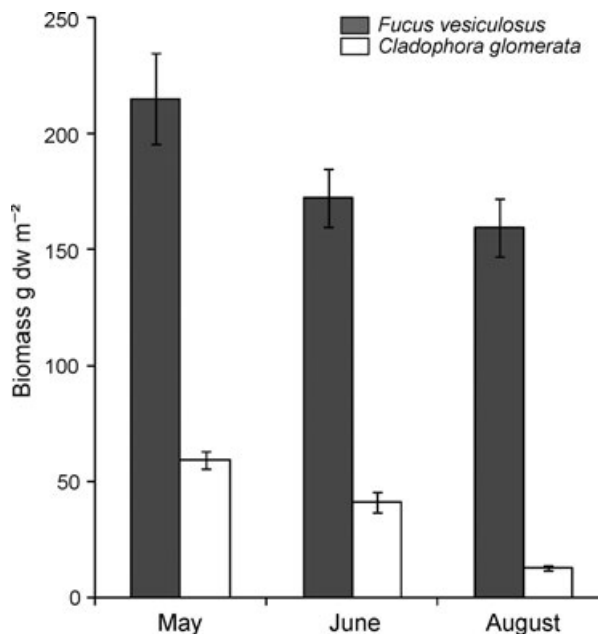
Fig. 1. Average biomasses of the *Fucus vesiculosus*- (F) and *Cladophora glomerata*- (C) dominated communities (means ± SE) (g·dw·m⁻²) in different months calculated on a sample basis.
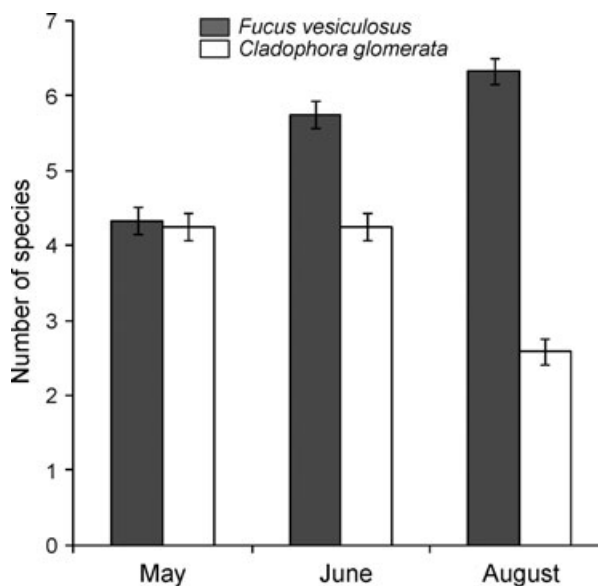
Fig. 2. Average number of species in the *Fucus vesiculosus* and *Cladophora glomerata* dominated communities (means ± SE) in different months calculated on a sample basis.

higher in the *C. glomerata* community than in the *F. vesiculosus* community (Fig. 3, repeated measures ANOVA, *post-hoc* Bonferroni test $P < 0.01$). When all samples were pooled together, the number of species within the studied macroalgal communities was almost equal (Table 1).
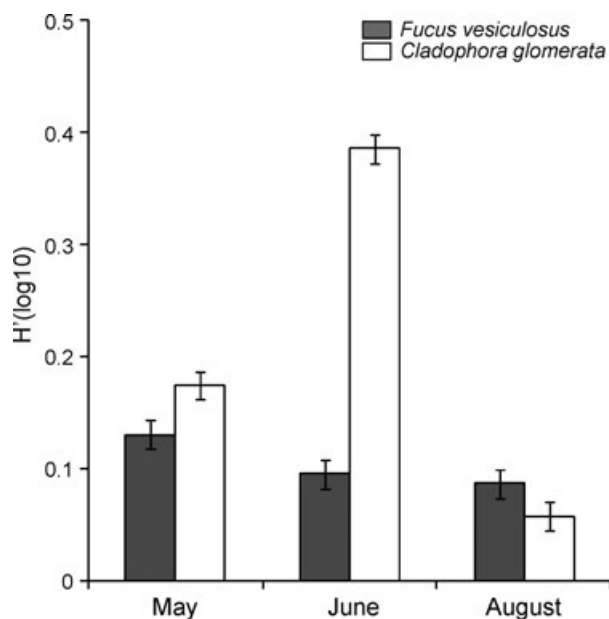
Fig. 3. Shannon diversity index on a log-base-10 scale in the *Fucus vesiculosus* and *Cladophora glomerata* dominated communities in different months calculated on a sample basis.

In terms of dominant species the *F. vesiculosus* and *C. glomerata* communities did not differ largely among months. Thus, seasonal differences were mostly due to the sporadic occurrence or absence of rare species (Table 1). On a sample basis, both studied communities had higher biomasses in May than in August (*post-hoc* Bonferroni test P < 0.001) (Fig. 1). Similarly, the *C. glomerata* community had a higher number of macrophyte species in May and June than in August, whereas the *F. vesiculosus* community had a lower number of macrophyte species in May than in June and August (Fig. 2; repeated measures ANOVA, *post-hoc* Bonferroni tests P < 0.001). The Shannon diversity of the *C. glomerata* community was highest in June, intermediate in May and lowest in August. The Shannon diversity value in the *F. vesiculosus* community was opposed to the seasonal patterns of species number (Fig. 3; repeated measures ANOVA, *post-hoc* Bonferroni tests P < 0.001). In May the studied communities had a similar number of macroalgal species, whereas in June and August the species number of the *F. vesiculosus* community exceeded the values in the *C. glomerata* community (Fig. 2). In May and August there was no difference in the Shannon diversity of the studied communities. In June, however, the *C. glomerata* community had higher Shannon diversity values compared with the *F. vesiculosus* community (Fig. 3; repeated measures ANOVA, *post-hoc* Bonferroni tests P < 0.001).

During the measurements of photosynthetic production, light levels within benthic chambers were between 273 and 1929 μmol m^{-2} s^{-1}. Light levels did not differ statistically among macrophyte communities and months (Fig. 4; Table 2; repeated measures ANOVA, *post-hoc* Bonferroni test P > 0.05).

The net photosynthetic production of macrophytes (NPP) was significantly affected by almost all studied factors and interactions. The NPP was mostly higher at the individual level than at the community level (Table 2; repeated measures ANCOVA, *post-hoc* Bonferroni test P < 0.05), estimated at 0.017–0.048 and 0.004–0.037 mg O$_2$ min^{-1} g^{-1} dw for *F. vesiculosus,* and at 0.011–0.400 and 0.005–0.309 mg O$_2$ min^{-1} g^{-1} dw for *C. glomerata*, respectively (Fig. 5).

There were clear differences in the NPP between macroalgal species level, with *C. glomerata* having significantly higher NPP compared with *F. vesiculosus* (*post-hoc* Bonferroni test: *C. glomerata* vs. *F. vesiculosus* P < 0.001). At the individual level *C. glomerata* had higher photosynthetic production than *F. vesiculosus* in all the months studied (*post-hoc* Bonferroni tests: P < 0.001), whereas at the community level the difference was statistically important only in August (*post-hoc* Bonferroni tests: P < 0.05). The individual NPP of *C. glomerata* was in the range 0.025–0.059, 0.011–0.098 and 0.082–0.400 mg O$_2$ min^{-1} g^{-1} dw in May, June and August, respectively. The individual NPP values for *F. vesiculosus* were 0.015–0.034 mg O$_2$ min^{-1} g^{-1} dw in May, 0.021–0.053 mg O$_2$ min^{-1} g^{-1} dw in June and 0.022–0.048 mg O$_2$ min^{-1} g^{-1} dw in August (Fig. 5). The community NPP of *C. glomerata* was in the range 0–0.023, 0–0.095 and 0.006–0.309 mg O$_2$ min^{-1} g^{-1} dw in May, June and August, respectively. The community NPP of *F. vesiculosus* was 0.001–0.037 O$_2$ min^{-1} g^{-1} dw in May, 0.001–0.028 O$_2$ min^{-1} g^{-1} dw in June and 0.0002–0.012 O$_2$ min^{-1} g^{-1} dw in August.

Fucus vesiculosus had quite stable NPP values, both at community and individual levels (*post-hoc* Bonferroni tests: difference among months P > 0.05). In contrast, *C. glomerata* had significantly higher production values at community and individual levels in August compared to May and June (*post-hoc* Bonferroni tests: August *vs.* May and June P < 0.001).

Gross photosynthetic production (GPP) showed similar responses to the studied factors, although the effects were more pronounced compared with NPP (Fig. 6, Table 3). The GPP was always higher at the individual than at the community level, estimated at 0.025–0.054 and 0.004–0.038 mg O$_2$ min^{-1} g^{-1} dw for *F. vesiculosus* and at 0.035–0.416 and 0.006–0.333 mg O$_2$ min^{-1} g^{-1} dw for *C. glomerata*, respectively.

The ratio of individual NPP to community NPP increased linearly with light intensities, whereas total macroalgal biomass had no separate and interactive effects on this ratio (Table 4). Nevertheless, the ratio varied more below 100 g m^{-2} of macroalgal biomass and

Table 1. Average biomasses of macrophyte species calculated from monthly pooled samples (means ± SE) (g dw m^{-2}) in the *Fucus vesiculosus*- and *Cladophora glomerata*-dominated communities.

Species	Taxonomic group	*Fucus vesiculosus* community			*Cladophora glomerata* community		
		May	June	August	May	June	August
Total biomass		215.333 ± 19.834	172.499 ± 12.439	159.726 ± 12.421	59.493 ± 3.518	41.311 ± 4.210	12.782 ± 1.090
Fucus vesiculosus	P	202.305 ± 20.021	163.737 ± 11.485	154.272 ± 12.676	5.902 ± 2.234	0.005 ± 0.003	0
Cladophora glomerata	C	2.953 ± 0.277	3.328 ± 0.726	0.997 ± 0.123	30.396 ± 2.602	17.712 ± 1.240	12.475 ± 1.110
Pilayella littoralis	P	7.022 ± 1.047	3.738 ± 0.740	3.117 ± 0.688	5.141 ± 0.509	10.299 ± 1.203	0.148 ± 0.081
Ceramium tenuicorne	R	1.813 ± 0.267	0.699 ± 0.080	0.238 ± 0.035	0.341 ± 0.062	9.994 ± 2.342	0.034 ± 0.012
Sphacelaria arctica	P	0.183 ± 0.102	0.033 ± 0.011	0	0	0.273 ± 0.115	0
Ruppia maritima	A	0	0.022 ± 0.008	0	0.042 ± 0.021	0.024 ± 0.007	0
Ulva intestinalis	C	0	0	0.015 ± 0.003	0.063 ± 0.015	0	0.089 ± 0.020
Dictyosiphon foeniculaceus	P	0	0.01 ± 0.005	0.06 ± 0.021	0	0.001 ± 0.000	0
Elachista fucicola	P	0.277 ± 0.135	0.576 ± 0.143	1.005 ± 0.163	0	0	0
Polysiphonia fucoides	R	0.005 ± 0.003	0	0.001 ± 0.001	0.004 ± 0.002	0	0
Polysiphonia fibrillosa	R	0	0	0.006 ± 0.003	0	0	0
Potamogeton pectinatus	A	0.016 ± 0.009	0.068 ± 0.024	0	0.021 ± 0.007	0.136 ± 0.032	0.036 ± 0.012
Furcellaria lumbricalis	R	0	0.003 ± 0.002	0.014 ± 0.006	0.003 ± 0.002	0.006 ± 0.003	0
Myriophyllum spicatum	A	0	0	0	0.042 ± 0.021	0	0
Zannichellia palustris	A	0	0	0	0.001 ± 0.000	0.003 ± 0.002	0
Chorda filum	P	0	0.001 ± 0.000	0	0.001 ± 0.000	0	0

The code of plant taxonomic groups is as follows: R = Rhodophyta; P = Phaeophyceae; C = Chlorophyta; A = Angiospermae.
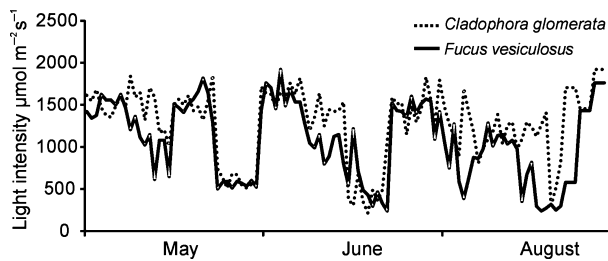
Fig. 4. The variation of light intensity (μmol m^{-2} s^{-1}) during photosynthetic production measurements of *Cladophora glomerata* and *Fucus vesiculosus* in May, June and August.

Table 2. RM factorial ANOVA analysis on the separate and combined effects of the studied factors and interactions on net photosynthesis.

Source	Numerator df	Denominator df	F	Significance
Intercept	1	86.682	13.6	<0.001
1: Community	1	38.381	55.8	<0.001
2: Organization	1	59.754	34	<0.001
3: Month	2	114.266	17.6	<0.001
1 × 2	1	38.686	12.7	0.001
1 × 3	2	81.952	24.3	<0.001
2 × 3	2	98.784	6.37	0.003
1 × 2 × 3	2	83.129	5	0.009
4: Light	1	110.03	3.75	0.055
5: Temperature	1	86.31	8.28	0.005
4 × 5	1	95.245	3.69	0.058

Factors and levels were as follows: months (levels: May, June, August), organizational level (levels: individual, community), community (levels: *Fucus vesiculosus*, *Cladophora glomerata*). Covariates: (light, temperature).
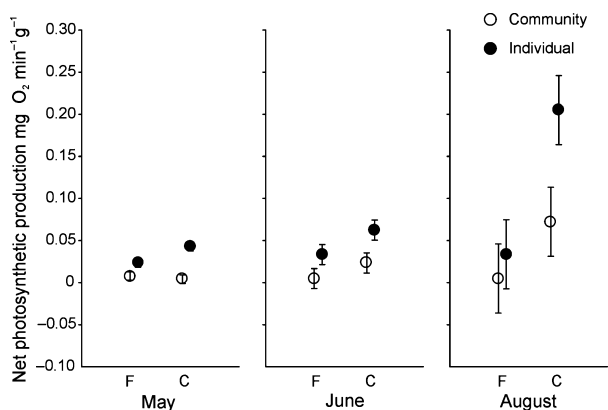
Fig. 5. The net photosynthetic production of macroalgae at individual and community levels in different months (means ± SE). F = *Fucus* community; C = *Cladophora* community.

levelled off at higher biomasses (Fig. 7). The GPP showed similar responses to increased light intensities as compared with the NPP.
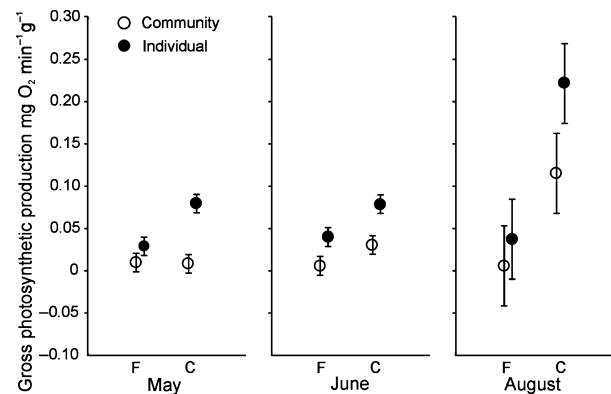
Fig. 6. The gross photosynthetic production of macroalgae at individual and community levels in different months (means ± SE).

Table 3. RM factorial ANOVA analysis on the separate and combined effects of the studied factors and interactions on gross photosynthesis.

Source	Numerator df	Denominator df	F	Significance
Intercept	1	71.401	7.71	0.007
1: Community	1	39.278	95.28	<0.001
2: Organization	1	61.801	35.35	<0.001
3: Month	2	114.522	12.16	< 0.001
1 × 2	1	39.717	12.71	0.001
1 × 3	2	83.390	19.78	<0.001
2 × 3	2	99.593	1.63	0.202
1 × 2 × 3	2	84.391	1.15	0.322
4: Light	1	100.857	2.31	0.132
5: Temperature	1	71.043	3.37	0.071
4 × 5	1	82.978	2.33	0.131

Factors and levels were as follows: months (levels: May, June, August), organizational level (levels: individual, community), community (levels: *Fucus vesiculosus*, *Cladophora glomerata*). Covariates: (light, temperature).

Table 4. Summary table of the linear regression analysis on the separate and combined effects of macroalgal biomass and light on the ratio of individual photosynthetic production to community photosynthetic production ($F_{3,36} = 3.66$, $P < 0.021$, $R^2 = 0.23$).

	Regression coefficients	Standard error of coefficients	t	P
Intercept	−1.029	1.745	−0.590	0.559
1: Macroalgal biomass	0.029	0.015	1.895	0.066
2: Light	0.004	0.001	2.900	0.006
1 × 2	<0.001	<0.001	−1.574	0.124

Discussion

We predicted that photosynthetic production is higher at the individual than the community level. The results of
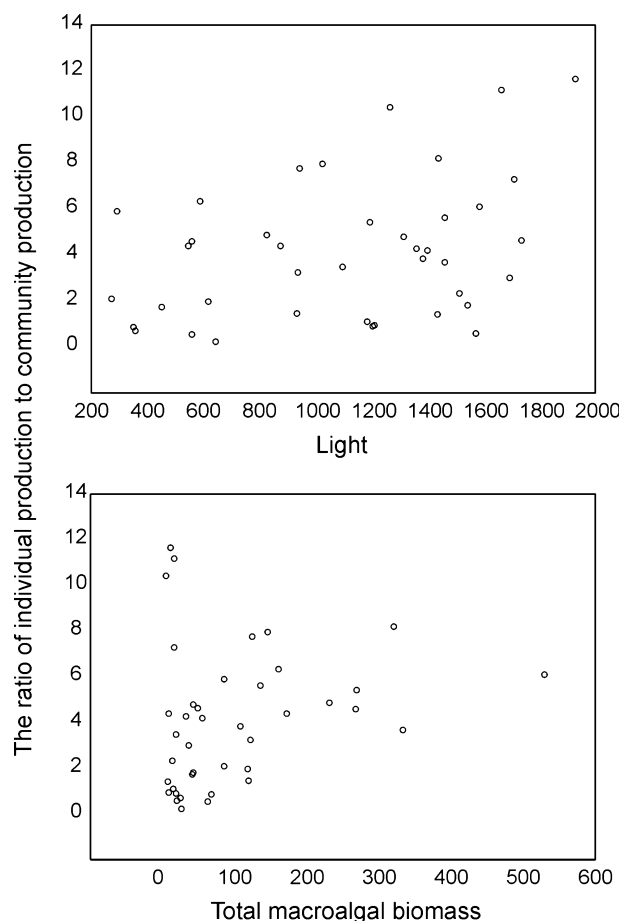
Fig. 7. Relationships between light, total macroalgal biomass and the ratio of individual photosynthetic production to community photosynthetic production.

our experiment agreed with this hypothesis and showed significantly larger individual level photosynthetic production compared with community level photosynthetic production. Likewise, our results confirm that photosynthetic rates were much more variable for individual thalli than communities of the same algae (Binzer & Middelboe 2005; Middelboe *et al.* 2006; Sand-Jensen *et al.* 2007).

At low light intensities a large difference between individual and community photosynthetic production is not expected, as light is not sufficient to induce a positive net photosynthetic production. With increasing light intensities the difference in algal photosynthetic production between community and individual levels is expected to increase due to the greater effect of shading at the community level. The observed relationship was linear and the values of community photosynthetic production did not level off even at high light intensities around 2000 μmol m^{-2} s^{-1}. This suggests that light saturation

and light inhibition were never observed and the observed macroalgal communities were always light-limited.

We also predicted that elevated algal biomasses increase light limitation as shown by larger differences in individual and community level photosynthetic production. The data did not fully agree with the hypothesis. Instead, the light limitation (i.e. assessed by a ratio of individual to community level photosynthetic production) seemed to be variable at low macroalgal biomasses and displayed uniformly high values at biomasses above 100 g m^{-2}. This suggests that dense macroalgal communities are characterized by near-constant light limitation of photosynthesis regardless of light intensities. This lack of a continuous relationship with macroalgal biomass and light limitation may arise from the morphology of macroalgae. Macroalgae are generally able to become saturated at lower irradiances than terrestrial plants because their non-rigid structure can ensure an even distribution of light among the photosynthetic tissue (Sand-Jensen & Krause-Jensen 1997; Beyschlag & Ryel 1998; Binzer & Sand-Jensen 2002). As the algae are in continuous motion, small changes in biomass might not affect light limitation in the canopy (Hurd 2000). Besides, the different parts of macroalgal thalli have variable capabilities to utilize irradiance depending on their absorption abilities, potential to carry out photosynthesis and capacity to distribute irradiance (Binzer & Middelboe 2005).

Finally, we predicted that owing to higher structural and species diversity, the *Fucus vesiculosus* community would have a more stable community photosynthetic production than the *Cladophora glomerata* community. The results of our experiment agreed with this hypothesis. Within a diverse community of *F. vesiculosus*, different species complement each other spatially and temporally and thus such a functional redundancy in the community has a positive and stabilizing effect on production (Middelboe *et al.* 2006; Sand-Jensen *et al.* 2007). In fact variable or high abundance of species can ensure stable and predictable community metabolism, because different species complement one another in utilizing all of the available light (Middelboe *et al.* 2006). Moreover, as compared with the light environment above algae, the natural variability in light intensity within the algal canopy is not large and therefore low variability in community photosynthetic parameters and production capacity is expected (Sand-Jensen *et al.* 2007). In addition, the perennial *F. vesiculosus* plants are relatively resistant to physical disturbances, independent of the direct resource acquisition and therefore vary less in their occurrence and biomass seasonally, as compared with *C. glomerata* (Pedersen & Borum 1996; Kiirikki & Lehvo 1997). On the other hand, the filamentous *C. glomerata* may occasionally bloom and have a high productivity (Littler & Littler 1984) but even

small physical disturbances may severely damage the photosynthetic tissues of the algae and result in a large decrease in the community photosynthetic production.

Reduced salinity is known to decrease the photosynthetic production of macrophytes (Pregnall & Rudy 1985; Koch & Lawrence 1987; Phooprong *et al.* 2007). As our measurements were conducted at a low salinity level (Kotta *et al.* 2008; Lauringson *et al.* 2009), the observed differences in the macroalgal photosynthetic production between individual and community levels are expected to be even larger in fully marine conditions. This is also confirmed when comparing our estimates with the published data on the macroalgal photosynthetic production. Although different studies report in different units, the photosynthetic production values are much lower in our study than those measured under fully marine conditions (Middelboe & Binzer 2004; Binzer & Middelboe 2005; Middelboe *et al.* 2006).

To conclude, the observed relationships give clear evidence that in shallow water, where light should be plentiful, macroalgal communities are nevertheless strongly light-limited. Although the photosynthetic production estimates at individual level are traditionally extrapolated to the community level, such estimates largely overestimate reality and give a false impression of potential photosynthetic production of macroalgae. In addition, this study adds to the knowledge of how the canopy-forming algae provide not only biodiversty (Kotta *et al.* 2000; Råberg & Kautsky 2007; Wikström & Kautsky 2007) but also stable photosynthetic production (i.e. food and habitat resource) through the large range of hydrographic conditions.

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ORIGINAL ARTICLE

New invasive copepod *Oithona davisae* Ferrari and Orsi, 1984: seasonal dynamics in Sevastopol Bay and expansion along the Black Sea coasts

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Abstract

Seasonal and interannual dynamics of abundance of the recent invader *Oithona davisae* (Copepoda: Cyclopoida) were investigated in Sevastopol Bay from October 2005, when the species appeared in the plankton, to December 2009. The study was based on bi-weekly plankton sampling at three stations located within and adjacent to Sevastopol Bay, Crimea, Northern Black Sea. The abundance of *O. davisae* increased steadily in Sevastopol Bay in 2006–2009. The species was more abundant at the centre of the bay than at its mouth. During the investigated period, an absolute maximum of the invader abundance at the centre of Sevastopol Bay was observed in early December 2008 (91,650 ind. m^{-3}) and at the mouth of the bay in late October 2007 (46,200 ind. m^{-3}). The maximum contribution of *O. davisae* to the total copepod abundance was about 99%. Expansion of the invasive copepod along the Black Sea coasts is reported. Changes in structure of the zooplankton community for the last 20 years that preconditioned the invasion of the new copepod species in the Black Sea as well as the changes in the community structure caused by *O. davisae* are discussed.

Introduction

The unique hydrological conditions of the Black Sea predetermined a poor diversity of its zooplankton fauna. Although the Black Sea fauna is mostly of Mediterranean origin, a combination of low winter temperatures, rather low salinity, and an anoxic layer below about 200 m depth represents an insurmountable ecological barrier for the bulk of the Mediterranean zooplankton species against penetration of the Black Sea (Zaika 2000; Isinibilir *et al.* 2011).

From time to time, Mediterranean species of copepods have been recorded in the Black Sea plankton near the Bosporus area (Pavlova 1965; Kovalev *et al.* 1976; Yuksek *et al.* 2002). However, none of these species has established in the Black Sea during the last decades. On the contrary, the recent zooplankton invaders are long-distance aliens (Gubanova *et al.* 2013). The copepod *Acartia tonsa* Dana, 1849, the ctenophores *Mnemiopsis leidyi* A. Agassiz, 1865 and *Beroe ovata*

Bruguiere, 1789 (Pereladov 1983, Belmonte *et al.* 1994; Konsulov and Kamburska 1998, Gubanova 2000) were most probably brought into the Black Sea in ballast waters of ships.

All the invasive species mentioned above have changed the structure of the Black Sea plankton community considerably. *Acartia tonsa* appeared in the plankton of Sevastopol Bay in the early 1970s and replaced the native species *Acartia latisetosa* Krichagin, 1873. Since 1976, this new species has been reported as the dominant copepod species in the coastal areas during summer (Gubanova *et al.* 2001). *Mnemiopsis leidyi* invasion increased the grazing pressure on zooplankton in late 1980s and early 1990s, especially during summer and autumn (Kamburska *et al.* 2003; Lebedeva *et al.* 2003). The invasion of *B. ovata*, preying on *M. leidyi*, reduced the abundance of the latter, the period of their reproduction and thus the overall grazing pressure on plankton during summer and autumn (Finenko *et al.* 2003; Hubareva *et al.* 2004; Gubanova *et al.* 2013).

The mesozooplankton of Sevastopol Bay, like that of other areas of the Black Sea, is represented by a relatively small number of taxonomic groups (Zernov, 1904; Greze *et al.* 1971). As few as seven species of Copepoda were found regularly in zooplankton samples from Sevastopol Bay until 2005: *Acartia clausi* Giesbrecht, 1889; *A. tonsa* Dana, 1849; *Calanus euxinus* Hulsemann, 1991; *Centropages ponticus* Karavaev, 1894; *Oithona similis* Claus, 1866; *Paracalanus parvus* Claus, 1863; and *Pseudocalanus elongatus* Boeck, 1865. With the exception of *A. tonsa,* all of these species are indigenous to the Black Sea.

Some specimens of a cyclopoid copepod new to the Black Sea were first found in Sevastopol Bay in December 2001. The species was identified as *Oithona brevicornis* (Zagorodnyaya, 2002). However, this discovery appears to represent an isolated record.

New specimens of the *Oithona* genus alien to the Black Sea were found in Sevastopol Bay only 4 years later (Gubanova & Altukhov 2007). The species was also identified as *O. brevicornis*. The same species has been routinely observed in samples taken since the mid-2000s (Altukhov & Gubanova 2006; Selifonova 2009). Recently, the species was re-identified as *Oithona davisae* (Temnykh & Nishida 2012). Thus, *O. brevicornis* and *O. davisae* are two different names for the same Black Sea species, but *O. davisae* is accepted as the correct name. From 2006 to 2009, the contribution of this species to the total copepod abundance in Sevastopol Bay increased to almost 99%, with its numbers rising up to about 90,000 ind. m^{-3}.

The aim of the present study was to investigate the invasion patterns, interannual variability, seasonal dynamics and spatial expansion of the alien copepod *O. davisae* in the Black Sea, and the impact they had on the zooplankton community.

Material and Methods

Sevastopol Bay (SW Crimea, Ukraine) is about 7000 m long and 850 m wide, with an average depth of 12 m. Regular studies of plankton communities in the bay waters were performed in 1976, 1980, 1989, 1990, 1995 and 1996. They were resumed in 2002 and are ongoing. Samples were usually collected twice a month (with gaps due to technical or meteorological conditions) at three stations located within and adjacent to Sevastopol Bay (St. 1–3, Fig. 1), Crimea, Northern Black Sea. Samples were collected by vertical hauls through the whole water column (from the bottom to the sea surface) using a Juday plankton net (mouth area 0.1 m^2 and mesh size 150 μm). Samples were taken in the morning and fixed with formaldehyde solution (4% final conc.). Zooplankton counts were made under an MBS-9 stereomicroscope using a Bogorov counting chamber.

From 2006 to 2008, *O. davisae* was found episodically (in about 10% of the collected samples) at St. 1. The data obtained at this station in 2009 were not processed completely. For these reasons, the results obtained at St. 1 have not been included in the diagrams in this paper.

To study *O. davisae* expansion along the Black Sea coast in 2008–2010, samples were collected at St. 5–11 (Fig. 1) by vertical hauls through the whole water column (from the bottom to the sea surface) using a closing Epstein plankton net (mouth diameter of 10 cm and mesh size of 55 μm). The unpublished data from St. 4 were kindly provided by Kremena Stefanova (IO BAS, Bulgaria) (Fig. 1). The data on the appearance of *O. davisae* at St. 12 and 13 are cited from Selifonova (2009).

Copepods (I–VI copepodites) were identified using the taxonomic keys of Shuvalov (1980), Nishida (1985) and
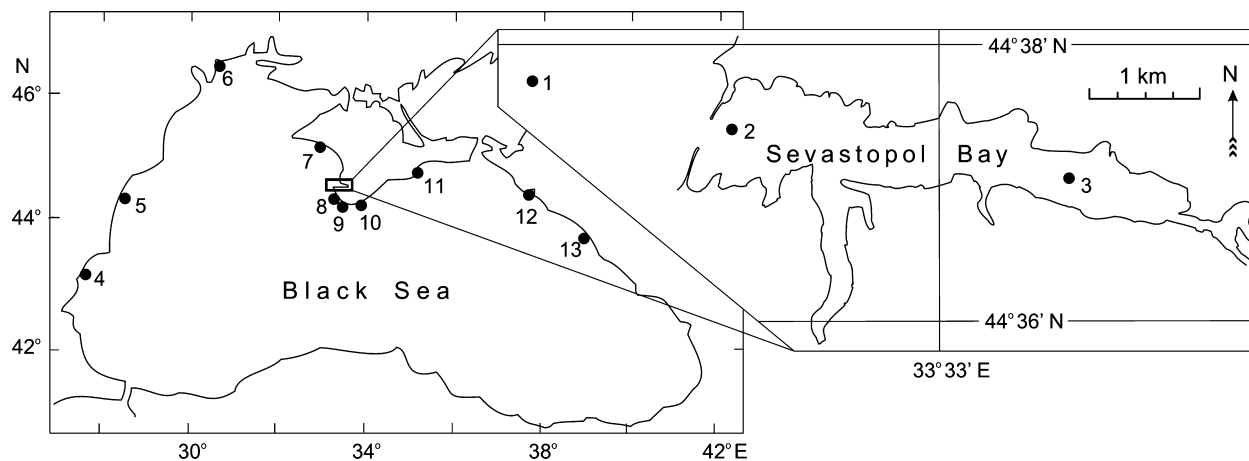
Fig. 1. Sampling stations in Sevastopol Bay and the Black Sea. Our data were obtained from St. 1–3 and 5–11; the data on the St. 4, 12 and 13 were obtained from other sources (see explanations in the text).

Sazhina (1969). Abundance was calculated per cubic meter assuming that the net filtered with 100% efficiency over the depth range. Appendages were dissected under a stereomicroscope and mounted on microscope slides for detailed examination.

As *O. davisae* has a relatively small body size, its early developmental stages (I–III copepodites) are underestimated when using a net equipped with 150-μm mesh size (Kovalev *et al.* 1977). For this reason, only adults and the IV–V copepodites were counted in this study and the I–III stages ignored.

Results

Seasonal dynamics of *Oithona davisae* in Sevastopol Bay in 2005–2009

After the first appearance of *O. davisae* in the bay from October 2005 to March 2006, the species was not found in the samples in April–May 2006 at any of the stations, demonstrating unstable dynamics. For the next years, its abundance steadily increased and never dropped down to zero. In general, its seasonal dynamics at both the bay stations were similar, although every year had its own specific pattern (Fig. 2).

At the mouth of Sevastopol Bay (St. 2), *O. davisae* attained a density of 264 ind. m^{-3} in November 2005. Density then declined until late August 2006 (no individuals were found in April–May 2006), after which intensive development of the population started again. The maximum abundance (21,050 ind. m^{-3}) was reached in late October 2006. After this, the abundance of the population decreased sharply. In 2007, the intensive development began a bit later, in September. The maximal abundance (46,200 ind. m^{-3}) was observed in late October 2007. A similar trend was observed in 2008 but the peak (23,500 ind. m^{-3}) was recorded later, in early November 2008, and the following decrease in the species abundance was not as abrupt as those observed in 2006–2007.

In January–May 2009, the number of individuals was relatively low. In June 2009, the number exceeded 10,000 ind. m^{-3}, whereas it did not reach 1000 ind. m^{-3} during the summer months in 2006–2008. Some decrease in abundance was observed in July 2009. The peak was recorded in late August (35,550 ind. m^{-3}). Until the end of 2009, the numbers of *O. davisae* did not drop below 1000 ind. m^{-3}.

In general, the population of *O. davisae* was more abundant in the centre of the bay (St. 3) than at its mouth (paired *t*-test, P < 0.001, *n* = 92). In 2005, the maximal abundance (2311 ind. m^{-3}) was recorded in late December. Between January and August 2006, the invader had lower abundances, with a small peak (620 ind. m^{-3}) in March. No individuals were found in late March or in the middle

of May 2006. A few specimens were found in April, June and July 2006. The annual maximum observed in late October was almost 20 times greater (42,667 ind. m^{-3}) than in 2005. Three distinct peaks in January, March and June 2007 against a background of rather small values were followed by a rapid development of the population through September, with the maxima occurring in October (51,667 and 43,555 ind. m^{-3}). In 2008, the character of the species dynamics from January to October was almost the same as in 2006–2007, but no considerable fall of abundance was observed in November. The annual peak of *O. davisae* abundance was reached in early December, amounting to 91,650 ind. m^{-3}. Such an extremely high value had never been reported for planktonic copepods in Sevastopol Bay. These values seem to be the highest copepod abundances ever recorded in the Black Sea.

In January and early February 2009, the *O. davisae* population exceeded 10,000 ind. m^{-3}. The annual minimum (1856 ind. m^{-3}) was recorded in late February. Intensive development of the population started substantially earlier in 2009 than in 2006–2008. The maximal annual abundance was observed in mid-June (71,167 ind. m^{-3}) and *O. davisae* remained very numerous until the end of year. A decrease in the abundance (down to 4800 ind. m^{-3}) was observed in mid-November 2009.

From 2006 to 2009, outside Sevastopol Bay, *O. davisae* was observed at St. 1 episodically at rather low abundances. However, a slight increase in the species abundance and frequency of appearances was noticed. Analysis of the samples collected in autumn 2010 showed that at St. 1, the abundance of *O. davisae* increased sufficiently and exceeded 7000 ind. m^{-3} in October.

In the period from 2006 to 2009, the average annual abundance of *O. davisae* increased from 1749 to 5543 ind. m^{-3} at the mouth of Sevastopol Bay (St. 2) and from 4347 to 22,284 ind. m^{-3} at the centre of the bay (St. 3). The average annual abundances of the other copepod species were less variable. At St. 2, the minimum (2138 ind. m^{-3}) and the maximum (3652 ind. m^{-3}) numbers were recorded in 2006 and 2008, respectively. At St. 3, the lowest (1968 ind. m^{-3}) and the highest (3377 ind. m^{-3}) values were obtained in 2008 and 2007, respectively. During the periods of maximum densities of *O. davisae*, its contribution to the total copepod abundance rose to 96.42% and 98.72% at St. 2 and 3, respectively. The contribution of the invader to the total average copepod abundance was considerably higher at the centre of the bay (St. 3) than at its mouth (St. 2) (Fig. 3).

Expansion of *Oithona davisae* along the Black Sea coasts

In autumn 2005, establishment of *O. davisae* in the Black Sea was reported simultaneously in Sevastopol Bay (our
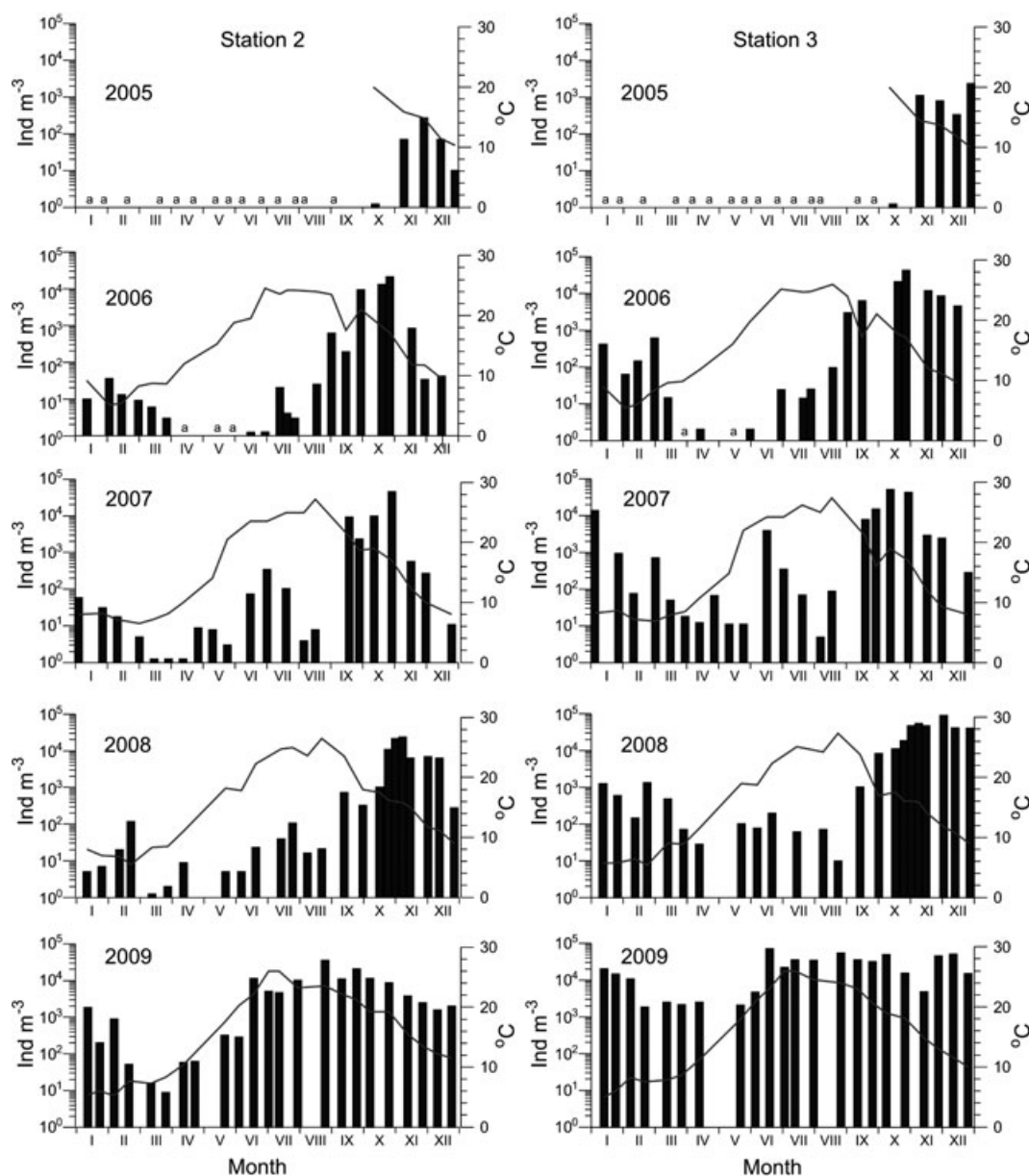
Fig. 2. Seasonal dynamics of *Oithona davisae* abundance (bars) and water temperature (lines) at the mouth (St. 2) and centre (St. 3) of Sevastopol Bay from 2005 to 2009. Samples marked 'a' are samples in which no individuals of *O. davisae* were found.

data, St. 1–3), Novorossiysk (St. 12) and Tuapse bays (St. 13) (Selifonova 2009). In October 2008, when *O. davisae* were extremely abundant in Sevastopol Bay, zooplankton samples were additionally taken in Balaklava Bay and the open waters outside it (St. 8) and no individuals of the species were found there. In October 2009, samples were taken at the same stations, *O. davisae* proving to be the dominant species in all of them. Then, in November–December 2009, *O. davisae* was found in all the samples from Yevpatoriya (St. 7), Yalta (St. 10) and Feodosiya (St. 11) coastal waters. The species was reported by

Kremena Stefanova (IO BAS, Varna, Bulgaria) from Varna Bay (St. 4) in August 2010. In autumn 2010, the invader was discovered in waters off Odessa (St. 6) and Constanta (St. 5).

Discussion

The present study has shown that *O. davisae* is very tolerant of variations in water temperature. For example, almost the same values of abundance (exceeding 10,000 ind. m^{-3}) were observed at St. 3 in February 2009 and
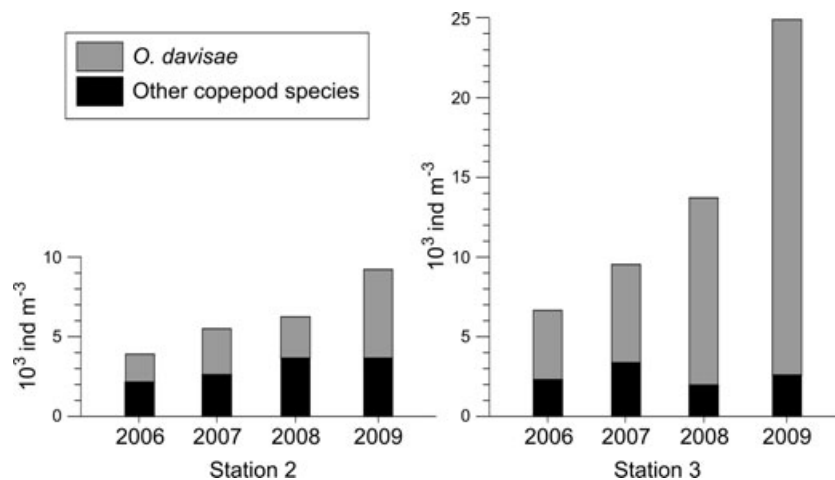
Fig. 3. Average annual contribution of *Oithona davisae* to the total copepod abundance at St. 2 and 3 in 2006–2009.

St. 2 in August 2009, with surface water temperatures of 8.2 °C and 23.2 °C, respectively. Maxima of abundances were also observed over a rather wide range of water temperatures (Table 1).

According to an experimental study, the salinity tolerance of *O. davisae* ranges from 3 to 40 psu (Svetlichny L. S. and Hubareva E. S., unpublished data). Thus, *O. davisae* has a high tolerance to different environment conditions, allowing it to survive and reproduce in the Black Sea. However, the success of the invasion was strongly influenced by the native community (Alimov *et al.* 2004). After occasional introduction, an invader must either find a niche that is not occupied or compete for an occupied one (Berg & Carton 1988; Di Castri 1990). Low resilience of the native zooplankton community to *O. davisae* invasion was possibly preconditioned by earlier invasions of the predatory ctenophores *M. leidyi* and *B. ovata* (Gubanova & Altukhov 2007). *Mnemiopsis leidyi* invasion resulted in a substantial increase in grazing pressure on zooplankton in the late 1980s and early 1990s, especially during summer and autumn (Kamburska *et al.* 2003; Lebedeva *et al.* 2003). One of the results of the destructive *M. leidyi* invasion was a complete disappearance of *Oithona nana*, which had dominated the copepod community until the 1990s (Gubanova *et al.* 2002).

The invasion of *B. ovata* preying on *M. leidyi* reduced the abundance of the latter, curtailing the period of their reproduction and thus reducing the overall grazing pressure on plankton during summer and autumn (Finenko *et al.* 2003; Hubareva *et al.* 2004; Gubanova *et al.* 2013). As a result, the abundances of the copepod species that reproduce in autumn increased in 2002–2004. The new copepod species, *O. davisae*, was introduced in the Black Sea in 2005. We suppose that it occupied the environmental niche of *O. nana* in Sevastopol Bay zooplankton. These related species are ecologically similar. They also have the same body size. Seasonal dynamics of *O. davisae* at St. 2 became more and more similar to those of *O. nana*, especially in the last years (2008 and 2009) (Fig. 4). Like *O. davisae*, *O. nana* is an eurythermic species present in the Black Sea plankton all year round. According to regular observations of the plankton dynamics in Sevastopol coastal waters between 1960 and 1970, the highest numbers of *O. nana* were recorded in September–November, and the minimal densities occurred between January and March (Greze *et al.* 1971). At the mouth of Sevastopol Bay in 1976 and 1980, the maximum abundances were observed in September. So, both the biological features of *O. davisae* and the susceptibility of the native zooplankton community to invasion favoured the success of the species' establishment in the Black Sea.

As the smallest planktonic copepod species in the Black Sea, *O. nana* was one of the preferred food items for fish

Table 1. *Oithona davisae* maximum abundances and water temperatures at the two stations in Sevastopol Bay from 2006 to 2009.

| Year | Station 2 | | | Station 3 | | |
	Max value (ind. m^{-3})	Date	T, °C	Max value (ind. m^{-3})	Date	T, °C
2006	21,050	27 October	17.1	42,667	27 October	17.1
2007	46,200	29 October	17	51,667	12 October	19
2008	23,500	7 November	15.8	91,650	1 December	11.7
2009	35,550	26 August	23.5	71,167	19 June	23.1

T, temperature.

Fig. 4. Seasonal dynamics of *Oithona nana* in 1976 and 1980 and *O. davisae* in 2008 and 2009 at the mouth of Sevastopol Bay.

larvae in the early developmental stages. The disappearance of *O. nana* at the end of the 1980s resulted in a dramatic decrease in the abundance of fish larvae (Tkach 1995).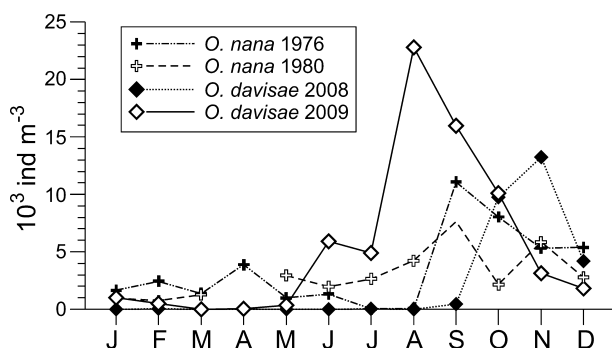 According to our data, the duration of the population increase of *O. davisae* has been extended not only in the bay but also in open coastal waters (Altukhov 2010). The food stocks necessary for the survival of fish larvae have become more abundant. Thus, an increase in abundance of larvae of the fish species that are spawning in bays and the coastal waters during the second half of the year may be expected.

It was noted (Berezina 2004) that if an alien species tolerant of recipient environments does not encounter a strong grazing/predation pressure, its abundance rises sharply. Afterwards, the growth is limited and the abundance reaches a plateau. If *O. davisae* follows the same pattern, a stabilization of its numbers may be foreseen owing to elevated predation pressure.

Conclusion

The new invasive species *O. davisae* has become one of the most abundant copepod species in Sevastopol Bay since its first appearance there in 2005. *O. davisae* usually reached peaks of abundance in autumn. The period of dominance of the species in the bay is extending, with the highest abundances being recorded in the central part of the bay. Species abundance increased not only in the bay but also in the open coastal areas. The invader has expanded along the Black Sea coast since 2009. The success of the *O. davisae* invasion appears to have been preconditioned by the biological features of the species and the vulnerability of the native zooplankton community. We hypothesize that *O. davisae* has occupied the niche of the indigenous copepod *O. nana,* which vanished in the late 1980s.

As a result of the *O. davisae* invasion, the total abundance of fodder zooplankton increased considerably. Like *O. nana,* the invader seems to be a preferred food item for early stages of fish larvae. Long-term studies at basin

scale are still needed to monitor continuously the structural changes in the Black Sea zooplankton community.

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ORIGINAL ARTICLE

Gaidropsarus granti from a Ligurian seamount: a Mediterranean native species?

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Abstract

Two specimens of the Azorean rockling *Gaidropsarus granti* (Regan 1903) were caught in the period August 1989–January 1990 on a seamount located about 29 nautical miles south of Genoa, Ligurian Sea. The records remained unpublished because of errors in the literature concerning the two Macaronesian species *Gaidropsarus granti* and *Gaidropsarus guttatus*. Old and recent records of *G. granti* are now used to characterize morphological and ecological traits of this species. In particular, *G. granti* is unmistakably adapted to a sedentary life on hard substrata, and all records occurred in offshore slope waters, frequently on banks and seamounts. Ecological characteristics and biogeographical considerations are introduced in the discussion about the origin of the Mediterranean records of *G. granti*, specifically whether this fish should be considered an introduced or a native species.

Introduction

Because of the difficulties of sampling, deep-sea hard bottoms are little known environments and this lack of knowledge applies to fish. About 28.6 nautical miles (nm) south of Genoa, the eastern wall of the submarine canyon of the Bisagno River rises in a seamount (Fig. 1) whose top reaches 550 m under the sea surface from a base at about 2200 m on the bottom of the canyon. Named 'secca di Ulisse' (Ulysses Seamount), this peak is a popular fishing site for semi-professional and recreational fishermen, who reach the seamount with gear called 'filaccioni', arrays of hooks around a weighted rope that connects them to a float.

The edible species obtained by this method include *Pagellus bogaraveo, Merluccius merluccius, Schedophilus ovalis, Centrolophus niger, Polyprion americanus, Epigonus telescopus, Palinurus mauritanicus, Paromola cuvieri.* An amateur fisherman, Bruno Fida, preserved for us two specimens of a *Gaidropsarus* (in his opinion a nonedible species), which were identified as *Gaidropsarus granti* (Regan 1903). This rare deep-living species was discovered about a century ago in the Azores and later was confused with the shallow-water species *Gaidropsarus*

guttatus (Collett 1890), known from Madeira, the Azores and the Canaries. These notes report the presence of *G. granti* in the northernmost area of the Western Mediterranean and clarify its morphological and ecological characteristics in comparison with the other Macaronesian species, *G. guttatus*. Several records of *G. granti* in Italian waters remain unpublished or are confused with other *Gaidropsarus* species, and there is current debate about whether *G. granti* should be considered an introduced or a native species (*cf.* Golani *et al.* 2002).

Material Examined

Two specimens, total length (TL) 25.5 and 27.8 cm, caught using filaccioni on the Ulysses Seamount between 550 and 700 m depth, in the period August 1989–January 1990, were preserved by freezing. After formalin fixation these fish are now in the present laboratory collection. Their main biometric and meristic characteristics are given in Table 1. A third specimen was obtained more recently by trawl (not used for radiograph).

The following summary description of these specimens is in accordance with *Gaidropsarus granti* (Regan 1903): a
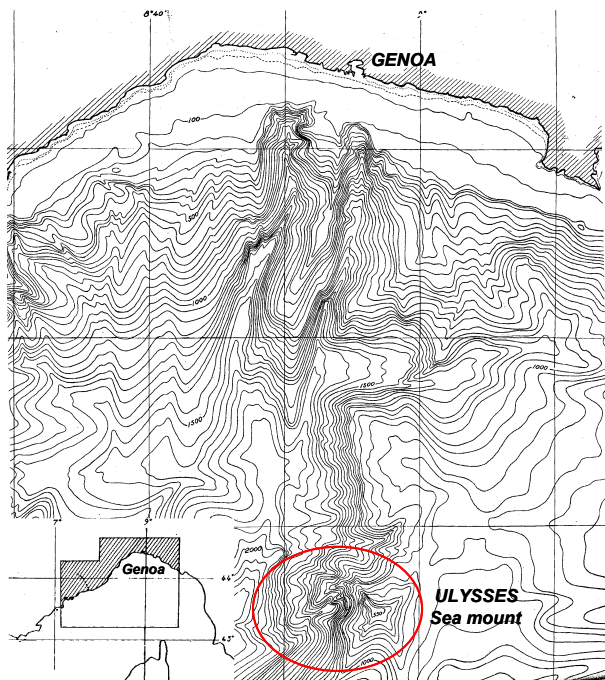
Fig. 1. Geographical location of the Ulysses Seamount.

Table 1. Biometric (measurements in cm) and meristic characteristics (N) of two specimens of *Gaidropsarus granti* caught on the offshore seamount of the Ligurian Sea.

	1st specimen	2nd specimen
Total length	25.5	27.8
Standard length	23.2	25.0
Head length	5.5	6.0
Eye longest diameter	0.9	0.9
Interorbital space	0.8	0.9
Pectoral length	3.2	3.5
Pelvic length	3.6	4.9
II Predorsal length	8.8	9.3
Preanal length	12.0	13.7
Vertebrae[a]	47.0	47.0
II Dorsal rays[a]	57.0	58.0
Anal rays[a]	46.0	45.0
Pectoral rays	22.0	21.0

[a]Counted on radiographs.

species with a short anterior ray of first dorsal fin (ray length less than eye diameter), with a large head (total length/head length ratio 4.7), 57–58 rays in the second dorsal fin, 45–46 in the anal fin, and 21–22 in the pectoral fin. Gillrakers in the form of dentated tubercles, five on the outer side of the first arch and seven on the inner side. The lateral line is inconspicuous, with 15 distinct units in the segment extending from the opercular rim to the bent (lateral line inflexion) included. Interorbital
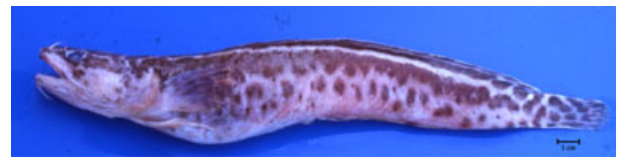
Fig. 2. *Gaidropsarus granti*, adult female, 27.8 cm TL, Ligurian Sea.

space is slightly less than the longitudinal diameter of the eye. Teeth lie in large dense bands of conical elements with large sparse back-deflected canines, in numbers varying with size/age. The colour is deep pink, mottled by brown irregular blots and lines. Dentition consists of bands of densely packed small conical elements and sparse larger canines on upper and lower jaws and vomer. In the present specimens the number of canines (by increasing size of fish) were: 3–5 in the upper jaw; 11–13 in the lower jaw and 3–4 on the vomer. The freshly caught fish were deep pink in colour, with brown blotches that formed asymmetrical patterns on the upper part of the body. In preserved specimens (Fig. 2) the pink colour vanishes, and is replaced by a cream base on which brown patterns become apparent. The lower surfaces are all cream, including the head. The upper part of the body has three longitudinal brown stripes, one dorsal and two dorso-lateral, separated by a light band, with a somewhat zigzagged contour. On the head the brown is interrupted by light spots and curved stripes; ventrally and in the tail region the dorso-lateral brown bands break into spots. Under the dorso-lateral brown band, are one or two irregular rows of spots, the most ventral smaller. Comparing the three available specimens, individual patterns are evident, particularly in the anterior half of the body. The dorsal fin is brown with a central longitudinal light band; pectoral and caudal fins cream with brown blotches; ventral fins all cream. The smallest specimen had red edges on dorsal and anal fins.

The first specimen appeared heavily infested by juvenile Nematoda; its gonads were very small and degenerated and included worms in the parenchyma. The second, and largest, fish had a spent-recovering ovary. Remains of crustacean decapods, in part still red in colour, were found in the guts of the two fish.

Discussion

Little is known about *Gaidropsarus granti*. It was described by Regan (1903) on the basis of two individuals, both 39 mm TL, which Mr O. Grant obtained in the Azores with the help of Major Chaves, director of the Museum of Punta Delgada. In the 1990s only one type specimen could be found in the British Natural History Museum. In 1993, upon our request for information, A.

Wheeler was kind enough to show us an extract from a revision he was preparing on *Gaidropsarus*, giving the following characteristics (Table 2): second dorsal fin 60, anal rays 52, pectoral rays 21–21, pelvic rays 8/8, vertebrae 14+33 (on the basis of radiographs). He added: 'Regan's second type specimen may have been returned to the Azores Museum. It seems to have disappeared' (letter of 4 May 1993).

The literature such as FNAM (Svetovidov 1986b), CLOFETA (Cohen 1990), FAO fisheries synopsis number 125 (Cohen *et al.* 1990) and FISHBASE (Froese & Pauly 2010) reports the capture of other individuals of *G. granti* in the Atlantic, but these reports derive from the confusion with another species. In fact, Collett, who had described *Gaidropsarus guttatus* based on two young specimens taken at Fayal during the Prince of Monaco's cruise around the Azores in 1887 (Collett 1890, 1896), came back to the subject in 1905, adding information taken from two other specimens: one collected at Gran Canaria in shallow water, studied by Steindachner (1891), and a second fish obtained from the above-mentioned Major Chaves of Punta Delgada Museum.

While Collett (1905) was writing about *G. guttatus*, Svetovidov (1973, 1986a,b) assigned the two specimens of Collett (1905) to *G. granti*; *ipso facto* the distribution area

of *G. granti* was extended to the Canary archipelago and the habitat to shallow waters. Also Brito (1991) notes that the fish in the Canary archipelago are in fact those Steindachner identified (1891). The decision of Svetovidov is somewhat incomprehensible; in fact, he did not examine the specimens, as he himself states (1986a) excluding *G. granti* from his key to the genus *Gaidropsarus*. He wrote: 'Not included in the Key, as it was not studied on the basis of specimens'. However in FNAM (Svetovidov 1986b) he prepared a key of genus *Gaidropsarus* in which the vertebral number 44 is assigned to this species. This point is a mistake (see Table 2) and must be disregarded.

The first fish (Table 2) that was collected at Gran Canaria had meristic data and colour very different from those of *G. granti*. The second fish (Table 2), a fully grown female of 32.2 cm TL, had a coloration somewhat different from that of the two individuals (18.3 and 21.3 cm TL) described by Collett (1890) as the species *G. guttatus*, but Collett (1905) also stated that 'the fins and the number of their rays exhibit no divergence' (Collett 1890; had given 2a Dorsal 50–54; Anal 43–46; Pectoral 16–17; Ventral 7). Thus this specimen was also definitely *G. guttatus*, not *G. granti*. On the basis of the old samples mentioned, the extension of the distribution of *G. granti* to the Canary Archipelago should be rejected. About a century later, a

Table 2. Main meristic data (numbers), morphological characteristics and habitat of two Macaronesian species of *Gaidropsarus*.

	2° dorsal	Anal	Pectoral	Ventral	Vertebrae	Total length (cm)	Colour	Habitat	References
G. granti									
type, Azores	60	48	21	7	–	39	Deep pink with brown blotches and stripes	Unknown	Regan (1903)
syntype, Azores	60	52	21	8	47	39		Unknown	Wheeler (*in litteris*, 1993)
Ligurian Sea 1	57	46	22	7	47	25.5		Seamount – 550–700 m	Present note
Ligurian Sea 2	58	45	21	7	47	27.8		Seamount – 550–700 m	Present note
Rhodes	56	47	20	7	–	31		Sand- 360–400 m	Zachariou-Mamalinga (1999)
Galician bank	56	51	21	7	–	33.8		Offshore bank 823 m	Bañón *et al.* (2002)
Sardinia 1	55	47	20	7	47	24.5		Slope mount – 120–136 m	Mura & Cau (2003)
Sardinia 2	60	49	20	7	47	31.9		Slope – 250 m	Pais *et al.* (2008)
FNAM	60	52	–	–	44	–			Svetovidov (1986a,b)
G. guttatus									
Azores (Fayal)	50–54	43–46	16–17	7	–	18.3; 21.3	Dark brown with yellow spots	Shallow water rocky bottoms	Collett (1890)
Gran Canaria	50	43	16	–	–	26			Steindachner (1891) and Collett, (1905)
Azores (S. Miguel)	[a]	[a]	[a]	[a]	[a]	32			Collett (1905)
FNAM	53–57	45–49	17–19	7	47–49	–			Svetovidov (1986a,b)

[a]'No divergences in respect of the types' (Collett 1905).

study of fish of the Canarian slope did not include any *Gaidropsarus* (Uiblein *et al.* 1998).

Therefore, when the FNAM was published (1986) the sole available data about *G. granti* were those given in the description of the type by Regan (1903) and/or possibly derived by the study of the syntype. Since recording the present specimens in the Ligurian Sea, new information on this species has been accumulated:

1 A specimen of *G. granti* was collected in the Eastern Aegean Sea, at Rhodes. The fish, 31 cm TL (meristics in Table 2), has been ascribed to an accidental transport through the Strait of Gibraltar (Zachariou-Mamalinga 1999) (Table 2).

2 The Department of Oceanography of Fayal, Azores sampled new specimens (unpublished); a sample was lent to us for comparative studies.

3 One individual was found on an offshore bank at 823 m depth (Table 2) in North Spain (Bañón *et al.* 2002). The fish might indicate a northward expansion of the distribution area in relation to ocean warming.

4 *Gaidropsarus granti* was last found in the Canary Archipelago: in fact, one specimen was fished north of Lanzarote at 300 m depth (Brito *et al.* 2002).

5 Two individual fish were caught in Sardinia (Mura & Cau 2003; Pais *et al.* 2008). The latter paper tried to confirm an Atlantic origin of the fish by means of its parasites. However, *Anisakis simplex sensu strictu* larvae previously assigned to Atlantic areas, were more recently found in the Central Mediterranean (Algeria, Tunisia and Libya) in fish characterized by pelagic feeding, such as *Scomber scombrus* and *Merluccius merluccius* (Farjallah *et al.* 2008).

Thus the Mediterranean hosts four species of *Gaidropsarus*, two of coastal shelf waters, namely *Gaidropsarus vulgaris* and *Gaidropsarus mediterraneus*, and two deep eurybathic species, *Gaidropsarus biscayensis* and *G. granti*. *Gaidropsarus biscayensis* lives on muddy bottoms at circalittoral levels (100–200 m) and along the slope to 750 m (from results from national fisheries surveys in Ligurian waters; bottoms deeper than 750 m were not explored). *Gaidropsarus granti* in the Ligurian Sea was found on the hard substrata of the Ulysses Seamount between 500 and 700 m depth, and more recently also at 500 and 300 m (unpublished specimens). In Sardinia it was found at 120–136 m and 250 m; interestingly, the former location was a slope mount named 'banghittu', isolated from the shelf (Mura & Cau 2003).

The Macaronesian species *G. guttatus* is similar to *G. mediterraneus* in having in common the meristics and the general dark brown coloration, and inhabiting rocky environments in shallow waters. These could probably be considered the same species with two different colour patterns, mottled and uniform brown. It is interesting that Mediterranean specimens of *G. mediterraneus* sometimes have irregular light spots on their dark brown dorsal and lateral surfaces, as we have verified in the collection of the Museum of Natural History of Genoa.

The peculiar coloration of *G. granti* makes this species unmistakable. Such a colour pattern has disruptive properties (an appropriate name should be 'leopard rockling') and suits a sedentary fish, which during the day rests on illuminated substrata. The top of banks and seamounts present such an environment. The presence in the stomach contents of crustacean decapods, all red in colour, recall pelagic prey and probably indicates important vertical displacements in relation to feeding, which could be nocturnal in the water column. as is the case in general for gadoid fish.

From a geographical point of view, available records of the fish are from the Atlantic (Azores, Lanzarote, Galician bank, an offshore Lusitanian area) and the Mediterranean [Aegean, Adriatic (G. Bello, personal communication), Tyrrhenian, Ligurian and Ionian Seas]. The specimens collected in the Mediterranean are smaller (maximum TL about 32 cm) than the Azorean ones (type and syntype 39 cm TL, Table 2), suggesting the existence of a Mediterranean population.

On the basis of this additional information, we face two possibilities:

1 Mediterranean specimens are not native.

2 Mediterranean specimens have been concealed till now by their unusual habitat such as offshore banks and deep-seamounts, but they are a native component of the Mediterranean fauna.

In terms of fish fauna, the biogeographical links between the Mediterranean and the Azores to date seem to be one-way, i.e. from the Mediterranean to the Azores. It is generally assumed that after the Messinian crisis, the Mediterranean Sea was recolonized by Atlantic species between about 5 and 4 million years ago, as soon as a permanent communication was restored in the area of Gibraltar. About the same age as the post-Messinian Mediterranean is assigned to the Azores archipelago, a complex of islands which are the result of the volcanic activity associated with the Azorean microplate, a hot point where North American, Euroasiatic and African plates come in contact (Morton *et al.* 1998). In fact, the oldest of nine oceanic islands, S. Maria and S. Miguel, are considered about 5 million years old.

Gibraltar Strait itself, where the old Mediterranean exported its species to, is at present surrounded by Lusitanian and Mauritanian areas (*sensu* Ekman 1953), which seem the most probable sources of the present fish fauna present in both the Mediterranean and the Azores.

The coastal fish fauna of the Azores includes about 100 species, 80% of which are found in the Mediterranean;

the main fishery resources, in terms on finfish, are included in this group (Morton *et al.* 1998). To explain this large Mediterranean affinity, apparently in contrast to the fact that the main surface currents that surround the archipelago flow from the West, we must recall that the Gulf Stream is recent, <3 million years old (Cronin & Dowsett 1996). The Macaronesian shallow water species *G. guttatus*, which is very close to *G. mediterraneus*, could be related to ancient Mediterranean links. At present, similar links pertain not to surface but to deep-sea fauna; in fact, Mediterranean deep waters flow out of Gibraltar and have been traced in large Atlantic areas, including the Mid-Atlantic Ridge, which forms the Azores. The deep-sea gadoid fishes of the Mediterranean are all present in the Atlantic, with the exception of *Lepidion lepidion*, which is still considered endemic. Many other fish previously supposed endemic have been found recently outside of Gibraltar, including *Rhincogadus hepaticus* and *Eretmophorus kleinenbergi* (Quignard & Tomasini 2000). So at present the hypothesis of a Mediterranean origin of the Azorean rockling cannot be rejected, nor can the idea that the Mediterranean population is native. The growing numbers of Mediterranean records related to the Atlantic ones reinforce this interpretation.

Acknowledgements

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ORIGINAL ARTICLE

Effects of ocean acidification on sponge communities

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Abstract

The effects of ocean acidification on lower invertebrates such as sponges may be pronounced because of their low capacity for acid–base regulation. However, so far, most studies have focused on calcifiers. We present the first study of the effects of ocean acidification on the Porifera. Sponge species composition and cover along pH gradients at CO$_2$ vents off Ischia (Tyrrhenian Sea, Italy) was measured at sites with normal pH (8.1–8.2), lowered pH (mean 7.8–7.9, min 7.4–7.5) and extremely low pH (6.6). There was a strong correlation between pH and both sponge cover and species composition. *Crambe crambe* was the only species present in any abundance in the areas with mean pH 6.6, seven species were present at mean pH 7.8–7.9 and four species (*Phorbas tenacior*, *Petrosia ficiformis*, *Chondrilla nucula* and *Hemimycale columella*) were restricted to sites with normal pH. Sponge percentage cover decreased significantly from normal to acidified sites. No significant effect of increasing CO$_2$ levels and decreasing pH was found on spicule form in *Crambe crambe*. This study indicates that increasing CO$_2$ concentrations will likely affect sponge community composition as some demosponge species appear to be more vulnerable than others. Further research into the mechanisms by which acidification affects sponges would be useful in predicting likely effects on sessile marine communities.

Introduction

Atmospheric CO$_2$ concentration is currently approximately 383 parts per million by volume (ppmv), the highest level for at least 650,000 years and, due to anthropogenic influences, is projected to increase by 0.5% per year throughout the 21st century (Guinotte & Fabry 2008). The oceans are a major sink for CO$_2$ and have absorbed approximately half of all anthropogenic emissions (Sabine *et al.* 2004). Since CO$_2$ dissolves in seawater, it shifts the carbonate system towards higher concentrations of CO$_2$, bicarbonate (HCO$_3^-$) and protons H$^+$, therefore decreasing the seawater pH and the carbonate ions used by calcifiers to build their shells and skeletons. Since the early 1900s, a 30% increase in H$^+$ concentration of surface waters has resulted in a pH decrease of 0.1 units, and a further drop in surface seawater pH of up to 0.5 units has been projected by 2100 (Caldeira & Wickett 2005).

The adverse effect of seawater acidified with CO$_2$ has been most apparent on marine calcifiers, such as coralline algae (Kuffner *et al.* 2008; Martin *et al.* 2008), foraminiferans (Moy *et al.* 2009; Dias *et al.* 2010), corals (e.g. Silverman *et al.* 2009), echinoderms (e.g. Michaelidis *et al.* 2005) and molluscs (Gazeau *et al.* 2007). Among these organisms, rates of calcification have been predicted to fall by up to 60% within this century, depending on the physiology of the species and their mineralogy (Kleypas *et al.* 2006). However, although it is in calcifiers that the effects are most obvious, studies using high CO$_2$ levels indicate that unifying principles define sensitivity to CO$_2$ in both calcifying and non-calcifying animals (Pörtner 2008). The effects of ocean acidification on lower invertebrates such as sponges are likely to be pronounced because of their low capacity for acid–base regulation (Pörtner 2008). As yet no studies have examined the effect of ocean acidification on the Porifera.

Recently, CO_2 vents have been used as natural laboratories to advance our understanding of ocean acidification at the ecosystem level (Hall-Spencer *et al.* 2008; Cigliano *et al.* 2010; Porzio *et al.* 2011). While most vents emit hot gases, including toxic sulphur compounds (Dando *et al.* 1999), vents off Ischia island near the volcano of Vesuvius in Italy eject >90% CO_2 at ambient temperature without toxic sulphur (Hall-Spencer *et al.* 2008), acidifying coastal seawater on the north and south sides of Castello Aragonese for centuries, maybe millennia (Tedesco 1996). Hall-Spencer *et al.* (2008) studied species community composition of the area along gradients of normal pH (8.1–8.2), lowered pH (mean 7.8–7.9, min 7.4–7.5) to extremely low pH 6.6. They recorded 64 species of plant, alga and animal, many of which were resilient to the acidified conditions. Within sponges, eight genera were found with an apparent decrease in their distribution from normal to extremely low pH values (Hall-Spencer *et al.* 2008, supporting information). However, no sampling or full quantitative analysis of sponge species composition was undertaken. Although other studies have examined the biodiversity of sponges associated with hydrothermal vent sites (Morri *et al.* 1999; Pansini *et al.* 2000), none has looked at the effect of small-scale variations in pH on sponge community composition.

The sponge fauna of Ischia is relatively well studied, with some 86 species reported from the area (Sara 1959). Both demosponges, which possess skeletons of siliceous spicules, and calcarea, which have calcium carbonate spicules, are present. Here we present the results of a complete survey of the sponge fauna at CO_2 vents, taking account of both qualitative (species composition) and quantitative (percentage cover of the substratum) aspects of the sponge community composition along pH gradients.

Methods

Study site and seawater carbonate measurements

Sponge community composition was measured during May 2008 on the north and south sides of the Castello Aragonese (40°043.849′ N, 13°57.089′ E) off Ischia in Italy (Fig. 1). Six sites were identified along gradients of increasing pCO_2 to the north and south of the Castello: two normal pH stations (N1 and S1; Fig. 1); two stations that had reductions in mean pH of 0.2–0.4 units (N2 and S2; Fig. 1) and two stations with reductions in mean pH of 0.6–1.5 units (N3 and S3; Fig. 1) which are more representative of the localised effects to be expected from deliberate CO_2 sequestration rather than from global ocean acidification. Sites were chosen on the basis of the carbonate chemistry of the vents, mapped out by Hall-Spencer *et al.* (2008), Rodolfo-Metalpa *et al.* (2010) and

Cigliano *et al.* (2010), who repeatedly measured pH_T (in total scale), total alkalinity (TA), temperature and salinity. Water samples were collected at 1 m depth using glass bottles, and the pH_T was measured immediately using a metre accurate to 0.01 pH units (Metrohm 826 pH mobile, Metrohm, Herisau, Switzerland) calibrated using TRIS/HCl and 2-aminopyridine/HCl buffer solutions (DOE 1994). Seawater samples were then passed through 0.45-μm pore size filters (GF/F Whatman, Maidstone, Kent, UK) and poisoned with 0.05 ml of 50% $HgCl_2$ (AnalaR: Merck, Darmstadt, Germany) to avoid biological alteration, and stored in the dark at 4 °C. Three replicate 20-ml sub-samples were analysed at 25 °C using a titration system composed of a pH meter with an ORION pH electrode (see Hall-Spencer *et al.* 2008 for further details). Parameters of the carbonate system [pCO_2, CO_3^{2-}, HCO_3^-, and saturation state of calcite ($\Omega_{calcite}$)] were calculated from pH_T, TA, temperature and salinity (38‰) using the free-access CO_2 Systat package (Systat Software Inc., Chicago, Illinois, USA) (Table 1).

Sponge community composition

Quantitative and qualitative measurements of sponge community composition were assessed at each of the six study sites (N1–N3, S1–S3). At each site, 10 quadrats of 30 cm^2 were sampled, deployed haphazardly on very steep or vertical bedrock faces in a depth range of 1–3 m. Each quadrat was photographed with a Nikon D70 camera (Nikon Inc., Tokyo, Japan.) in an Ikelite housing using an 18–70 mm zoom lens for quantitative sponges cover. The number of species in each quadrat was counted and a small sample of each species was scraped off from the rock using a knife and placed in a ziplock bag. Samples were preserved in 70% denatured ethanol and examined microscopically to confirm identification (see Picton & Goodwin 2007 for detailed methodology).

Change in species composition along a pH gradient (7.09–8.14 pH) was examined on a 240-m transect. Three 30-cm^2 quadrats were haphazardly deployed at 25 stations spread at 10-m intervals. Quadrats were deployed on very steep or vertical bedrock faces between 1 and 3 m in depth and the composition of sponge communities was measured as described above. Only sponges on the north side of the Castello Aragonese were quantified in this way. Percentage sponge cover per square meter was calculated using IMAGEJ (Rasband 1997–2008). Percentage data were arcsine-square root-transformed. Differences in percentage cover were tested with the Kruskal–Wallis test.

Ten choanosomal style spicules from five samples of *Crambe crambe* were measured for each of the southern sites (S1–S3). The aim of this was to examine any effects of increasing CO_2 and decreasing pH on spicule form.
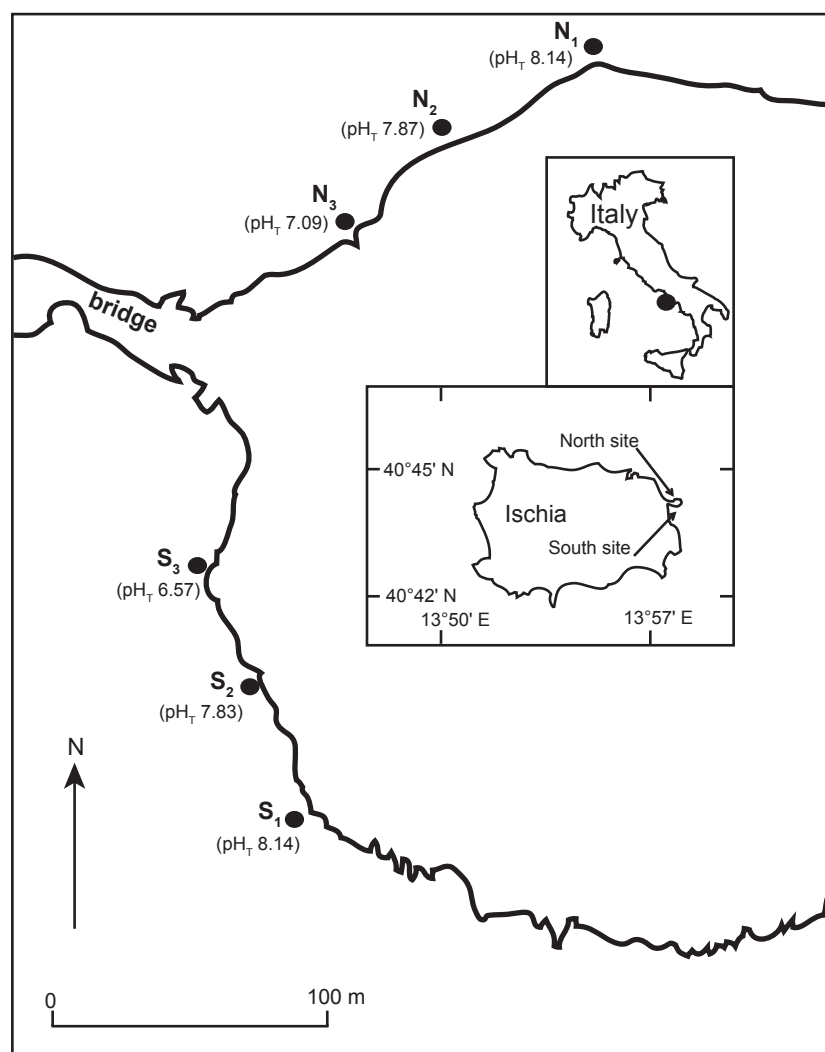
Only *C. crambe* was examined as it was the only species present at all three sites. The species has siliceous spicules.

Results

There was a significant difference in total percentage sponge cover on both south sites (Kruskall–Wallis H = 16.250, P < 0.0003) and north sites (Kruskall–Wallis H = 16.979, P < 0.0002). The difference was most marked between the highly acidified sites (N3, S3) and the control sites (N1, S1) (Figs 2 and 3).

In total 14 sponge species were recorded from the study area (Table 2). Species composition varied between sites. The only species present in any abundance in the extremely acidified sites (S3, N3) was *Crambe crambe*, although small patches of *Microciona* cf. *toxitenuis* and *Spirastrella cuncatrix* were also present. A greater variety of sponges was present at sites of intermediate pH (mean 7.8–7.9). Four species (*Phorbas tenacior*, *Petrosia ficiformis*, *Chondrilla nucula* and *Hemimycale columella*) were restricted to the normal pH sites (N1, S1).

Species composition on the north side of Castello Aragonese varied along the transect with very little sponge cover in the most acidified areas (Fig. 4). *Crambe crambe* was the most abundant species and was the only species present in the most acidified areas (Table 2).

There was no significant difference in mean spicule morphology or length (Kruskall–Wallis H = 3.86, P < 0.145) between sites for *Crambe crambe*.

Discussion

Whereas vertebrates and higher invertebrates have some capacity for acid–base regulation, sponges lack intercellu-

Table 1. Summary of pH_T (in total scale), and carbonate chemistry data from 2007 to 2008 at CO_2 vents off Ischia (from Hall-Spencer *et al.* 2008; Cigliano *et al.* 2010; Rodolfo-Metalpa *et al.* 2010). Water samples were taken from rocky shore zones N_1–N_3 and S_1–S_3 NB: min pH had the max pCO2 levels and vice versa.

Site	pH_T	pCO_2 (µatm)	CO_3^{2-} (µmol·kg^{-1})	HCO_3^- (µmol·kg^{-1})	Ω_{calc}	Ω_{arag}
N_1						
Min	7.96	286	190	1810	4.48	2.92
Mean (n = 18)	8.13	353	263	1949	5.93	3.86
Max	8.19	552	370	2830	6.58	4.28
N_2						
Min	7.49	309	70	1850	1.55	1.00
Mean (n = 24)	7.85	854	159	2127	3.71	2.41
Max	8.17	1908	270	2450	6.20	4.03
N_3						
Min	6.31	654	5	2090	0.11	0.07
Mean (n = 23)	7.13	7553	48	2392	1.12	0.73
Max	7.90	29,885	170	2480	3.89	2.53
S_1						
Min	8.09	304	240	1830	5.61	3.62
Mean (n = 12)	8.13	347	258	1920	6.02	3.91
Max	8.17	395	280	2000	6.50	4.24
S_2						
Min	7.27	314	50	1840	1.11	0.73
Mean (n = 23)	7.79	1119	148	2149	3.49	2.27
Max	8.16	3278	270	2470	6.35	4.14
S_3						
Min	6.07	886	3	2220	0.07	0.04
Mean (n = 35)	6.68	18,313	21	2462	0.46	0.30
Max	7.79	51,997	140	2490	3.27	2.13
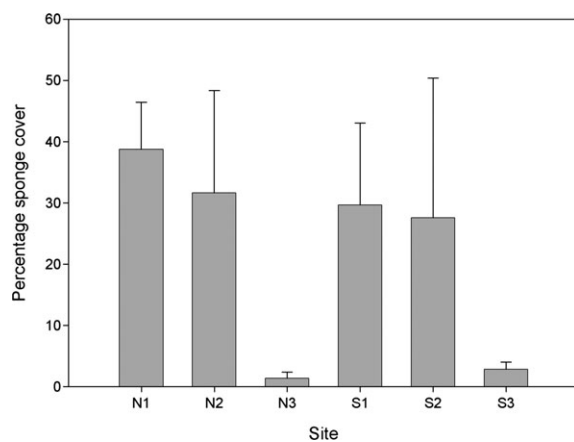
Fig. 2. Sponge percentage cover per square meter (mean + SE, n = 10) at sampling stations along the rocky cliff of Castello Aragonese. North stations: N1 (mean pH 8.14), N2 (mean pH 7.87) N3 (mean pH 7.09). South stations: S1 (mean pH 8.14), S2 (mean pH 7.83), S3 (mean pH 6.57).

lar junctions and other ultrastructural connections which allow the epithelial layers of other animals to act as barriers (Pörtner 2008). Their interstitial fluid is very similar to that of their environment (Ruppert & Barnes 1994). Consequently they are unable to regulate for changes in

acidity and may be particularly vulnerable to alterations in environmental conditions.

Sponge community species composition and abundance has been shown to be affected by substratum inclination (Preciado & Maldonado 2005), exposure to light (Jokiel 1980), degree of siltation (Brien *et al.* 1973; Bell & Barnes 2000b), exposure to predators (Dunlap & Pawlik 1996) and algal abundance (Bell & Barnes 2000a, 2002). Differences in these environmental factors were controlled for as far as possible by sampling from similar habitats at all sites (vertical rock faces between 1 and 3 m in depth). However, orientation of rock face and therefore exposure to light could not be controlled for. Species abundance and sponge cover were slightly lower on the south sampling sites, possibly due to the greater light exposure. Despite this, a decrease in sponge abundance with increased pH was observed on both south and north sites, suggesting that this is the dominant factor affecting sponge community composition in the study area. There has been little research into the effects of pH on sponge metabolism but there is some evidence that it may affect reproductive success and regeneration. Changes in environmental pH have been shown to affect the calcium-induced cell aggregation of sponge cells, possibly by altering the level of intracellular Ca^{2+} or by affecting cell
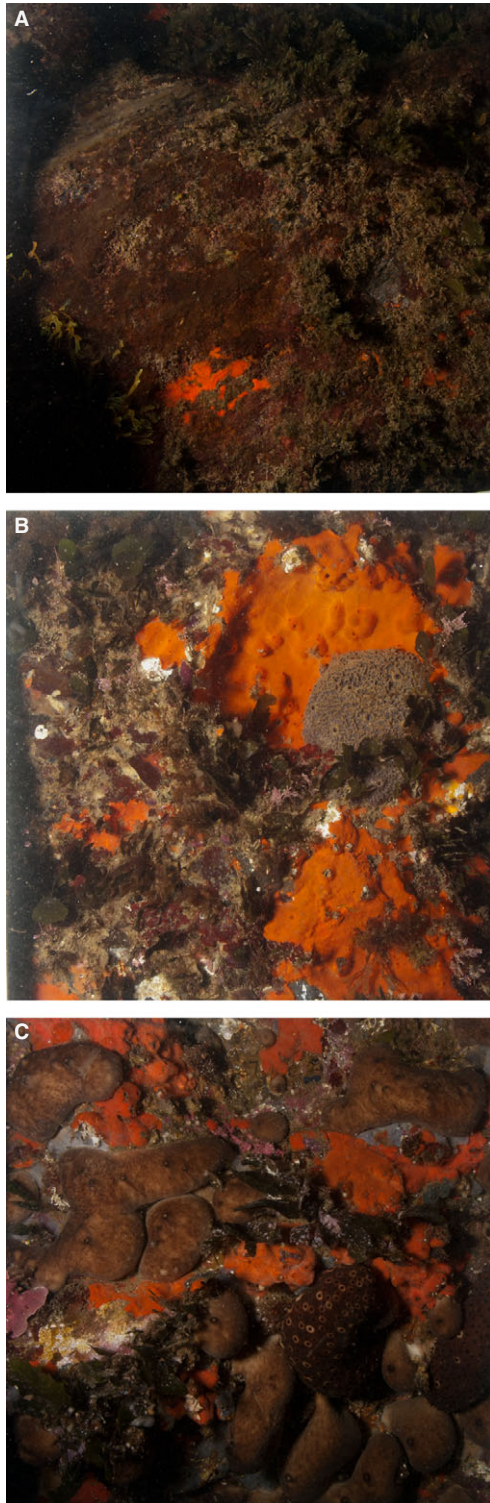
Fig. 3. Representative 25-cm² sponge cover quadrats from north side transect. (A) Quadrat 1, N3 (*Microciona* cf. *toxitenuis*). (B) Quadrat 9 N2 (*Crambe crambe*, *Ircinia variabilis*, *Chondrosia reiniformis*, *Phorbas tenacior*). (C) Quadrat 9, N1 (*Crambe crambe*, *Phorbas tenacior*, *Chondrosia reiniformis*).

permeability (Phillip 1997). Re-aggregation is thought to be one process involved in sponge regeneration (Bergquist 1978). It is possible that the difference in species composition between sites reflects differential effects on re-agreggation mechanisms and consequently potential to recover from disturbance.

Exposure to low pH (5.8–6.5) has been found to affect hatching rate and hatchability of gemmules in the freshwater sponge *Ephydatia mülleri* (Benfey & Reiswig 1982). Larval phases of marine organisms may be particularly vulnerable to the effects of even small alterations in pH, with increases resulting in larval morality and developmental abnormalities (Dupont *et al.* 2008) and a significant effect of pH on settlement has been shown for other groups at the study site (Cigliano *et al.* 2010). A variety of developmental forms was represented by the species present. *Crambe crambe*, the dominant species at the acidified sites, reproduces sexually with internal fertilisation, lecithotrophic larvae of the parenchymella type are released, spending 24–72 h in the plankton before settlement (Uriz *et al.* 1998). A range of reproductive modes was present in those species only found at the normal pH sites, including both direct development with no larval phase in *Petrosia ficiformis* (Gil 2007) and development via a non-tufted parenchymella larvae *Phorbas ficiticius* (Mariani *et al.* 2006). Consequently, it does not seem that mode of development can account for the difference in distribution between species; other factors may be affecting larval settlement efficiency.

Crambe crambe is one of the most widespread littoral sponges in the Mediterranean (Uriz *et al.* 1992). It is an opportunistic species with high reproductive output, and larval swimming behaviour which results in wide dispersal (Uriz *et al.* 1998). A trade-off between defence and growth rate has been found to occur, with allocation to chemical and physical defences being negatively correlated with reproduction and growth (Becerro *et al.* 1997) and spatial competition being a key factor. The absence of competitors in the acidified sites may favour *C. crambe* by enabling it to devote more resources to growth; it was the only species to decrease in abundance at the normal pH sites. There was no noticeable effect of acidity on spicule formation in *C. crambe*. Spicule formation has been found to be affected by other environmental factors such as water temperature and silica concentration (Elvin 1971; Bavastrello *et al.* 1993). During the late Permian mass extinction, possibly linked to elevated CO_2 levels, in South China there was a 88–92% decrease in spicule diversity: the surviving spicule forms were smooth and simple (oxeas, strongyles, oxy-orthpentactines and oxy-orthohexactines; Liu *et al.* 2008), mirroring the trend for simplified skeletons in other groups (Knoll *et al.* 1996). It is possible that CO_2 affects silica formation, preventing

Table 2. Sponge species recorded on the north (N1–N3) and south side of Castello Aragonese along gradients of pH. Mean percentage cover/m² (n = 10).

Species/Area	N1 (pH 8.14)	N2 (pH 7.87)	N3 (pH 7.09)	S1 (pH 8.14)	S2 (pH 7.83)	S3 (pH 6.57)
Crambe crambe	⬤	✹	◦	✹	✹	•
Chondrosia reiniformis	⬤	•		•		
Phorbas tenacior	•	◦				
Scalarispongia scalaris	◦	•			•	
Petrosia ficiformis	•			◦		
Ircinia variabilis	◦	•			•	
Sarcotragus sp.		•				
Microciona cf. *toxitenuis*			◦			
Hemimycale columella				◦		
Spirastrella cuncatrix						◦
Phorbas fictitius						
Chondrilla nucula				•		
Haliclona mediterannea		◦				
Eurypon cf. *cinctum*	◦					
Total number of species	6	7	2	5	3	2

◦ = <1%; • = 1–5%; ● = 5–10%; ⬤ = 10–20%; ✹ = 20–30%.
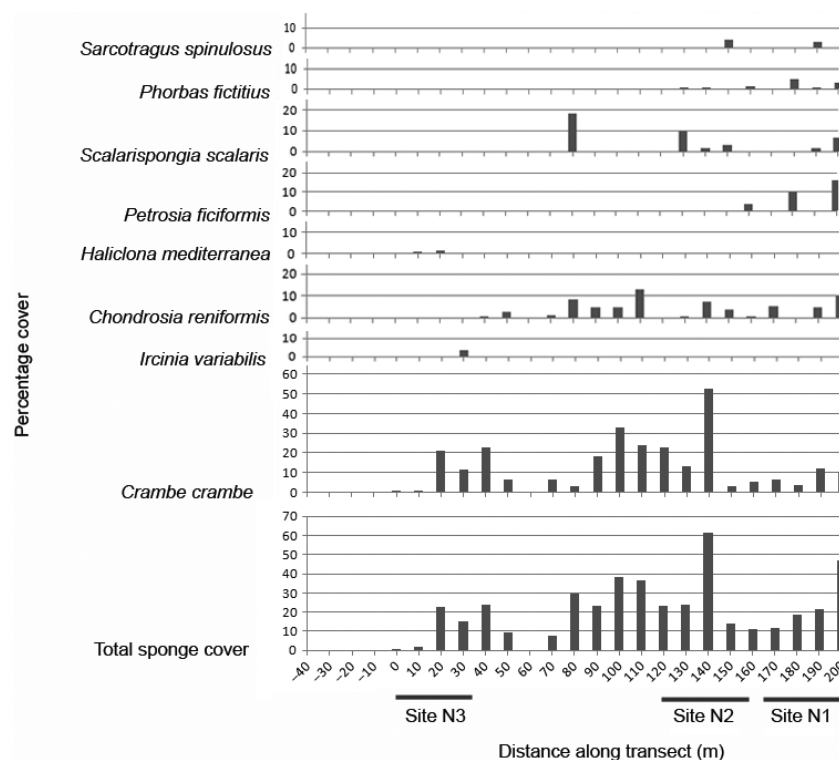
Fig. 4. Mean cover of sponge species (n = 3) from quadrats taken at 10-m intervals along a transect along the north wall of Castello Aragonese. The transect included sampling stations N1 (mean pH 8.14), N2 (mean pH 7.87) and N3 (mean pH 7.09).

the formation of complex spicules and consequently preventing species with more complex skeletons from colonising the vent sites. Experimental studies would be useful in determining whether this is the case.

Other studies on the vent sites have noted a shift from typical rocky shore communities with abundant calcareous organisms at the control sites to communities lacking scleractinian corals with significant reductions in sea

urchin and coralline algal abundance (Hall-Spencer *et al.* 2008). Although total algal cover remained relatively consistent throughout the sites, there was a shift from calcareous algal dominance to dominance by non-calcareous algae at the low pH sites (for example *Caulerpa, Cladophora, Asparagopsis, Dictyota* and *Sargassum;* Hall-Spencer *et al.* 2008; Porzio *et al.* 2011). However, many of the sites at which no sponge cover was recorded lacked macroorganisms, consequently greater competition from non-calcareous than calcareous algae is unlikely to be a controlling factor.

Studies of other hydrothermal vent communities in the Mediterranean have indicated that although sponge species biodiversity can be higher at vent than non-vent sites (Morri *et al.* 1999; Pansini *et al.* 2000), proximity to vents had no influence on sponge percentage cover (Pansini et al. 2000). In these studies no direct measurements of pH or attempts to correlate sponge biodiversity with pH were made. The hydrothermal vent fluids were recorded as having pH varying from pH 5.2 to 7.1 (Dando *et al.* 1999) but when diluted by the surrounding seawater the pH is likely to be higher. The study sites were large, encompassing a depth range of several metres and consequently even those in closest proximity to the vents will have encompassed a range of pH conditions.

Elevated CO_2 levels have been proposed as a causative factor for the late Permian mass extinction (Knoll *et al.* 1996; Pörtner *et al.* 2004). The extinction was particularly pronounced among reef-building metazoans such as calcareous sponges and rugose corals (Pruss & Bottjer 2005) and other organisms with carbonate skeletons (Knoll 2003; Dias *et al.* 2010). Disassociation of skeletal elements makes the study of siliceous species more difficult, but in South China extinction rates of 88–92% of spicule forms have been shown at the Permian–Triassic boundary, indicating extinction of the majority of siliceous sponge species, including all hexactinellids and species with tetraxons (Liu *et al.* 2008). Although the presence of a calcified skeleton is recognised as a factor in sensitivity to elevated CO_2 (Knoll *et al.* 1996; Pörtner *et al.* 2004), other aspects, also found in siliceous sponges, such as low-metabolic rate, absence of a circulatory system and elaborated gas-permeable surfaces, may also result in increased vulnerability (Knoll *et al.* 1996). Sponges are important functional components of marine ecosystems; playing roles in substrate modification and bentho-pelagic coupling, as well as having a large number of associations with species from other phyla (Bell 2008). Consequently, a future CO_2 rise may have significant ecological effects on sponges. Our study indicates that some demosponge species are likely to be more vulnerable than others and further research into the underlying mechanisms would be useful in predicting likely outcomes.

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ORIGINAL ARTICLE

Long-term patterns in the establishment, expansion and decline of invading macrozoobenthic species in the brackish and marine waters of Southwest Netherlands

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Abstract

The fluctuations in densities or biomass of a number of invading and native polychaete and mollusc species in the Southwest Netherlands were compared over a period of 20 years. For recent invaders a lag phase of 7–10 years occurred after their first appearance, followed by an exponential increase in abundance or biomass for 2–3 years. High numbers and biomass then continued for about 5 years, followed by a strong decline. The total sequence from introduction to decline lasted about 15 years. The densities or biomass of invaders appearing decades or even centuries ago in the Delta area have fluctuated in a similar manner to those of native species, indicating that the densities or biomass of invading species after a 15-year period of strong changes become governed largely by the same environmental factors as native species. The conclusion may be that after some decades, invading species can become part of a balanced co-existence with the native species, and that this may yield a net gain in the overall diversity.

Introduction

An increasing number of invading species has been observed worldwide, and consequent problems have been acknowledged (Streftaris *et al.* 2005; Simberloff 2011). Studies on invading marine species are far less numerous than studies of freshwater and terrestrial species; a Science Direct query for 'Invad* and Exotic' resulted in 52,262 papers, of which only 1016 (2%) discuss the marine realm. However, in the last two decades reports on the appearance and effects of invading and exotic species in the marine realm have also become increasingly numerous (of the 'marine' papers, 82 were published from 1990 to 1994, 134 from 1995 to 1999, 265 from 2000 to 2004, and 457 from 2005 to 2009). The reports mainly acknowledge the rate of range expansions, spatial patterns (globalization of ecosystems), the role of transport vectors, the influence of environmental factors (impact of climate change), the availability of resources (space and food), the impact on other species in the community and on ecosystem services and the characteristics of successful invaders (see Sakai *et al.* 2001; Stachowicz *et al.* 2002; Troost 2010; and references therein). However, an understudied topic is the long-term population dynamics of an invading species, i.e. the development of a species' population after its introduction or appearance, its (vigorous) increase and eventually its decline and subsequent stabilization or disappearance. A few studies (Stachowicz & Byrnes 2006; Branch *et al.* 2008; Rius *et al.* 2009) mention changes in densities of invading species over a period of a couple of months or years, yet are restricted in their time span and thereby do not show the process to its full extent. The reason might be that it takes long-term sustained observation over many years to assess the full process from appearance to decline. These long-term datasets from sustained observation have been scarce until recently, as it is only during the last two decades, due to national legislation or European

regulations (the Water Framework Directive for example), that a stronger emphasis has been placed on sustained monitoring, which may be able to provide such long-term datasets.

The large-scale monitoring of macrozoobenthic communities of soft sediments during the last 20 years in the waters of the Southwest Netherlands gives an insight into the development of populations, including those of the invading species. The aim of the current study is to evaluate the development over time, from arrival to eventual decrease, of a number of invading species that have recently entered the Dutch Delta area in comparison with both native species and those species that invaded the area many decades or centuries ago.

Material and Methods

The study area is the main outlet of the rivers Rhine, Meuse, and Scheldt in the southwest of the Netherlands known as the Delta area (Fig. 1). After disastrous flooding in 1953, it was decided to turn the estuarine Delta areas into a series of semi-enclosed, more or less stagnant, marine, brackish and freshwater basins (Wijnhoven et al. 2008). These basins include the Oosterschelde sea-arm, which was turned into a semi-enclosed marine bay by the building of a storm-surge barrier, and Lake Grevelingen, which was initially turned into a brackish lake and is now a saltwater lake (Nienhuis & Smaal 1994; Engelsma et al. 2010).

Since 1959, samples of macrozoobenthos have been taken on a regular basis from the various basins of the Delta by researchers of the NIOZ and their predecessors. All these data are stored in the database of the Monitor Taskforce of the NIOZ (BIS; Benthos Information System, database, version 1.20.0) (Wijnhoven et al. 2008).
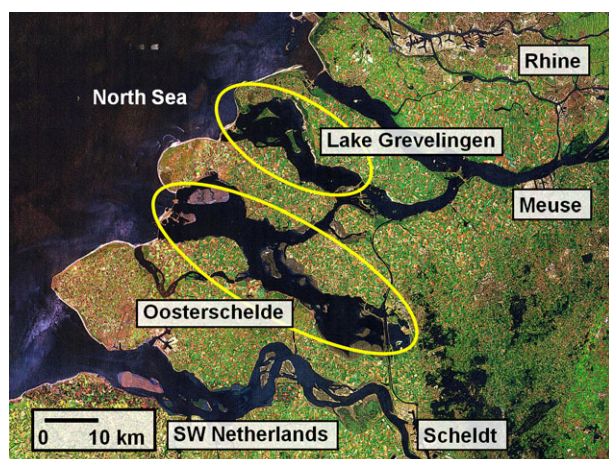
Fig. 1. The location of the study areas in the southwest of the Netherlands.

This database currently contains more than 80,000 samples, largely related to the Dutch Delta area. This makes the database highly suitable for analysis of the arrival and development of invading species in the different Delta waters. Since 1990, within the framework of the Monitoring Programme of the National Water Systems (MWTL), the Monitor Taskforce of the NIOZ has monitored the benthic macrofaunal communities of the Dutch Delta in a more standardized way (e.g. Engelsma et al. 2010; Wijnhoven et al. 2010). Samples are taken twice a year, in spring and autumn, for a minimum of 120 samples from the Oosterschelde and 60 from Lake Grevelingen. Three sediment cores (Ø 8 cm) are taken from each station and merged into a pooled sample (total surface area 0.015 m^2). Sediment cores are collected directly in the intertidal zone at low water, whereas subtidal cores are taken using a Reineck box corer deployed from a ship. The contents of the cores are gently washed in a 1-mm sieve and the material retained on the sieve is contained, stained with Rose-Bengal and preserved with a 4% buffered formalin solution. The macrofaunal groups are identified to the highest possible taxonomic level.

For the present study all available data on the viewed species were clustered per half year, taking all data for the two different seasons separately but averaging overall strata and/or sub-regions in the basins, and recalculating the data into species densities or biomass (ash-free dry weight) m^{-2}. To identify the dates of first recordings of species our data have been combined with records available in the literature.

The long-term changes in densities of two groups of species have been compared. First, a group of smaller polychaetes were compared – the recently invading *Syllis gracilis* and *Tharyx marioni* that entered the Delta area in the 1950s, and the native *Exogone naidina*. *Syllis gracilis* was observed in the Netherlands for the first time once in the Oosterschelde in 1940 (Korringa 1951; Wolff 2005). However, the species was not found again until 1990 when it was rediscovered in Lake Grevelingen (own monitoring data available in the BIS database). *Tharyx marioni* was first described for the Netherlands by Korringa in 1951 (at that time called *T. multibranchiis*) and has been common in all Dutch Delta waters including Lake Grevelingen and the Oosterschelde since the 1960s (Wijnhoven & Hummel 2009). *Exogone naidina* is an abundant species native to the Netherlands.

Secondly, from a group of medium-sized suspension feeding bivalves, the recent invader jackknife clam, *Ensis directus*, was compared with the soft-shell clam, *Mya arenaria*, which invaded the Netherlands some centuries ago (Lasota et al. 2004; Wolff 2005), and the native cockle *Cerastoderma edule. Ensis directus*, originally an American species, was first sighted alive in Dutch waters

in 1981 in the Wadden Sea (Armonies & Reise 1999; Wolff 2005). During the 1980s the species expanded along the North Sea coast to the southern part of the Netherlands. In 1989 the first specimen of *E. directus* in Dutch Delta waters was found in the Oosterschelde. In 1991 the species was also found in Lake Grevelingen (Wijnhoven & Hummel 2009). *Mya arenaria* may already have been introduced from America to Europe in the 13th century, but the first official observation is from 1765 (Streftaris *et al.* 2005; Wolff 2005). This species is now common in all Dutch Delta waters, as is the native *C. edule* (Wijnhoven & Hummel 2009).

Results

Two recently invading species, the polychaete *Syllis gracilis* (Fig. 2a) and the mollusc *Ensis directus* (Fig. 2b), showed a similar pattern of changes in density in Lake Grevelingen as well as the Oosterschelde. After a lag period with very low densities or biomass for 7–10 years after their first recordings, the densities and biomass increased exponentially to high levels over a period of 2–3 years. The high densities and biomass remained for another 3–5 years, after which the populations exhibited marked declines.

The densities and biomass of the invaders that arrived decades or centuries ago in the Delta area have fluctuated in a similar (erratic) way as those of the native species (Fig. 2a and b). These seemingly erratic changes are a normally occurring phenomenon among the benthic species in areas like the Dutch Delta (Coosen *et al.* 1994; Seys *et al.* 1994).

The total sequence of events from the first appearance of an invading species, through the time-lag, the exponential increase phase and then levelling off and decrease, lasted for about 15 years and was followed by a phase of balanced co-existence among the native species. This sequence can be visualized in a generalized pattern of population development for invasive species after arrival in a new environment (Fig. 3).

Discussion

The kind of long-term sustained monitoring programmes followed in the Southwest Netherlands, with samples taken each half year at many (60–120) stations per basin, has been instrumental in obtaining a sufficiently long record to be able to compare the long-term population dynamics of newly invading species from arrival to expansion and subsequent decline, coupled with the fluctuations of other macrobenthic species.

A lag phase between initial colonization and the onset of rapid population growth, followed by an exponential increase, is a common phenomenon in plants (Sakai *et al.* 2001 and references therein), but to our knowledge it has not been described before for marine macrozoobenthos. Here we have shown a 7- to 10-year lag phase and a 2- to 3-year exponential increase phase. The lag phase for plants has been attributed to the sorting and evolution of the best adaptive ecotypes of a species following a (multiple) colonization effect (Sakai *et al.* 2001), and the same may hold true for the studied species here.

The sequence of events in the densities and biomass of recent invaders, together with the observed fluctuations of earlier invaders resembling the pattern of native species, indicates that after a lag phase and exponential growth, invading species gradually become governed by the same (environmental) factors determining their population densities. Annual changes in densities and biomass are a regular phenomenon among the benthic species in areas like the Dutch Delta, primarily caused by the interplay of environmental variables and biotic interactions such as temperature, currents, food concentration, and parasite or predator occurrence, all affecting processes like reproduction, growth, dispersal or competitive capacities of species (Coosen *et al.* 1994; Seys *et al.* 1994). Moreover, in the Dutch Delta, anthropogenic impacts such as the control of the sluice regime for Lake Grevelingen, which affects the water exchange with the North Sea, have an impact on the densities of benthic species (Wetsteijn 2011). Both *Cerastoderma edule* and *Mya arenaria* showed increased densities after a period of higher water exchange. Minor fluctuations in densities or biomass are generally the result of differences in timing or are the effect of increased abiotic environmental parameters such as temperature, salinity, water currents, turbidity and nutrient levels on biotic processes such as reproduction, growth, dispersal and competitive capacities.

A lack of competitors and natural enemies (including diseases) is frequently mentioned as a reason for the exponential growth of invaders (e.g. Bax *et al.* 2001; Troost 2010). The adaptation of predators to consume the new invaders and the evolution of parasites may then be important factors in the subsequent decline in the density of invaders and result in them becoming regulated similarly to native species.

The information on the duration of the lag phase, expansion, and period with high numbers and biomass, as observed in our study for two species, may contribute to models on the rate of species introductions and the success of these biological invasions, as described by Wonham & Pachepsky (2006) and Miller *et al.* (2007). However, it might be expected that the duration of the lag phase and the period with high invader densities is environment-specific, as indicated by
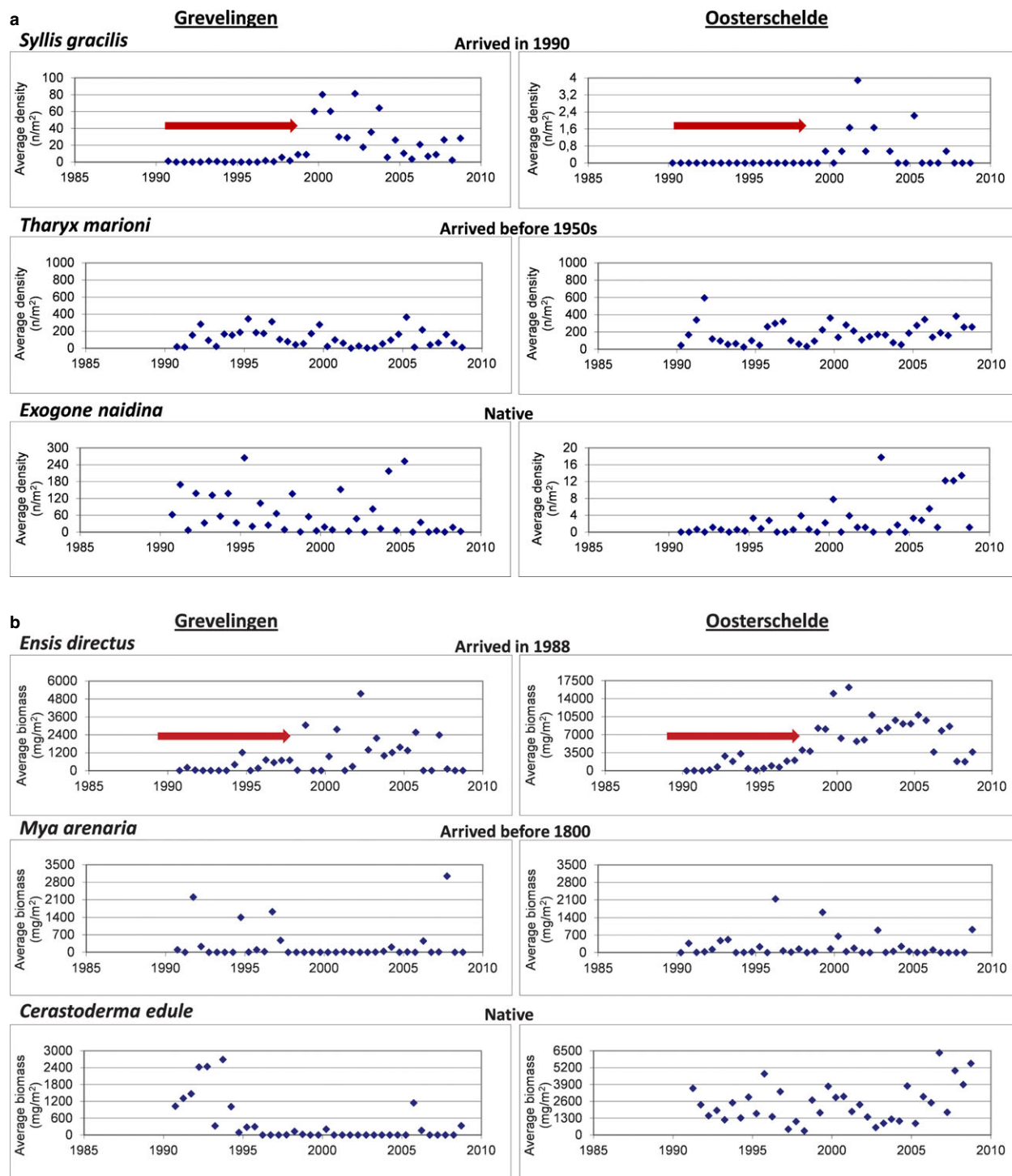
Fig. 2. The fluctuations in densities of a recent invading polychaete (a) and in biomass of a recent invading mollusc (b) compared with the fluctuations in earlier invading and native species.

observed differences in susceptibility between systems (Vermonden *et al.* 2010) and observations of recurrent and consecutive invasions in certain systems (e.g. Leuven *et al.* 2009).

We conclude that invasions can eventually result in a balanced co-existence with the native species, and that this may yield a localized net gain in diversity, as has also been suggested by Stachowicz & Byrnes (2006).
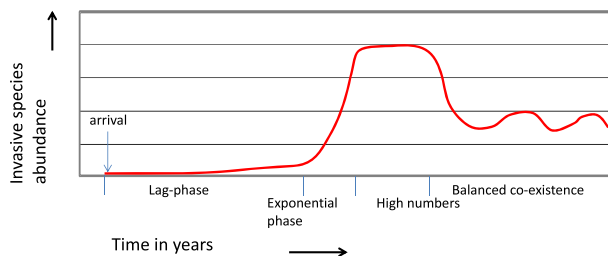
Fig. 3. Conceptual pattern of population development for invasive species after arrival in a new environment.

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ORIGINAL ARTICLE

Diet of mussels *Mytilus trossulus* and *Dreissena polymorpha* in a brackish nontidal environment

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Abstract

Benthic suspension feeders have traditionally been considered an important link between pelagic and benthic food webs in shallow seas. Their ecological impact depends highly on the removal rate of pelagic production from the water column. Besides phytoplankton, benthic suspension feeders can feed on microphytobenthos and detritus suspended in the water column by wind-waves or tidal forces. The share of the pelagic component in the diet of benthic suspension feeders is poorly studied in the non-tidal environment. We examined the diatom food items of two bivalve species, *Mytilus trossulus* Gould and *Dreissena polymorpha* Pallas, at four shallow locations in the Gulf of Riga basin, in the Baltic Sea, differing in hydrographic characteristics. The share of pennate benthic diatoms was always higher in mussel food than in ambient water, whereas pelagic species were under-represented. Mussels never consumed the dominant genera that were in the water column, *Cylindrotheca* and *Leptocylindrus*. There were no differences in the main food source between the two mussel species. Our results suggest that the impact of benthic suspension feeders on pelagic foodwebs and their role in promoting benthic–pelagic coupling is of a minor importance in the study area, whereas the impact on benthic food webs may be mainly by the re-arrangement of energy within benthic pathways.

Introduction

Benthic suspension feeders are an important functional guild in aquatic ecosystems (e.g. Cloern 1982; Higgins & Vander Zanden 2010). Suspension feeding bivalves can reach extremely high biomasses and, in favourable conditions, often dominate communities of the shallow coastal sea (e.g. Kotta & Witman 2009). The energy fluxes in coastal sea ecosystems cannot be understood without a sufficient knowledge of the food sources that benthic suspension feeders exploit (Tomczak *et al.* 2009). The classical model of a coastal foodweb includes pelagic primary producers as the main source of energy for both the pelagic and benthic suspension feeders, while the latter constitute an important energy pump from the water column to the sea floor. Benthic–pelagic coupling occurs when benthic animals remove planktonic production from the water column and move it to

the sea floor in the form of faeces and pseudofaeces (Newell 2004).

However, this point of view has been undermined by several recent studies from tidal systems, which have suggested that microphytobenthos is an important, or even the main, component of the food available to benthic suspension feeders (Kang *et al.* 1999; Machas *et al.* 2003; Kasim & Mukai 2006). Microphytobenthos can exceed estuarine phytoplankton both in production and biomass (Shaffer & Sullivan 1988; De Jonge & van Beusekom 1992). Also, it attains the highest biomass in the top few millimetres of the sediment, which can be easily resuspended by hydrodynamic forces (MacIntyre *et al.* 1996; Lucas *et al.* 2000; Guarini *et al.* 2008). However, studies mostly using stable isotope analyses have found fundamental differences in the origin of organic matter in suspension-feeding pathways among different estuarine systems and even within the same estuarine system

(Ruckelshaus *et al.* 1993; Yokoyama *et al.* 2005, 2009; Kanaya *et al.* 2008; Dang *et al.* 2009). Benthic suspension feeders have been found to rely on both benthic and pelagic microalgae and may at times rely almost entirely on one or the other food source (Kamermans 1994; Riera & Richard 1996; Herman *et al.* 2000; Kang *et al.* 2006, 2007). Additionally, in some estuaries, detritus of macrophyte, riverine or terrestrial origin is the prevailing food source (Kasai & Nakata 2005; Kanaya *et al.* 2008). Observed differences among and within estuaries may be attributed to the spatial patchiness of different food sources. This, in turn, is related to physical characteristics of estuaries (riverine inputs and water residence time, marine impact, tidal range, area of tidal flats, turbidity) that determine the amount and spatiotemporal distribution of food, including the biomass and resuspension rates of microphytobenthos. However, no general patterns have been described to date. To complicate the issue even more, isotopic signatures of different suspension feeder species have been found to differ even at the same place and time, indicating either a reliance of species on different food sources (Dubois *et al.* 2007) or on some unknown attributes related to the methodology. Small-scale spatial variability in signatures within species also remains to be explained (Dubois *et al.* 2007). To conclude, the causes of the striking variability in the main food pool of benthic suspension feeders are still not fully understood, and the environmental factors influencing it remain unexplained.

Most of the studies discussed above have been conducted in macrotidal estuaries, and a few studies have been conducted in tidal bays. Benthic suspension feeders also inhabit microtidal and nontidal environments, where they may constitute a large share of zoobenthic biomass and consequently form an important part of the ecosystem (Kautsky & Kautsky 2000; Higgins & Vander Zanden 2010). Microtidal and nontidal waterbodies are often in close contact with extensive human populations, thereby having high economic and aesthetic value, as well as high rates of exploitation and human impact relative to the surface area. It is, therefore, of the utmost importance that we get better understanding of the functioning of such ecosystems.

Benthic suspension feeders are important in non-tidal habitats as a food source for higher trophic levels (Öst & Kilpi 1998; Lappalainen *et al.* 2005), as autogenic engineers (Ward & Ricciardi 2007), and by removal of phytoplankton from the water column (e.g. Budd *et al.* 2001; Petersen *et al.* 2008). Their feeding activity, in turn, also impacts benthic communities (Kotta *et al.* 2009). Despite their obvious importance, little is known about either the food sources of benthic suspension feeders in nontidal systems or the potential spatial variability of their feeding.

Even if resuspension is controlled by strong tidal forces, the influence of wind on the resuspension of benthic algae can be detectable and significant (Demers *et al.* 1987). In shallow areas of large non-tidal systems such as the Baltic Sea, the resuspension of microphytobenthos may be almost completely wind-driven. Epibenthic suspension feeders form distinctive populations with high biomasses in a number of coastal areas in the Baltic Sea, offering an excellent nontidal model system for the studies on benthic–pelagic coupling in wind-driven conditions. The roles of benthic suspension feeders in the ecosystem of the Baltic Sea are not very well documented.

Here, we aimed to test the impact of benthic suspension feeders on pelagic food webs and their role in promoting benthic–pelagic coupling in a shallow non-tidal water body. This was done by comparing the different diatom species in the water column with the diets of the mussels *Mytilus trossulus* Gould and *Dreissena polymorpha* Pallas in a shallow gulf in the Eastern Baltic Sea. Our main hypotheses were: (i) benthic suspension feeders, by actively feeding on phytoplankton, facilitate benthic–pelagic coupling in a non-tidal ecosystem and (ii) the observed relationship may vary according to spatial variability in environmental conditions. We predicted that the species composition of microalgae in the diets of benthic suspension feeders resembles that of the water column and the proportion of the diet comprising plankton is related to local hydrographic conditions.

Study area

The study area is located in the Gulf of Riga, Southeastern Baltic Sea (Fig. 1) and represents a non-tidal, brackish basin. The study was conducted in the unstratified

Fig. 1. Study area. Experimental sites 1, 2 and 3 represent sediment accumulation areas, site 4 represents an erosion area.

coastal zone in the northern part of the Gulf with a mean depth of 2 m. Current- and wind-induced water velocities are very low in the area compared with those in oceans, usually falling between 0 and 10 cm s^{-1} in the summer period (Kotta *et al.* 2008). Experimental sites had dissimilar hydrographic regimes; three study sites were depositional areas with silty sand and one site was erosional area with a gravel bottom.

Material and Methods

Two epibenthic suspension-feeding bivalves can be found in the Baltic Sea, both being common in the study area. The most widespread mussel species inhabiting the Baltic Sea was formerly known as *Mytilus edulis* L. and was later renamed *Mytilus trossulus* Gould. While the naming debate is not over yet, this species will be referred to as *Mytilus trossulus* in the present study. The bay mussel, *M. trossulus*, is the dominant suspension feeder on hard bottoms in the whole Baltic Sea, often forming dense banks in shallow coastal waters (Kautsky & Evans 1987). This species is of marine origin, and lower salinity limits both its performance and distribution within the Baltic Sea (Westerbom 2006). The zebra mussel, *Dreissena polymorpha* Pallas, is an invasive species from the Ponto-Caspian region that has been in the Baltic Sea for almost 200 years (Thienemann 1950). This species, although tolerant to salinity fluctuations, is confined to bays with salinity below 6 and is absent from the northern part of the basin. The species is primarily found in river estuaries, where it can build up remarkable biomasses (Järvekülg 1979; Olenin & Daunys 2005).

Field experiments were carried out on two dates within the warm season from May to September. The two bivalve species, *M. trossulus* and *D. polymorpha*, represented benthic suspension feeders. Mussels were allowed to adapt to the ambient conditions for 12 h before the start of the experiment. Mussels were located at 70 cm depth (30 cm above the sea bottom) at a total depth of 1 m. The height of the incubation unit from the bottom was comparable to the height mussels attached to boulders or macrovegetation commonly inhabited. Three mussels were placed in a net cage on the top of the funnel. Mussel biodeposits fell into the collecting vial below (Fig. 2; Kotta & Møhlenberg 2002). Five or six concurrent replicates were run at each site twice using different mussels. Each incubation lasted 4 h. The average shell length of mussels was 18.3 mm ± 2.5 SE for *M. trossulus* and 15.0 mm ± 2.3 SE for *D. polymorpha*, which corresponds to the mean size of mussels in the field. The taxonomic composition of diatoms in the water column and in mussel faeces was used to evaluate the contribution of planktonic food items in the diet of mussels.
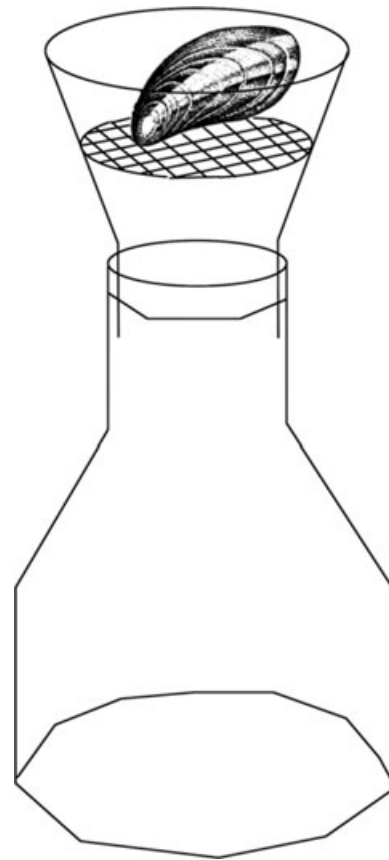
Fig. 2. A scheme of the experimental set-up.

At the beginning and end of each experiment, water samples were taken within 5 cm from the funnel containing the mussels to measure chlorophyll *a* (Chl-*a*) and planktonic diatom assemblages. The near-bottom temperature and salinity were monitored using CTD (conductivity, temperature, depth) profiling and current velocities with a JFE miniature electromagnetic current recorder. Salinity was measured on the practical salinity scale.

Water samples were immediately filtered through Whatman CF/F filters. The sedimented material in the vials was sorted under a dissecting microscope and faeces were collected with a pipette within 4 h of retrieval. Pseudo-faeces were not produced during the experiments. Filtered material was extracted in the dark with 96% ethanol overnight. Chl-*a* was quantified spectrophotometrically, correcting for phaeopigments (Pha-*a*) (Strickland & Parsons 1972). The values of Chl-*a* equivalent or total Chl-*a* (Chl-*a* eq) were calculated as Chl-*a* eq = Chl-*a* + 1.52 × Pha-*a*.

Water samples for diatom species analysis were fixed with acid Lugol's solution. A 50-ml aliquot of each sample was allowed to settle for 24–48 h in a combined chamber and cylinder, and cells were counted with inverted microscope (Olympus IMT-2), following the

method of Utermöhl (1958). A magnification of 200× was used for larger cells and cells along one or two diagonals were counted, depending on the density of the sample. The abundance of smaller cells was estimated by examining 20 or 30 objective fields using 400× magnification. Faecal samples for diatom species analysis were fixed with 4% formaldehyde solution. However, formaldehyde was a relatively inconvenient fixative for our purpose, as formalin-fixed samples tended to form lumps; due to this, a few samples were spoiled and were therefore excluded from the analysis. The following procedures were based on the method described by Vilbaste *et al.* (2000). Samples were treated with HCl to remove carbonates (0.5 ml for a 5-ml sample) and washed carefully by centrifuging with distilled water (three times, 10 min, 1520.5 *g*). Subsequently, one sample volume of concentrated H_2SO_4 was added to every sample. Samples were heated at 150–200 °C until all organic substances were removed from the frustules. Samples were then cooled down and bleached by adding tiny amounts of $KMnO_4$ until the solution was coloured light violet. Samples were then heated up again and, if the coloration disappeared, $KMnO_4$ was added again. The process was repeated until the light violet coloration was found to persist. After that, a small amount of crystalline oxalic acid ($H_2C_2O_4$) was added to the solution to decolour samples. Then, samples were washed with distilled water and centrifuged five times. Samples were allowed to settle in phytoplankton sedimentation chambers and counted, similar to water algal samples. For faecal samples, 400× times magnification was used for counting, and 1000× magnification was occasionally used for identification. On average, 45 objective fields were examined at 400× magnification per sample and, on average, 165 algal cells were counted in every faecal sample (min. 18 cells, max. 760 cells, n = 49 samples). Identification of diatoms was performed to the closest taxon possible – to genus, species or subspecies level – according to the following references: Snoeijs (1993), Snoeijs & Vilbaste (1994), Snoeijs & Potapova (1995), Snoeijs & Kasperoviciene (1996), and Snoeijs & Balashova (1998). Unidentified diatoms were excluded from further analyses. Diatoms were grouped as benthic or planktonic taxa according to Hällfors (2004). To detect any size-related preferences, we measured the lengths of the diatoms. Cell sizes were measured for all counted cells using an ocular scale and volumes were calculated from cell geometry (HELCOM 1988; Hillebrand *et al.* 1999) or using standard size-classes (Olenina *et al.* 2006). Wet weight biomasses were calculated for individual taxa and for the total biomass in all samples (http://helcom.fi/Documents/Action areas/Monitoring and assessment/Manuals and Guidelines/Manual for Marine Monitoring in the COMBINE Programme of HELCOM.pdf). Relative abundances or biomasses of diatoms were used in statistical analyses.

Multivariate data analyses on the relative abundances of diatom taxa were performed using the statistical package PRIMER version 6.1.5 (Clarke & Gorley 2006). Similarities between each pair of samples were calculated using a zero-adjusted Bray–Curtis coefficient. The coefficient is known to outperform most other similarity measures and enables samples containing no organisms at all to be included (Clarke *et al.* 2006). PERMANOVA (Anderson *et al.* 2008) was used to test for statistical differences in abundance distributions of taxa in diatom communities between water and faecal samples and among sites. Significant differences in the species composition and dominance structure of diatoms between faecal and water samples indicated the dissimilarity between mussel diet and pelagic microalgal communities. The biomass ratio of benthic algae in faeces was divided using the same ratio in the ambient plankton, further interpreted as an index of independence of mussels from the pelagic food pool. This index was further investigated using ANOVA analysis (StatSoft 2007). A global BEST match permutation test was run to examine the relationship between the biomass ratio of benthic diatoms in faeces and cell lengths of diatoms in ambient water.

Results

The mean values of water salinity, temperature, velocity and Chl-*a* content are presented in Table 1. A total of 86 diatom taxa was recorded in water and faecal samples, including subspecies. Among these, 26 taxa were present only in water and 55 taxa only in faeces. Based on the literature, 17 taxa were classified as planktonic and 69 as benthic. Diatom communities in the water consisted of both planktonic and benthic taxa and the community

Table 1. Environmental factors during the experiments.

Site/time	T	S	Velocity	Chl-*a*
1 (accumulation area)				
May	14.7	3.8	0.0 ± 0.0	4 ± 1
July	25.6	4.5	6.7 ± 14.4	4 ± 1
2 (accumulation area)				
May	13.8	2.0	2.7 ± 3.0	21 ± 5
July	22.7	1.5	4.3 ± 10.7	8 ± 0
3 (accumulation area)				
May	14.5	2.5	2.9 ± 2.6	9 ± 1
July	27.2	4.9	1.0 ± 0.3	11 ± 6
4 (erosion area)				
May	14.7	3.8	10.0 ± 26.3	18 ± 1
July	28.1	4.2	0.0 ± 0.0	21 ± 16

T = temperature (°C); S = salinity (psu); Velocity = water current speed (cm s^{-1}); Chl-*a* = water chlorophyll *a* (mg m^{-3}), '±' SE values.

composition differed between sites (PERMANOVA pair-wise tests, P < 0.01) and between depositional and erosional areas (PERMANOVA, Tables 2 and 3).

The biomass ratio of benthic to planktonic diatom taxa was always higher in mussel faeces than in ambient planktonic communities (ANOVA, $F_{1,63} = 26.99$, P < 0.001; Fig. 3). The ratio of this benthic diatom dominance in faeces to water (i.e. the index of independence of mussels from the pelagic food pool) varied between sites (ANOVA, $F_{3,29} = 6.2$, P = 0.002). The abundance distributions in diatom communities also distinguished between mussel faeces and ambient plankton (PERMANOVA, Table 2) and this effect was consistent over all studied locations despite sedimentation characteristics (PERMANOVA pair-wise tests for all sites, P < 0.005; Table 3, Fig. 4). The two most common taxa in water, *Cylindrotheca* and *Leptocylindrus*, were absent in faecal samples. On the other hand,

most of the diatom species consumed by mussels were absent in water samples (Fig. 5).

The biomass ratio of benthic algae in mussel faeces was related to site (ANOVA, $F_{3,33} = 16.51$, P < 0.001). The

Fig. 4. MDS analysis visualizing differences in the dominance structure of diatom abundances between water and mussel faeces and among two hydrographic regimes.

Table 2. PERMANOVA test analysing the difference in the dominance structure of diatom abundances between water and faecal samples (factor: origin) and among accumulation and erosion areas (factor: hydrographic regime).

Factors and interactions	df	SS	Pseudo-F	P-level
Origin	1	21,819	8.35	0.001
Hydrographic regime	1	13,335	5.1	0.001
Origin × hydrographic regime	1	15,484	5.92	0.001
Total	64			

SS = sum of squares.

Table 3. PERMANOVA pair-wise *a posteriori* tests analysing the difference in the dominance structure of diatom abundances between water and faeces and among two hydrographic regimes.

Pair-wise tests	P-level
Water *versus* faeces in accumulation areas	0.001
Water *versus* faeces in erosion area	0.005
Accumulation *versus* erosion area in water	0.001
Accumulation *versus* erosion area in faeces	0.001

Fig. 3. The relative share of the biomass of benthic to planktonic diatom species in faeces compared to water (% wet weight).

Fig. 5. Relative abundances of dominant diatom taxa in faeces and water in accumulation and erosion areas.
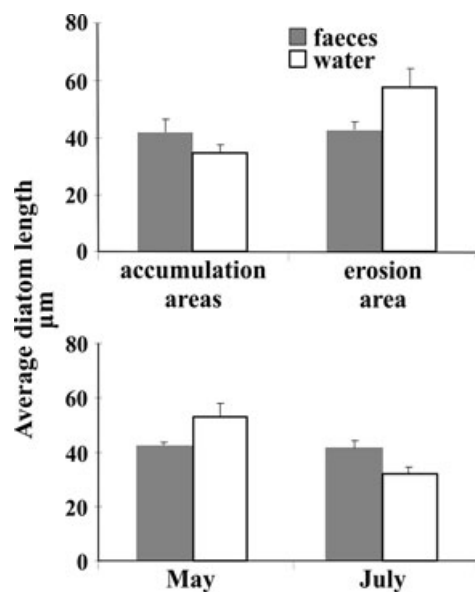
Fig. 6. Average length of diatoms in water samples and mussel faeces.

abundance dominance of diatoms ingested by mussels differed between all locations (PERMANOVA pair-wise tests, P < 0.02) and between depositional and erosional areas (PERMANOVA, Table 3). The feeding was not related to the bivalve species, as both *Mytilus trossulus* and *Dreissena polymorpha* fed on the same diatom species (PERMANOVA, P > 0.05).

Cell lengths of diatoms were different in faeces and water (PERMANOVA, $F_{1,64}$ = 25.41, P = 0.001). The average length of diatoms in water varied more than in faeces, indicating a possible preference towards certain size classes (Fig. 6). The share of benthic diatoms in faeces was not related to cell lengths of diatoms in water (BEST, P > 0.05).

Discussion

The main finding of the present study was that the diatom composition in the diet of mussels was significantly different from that in the water column. This indicates a relatively weak linkage between mussels and the pelagic food pool in the study area. Suspension feeding bivalves have been shown to be highly important in coupling pelagic and benthic processes by removing phytoplankton from the water column and depositing faeces on the bottom, which can result in strong effects on both pelagic and benthic foodwebs (Newell 2004). Documented impacts of recent invasions of zebra mussels have shown dramatic re-arrangements of energy pathways in a number of nontidal ecosystems, with losses of energy from

pelagic and an increased input to benthic foodwebs (Higgins & Vander Zanden 2010). The impact of bivalve suspension feeders on pelagic foodwebs may occur via both consumptive and competitive pathways (Wong *et al.* 2003; Prins & Escaravage 2005). However, in some cases no impoverishment of pelagic foodwebs has been observed (Wu & Culver 1991; Noonburg *et al.* 2003). This has been assumed to be related to poor water exchange between near-bottom and upper water layers in slightly deeper limnic systems (Ackerman *et al.* 2001). Nevertheless, in a marine, open, sandy sublittoral, with a well-mixed water column, benthic suspension feeders still appeared to be uncoupled from the pelagic primary production (Sasaki *et al.* 2004). Impacts of suspension-feeding zebra mussels on pelagic ecosystems were found to be substantial across large gradients of ecosystem size and trophic status, and generally larger in shallow, nonstratified ecosystems (Higgins & Vander Zanden 2010). MacIsaac *et al.* (1999) suggested that zebra mussels strongly enhance benthic–pelagic coupling in shallow lakes. Similarly, our study was conducted in a shallow and nonstratified ecosystem, inhabited by widespread benthic suspension feeder communities. Still, no causal relationships between the biomass of benthic suspension feeders and the status of adjacent pelagic communities have been described in this ecosystem to date. On the contrary, as demonstrated here, benthic suspension feeders are not likely to exert a strong impact on pelagic foodwebs in the studied ecosystem.

Commonly, removal of energy from pelagic foodwebs has been coupled with energetic inputs to the benthic system. Benthic macroalgae have been shown to profit from the presence of benthic suspension feeders both in tidal areas (Pfister 2007; Aquilino *et al.* 2009) and in our study area (Kotta *et al.* 2009). However, as suggested by the results of the present study, this phenomenon may not necessarily indicate the removal of energy from the pelagic and input to the benthic system. In our study area, the enhancement of benthic vegetation by benthic suspension feeders may rather indicate a re-arrangement of energy within the benthic system. Such a concept might elucidate a potential, yet rarely considered, role of benthic suspension feeders in modifying competitive interactions between different phytobenthic groups, e.g. by favouring macrovegetation over microphytobenthos.

Studies in tidal systems have revealed a large variability in food sources of benthic suspension feeders occurring at various scales (Riera & Richard 1996; Yokoyama *et al.* 2005, 2009; Riera 2007; Dang *et al.* 2009). Local hydrographic conditions may influence the resuspension and lateral advection of sediment and, therefore, generate spatial variability in the food source of benthic suspension feeders. The extreme importance of hydrodynamic

processes on benthic suspension feeders was demonstrated in two shallow micro- and non-tidal waterbodies of opposite hydrographic regimes (Sarà 2006). Resuspended sediments were present in the water column and constituted the main energetic pathway in a closed and completely wind-driven pond, but in the lagoon, which was open to substantial horizontal water movement, phytoplankton was the main suspended organic matter and also the main energetic pathway for benthic suspension feeders. Differences between locations based on sedimentation characteristics were much less extreme in the present study, and all locations were open to horizontal flow. Flow regime depended mainly on fetches and wind conditions, and three of our study locations fell in the range of accumulation areas, and only one location represented an erosion area. Accordingly, we cannot draw major conclusions about the generalizability of our results along the natural sedimentation gradient. Nevertheless, we would like to point out that the observed uncoupling between mussels and the pelagic food pool was consistent over all studied locations. Therefore, our results may hint that the main source of energy for mussels does not vary spatially within the range of variability in hydrographic characteristics recorded in the present study.

Temporal patterns in the availability of different food sources may also be of importance, as benthic suspension feeders in tidal systems have been found to use extensively pelagic food as a source during periods of lush pelagic food connected to seasonal phytoplankton blooms, especially the spring bloom, and to rely on other food sources during the rest of the year (Page & Lastra 2003; Lefebvre *et al.* 2009). Seasonal phytoplankton blooms in the present study area usually take place in early spring and late autumn when seawater temperatures are low at 2–7 °C. The feeding activity of studied mussels is low under 5–6 °C (Kotta *et al.* 2005). Summer blooms of cyanobacteria mainly form in the open areas of the Baltic Sea basin and are not found in the shallow coastal sea where the present study was conducted. The amount of phytoplankton in the water column is, therefore, relatively stable during the warm season in the study area (Fig. 7; Lauringson *et al.* 2009). Pervasive differences between ingested and water column diatom composition indicate that the extent of the reliance of mussels on the pelagic food source may have been unrelated to the recorded variability in water Chl-*a* content.

The abundance of certain benthic diatom species in the water column may be explained by their possible tychopelagic life mode in the study area. However, several benthic species were absent from water samples while still being abundant in mussel faeces. This leads to an intriguing question about the process that enabled mussels to feed on algae seemingly absent from the suspension. The
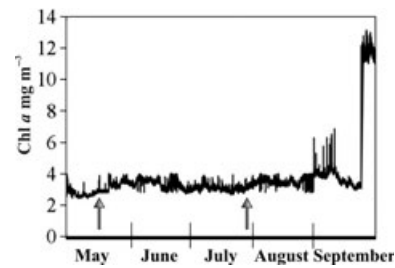
Fig. 7. Logger recordings for water Chl-*a* content at site 1 in 2003. Arrows indicate the dates of experiments. Modified from Lauringson *et al.* (2009).

questionable food source mainly comprised pennate benthic diatom species, which could have been resuspended by wind–wave action. Wind–waves reaching the bottom cause shear stress that peels off the top layer of the sediment together with the highly nutritious microalgal cover (Miller *et al.* 1996). Thereby, the shallow-water benthos is characterized by a thin near-bottom water layer with very high turbidity (Muschenheim 1987). This near-bottom 'layer' probably represents a highly dynamic system, where the wave action induces periodic resuspension events followed by resedimentation events. The thickness of the layer can possibly also display high spatial variability caused by bottom roughness, where any kind of obstructions like boulders can cause turbulent events and modify the strength of wave-induced stress to the bottom. Such variability in turbulence and in microalgal resuspension conditions has a direct effect on benthic suspension feeders. The amount of particles in water has been shown to decrease rapidly with increasing distance from the bottom, especially heavy particles such as sand grains and big algal cells (Muschenheim 1987). Microalgal particles have also been shown to settle extremely rapidly after resuspension events if the flow is switched down (Lucas *et al.* 2000, 2001), which may happen in a similar manner between wind-induced pulses of flow. Mussels have been shown to use up to a 7-cm-thick bottom water layer for feeding (Muschenheim & Newell 1992); however, mussels located on boulders, plants and other higher physical structures can feed at much higher positions and can reach upper water layers. Mussels in the present study were located at a height of 30 cm from the bottom. Thereby, the supply of particle-rich near-bottom water layer may have been facilitated by turbulent forces around experimental units similar to the forces around boulders and other protruding structures on the sea floor. Periodic resuspension events may have been occurred at a regular basis as a result of wind–waves. Bivalves may be expected to have higher consumption rates at the pulses of resuspended sediment. These differences may have been further accentuated by the selective capacities of

bivalves (Ward & Shumway 2004). For unknown reasons, certain taxa commonly occurring in the water column were completely absent in the diet of mussels in the present study, despite being within the size range of edible particles. The most important of these taxa, *Leptocylindrus* spp. and *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin, have a similar morphology with high length-to-width ratio (common dimensions of cells were 5–10 × 32–84 μm for *Leptocylindrus* and 1.5–5 × 32–105 μm for *C. closterium* in our samples). Suspension-feeding bivalves have been shown to select particles based on size (Ten Winkel & Davids 1982; Ward & Shumway 2004) and epicellular chemical substances may also be important selection cues (Ward & Targett 1989; Beninger & Decottignies 2005; Espinosa *et al.* 2010). Cells of *Cylindrotheca* spp. have been found to be selectively rejected by cockles fed on a mixed diet of diatoms (Rueda & Smaal 2002), and Rouillon *et al.* (2005) found *Leptocylindrus danicus* Cleve and *C. closterium* to be absent in stomach contents of blue mussels at two contrasting locations, while both species were important planktonic taxa in these habitats. Nonetheless, behind the apparent absence may have also stood a possible independence of mussels from the pelagic food pool and a use of another food source, instead of selective rejection. Indeed, our results may not necessarily suggest selective feeding. Any conclusions related to the selectivity of feeding by mussels in the present context would presume detailed information about the composition of all available sources of food and perhaps further experimental manipulations. To clarify the mechanism of feeding of mussels on items not represented in our water samples, measurements of temporal variability in near-bottom water Chl–*a* content, with a resolution similar to the scale of wave frequency, might be beneficial.

In wind-driven systems, predominant resuspension events follow much finer temporal scale compared with macrotidal estuaries. The significance of wind–wave-generated resuspension for benthic suspension feeders may be masked by the resuspension or flushing due to tidal forces in tidal systems, and is perhaps more easily detectable in nontidal systems. Wind-driven resuspension processes of microphytobenthos may also be significant in macrotidal systems (Demers *et al.* 1987; De Jonge & van Beusekom 1995) and it is possible that such wind-driven processes may contribute to the variability in food sources of benthic suspension feeders in tidal systems as well.

The current study was carried out in an area with a monotonously flat topography where bottom slopes rarely exceed a few degrees at scales from tens to hundreds of metres. Bottom slope may strongly impact the hydrodynamic conditions of the location, including water exchange in the near-bottom water layer (Slinn & Riley 1996). Due to its flat topography, the study area is presumed to have a relatively weak supply of fresh phytoplankton from the open sea to near-coastal areas that can be reached by benthic suspension feeders. The consequent temporal scarcity of lush planktonic food may, in turn, decrease the reliance of benthic suspension feeders on the pelagic food source. Observed feeding habits probably reflect the utilization of the richest food source available. To test the generalizability of our finding and the possible variability in food sources of benthic suspension feeders within the studied ecosystem, it seems essential to cover larger gradients in topographic conditions, including steep bottom slopes inhabited by extensive mussel beds in other parts of the Baltic Sea.

Conclusions

Feeding strategies of benthic suspension feeders in non-tidal systems are generally poorly investigated. It still remains to be shown how benthic suspension feeders exploit various local food pools available in such environments. We found that benthic suspension feeders were poorly utilizing the pelagic food pool in a flat shallow non-stratified, non-tidal coastal area. The diets of zebra and blue mussels were quite similar and clearly distinguished from diatom communities in the water column in both May and July. This effect was consistent within the four studied locations with different hydrographic regimes. Most diatom taxa consumed by mussels tended to be absent in the water samples taken, indicating an unknown mechanism for food supply, perhaps in short pulses related to wind–wave action. Our results suggest a need to re-evaluate the role of benthic suspension feeders as consumers of phytoplankton in shallow nontidal ecosystems.

Acknowledgements

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ORIGINAL ARTICLE

Environmental factors contributing to the development of brown muscle disease and perkinsosis in Manila clams (*Ruditapes philippinarum*) and trematodiasis in cockles (*Cerastoderma edule*) of Arcachon Bay

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Abstract

The aim of the present study was to identify environmental factors that could explain the distribution of different pathologies of commercially exploited intertidal bivalves in an Atlantic lagoon, Arcachon Bay. In particular, the role of the salinity gradient as a driver was explored. The Manila clam *Ruditapes philippinarum* experienced two severe pathologies, perkinsosis, which is due to a protozoan parasite, and brown muscle disease (BMD), the etiological agent of which remains unknown. *Perkinsus olseni* infection was very low in a small low-salinity area but, at the scale of the entire lagoon, was more influenced by organic matter content in the sediment and by emersion time. BMD prevalence was also 2.6 times higher in the higher organic content area but was also negatively correlated with salinity. The sympatric cockle (*Cerastoderma edule*) was affected by eight trematode species. These parasites have a complex life cycle that generally involves three free-living host species. The distribution of the different trematode communities was rather patchy within Arcachon Bay with no clear relationship with measured environmental factors. The dominance of particular trematode species could be due to the presence of other hosts involved in their life cycle, making it more difficult to detect a major environmental driver. This survey demonstrated that salinity is not a major factor explaining disease distribution in this temperate lagoon. This result has consequences for the research of 'refuge areas' (free of diseases) or 'hot spots' (heavy infection) for species of high economic value.

Introduction

In coastal ecosystems, salinity is generally a strong driver of community structure (Blanchet *et al.* 2005). This is particularly true in systems such as estuaries where the gradient is obvious (Wolff 1973; Ysebaert & Herman 2002; Rybarczyk & Elkaim 2003). Typically, three types of faunal communities are encountered, associated with euhaline, polyhaline, mesohaline and oligohaline waters, respectively. All of these have both pelagic (Herman *et al.*

1968; Orsi & Mecum 1986; Baretta & Malschaert 1988; David *et al.* 2006) and benthic components (Attrill & Rundle 2002; Ysebaert & Herman 2002). Parasite species may also display strong relationships to salinity gradients when their life cycles involve free-living stages sensitive to water mass characteristics. This has been described in protozoans such as *Perkinsus* spp. that parasitize numerous mollusc species (Ahn & Kim 2001; La Peyre *et al.* 2006). When the host dies, the parasite transforms into a stage, the hypnospore, that spends some time in the

water before being inhaled by a new host (Choi & Park 2010). The various species of the genus *Perkinsus* are known to be limited by low salinity (Leite *et al.* 2004). Most trematode species also display free-swimming larval stages in their life cycles, usually including two such stages. The parasite reproduces sexually in the definitive host. Eggs are emitted into the water with faeces and develop into miracidium larvae which infect the first intermediate host, always a mollusc. Asexual reproduction in this host leads to the formation of a new type of larvae, cercariae, which are shed into the water and swim or drift before infection of the second intermediate host. There, larvae remain in a latent stage, the metacercariae, and wait for their host to be preyed upon by the final host in order to complete their life cycle. Many studies have demonstrated that these swimming larvae are sensitive to salinity (Mouritsen 2002; Koprivnikar *et al.* 2010), suggesting that this factor could contribute to explaining the structure of parasite communities.

However, in lagoons, salinity gradients are not always so pronounced and other structuring factors may interfere with the process. These include emersion rate, seagrass occurrence, and sediment grain size (Bachelet *et al.* 1996; Marzano *et al.* 2003; Blanchet *et al.* 2004). This results in a mosaic of communities where the major structuring factors are not always easy to detect. In relation to parasites, there is little knowledge on the factors that drive their distribution in these sheltered areas. For fishermen, such knowledge could contribute to identifying 'refuge areas' that are more or less exempt from infectious diseases (Hoffmann *et al.* 2009).

Arcachon Bay is a good system to illustrate and study these questions. It is a typical tidal lagoon with a cape sheltering most of the system and with freshwater inputs. Previous studies performed on free-living intertidal fauna demonstrated that the salinity gradient was not the only driver of the distribution of pelagic (Vincent 2002) and benthic communities (Bachelet & Dauvin 1993; Blanchet *et al.* 2004). Arcachon Bay also supports the highest production of Manila clam *Ruditapes philippinarum* in France and periodically sustains a small cockle (*Cerastoderma edule*) fishery also.

Both bivalves are susceptible to pathological conditions. Perkinsosis (*Perkinsus olseni*) is very prevalent in Manila clams in the whole bay, with no detected gradient (Dang *et al.* 2010). In 2005, a new disease of the bivalve, brown muscle disease (BMD), was described for the first time (Dang *et al.* 2008). The infectious agent is still unknown, although a virus is suspected (Dang *et al.* 2009). Finally, trematodes are abundant, mostly in cockles. An assemblage of 13 trematode species was identified in previous studies (Desclaux *et al.* 2002; de Montaudouin *et al.* 2009) but the distribution of these parasites at the lagoon scale remains unknown. Trematodes induce a less severe impact on their cockle hosts than that observed in Manila clams infected with perkinsosis and BMD, except when the intensity (number of parasite individuals per infected host (Bush *et al.* 1997)) becomes high in cockles infected as second intermediate hosts (Desclaux *et al.* 2004) or when the cockle is the first intermediate host (Jonsson & André 1992; Thieltges 2006).

Our specific aims were: (i) to describe a series of biotic and abiotic factors in Arcachon Bay in order to identify homogeneous entities, i.e. groups of stations that were defined by similar environmental characteristics with (salinity, temperature, emersion rate, grain-size median, seagrass biomass); (ii) to assess levels of infection in cockles and clams; and (iii) to identify environmental factors correlated with the various diseases. The general aim was to explore whether it is possible to identify 'hot spots' (accumulation of infections) or 'refuge areas' (sites free of pathogens) by measuring biotic and/or abiotic factors.

Study Site

Arcachon Bay (44°40′ N, 1°10 W) is a macrotidal (tidal range = 0.9–4.9) coastal lagoon situated on the south-western coast of France. This 156-km^2 ecosystem is connected to the Atlantic Ocean by a 2- to 3-km-wide and 12-km-long channel. Channels represent 41 km^2 of the lagoon surface and penetrate between large intertidal areas (115 km^2). A significant proportion of these tidal flats (46 km^2) is covered by *Zostera noltii* seagrass beds (Plus *et al.* 2010). Arcachon Bay receives freshwater inputs from the northeast and south but mainly from a river (Leyre) located in the southeastern end of the lagoon. The balance between marine and freshwater inputs and the slow renewal of water by tides induce salinity and temperature gradients (Robert *et al.* 1987).

Material and Methods

Sampling procedure

In October and November 2009, a total of 39 stations were sampled along two axes (i.e. two sub-areas) drawn between the most seaward part of the lagoon and the most landward, within the Manila clam and cockle habitats (Fig. 1). Stations were sampled at low tide. Six 0.25-m^2 quadrats were sampled by hand to collect clams and cockles. When the number of collected individuals was insufficient, additional individuals were collected haphazardly in the immediately surrounding area. After all stations had been sampled, we selected individuals
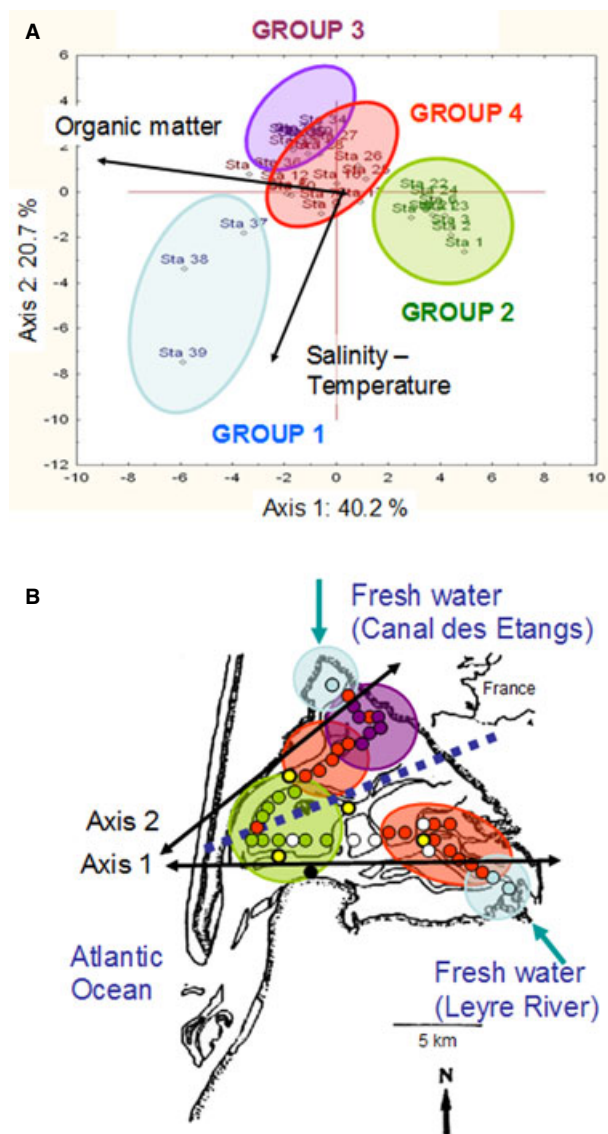
Fig. 1. Principal component analysis (PCA) based on 21 environmental factors (A) from 33 stations (B). Four groups can be separated. Organic matter content in the sediment contributed to Axis 1, and salinity and temperature contributed to Axis 2. Group 1: blue; Group 2: green; Group 3: violet; Group 4: red. White: no values. Dots with the above-mentioned colours represent stations where bivalves were collected. Yellow dots represent stations that were surveyed within the ARCHYD network to obtain ground-truth values of salinity and temperature for model validation. Black dot shows the Eyrac tide gauge to calibrate sea surface height with the model and to deduce emersion time.

with a shell length of 28–36 mm, a length range that was common to all stations, for further analysis. Sediment was sampled for analysis of organic matter and median grain size. Pictures were taken for analysis of seagrass biomass (see below) using a Fujifilm FinePix S9500 camera at a resolution of 1600 × 1200 pixels.

Environmental factors

Grain size and organic matter
At each station, the upper 3 cm of sediment was collected and subsequently analysed for grain-size distribution (wet sieving) and organic matter (loss of weight of dry sediment at 450 °C for 4 h).

Biomass of seagrass foliage
A rapid but reliable method of assessing the biomass of *Zostera noltii* leaves was developed for this study, during a preliminary sampling trip. Fifteen 15 × 15 cm quadrats were carefully laid over the sediment surface at low tide. These quadrats were visually selected to represent a large range of vegetation cover, from 0% (bare sediment) to 100%. For each quadrat, a numerically identified photograph was taken from directly overhead, 1 m above the surface.

All leaves in the quadrat were cut at their base with scissors. Back at the lab, each sample was washed, weighed (fresh weight) and dried at 60 °C for 48 h to obtain a dry weight.

On each photograph, polygons corresponding to bare sediment were drawn using image analysis software (ENVI 4.2, ITT Corp., White Plains, NY, USA). The surface of these polygons was automatically calculated and the seagrass cover was deduced from the quadrat surface. The leaf dry weights and the surface covers were correlated following logarithmic transformation of both variables (and after removing one picture without grass). The relationship was described by the following equation:

$$\ln(DW) = 1.450 \times \ln(S) - 1.733, \text{ with } R = 0.98 \, (n = 14 \, \text{pictures})$$

where $\ln(S) = 0.690 \times \ln(DW) + 1.195$

DW is the dry weight in $g \cdot m^{-2}$ of *Z. noltii* leaves and S is the percentage of sediment covered by *Z. noltii*. The biomass in fresh weight (FW) could also be obtained:

$$FW = 13.2 \times DW, \, R^2 = 0.95 (n = 15)$$

Therefore, from a numerically identified picture, it was possible to obtain a biomass with reliable precision. This method was more precise for lower seagrass covers; as vegetation cover approaches 100%, the method becomes less precise because 100% cover may correspond to many biomass values, depending on seagrass bed thickness.

The drawing of polygons allowed us to obtain the relationship between leaf biomass and seagrass cover but was highly time-consuming for routine survey. Consequently, we used a line-drawing method to estimate foliage biomass more rapidly. This method consisted of drawing three equidistant lines across each numerical image and

counting the intersections between lines and leaves. The 15 × 15 cm frame was placed with leaves perpendicular to one of the square sides and lines were drawn at right angles to leaves. The best correlations were found after logarithmic transformation of both variables (intersection and biomass) and described as follows:

ln(DW) = 1.514 × ln(mean number of intercepts per line) − 1.9113, with R = 0.98 (n = 14)

This method was utilized to determine aerial biomass in the 39 investigated stations (10 frames per station). Less than 10 min per photograph, including line drawing, were necessary to estimate aerial biomass.

Temperature, salinity and emersion time

Temperature, salinity and emersion rates for each sampling site were obtained by means of a mathematical model (MARS; Lazure & Dumas 2008) that had been previously applied and validated on Arcachon Bay (Plus *et al.* 2009). This three-dimensional hydrodynamic model calculates free surface height variations, current speed and direction, water temperature and salinity, at a 235-m resolution on the horizontal plane (10 meshes on the vertical) and at time steps ranging from 10 to 60 s. The model was used during a 3-year period (November 2006 to November 2009), and the following parameters were recorded for each site:

1 Temperature and salinity minima, maxima and means.
2 Percentage of time spent in emersion.
3 Frequency histograms for temperature (percentage of time spent at temperatures below the following thresholds: 8, 12, 16 and 20 °C). These threshold values cover the range of values found in this area and allow detection of particularly low and high temperatures from 'rare events'.
4 Frequency histograms for salinity (percentage of time spent at salinities below the following thresholds: 13, 28 and 34). These threshold values cover the range of values found in this area and allow detection of particularly low and high salinities from 'rare events'.

Boundary conditions were provided by the global tidal solution FES99 (Lefèvre *et al.* 2002) and the atmospheric forcing parameters – air temperature, atmospheric pressure, nebulosity, relative humidity and surface wind stress – were provided by the ARPEGE model (Centre National de Recherche Météorologique, Toulouse, France).

Comparisons between available ground-truth values and model simulations were performed to validate the mathematical model for the study period (from November 2006 to November 2009). Empirical temperature and salinity data were taken from the ARCHYD database (Ifremer), selecting four stations located along the clam sampling axes (Fig. 1). Sea surface height (SSH) observations at the Eyrac tide gauge (Fig. 1) were provided by

the REFMAR website (refmar.shom.fr) and remain the property of SHOM (Naval Hydrographic and Oceanographic Service) and the Gironde DDTM (Sea and Territory Departmental Directorate). Model evaluation was performed following Piñeiro *et al.* (2008), regressing observed *versus* predicted values and testing the significance of slope = 1 and intercept = 0. This analysis was complemented by RMSD (root-mean squared deviation) and EFF (model efficiency) calculations:

$$RMSD = \sqrt{\frac{\sum_{i=1}^{n}(Y_{imod} - Y_{iobs})^2}{n}}$$

$$EFF = 1 - \frac{\sum_{i=1}^{n}(Y_{imod} - Y_{iobs})^2}{\sum_{i=1}^{n}(Y_{imod} - Y_{iobs})^2}$$

where Y_{imod} and Y_{iobs} are respectively the predicted and observed values and n is the total number of values.

Table 1 summarizes the results of the model validation. Observed *versus* predicted values regressions showed that the model behaved satisfactorily. Best model performances were obtained for sea surface elevation and temperature, with a very high percentage of variance in observed values explained by the model (respectively 97% and 98%). The worst model performance was obtained for salinity but the coefficient of determination still remained high (85%). All tests for slope = 1 and intercept = 0 were passed and model efficiency was near 1.

Table 1. Regression parameters (slope a and bias b, for the $Y_{obs} = aY_{mod} + b$ equation), coefficient of determination (r^2), Theil's partial inequality coefficients (U_{bias}, U_{slope} and U_{error}, are the proportions of observed variance not explained by the predicted values but due to, respectively, mean differences between observed and predicted values, slope error and unexplained variance), root mean-squared deviation (RMSD, expressed in the same units as the variables) and model efficiency (Eff; the closer Eff is to 1, the better the model), for observed *versus* predicted variables (temperature – TEMP, salinity – SAL, sea surface height SSH).

	SSH	Temperature	Salinity
a	1.008	0.958	0.910
Significance of test a = 1	0.20	0.09	0.18
b	0.016	0.376	3.086
Significance of test b = 0	0.33	0.35	0.16
Degree of freedom	915	34	34
r^2	0.97	0.98	0.85
U_{bias} (%)	0.034	0.125	0.039
U_{slope} (%)	0.002	0.071	0.049
U_{error} (%)	0.964	0.804	0.912
RMSD	0.19	0.78	0.79
EFF	0.96	0.98	0.84

Theil's partial coefficients show that most of the errors in model predictions were due to unexplained variance and not to bias or to misdirection. Temperature, salinity and SSH root-mean squared deviations were 0.78 °C, 0.79 and 19 cm, respectively.

Bivalve models and associated pathology

Manila clam and Perkinsus

All collected Manila clams (*Ruditapes philippinarum*) belonging to shell length class 28–36 mm were opened (34 stations of 39 investigated stations harboured Manila clams with adequate shell length) and gill samples were excised. Five gills from clams with identical lengths were pooled and weighed for analyses of *Perkinsus* infection levels as determined by the FTM (fluid thioglycolate medium) assay (Ray 1966). Depending on clam availability, between two and six pools (except one pool in a single station, no. 21) were obtained. For induction of prezoosporangia (hypnospores), gill samples were placed in separate 15-ml tubes containing 9.5 ml FTM supplemented with 66 $\mu g \cdot ml^{-1}$ streptomycin, 32 $\mu g \cdot ml^{-1}$ penicillin G and 40 $\mu g \cdot ml^{-1}$ nystatin (final concentrations), to prevent bacterial and fungal activity. The tubes were incubated at room temperature for 7 days, in the dark. After incubation, the samples were stored at 4 °C until hypnospore enumeration. To lyse tissues, samples were centrifuged at 664 *g* for 10 min. Pellets were added with 5 ml 2 N NaOH, and incubated at 60 °C for at least 1 h. This step was repeated before pellets were rinsed twice with 10 ml 0.1 M phosphate-buffered saline (PBS). Final pellets were resuspended in 1 ml PBS and hypnospores were counted twice using a Malassez counting chamber.

The concentration of *Perkinsus* was correlated to the different variables of the environment (Pearson correlation, after verifying normality of residuals), and was compared among the four spatial groups (Kruskal–Wallis test due to heteroscedasticity) and between the two axes, i.e. between the two sub-areas of the lagoon (Student's *t*-test) (STATISTICA 7 software, Statsoft Inc., Tulsa, Oklahoma, USA).

Manila clam and BMD

All collected clams belonging to the 28- to 36-mm shell length class were opened and an index of the pathology (muscle print index, MPI) estimated. On the posterior adductor muscle (the only affected one), the MPI was used to designate the surface colonized by the brown muscle print on a scale of 0–4 as follows: 0 (healthy), 1 (0–25%), 2 (25–50%), 3 (50–75%) and 4 (75–100%). When both valves displayed different pathology indices, the highest category was selected to characterize the stage of BMD. Prevalence was defined as the percentage of infected hosts (Bush *et al.* 1997).

Cockles and trematodes

When possible, five cockles between 13- and 29-mm shell length per station were opened. The flesh was separated and squashed between two large glass slides. Trematodes were identified and counted under a stereomicroscope (de Montaudouin *et al.* 2009). Trematode abundance was defined as the mean number of metacercariae per individual host, and prevalence as the percentage of infected hosts (Bush *et al.* 1997). A correspondence analysis was performed on data which consisted of a '35 stations × eight trematode species' matrix when each species occurred. Data (averaged metacercariae abundance) were $\log_{10}(x + 1)$-transformed. In the case of *Bucephalus minimus*, it was not possible to separate and count sporocysts and a value of 1 was arbitrarily imposed in the matrix. Particular attention was devoted to the identification of the 'contributive' taxa. A taxon was considered 'contributive' when its contribution to the dimension's inertia was at least twice the mean theoretical contribution of a taxon. Considering that the eight taxa of the matrix contributed to 100% inertia, a 'contributive' taxon inertia should arbitrarily be over $(100/8) \times 2 = 25\%$.

Results

Environmental factors

The principal component analysis separated four spatial groups (Fig. 1A). Group 1 isolated a small number of stations at the mouth of the two freshwater inputs (Canal des Etangs and Leyre) (Fig. 1B). This group was characterized by low mean salinity (22.6), high frequency of T < 16 °C (50%), high organic matter and silt and clay contents in the sediment (7.8% and 38.5%, respectively), null emersion time, and low seagrass coverage (7%) (Table 2). Group 2 gathered stations in oceanic position with high median grain size (191 μm), high mean salinity (32.8), mean temperature similar to elsewhere in the lagoon (17 °C) but with low occurrence of cold events, i.e. low frequency of T < 8 °C (4.2%), low organic matter and silt and clay content in the sediment (3.2% and 16.3%, respectively) (Fig. 1, Table 2). Groups 3 and 4 displayed medium mean salinity (30.1), medium frequency of T < 8 °C (7.7%) (Table 2). Axis 2 displayed a higher percentage of silt and clay, organic matter in the sediment and emersion time and more extreme water temperatures than Axis 1 (Table 3).

Manila clam and perkinsosis

The mean concentration of *Perkinsus* in the bay was 62 000 cells g^{-1} (gill fresh weight, FW) and could reach 209 000 cells g^{-1} (gill FW) (station 33, Axis 2, Fig. 2). It

Table 2. Different characteristics of the environment averaged for each of the four groups that were defined by the principal component analysis (Fig. 2). ANOVA (F-value) or Kruskal–Wallis tests (H) were performed to compare values among groups.

	Group 1	Group 2	Group 3	Group 4	F	H	P
Sediment							
Median (µm)	$103^b \pm 52.8$	$\mathbf{191.2^a} \pm 85.3$	$96.5^b \pm 37.8$	$108.3^b \pm 22.5$		13.23	***0.004***
Silt (%)	$38.5^a \pm 11.3$	$\mathbf{16.3^b} \pm 16.3$	$38.8^a \pm 3.2$	$29.5^a \pm 8.3$	8.32		***<0.001***
Organic matter (%)	$7.8^{b,c} \pm 2.6$	$\mathbf{3.2^a} \pm 2.5$	$\mathbf{9.1^c} \pm 1.3$	$\mathbf{6.2^b} \pm 2.5$	7.91		***0.000***
Seagrass cover (%)	$6.2^a \pm 10.8$	$72.2^b \pm 22.7$	$40.1^{a,b} \pm 23.4$	$44.9^{a,b} \pm 26.7$	6.24		***0.002***
Salinity							
Mean	$\mathbf{22.5^c} \pm 3.7$	$\mathbf{32.8^b} \pm 0.3$	$30.3^a \pm 0.3$	$29.9^a \pm 1.4$		23.16	***<0.001***
F < 13	$16.4^a \pm 8.7$	$0.0^b \pm 0$	$0.1^{a,b} \pm 0.3$	$0.3^{a,b} \pm 0.9$		14.64	***0.002***
F < 28	$\mathbf{66.6^a} \pm 19.0$	$1.8^b \pm 0.7$	$20.1^b \pm 3.9$	$22.7^b \pm 13.1$		22.87	***<0.001***
F < 34	$99.9^a \pm 0.0$	$\mathbf{75.1^b} \pm 7.8$	$99.8^a \pm 0.1$	$98.8^a \pm 1.5$		21.92	***<0.001***
Temperature							
Mean	$16.7^a \pm 0.2$	$16.7^a \pm 0.1$	$17.0^b \pm 0.1$	$17.0^b \pm 0.1$	31.80		***<0.001***
Minimal	$1.2^{b,c} \pm 0.4$	$\mathbf{3.1^a} \pm 0.9$	$0.8^c \pm 0.5$	$1.9^b \pm 0.9$	9.55		***<0.001***
Maximal	34.4 ± 0.5	33.1 ± 2.2	34.1 ± 2.1	32.2 ± 2.6	1.36		0.274
F < 8 °C	$9.5^a \pm 0.3$	$\mathbf{4.2^b} \pm 1.1$	$7.9^a \pm 0.2$	$7.5^a \pm 0.7$		23.74	***<0.001***
F < 12 °C	$31.6^a \pm 0.7$	$\mathbf{27.1^b} \pm 0.2$	$28.0^a \pm 0.2$	$28.1^a \pm 0.5$		23.11	***<0.001***
F < 16 °C	$50.4^a \pm 0.7$	$48.4^{a,b} \pm 0.4$	$48.0^b \pm 0.2$	$48.3^{a,b} \pm 0.3$		12.13	***0.006***
F < 20 °C	$62.7^{a,c} \pm 1.3$	$63.1^a \pm 1.4$	$59.5^b \pm 0.3$	$59.9^{c,b} \pm 0.7$		23.07	***<0.001***
Current F < 0.25 m s^{-1}	93.3 ± 6.9	95.6 ± 4.5	91.3 ± 10.4	82.2 ± 15.7	4.97		0.174
% Emersion time	$0.0^a \pm 0$	$30.9^b \pm 16.9$	$32.9^b \pm 11.4$	$13.0^a \pm 15.1$	6.26		***0.002***
Clam density (ind. m^{-2})	$16.2^{a,b} \pm 28.1$	$6.3^a \pm 7.9$	$51.4^b \pm 41.7$	$23.9^{a,b} \pm 18.6$		10.84	***0.012***
Distance (km)							
Leyre	$5.9^{a,b} \pm 8.1$	$13.8^a \pm 1.1$	$12.4^{a,b} \pm 1.3$	$8.2^b \pm 4.3$		10.66	***0.013***
Canal des Etangs	$9.8^{a,b} \pm 8.1$	$8.2^{a,b} \pm 0.9$	$3.3^a \pm 1.3$	$8.2^b \pm 4.1$		9.07	***0.028***
Atlantic Ocean	$12.7^a \pm 1.9$	$\mathbf{3.0^b} \pm 1.0$	$9.6^a \pm 0.4$	$9.5^a \pm 1.9$		22.06	***<0.001***

Superscript letters gather groups that are similar for a given parameter, values that are different from values of the three other groups (P < 0.05) are in bold and/or italics.

was positively correlated to organic matter concentration in the sediment, percentage of emersion, and distance to Leyre River and was negatively correlated to frequency of T < 16 °C and distance to Canal des Etangs (Table 4). There was a significant difference of *Perkinsus* concentration among spatial groups (Kruskal–Wallis, d.f. = 3, H = 22.58, P < 0.001), Group 1 (7400 cells g^{-1} gill FW), being different from Groups 2, 3 and 4, which displayed similar infection (70 193 cells g^{-1} gill FW). *Perkinsus* abundance was three times higher in Axis 2 (mean = 95 701 cells g^{-1} gill FW) than in Axis 1 (mean = 28 917 cells g^{-1} gill fresh weight) (Student's *t*-test, df = 33, t = −4.62, P < 0.001).

Manila clam and brown muscle disease

BMD prevalence per station in Manila clams throughout the bay was 11% (Fig. 3). BMD prevalence and MPI were positively correlated with silt and organic matter contents in the sediment and with salinity frequency S < 34. They were negatively correlated with minimal temperatures. In addition, MPI was positively correlated with mean water temperature and with Manila clam density (Table 4). However, the influence of freshwater input was not

similar between Canal des Etangs and Leyre. There was no difference of prevalence between spatial groups (one-way ANOVA, $F_{3,28} = 2.03$, P = 0.13). However, muscle print index increased from the most oceanic spatial group (G2) ($MPI_{mean} = 0.8$) to the more continental ones (G3 and G4) ($MPI_{mean} = 2.4$) groups (one-way ANOVA, $F_{3,28} = 11.74$, P < 0.001). Prevalence was 2.6 times higher along Axis 2 (16.1%) (see Fig. 1 for localization of both axes) than along Axis 1 (4.5%) (Mann–Whitney *U*-test, Z = −2.70, P = 0.007) (Fig. 3). MPI was similar in both axes (1.84) (Mann–Whitney *U*-test, Z = −1.55, P = 0.12).

Cockles and trematodes

Cockles were present in 35 of the 39 stations investigated. Eight trematode taxa were found. Seven species utilize the cockle as the second intermediate host. One of them belongs to the genus *Himasthla* and could be a complex of two species, *Himasthla quissetensis* and *Himasthla continua*. The 8th species, *Bucephalus minimus*, utilizes the cockle as the first intermediate host with a global prevalence of 13% (n = 144 cockles). Dimension 1 of the correspondence analysis (44.6% of inertia) discriminated *Himasthla interrupta*, whereas dimension 2 (23.6%

Table 3. Different characteristics of the environment in both axes (Fig. 1). Student's *t*-test (*t*-value) was performed to compare values between axes.

	Axis 1	Axis 2	*t*	P
Sediment				
Median (μm)	131.9	127.4	0.20	0.838
Silt (%)	23.5	32.1	−2.12	**0.041**
Organic matter (%)	4.3	7.3	−3.11	**0.004**
Seagrass cover (%)	44.6	51	−0.60	0.550
Salinity				
Mean	29.5	30.9	−1.38	0.178
F < 13	2.6	0.8	1.02	0.315
F < 28	25.2	15.2	1.49	0.144
F < 34	91.2	92.3	−0.27	0.790
Temperature				
Mean	16.9	16.9	0.30	0.765
Minimal	2.5	1.7	**2.28**	**0.029**
Maximal	32.1	33.7	−2.05	**0.048**
F < 8 °C	6.6	6.9	−0.39	0.700
F < 12 °C	28.2	27.9	0.69	0.489
F < 16 °C	48.6	48.3	1.33	0.191
F < 20 °C	61.3	60.9	0.57	0.573
Current F < 0.25 m·s⁻¹	85.3	91.9	−1.53	0.135
% Emersion time	11.4	28.3	−3.16	**0.003**
Clam density (ind. m⁻²)	17.6	27.7	−1.11	0.273
Distance (km)				
Leyre	7.2	13.3	−5.10	**<0.001**
Canal des Etangs	10.9	4.5	7.83	**<0.001**
Atlantic Ocean	8.4	7.4	0.83	0.414

Values that are different (P < 0.05) are in bold.

Fig. 2. Abundance of *Perkinsus olseni* (cells g⁻¹ gill fresh weight) per station in Arcachon Bay.

inertia) discriminated *Curtuteria arguinae* and *Himasthla* spp. (Fig. 4A).

Three trematode communities could be identified in the bay (Fig. 4B). *Himasthla interrupta* was present in the northern part of the bay only (mean abundance per station comprised between 3 and 22 metacercariae per cockle) and was accompanied by a high *B. minimus* prevalence (24% with n = 25 cockles), *Curtuteria arguinae* was the dominant trematode in the southwest end of the bay, i.e. the most oceanic part (mean abundance per station was 9–43 metacercariae per cockle, n = 34 cockles), often accompanied by *Diphterostomum brusinae*. The southeastern end of the bay was characterized by a higher abundance in *Himasthla* spp. (mean abundance per station was 9–43 metacercariae per cockle, n = 34 cockles) which represented the only trematode taxon present there.

Discussion

The aim of the study was to correlate the distribution of three types of bivalve diseases within a lagoon (Arcachon Bay) in order to identify the main drivers. Salinity is often cited as a strong contributing factor (Ahn & Kim

2001; Elandalloussi *et al.* 2008) but many confounding factors may intervene along the gradient. The unusual feature of Arcachon Bay was the presence of two major freshwater inputs that induce a bi-directional salinity–temperature gradient, east–west and north–south. We demonstrated that the effect of salinity was restricted to a very narrow area around the mouth of the river, for perkinsosis only. Indeed, *Perkinsus* concentration was significantly lower than elsewhere only in Group 1, at three stations near river mouths. The distribution of all diseases was explained less in the 'oceanic *versus* continental' axis than between either northwest or southwest sub-areas of the lagoon. This suggests that factors other than salinity are contributory.

The principal component analysis discriminated groups of stations in accordance with what could be expected in such an ecosystem: an oceanic-influenced group (Group 2), a continent-influenced group (Group 1) and two intermediate groups (Groups 3 and 4) (Bouchet 1993).

The mean abundance of perkinsosis infection was high compared to previous studies (Lassalle *et al.* 2007) but was lower than that assessed in Arcachon Bay in 2006 [96,000 cells g⁻¹ (gill FW) (Dang *et al.* 2010)]. This difference can be explained by the distribution of sampling stations, which in the present study took covered a
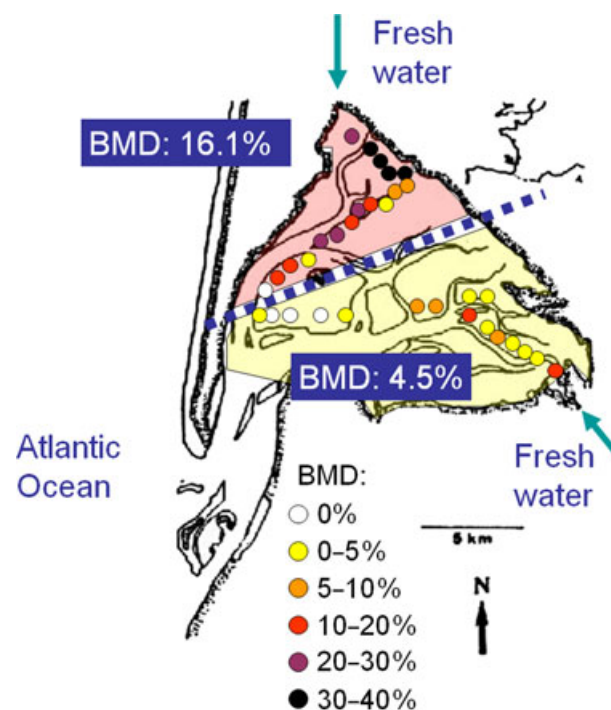
Table 4. Correlation between (1) *Perkinsus* concentration, brown muscle disease (BMD) prevalence, BMD's muscle print index (MPI) and (2) different parameters of the environment (n = 34).

| | *Perkinsus* concentration (cells g^{-1} of gills) | | BMD | | | |
| | | | Prevalence (%) | | MPI | |
	R	P	R	P	R	P
Sediment						
Median (μm)	−0.04	0.829	−0.27	0.141	−0.28	0.119
Silt (%)	0.25	0.149	**0.45**	**0.010**	**0.39**	**0.025**
Organic matter (%)	**0.35**	**0.040**	**0.52**	**0.003**	**0.42**	**0.015**
Seagrass cover (%)	0.09	0.594	0.07	0.713	−0.35	0.051
Salinity						
Mean	0.29	0.101	−0.10	0.580	−0.26	0.145
F < 13	−0.31	0.073	0.08	0.672	−0.03	0.865
F < 28	−0.27	0.127	0.07	0.681	0.29	0.098
F < 34	0.03	0.875	**0.37**	**0.039**	**0.72**	**<0.001**
Temperature						
Minimal	−0.29	0.093	**−0.37**	**0.034**	**−0.46**	**0.008**
Maximal	0.29	0.090	0.12	0.514	−0.02	0.900
Mean	0.09	0.614	0.12	0.6644	**0.66**	**<0.001**
F < 8 °C	0.00	0.990	0.32	0.071	**0.65**	**<0.001**
F < 12 °C	−0.26	0.131	0.18	0.330	0.21	0.245
F < 16 °C	**−0.35**	**0.043**	−0.00	0.987	−0.24	0.176
F < 20 °C	−0.17	0.344	−0.23	0.213	**−0.73**	**<0.001**
Current F < 0.25 m·s^{-1}	0.09	0.626	−0.16	0.390	−0.20	0.270
% Emersion time	**0.34**	**0.048**	0.13	0.491	0.03	0.875
Clam density (ind. m^{-2})	0.10	0.569	−0.12	0.502	**0.37**	**0.037**
Distance (km)						
Leyre	**0.43**	**0.011**	0.28	0.114	−0.28	0.113
Canal des Etangs	**−0.51**	**0.002**	**−0.60**	**<0.001**	−0.20	0.260
Atlantic Ocean	−0.14	0.422	0.20	0.264	**0.62**	**<0.001**

R, Pearson correlation coefficient.
Significant correlations (P < 0.05) are in bold.

Fig. 3. Brown muscle disease (BMD) prevalence per station in Arcachon Bay. Mean BMD prevalence per axis (i.e. sub-area) is noted.

broader area and included stations with low infection. Salinity is an important factor structuring the abundance of *Perkinsus* spp. in some locations (Leite *et al.* 2004). For *Perkinsus olseni*, the optimal salinity range is 25–35 (Auzoux-Bordenave *et al.* 1995) and high infection generally corresponds to high salinity (Burreson & Ragone Calvo 1996; Cigarría *et al.* 1997; Park & Choi 2001). In Arcachon Bay a similar gradient was obtained previously (Dang *et al.* 2010) but was mainly due to stations sampled near freshwater inputs. In the present study, the refuge function of low-salinity areas was evident (Group 1) but concerned <5% of the axis lengths. It should be

determined whether these low salinity areas are effective for commercially relevant growth rates in the shellfish. The salinity gradient of these areas was not high enough to induce a perkinsosis infection gradient. Unexpectedly, the highest difference in perkinsosis infection was observed between Axes 1 and 2. The environmental characteristics that were significantly different between axes involved sediment and emersion. The highest content in silt and clay, and organic matter in the sediment coincided with the highest emersion rate, the highest extreme temperatures and eventually the highest perkinsosis infection (Axis 2). Higher infection in muddy sediment than in sands has already been reported (Choi *et al.* 2002). Our hypothesis is also that the highest emersion rate (higher average hypsometric level) is due to (and/or is the consequence of) a smaller input of oceanic water during flood-tides, a lower water turnover and a higher sedimentation of fine particles. This lower turnover is consistent with an unpublished report that one-third of the water mass transits through Axis 2 against 2/3, through Axis 1 (SOGREAH 2001). That would increase the retention of *Perkinsus* hypnospores and facilitate infection between clams.

A similar observation was made with brown muscle disease (BMD). Interpretation is more difficult due to the lack of knowledge concerning the infectious agent of this disease, which might be a virus (Dang *et al.* 2009). Here
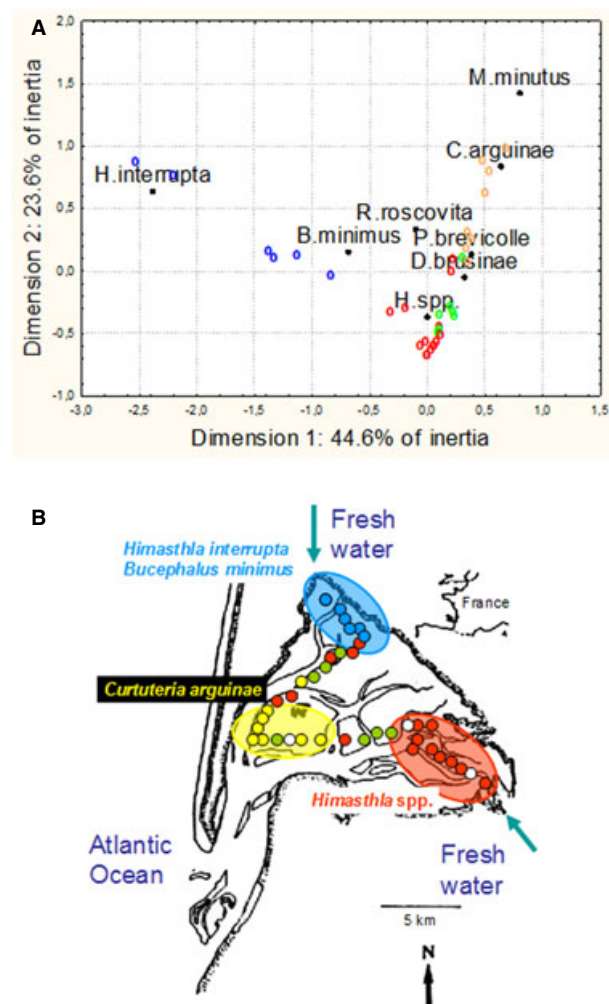
again, Axis 2 displayed higher prevalence in relation to sediment characteristics (but similar muscle print index MPI). This axis was also characterized by higher clam densities (+60%, although $P > 0.05$), which could facilitate disease transmission.

The situation of trematodes was different because many species were involved and the distribution corresponded to a community analysis pattern. At the lagoon scale, three communities were discriminated by one or two dominant trematode species, and these were related to the presence of their other hosts (Sapp & Esch 1994; Hechinger & Lafferty 2005; Byers *et al.* 2008), the first intermediate and definitive hosts, which are sensitive to environment characteristics. Trematode communities

were also different between the two areas of freshwater input (Leyre River and Canal des Etangs).

Our analyses were all correlative. Relationships between causes and consequences were not demonstrated but the identification of two different sub-ecosystems arose, independently of salinity gradient. For Manila clams, the notion of 'refuge' concerns a very small area and is not relevant for BMD. Hence, this is not interesting in terms of fishery management. However, the difference in infection between the two axes is important and suggests that fisheries models should be developed by sub-areas and not at the whole ecosystem scale (Bald *et al.* 2009). Trematode communities in cockles have a patchy distribution but the level of infection remained low compared with known pathological thresholds (Desclaux *et al.* 2004; Gam *et al.* 2009) and should, therefore, have a low impact in this system.

Acknowledgements

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ORIGINAL ARTICLE

Biodiversity in transitional waters: steeper ecotone, lower diversity

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Abstract

Benthic communities were studied in four transitional water ecosystems in the Mediterranean, located in Albania (Narta), Greece (Logarou) and Italy (Grado-Marano and Margherita di Savoia), with different degrees of salinity ranges, in order to investigate biodiversity trends across a scale of environmental stress. The intensity of natural stress in the transition zones, from the marine-based to the land-based influence, varied from gradual to sharp. The spatial variability in the physical environment had a stronger effect on species richness than did the temporal fluctuations. The sharper the spatial variations of salinity, the lower the number of species and the diversity level of the system. The differences in intensity of natural instability were also reflected by the presence of different sets of species, with the euryhaline species developing large populations and dominating the more enclosed systems, whereas the marine component of the fauna plays the most important role in increasing the level of benthic diversity.

Introduction

Coastal lagoons are ecotones between marine and terrestrial environments, receiving variable amounts of fresh water. Mediterranean lagoons are usually shallow water bodies and, due to their geo-morphological and hydrological characteristics, environmental conditions in them undergo frequent fluctuations on a spatial and seasonal basis.

Seawater renewal, depending on the degree of enclosure of the lagoon, affects most environmental variables and has a prominent role in the organization of biological communities. Natural instability leads to wide variations in species diversity. The recurring pattern of species richness, decreasing from the marine regions to the inner parts of the lagoon, has been widely documented (Guélorget & Perthuisot 1983; Lardicci *et al.* 1993; Koutsoubas *et al.* 2000; Bazaïri *et al.* 2003; Reizopoulou & Nicolaidou 2004). A strong relationship is documented between

diversity and confinement (*sensu* Guélorget & Perthuisot 1992), as environmental instability increases in relation to the degree of isolation from the marine influence (Reizopoulou & Nicolaidou 2004).

In coastal ecosystems with poor seawater inflow, several lagoon specialist species may tolerate extreme environmental conditions, and potentially develop large populations in a wide range of salinity gradients. Bamber *et al.* (1991) stated that lagoon specialists are better adapted to the environmental variability, most likely afforded by a degree of genetic plasticity; however, in salinity extremes a drop of species richness is expected (Cognetti 1992).

It is essential to investigate and compare spatial and temporal patterns of biota in coastal lagoons with various degrees of natural stress, in order to distinguish and classify the principal factors structuring communities, the role of critical extremes, and the degree of instability as a predictor of species distribution.

The present study is an attempt to link the degree of natural variability with biota variations in coastal transitional ecosystems, investigating the spatial and temporal variations of the zoobenthic communities along a range of environmental gradients in four saline and brackish Mediterranean lagoons.

Material and Methods

Sampling was carried out in four Mediterranean transitional ecosystems (Fig. 1) with a variable degree of enclosure and marine influence that was reflected by the salinity ranges within each system.

Grado-Marano, a lagoon characterized by high exchange with the sea, is situated in the North Adriatic Sea. Sampling was performed in the Marano basin. Grado-Marano has estuarine features, receiving fresh water input and being subjected to tides. The lagoon is affected by chemical pollution and eutrophication (Ianni *et al.* 2008; Ponti *et al.* 2008).

Logarou is on the north coast of Amvrakikos Gulf with inflow from the Louros and Aracthos rivers. The lagoon is separated from Amvrakikos by a narrow barrier, with openings allowing limited water exchange with the marine environment.

Margherita di Savoia is located along the Adriatic coast in Southern Italy and Narta in the Adriatic Sea in Western Albania.

Sampling in the lagoons was mostly performed in the bare sediment habitats during autumn of 2004 and spring/early summer of 2005. Six stations were sampled in Narta and eight stations in each of Margherita di Savoia, Grado-Marano and Logarou. Five replicate benthic samples were taken at each station with a box corer sampling 0.03 m² of the bottom. The samples were sieved through a 0.5-mm-mesh sieve and stained with Rose Bengal. Samples were preserved in 4% formalin. In the laboratory, the macrofauna was sorted, identified to a species level where possible, and counted. Temperature, salinity and dissolved oxygen were measured just above the bottom by temperature/salinity and oxygen probes at all stations for each study area over the two sampling occasions.

Spearman's rank correlation was employed to investigate the possible correlation between biotic and abiotic parameters. Community structure is described by multi-dimensional scaling (MDS) based on a similarity matrix constructed using the Bray–Curtis similarity index. The data were first transformed by $Y = \log(x + 1)$. The ANOSIM test was applied to the species abundance matrix testing the null hypothesis that there were no significant
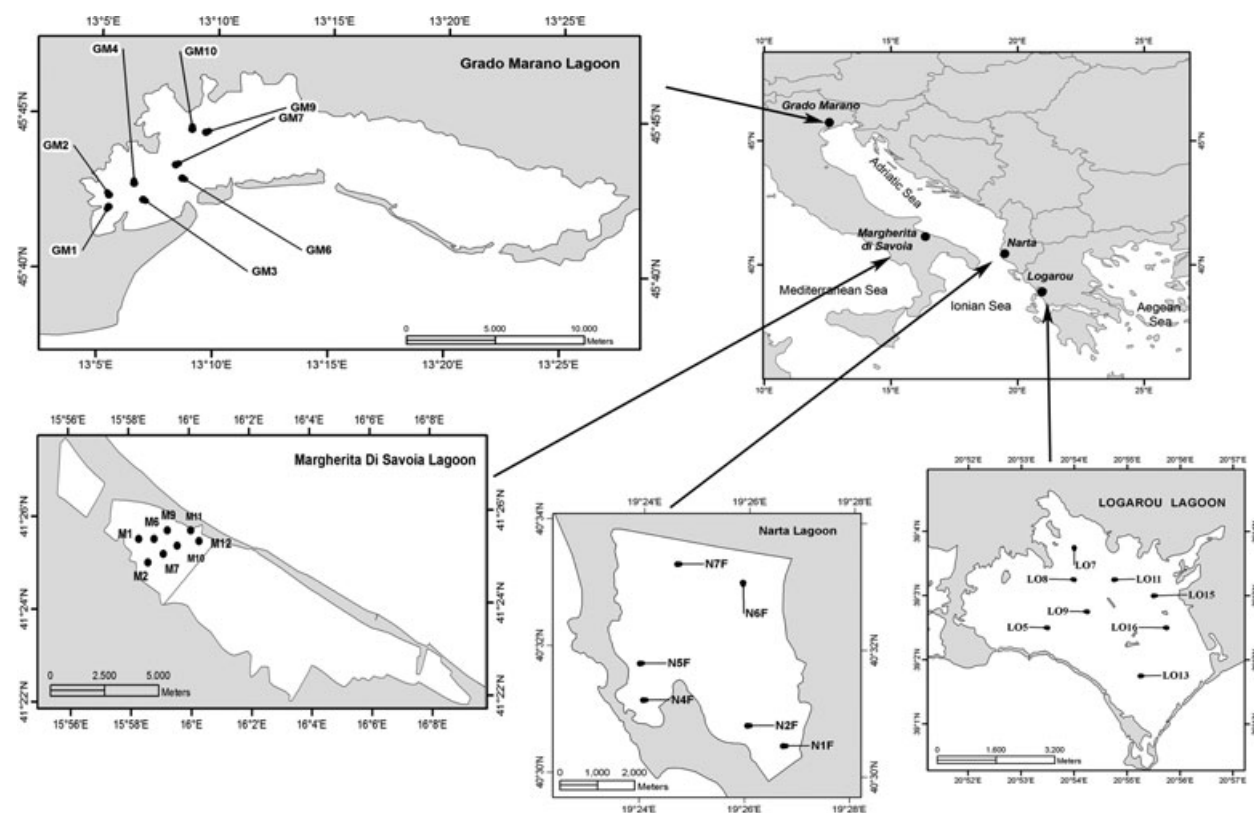

Fig. 1. Maps of the study sites.

differences among study areas and sampling times. Statistical analyses were performed using the PRIMER v5 software package.

Results

The surface area, tidal range and variations of the environmental parameters for each lagoon over the sampling seasons are shown in Table 1. In general, Grado-Marano and Logarou had high temporal ranges of the abiotic variables, whereas Margherita di Savoia and Narta mostly had high spatial ranges of environmental conditions. Salinity ranges in Margherita di Savoia and Narta reflected a strong spatial gradient, whereas Grado-Marano and Logarou demonstrated more marked seasonal changes.

Differences in the variation of abiotic parameters among the study areas could be attributed mostly to the degree of communication with the sea, the tidal range, the climatic factors including rainfall and wind, and the hydrogeomorphological characteristics, including depth.

Multidimensional scaling analysis based on species abundance identified distinct benthic assemblages associated with the different lagoon sites (Fig. 2). Seasonal differentiation was more evident in Narta, whereas Logarou, with high temporal variation in salinity, demonstrated a low seasonal variability in community structure. An ANOSIM test applied to the matrix of species abundances indicated that the lagoons differ significantly at a 0.001 level, except for Margherita di Savoia and Narta, where the significance level was 0.05. Logarou and Margherita di Savoia were the most different (R = 0.87, P < 0.001). The comparisons between autumn and spring for each lagoon showed a seasonal difference at the 0.05 level in all the study areas. The greatest seasonal effect was observed in Narta (R = 0.63, P < 0.05).

The species matrix compiled from the whole dataset can be classified in three major groups: euryhaline species (including lagoon specialists and species tolerant of broad salinity and temperature ranges), opportunistic species and marine species.

The species composition is similar to that of other Mediterranean transitional waters, with wide distribution of euryhaline species such as *Abra segmentum*, *Cerastoderma glaucum*, *Hediste diversicolor*, *Corophium* sp., *Gammarus* sp. and *Nephtys hombergii* (Guélorget & Perthuisot 1983; MarLIN 2006); however, the dominance of these species differed among the lagoons. The dominant species (>3%) for each study site are shown in Table 2.

Figure 3 shows the main groups of species forming the benthic communities: euryhaline, opportunistic and marine species. In the more eutrophic lagoon, Grado-Marano, the community shifts between opportunists and

Table 1. Surface, tidal range and variation of abiotic parameters for each study area over the sampling occasions.

| Lagoon	Surface (km²)	Tidal range (m)	Salinity Autumn Range	Mean (SD)	Spring Range	Mean (SD)	Temperature (°C) Autumn Range	Mean (SD)	Spring Range	Mean (SD)	Oxygen (mg l⁻¹) Autumn Range	Mean (SD)	Spring Range	Mean (SD)
Grado-Marano	142.0	0.65	23.0–30.0	26.5 (3.3)	17.4–27.2	22.3 (4.6)	5.3–7.7	6.5 (1.0)	19.9–21.9	20.9 (0.7)	9.7–10.6	10.2 (0.4)	6.3–7.9	7.1 (0.7)
Logarou	24.2	0.40	36.3–38.8	37.5 (0.8)	26.4–30.9	28.7 (1.7)	22.2–23.5	22.9 (0.4)	25.5–29.1	27.3 (1.4)	5.1–5.6	5.4 (0.2)	8.2–9.5	8.85 (0.8)
Margherita di Savoia	12.0	0.10	37.1–52.0	44.6 (5.4)	26.2–48.6	37.4 (3.2)	8.2–14.2	11.2 (2.9)	19.1–23.5	21.3 (1.9)	3.7–8.4	6.1 (2.0)	5.3–7.0	6.15 (0.7)
Narta	29.9	0.10	37.6–54.3	44.7 (7.7)	26.1–49.6	36.5 (10.5)	6.3–7.5	6.8 (0.4)	15.9–24.0	18.8 (3.5)	10.2–12.5	11.3 (1.0)	6.6–9.7	7.7 (1.3)

SD, standard deviation.
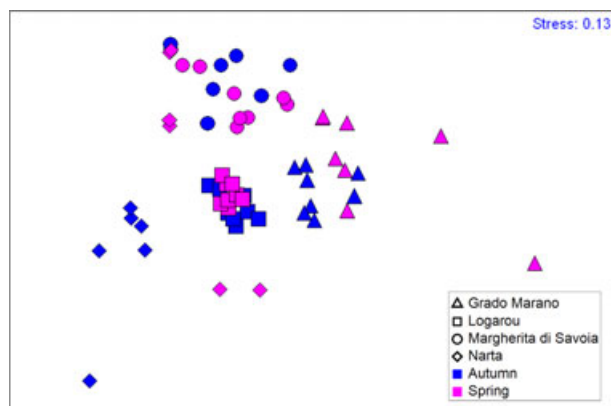
Fig. 2. Multidimensional scaling plot based on species abundance.

tolerant euryhaline species, depending on the seasonal variation of the environmental parameters and probably on the intensity of the eutrophication phenomena. The opportunistic group in Grado-Marano is largely composed of *Cirratulus* sp., which in autumn reached up to 90% of the total community abundance. In the more confined and undisturbed lagoons, euryhaline species form a predominant part of the community in both seasons.

The number of species forming each group is shown in Fig. 4. In Grado-Marano the higher number of species and the resulting higher diversity are associated with the marine influence, as evidenced by the elevated number of marine species. The rest of the lagoons dominated by the euryhaline species reflect their higher degree of enclosure.

The overall data analysis (Fig. 5) confirmed a large-scale pattern related to the degree of environmental changes, with mean species richness (P = 0.0001, R = −0.86) and mean diversity (P = 0.0002, R = −0.70) across lagoons being strongly correlated with the salinity variance, expressed as the standard deviation of the salinity values within each lagoon for each sampling occasion. The stronger the spatial salinity gradient, the lower the diversity and number of species of the ecosystem. Abundance and salinity variance showed a weak relationship (P = 0.03, R = −0.44). The correlations among the benthic descriptors and the mean values of salinity were not statistically significant.

Discussion

The contribution of different variables in structuring the natural gradient of transitional waters depends on the main hydrodynamic energy source of the system (Tagliapietra *et al.* 2009). The chemical and physical gradients influence the biota in many ways (McLusky 1993), so in coastal lagoons, species richness is not dependent on

Table 2. Dominant species (>3%) for each study area.

Species	Grado-Marano	Logarou	Margherita di Savoia	Narta
Polychaeta				
Armandia cirrhosa		+		
Capitella capitata			+	
Capitellidae	+			
Cirratulus sp.	+			
Glycera convoluta	+			
Glyceridae				+
Hediste diversicolor	+		+	
Hydroides dianthus		+		
Lumbrineridae			+	
Malacoceros fuliginosus	+		+	
Mediomastus sp.	+			
Micronephthys sp.	+			
Naineris laevigata		+	+	
Nephtys hombergii	+	+		
Nephtyidae				+
Nereis sp.	+			
Paraonidae	+			
Phyllodocidae				+
Prionospio caspersi	+			
Spio decoratus	+			
Streblospio shrubsolii	+			
Mollusca				
Abra alba	+			
Abra prismatica	+			
Abra segmentum	+	+	+	
Acanthocardia paucicostata	+			
Cerastoderma glaucum	+	+	+	+
Cyclope neritea		+		+
Cypraeidae			+	
Hydrobia acuta				+
Hydrobia ventrosa			+	
Loripes lacteus	+		+	
Mysella bidentata	+			
Pirenella conica				+
Scrobicularia cottardi				+
Tellina sp.			+	
Tellina tenuis	+			
Venerupis aurea				+
Ventrosia ventrosa				+
Crustacea				
Ampelisca diadema	+			
Ampelisca sp.	+			
Amphilochus sp.	+			
Corophium acherusicum		+		
Corophium sp.	+		+	
Cumacea	+			
Dexamine spinosa	+			
Elasmopus sp.	+			
Gammaridae	+			
Gammarus aequicauda	+			
Gammarus insensibilis		+	+	
Gammarus sp.				+
Idotea balthica		+		

Table 2. Continued

Species	Grado-Marano	Logarou	Margherita di Savoia	Narta
Iphinoe serrata		+		
Lekanesphaera hookeri			+	
Lekanesphaera monodi		+		
Microdeutopus gryllotalpa		+	+	
Paramysis helleri	+			
Phtisica marina	+			
Upogebia pusilla	+			
Miscellanea				
Amphiura chiajei	+			
Insecta larvae			+	+
Oligochaeta	+	+	+	+
Ophiuridae			+	
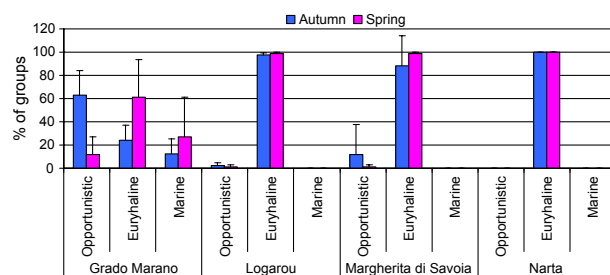
Fig. 3. Percentage abundance of groups of species for each study area over the sampling occasions.
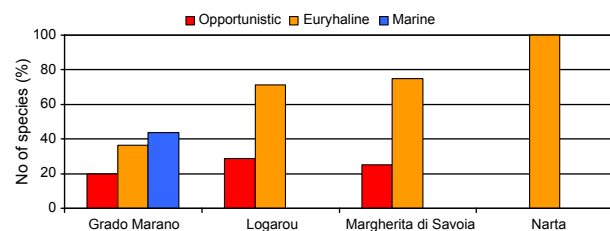
Fig. 4. Percentage of number of species forming the benthic community for each study area.

Fig. 5. Mean diversity (H'), mean number of species (S) and mean abundance (N) *versus* salinity variance over the sampling occasions.

salinity alone but is the result of a complex of factors, described by the term 'confinement' – the time required to renew the marine element (Guélorget & Perthuisot 1983; Reizopoulou & Nicolaidou 2004).

In estuaries, the salinity variation is considered a major environmental factor in structuring species distribution (Holland *et al.* 1987; Attrill 2002; Ysebaert *et al.* 2003; Wijnhoven *et al.* 2008). Attrill & Rundle (2002) investigating gradients in estuarine systems proposed a two-ecocline model, considered to overlap the gradients from river to mid-estuary for the freshwater species, and from sea to mid-estuary for the marine species. In coastal lagoons it is mainly the marine influence that structures the environmental gradient, strongly determined by the sea–land axis, with species richness following a single-scale pattern.

In the present study, the influence of seawater was the primary factor that determined the diversity level of the ecosystems studied, whereas the degree of isolation of each lagoon was better reflected in the variance in salinity.

The differences in natural stress were also reflected by the presence of different sets of species. The main groups forming the communities studied were the widely occurring group of euryhaline species comprising the lagoon specialists (e.g. *Abra segmentum*), with a high tolerance to osmotic stress, inhabiting the transition zone. The presence of the marine group, with more restricted ranges, inhabiting the edge of the system in Grado-Marano, increased the species richness of the ecosystem.

The ecological relevance of the relationships between marine water movements within the system and biotic

zonation was emphasized by Millet & Guelorget (1994). In the four Mediterranean lagoons examined, there was a positive linear association of species richness and community diversity with the sharpness of salinity drop within each water body, indicating their dependence on the degree of marine influence.

The sharper the spatial salinity gradient, the lower the mean number of species and diversity level of the ecosystem. Abundance followed the same pattern; however, the correlation with salinity variance was weak. Abundance is probably related more to food availability and biological seasonal cycles, whereas species richness is mainly related to the intensity of the gradients of environmental stress.

Temporal variations seem to have less of an effect on the community structure of Logarou, with higher seasonal salinity changes, which demonstrated the lowest temporal community variation as indicated by the MDS plot. Temporal changes of species richness and community structure are often found to be non-significant in coastal lagoons (Nicolaidou *et al.* 2006; Nicolaidou 2007). This lack of seasonality has been attributed mainly to the continuous reproduction of some abundant species and to species interactions (Nicolaidou 2007).

Environments that exhibit a low number of species and a low diversity are often considered impacted. In transitional water ecosystems, however, this is a natural stress resulting from (i) the edge-point or extreme values of some environmental parameters, such as salinity, that benthic species are required to endure to survive and (ii) the areas of variation of environmental parameters causing the natural instability imposed on the organisms. The latter is reflected in the gradient zone, and the structure of this zone has proved to be the most important factor in the distribution of biological communities. This transition zone from the marine-based to the land-based influence may vary from gradual to sharp, and natural stress levels can be expressed by the intensity of variation of the physical environment controlled by the hydrodynamic status of each system.

Diversity measures and biotic indices that combine them have not proved very efficient for transitional waters because these ecosystems naturally host species tolerant to natural stressors (Reizopoulou & Nicolaidou 2007; Zettler *et al.* 2007; Simboura & Reizopoulou 2008; Texeira *et al.* 2008). This situation has been outlined as the 'Paradox of Estuarine Quality' and may lead to misclassifications (Dauvin 2007; Elliott & Quintino 2007).

Establishing patterns of diversity changes across gradients of changing environment over large geographical areas could help test predictions on the extent of natural stress *versus* anthropogenic disturbance. Species richness and community diversity in transitional waters are strictly related to the environmental gradients overlapping anthropogenic degradation, therefore indices used for ecological quality assessment that include diversity indices should also take into account the degree of natural instability.

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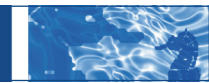
ORIGINAL ARTICLE

Spatial and temporal patterns of bryozoan distribution and diversity in the Scottish sea regions

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Abstract

Diversity and distribution patterns of sessile benthic fauna over space and time are often incompletely known. Understanding the factors that govern these patterns is important for informing marine spatial planning, and monitoring the impacts of climate change and habitat alteration in the marine environment. Historical and contemporary records of bryozoans from Scotland were mapped to produce a GIS distribution layer and were assessed in conjunction with JNCC GIS benthic habitat layers. Bryozoans have predominantly been recorded from the west coast, Orkney, Shetland and Western Isle archipelagos. The greatest number of bryozoan species occurred in high energy, rocky environments; few species have been identified from the continental slope, which also has the least number of records. The biodiversity, as measured by the average taxonomic distinctness and variation in taxonomic distinctness, was assessed for sampling sites and sub-regions within Scotland. High values were concentrated around the Orkney Isles and Southwest Scotland. This is the first known attempt at a comprehensive analysis of changes in Northern European bryozoan diversity and distribution from the 1700s through to the present day, but overall temporal patterns remain uncertain due to differences in the availability of records through time. This study provides an example of the application of methods that may be used to assess benthic diversity to identify potential sites for marine conservation or marine protected areas. As benthic data layers improve, and sampling is extended to unstudied areas, greater insight into the relationship between the physical environment and diversity and distribution patterns of benthic fauna will be gained. This study serves as a baseline for long-term monitoring of biodiversity changes in this poorly studied Phylum.

Introduction

Understanding the factors governing the distribution and spatial patterns of biodiversity of benthic organisms is vital for assessing and mitigating against anthropogenic impacts to the ocean. The lack of local faunistic inventories and limited information on historical biodiversity often prevents rigorous assessments of spatial and temporal changes. Inconsistencies in sampling effort and quantification of abundance make comparisons between geographic regions and time periods difficult.

Bryozoans are sessile colonial invertebrates that inhabit all of the world's oceans and are abundant on rocky shores. They play a significant role in marine ecosystems through contributions to temperate and tropical carbonate sediments (Maxwell 1968; Nelson *et al.* 1988); provision of food sources for other marine species (Lidgard 2008); and provision of three-dimensional structures, attachment surfaces and nursery grounds for other marine species, including some of commercial importance (Bradstock & Gordon 1983). More recently, bryozoans have been recognised as a valuable source of bioactive

compounds (Narkowicz *et al.* 2002; Lopanik *et al.* 2004; Sharp *et al.* 2007).

Previous studies of diversity and distribution

The diversity and distribution of bryozoans have been assessed previously in New Zealand (Rowden *et al.* 2004), Australia (Gordon 1999), the North Atlantic (Clarke & Lidgard 2000), the Southwest Atlantic (López Gappa 2000) and areas of the British Isles (Eggleston 1972; Grant & Hayward 1985). Factors influencing the distribution and diversity have been shown to include: substrate availability (Eggleston 1972), habitat heterogeneity (Clarke & Lidgard 2000), hydrodynamic factors such as current strength and tidal streams (Eggleston 1972), temperature (Ryland 1963) and depth (Grant & Hayward 1985). In addition to these patterns, it has been suggested that the distribution and diversity of bryozoans may reflect that of other benthic marine species (Schopf 1969; López Gappa 2000) and hence bryozoans may serve as a useful indicator taxon for selecting areas to protect marine biodiversity (Rowden *et al.* 2004).

Most previous work on bryozoan diversity has considered either species richness or evenness, or a combination of these (Clarke & Lidgard 2000; López Gappa 2000). As indices of biodiversity, both measures may disregard many aspects such as the diversity of ecosystem functions provided by the biological community (functional diversity) (Hooper *et al.* 2002) and the taxonomic spread of individuals contributing to the assemblage. Species richness, furthermore, may not respond to environmental degradation monotonically, and may in fact increase under moderate levels of disturbance (Wilkinson 1999). These indices are heavily biased by sampling effort and any variation in the methods used to estimate the abundance of colonial species. The use of these methods frequently results in exclusion of historical species lists due to a poor or unknown sampling effort, yet such lists may serve as a vital source of data on past faunal conditions and can greatly assist in the assessment of biodiversity (Ponder 1999).

There has been an increasing move towards assessing biodiversity using information on the relatedness of species (Clarke & Warwick 1998). Two indices that reflect the phylogenetic relationships are the average taxonomic distinctness of species (AvTD) (Warwick & Clarke 1995) and the variation in taxonomic distinctness (VarTD) (Clarke & Warwick 2001). These indices overcome many of the problems associated with species richness and evenness, and Clarke & Warwick (2001) claim that they provide a robust measure of biodiversity, with low values reflecting degraded environments and high values in pristine environments. They can be used in conjunction with presence/absence data and allow for the inclusion of historical species lists in biodiversity assessments. Both indices were used in the present study to evaluate the diversity of bryozoans from Scottish sea regions.

Objectives

The aim of this study was to assess the distribution and diversity of bryozoans in Scotland in relation to spatial and temporal patterns.

This study has the following objectives:

1 To study the distribution of Bryozoa in Scotland and investigate the relationship(s) between the distribution and the physical characteristics of the area.
2 To establish the average taxonomic distinctness (AvTD) and variation in taxonomic distinctness (VarTD) as indices of bryozoan biodiversity in Scotland and Scottish sub-regions.
3 To investigate temporal patterns in bryozoan diversity in Scotland.

The wider implications of this study include contributing to the assessment of the current state of UK seas as required by the EU Marine Strategy Framework Directive, and providing baseline data on Scottish bryozoans, against which changes can be monitored. In the long-term, such data may be used to inform marine spatial planning and the conservation of the Scottish marine environment as required by the Marine (Scotland) Act 2010. This study will form the basis of a much wider project on bryozoan diversity in Britain and Ireland.

Study Area

Scottish bryozoans were defined as occurring from 54°38′2″ N to 60°51′38″ N and from 0°46′50″ W to 13°40′13″ W.

Sources

Historical records were obtained from the Bryozoa collection at the Natural History Museum, London. The collection includes records from the dredging surveys commissioned by the British Association for the Advancement of Science throughout the 1860s; records from the expedition of HMS *Porcupine*, which visited Shetland in the summer of 1869; the collections of Rev. A. Norman; and the collections of George Barlee, among other historical Scottish records. Contemporary records were sourced primarily from the reports of the Marine Nature Conservation Review (MNCR), which sampled the marine fauna of the UK between 1987 and 1998 (Hiscock 1996). Additional records were sourced from selected literature, a field survey conducted in Orkney

between 26 and 30 June 2010 and the National Biodiversity Network gateway (which includes data collected by the Joint Nature Conservancy Council (JNCC); Scottish Natural Heritage (SNH); MarLin; Seasearch and private contract surveys) (Table 1). Records from North Liverpool Bay were included due to their proximity to the Scottish border.

Records for which the location was uncertain or not provided, and/or the species seemed likely to be wrong, based on its generally accepted distribution (e.g. tropical or Antarctic), were not used. Other records that had only been documented in Scotland by one source, with an unknown or non-expert identifier, were also excluded from all analyses. Incomplete records (no information on the date of collection or not identified to species level) or those based on unpublished data where the methods and identifier were unknown, were included in the distribution analysis but excluded from the diversity analysis.

Material and Methods

Data on location, date of collection, and depth were extracted from the records. All records were adjusted to modern taxonomic usage, using the *Synopses of the British Fauna* (Hayward 1985; Hayward & Ryland 1985, 1998, 1999), the World Register of Marine Species (WoRMS) and through personal communication with M. Spencer Jones (curator of Bryozoa at the NHM). Depth data were converted to metres below chart datum, and the average depth taken for sites sampled at a range of depths. Intertidal records were assigned a depth of 0 m, as most bryozoan species are found in the mid- to lower intertidal (excluding rock pools). This approach is consistent with other assessments of bryozoan diversity (e.g. Rowden *et al.* 2004). Sampling methods for the data vary, and include collections made by hand in the intertidal and via SCUBA diving, dredges, grabs and trawls in the subtidal.

Table 1. Sources of Scottish bryozoan records.

| Source	References
MNCR	Bennett (1989); Connor (1989, 1990, 1991); Covey (1990); Davies (1989a,b,c, 1990, 1991); Hiscock & Covey (1991); Holt & Davies (1991); Howson (1989a,b, 1990, 1991)
NBN gateway	www.nbn.org.uk
Literature	Chumley (1918); De Kluijver (1993); Souto *et al.* (2007)
Synopses	Hayward (1985); Hayward & Ryland (1985, 1998, 1999)
Museum Collections	Natural History Museum, London

Distribution analysis

Records were mapped with ARCMAP version 9.2 to produce a GIS bryozoan distribution layer for Scottish sea regions. Species presence was examined in conjunction with other GIS layers on substrate, energy and biozone; these were obtained from the predictive MESH-EUNIS model of the Mapping the European Seabed Habitats (MESH) project. For a full explanation of the MESH-EUNIS layers, see Coltman *et al.* (2008).

Biodiversity analysis

Two indices of biodiversity (AvTD and VarTD) were calculated for all sampling sites with 10 or more bryozoan species. Average taxonomic distinctness is a measure of the average path length between every pair of species in a sample, traced through a taxonomic tree (Clarke & Warwick 1998). It represents the degree to which the species in a sample are taxonomically related. The AvTD index of biodiversity assumes that there is an increase in taxonomic distinctness with increasing environmental stability, and that in perturbed situations, communities are held in an early successional stage with an assemblage of closely related species (Warwick & Clarke 1995). Variation in taxonomic distinctness is a measure of the degree to which taxa are evenly or unevenly spread across the full taxonomic tree (Clarke & Warwick 2001). The index represents the variance of the path lengths connecting every pair of species in a sample.

The main advantage of using these indices is that they are not, on average, dependent on sampling effort, unlike many other commonly used measures of biodiversity (Clarke & Warwick 1998). This independence allows for comparison across studies with differing and uncontrollable degrees of sampling effort, as is the case with the current dataset. In particular, the sampling effort for the historical data is uncertain and many records represent diffusely collected, single specimens. However, a small bias is manifested at very low species numbers (Clarke & Warwick 1998, 2001); therefore, only sites with 10 or more species were included in the analyses. This led to the exclusion of many of the historical bryozoan records. To overcome this problem and utilise all available data, records were also classified into one of 12 sub-regions, primarily defined according to the MNCR coastal sectors with additional regions defined at Rockall, St. Kilda and an east coast offshore region (Fig. 1). The AvTD and VarTD were then calculated for each Scottish sub-region.

The relative importance of local (ecological) and regional (evolutionary) factors in determining the biodiversity of bryozoans at any one location was assessed. If
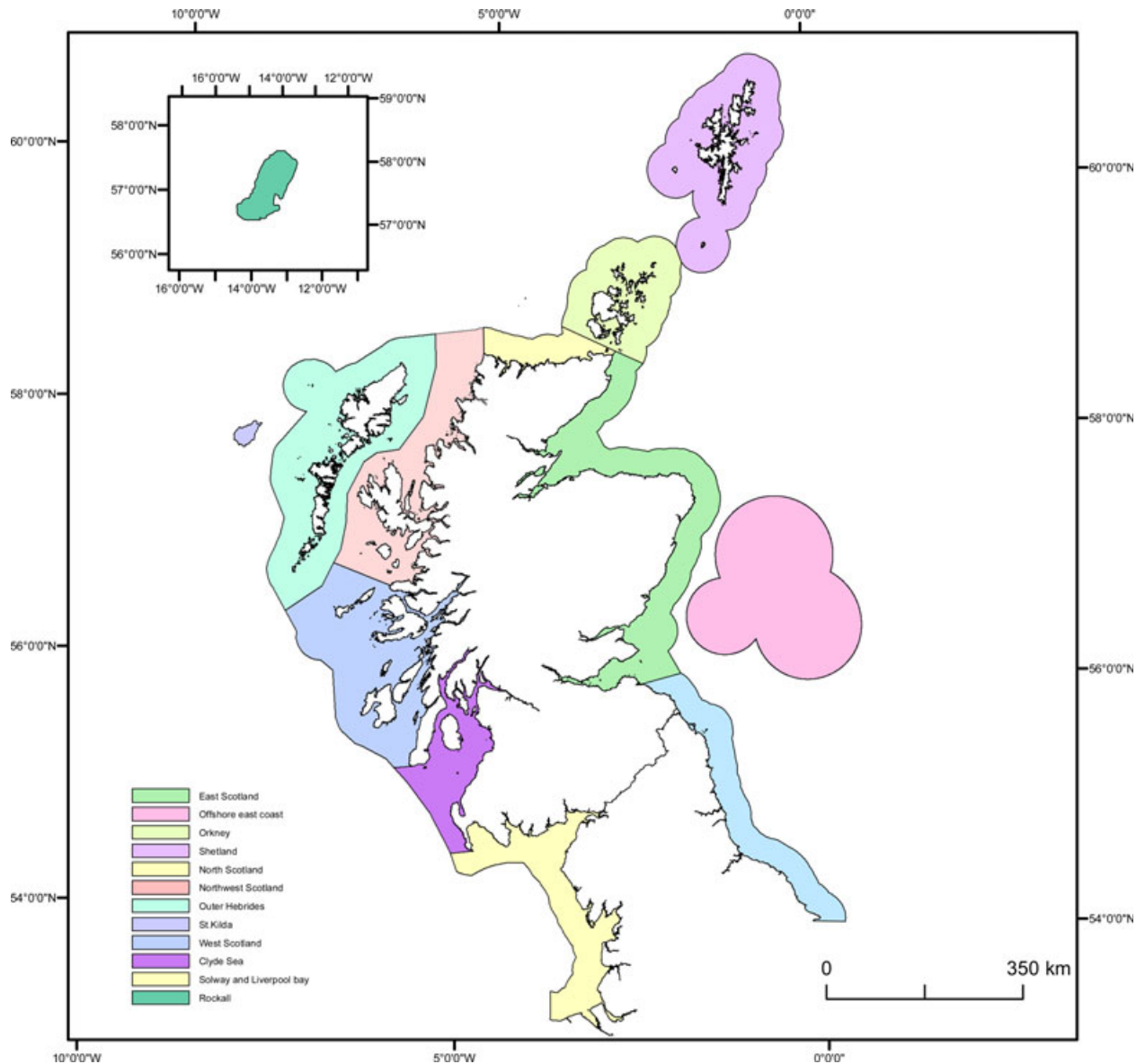
Fig. 1. Scottish sub-regions used for biodiversity and distribution analysis of bryozoans. Regions are based on the MNCR coastal sectors with additional regions defined at Rockall, St. Kilda and an east coast offshore region.

evolutionary mechanisms are responsible for the biodiversity in an area, it would be expected that the species assemblage at that location is merely a random selection of species from the regional species pool (Clarke & Warwick 1998). The observed AvTD and VarTD for Scottish sites were plotted as points on a probability funnel, which shows the mean (expected) AvTD/VarTD and 95% probability intervals. The expected mean and probability intervals were calculated from 1000 random selections of n species (where n = the number of species present at the sample site) from the regional species pool. Where the observed values fall outside of the 95%

confidence funnel, the biodiversity represents a significant departure from the null distribution and suggests that local, ecological factors are modifying the expected biodiversity.

The taxonomic categories used to calculate AvTD and VarTD were: species, genus, family, suborder, order and class. The weightings of path lengths between hierarchical taxonomic levels were standardised, such that two species connected at the highest possible level were assigned a path of 100, with all steps between each level having equal weighting (Clarke & Warwick 1998). The tree for which the AvTD and VarTD are based should represent

the assumed regional species pool, from which bryozoans in Scottish sea regions are theoretically drawn. Deciding what constitutes a regional species pool can be problematic and is a drawback of the taxonomic distinctness method. It may be reasonably assumed that the species for Scotland would consist of Bryozoa present in the two biogeographic regions in which Scotland lies, the North Sea and the Celtic Sea (Spalding *et al.* 2007); however, a complete species list for these two areas was not readily available. To address this issue, a list of British bryozoans, compiled from *Synopses of British Fauna* (Hayward 1985) and from Hayward & Ryland (1985, 1998, 1999), and a list of Bryozoa from the Southern Irish Sea (Robinson *et al.* 2009) were defined as the regional species pool. This tree includes the majority of species expected from the North and Celtic Seas.

Records were grouped into five time periods (1792–1849, 1850–1950, 1950–75, 1975–2000, and 2000–2010). The AvTD and VarTD of each time period were calculated to investigate temporal patterns in bryozoan diversity.

Results

From a total of 17,371 records extracted from the various sources, 214 species of Bryozoa were identified from 3139 localities in Scottish sea regions. The species represented 121 genera belonging to 59 families across all the extant orders. The most commonly occurring species in Scotland is *Membranipora membranacea*, which has been recorded at 1266 locations. The earliest Scottish specimens were collected by Robert Brown in 1792, *Carbasea carbasea* from Leith (NHMUK 2011.1.28.1) and *Bugula plumosa* from Newhaven (NHMUK 2011.1.28.2) (Fig. 2).
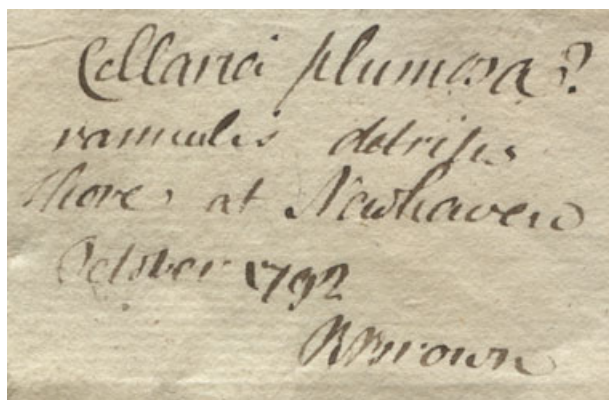
Fig. 2. A museum label identifying one of the earliest bryozoan specimens from Scotland 'Cellaria plumosa? ramulis detritus Shore at Newhaven October 1792 R Brown'.

Bryozoan distribution

Bryozoans have been recorded from all Scottish coasts and archipelagos (Fig. 3). The majority of records are from the west coast and in the Shetland, Orkney and Western Isles archipelagos, with notable gaps on the eastern and northern coasts. There are comparatively few bryozoan records from offshore areas. Figure 4 illustrates the distribution and intensity of sampling from the Marine Nature Conservation Review (from which the majority of bryozoan records were sourced). Sites are distributed throughout the entire Scottish sea region, but again there is an emphasis on the west coast, with fewer samples on the east and north coasts. The distribution of bryozoans in Scottish sea regions is mapped with GIS layers for biological zones (Fig. 5), energy levels (Fig. 6) and substrate types (Fig. 7). The majority of bryozoans have been recorded from the infralittoral to the deep circalittoral, with a few records from the slope close to Rockall (Table 2).

Spatial patterns of bryozoan diversity

A regression of AvTD against VarTD for all sites with 10 or more species indicates a significant positive linear correlation between the two indices ($y = 10.17x - 367.77$, $r^2 = 0.4347$, $P < 0.001$). The 95% probability ellipses for all sites with 10 or more species show broadly the same pattern for the VarTD (Fig. 8) and the AvTD (Fig. 9). In view of this correlation, it is only necessary to consider the AvTD biodiversity index from this point forward.

Figure 10 illustrates the measures of AvTD for sites with 10 or more species within the Scottish sea region. Values vary across the region from 45.08 in Loch Sween, West Scotland, to 70.51 in Sgeir Dhonncha, West Scotland. High values are particularly concentrated in Orkney and the southwest, whereas low values tend to occur in Shetland and between the Outer Hebrides and the mainland. Biodiversity appears independent of water depth, with intertidal sites and sites deeper than 100 m showing similar values of AvTD (Fig. 11).

Figure 12 shows the AvTD for each of the Scottish sub-regions, based on the combined bryozoans for each area. The AvTD for Scottish sub-regions varies over a narrow range. The highest value, 67.97, occurs in Rockall, followed by the Outer Hebrides, West Scotland and the Clyde Sea. North Scotland, Shetland and St. Kilda have the lowest values at 60.36, 60.80 and 60.80, respectively.

The results of the analysis to test whether the AvTD at any one site within Scotland is statistically different from the regional mean are displayed in Fig. 9. The majority of sites have a bryozoan composition representative of the region as a whole; however, a number of
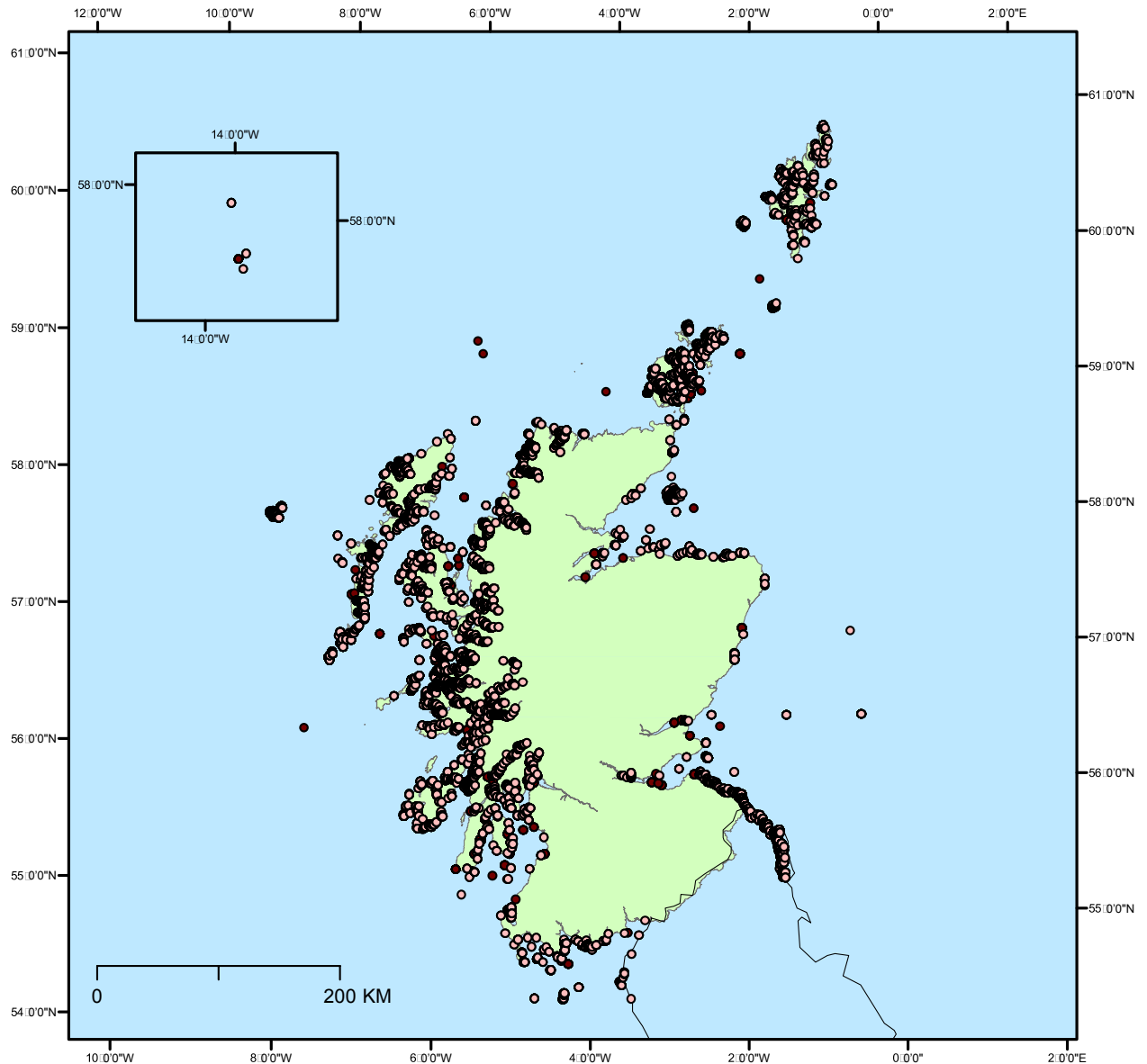
Fig. 3. Locations from which bryozoans have been recorded in Scotland. Records cover the timeframe 1792–2010 and were sourced from the NHM, the MNCR, the NBN gateway, selected literature and a field survey. Lighter circles represent precise records, whereas darker circles are an approximate location. Inset showing Rockall.

sites show a significant departure from the hypothetical mean AvTD in both a negative (nine sites) and positive (eight sites) direction. Figure 10 shows the location of these sites. Six of the eight sites with a significantly higher AvTD are located in Orkney. The other two sites are within the West Scotland sub-region. Two of the nine sites with values of AvTD less than the theoretical mean are also present in the West Scotland sub-region. Four sites with lower AvTD than expected are located in Shetland, with the remaining sites in the Clyde Sea and East Scotland.

Temporal patterns in bryozoan diversity

Table 3 shows the AvTD for bryozoans in Scotland from 1792 to 2010. AvTD peaks between 1792 and 1849 with a second peak between 1975 and 2000. AvTD is lowest in the present time period (2000–2010).

Discussion

The aim of this study was to assess the distribution and biodiversity of bryozoans in Scotland, using a combination
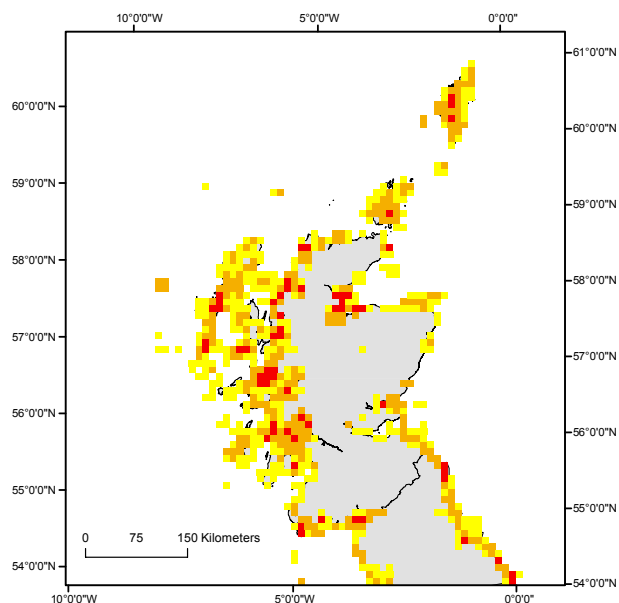
Fig. 4. Sampling sites of the Marine Nature Conservation Review. Colour represents the intensity of sampling: red = 43–489 samples per 10 km^2; orange = 8–42 samplers per 10 km^2; yellow = 1–8 samples per 10 km^2. Taken from the NBN gateway website.

of mapping techniques and biodiversity indices. The results provide baseline data on Scottish bryozoans and highlight the regions and habitats that remain poorly studied.

Ecological insight into the distribution and diversity of bryozoans is somewhat confounded by variations in data quality and availability. The MESH-EUNIS data layers have a fairly coarse resolution because of underlying limitations in seabed substratum data from the British Geological Survey. Resolution is lowest in coastal areas (0–3 m) and on rocky substrates, both of which contain a high proportion of the bryozoan records. Moreover, the layers are based on a predictive model and therefore may not accurately represent the benthic habitat of all areas. This, along with the fact that the location of many bryozoan records is only approximate, and that the benthic habitat varies over a relatively small spatial scale, means that the species numbers in each marine zone may not be fully representative of the true distribution of bryozoans in Scotland. It may well be expected that given the modest sampling effort, bryozoan diversity is underestimated in almost all locations.

The inclusion of museum specimens in the present dataset provides information on the historical state of diversity. Museum data has the advantage of being able to confirm species identifications. The date of museum specimens, however, can be misleading, reflecting the date when the specimen was registered, rather than collected. Furthermore, changes in taxonomic usage and place names, and imprecisely defined locations or sampling

methods can restrict comparisons of present day and historical data. Alternatively, present-day survey data will tend to provide accurate information on the location, date and depth of sampling; however, specimens are rarely retained, preventing the identification being checked at a later date.

Comparison of diversity (as measured by AvTD/VarTD) with other studies, and therefore ecological interpretation of the results, is limited, as few studies have considered the relationship of AvTD/VarTD to physical characteristics of the environment (e.g. Ellingsen *et al.* 2005). Fewer still have considered the average taxonomic distinctness of bryozoans (e.g. Rowden *et al.* 2004). Some insight may be gained by considering factors that influence species number, but it is unclear whether species richness and AvTD/VarTD are governed by similar environmental parameters. Preliminary work shows that species richness and taxonomic distinctness relate to different environmental gradients (Ellingsen *et al.* 2005; Heino *et al.* 2005). Assemblages with lower species richness do not necessarily have a lower AvTD than those of many other species, e.g. when there is a limited number of species, but the species represent a broad phylogenetic range.

Despite these problems, the results give an indication of the broad-scale patterns in bryozoan occurrence and enable the identification of localities and habitats that represent future sampling priorities in Scotland.

Bryozoan distribution

The location of bryozoan records largely mirrors the MNCR sampling sites, suggesting that the observed distribution reflects a greater sampling intensity on the west coast and the archipelagos of Shetland, Orkney and the Western Isles. Few species have been recorded from offshore areas, and again this is likely to reflect a bias towards sampling coastal areas, which are both easier and cheaper to access.

Patterns of offshore *versus* near-shore species richness remain ambiguous, with several studies finding fewer species in offshore shallow benthic areas compared with near-shore areas (e.g. Barnes 2000), and others finding the reverse of this pattern (Schopf 1969). Offshore seamounts in the north of Scotland may be expected to support bryozoan species, as they provide suitable substrate and are generally associated with high primary productivity and current speeds (Rogers, 1994). There are so few records of offshore bryozoans in the present study that no conclusions can be drawn regarding offshore *versus* coastal patterns.

Limited surveys have been conducted in areas of the eastern and northern coasts where bryozoans appear to be absent. Potentially, therefore, the distribution of
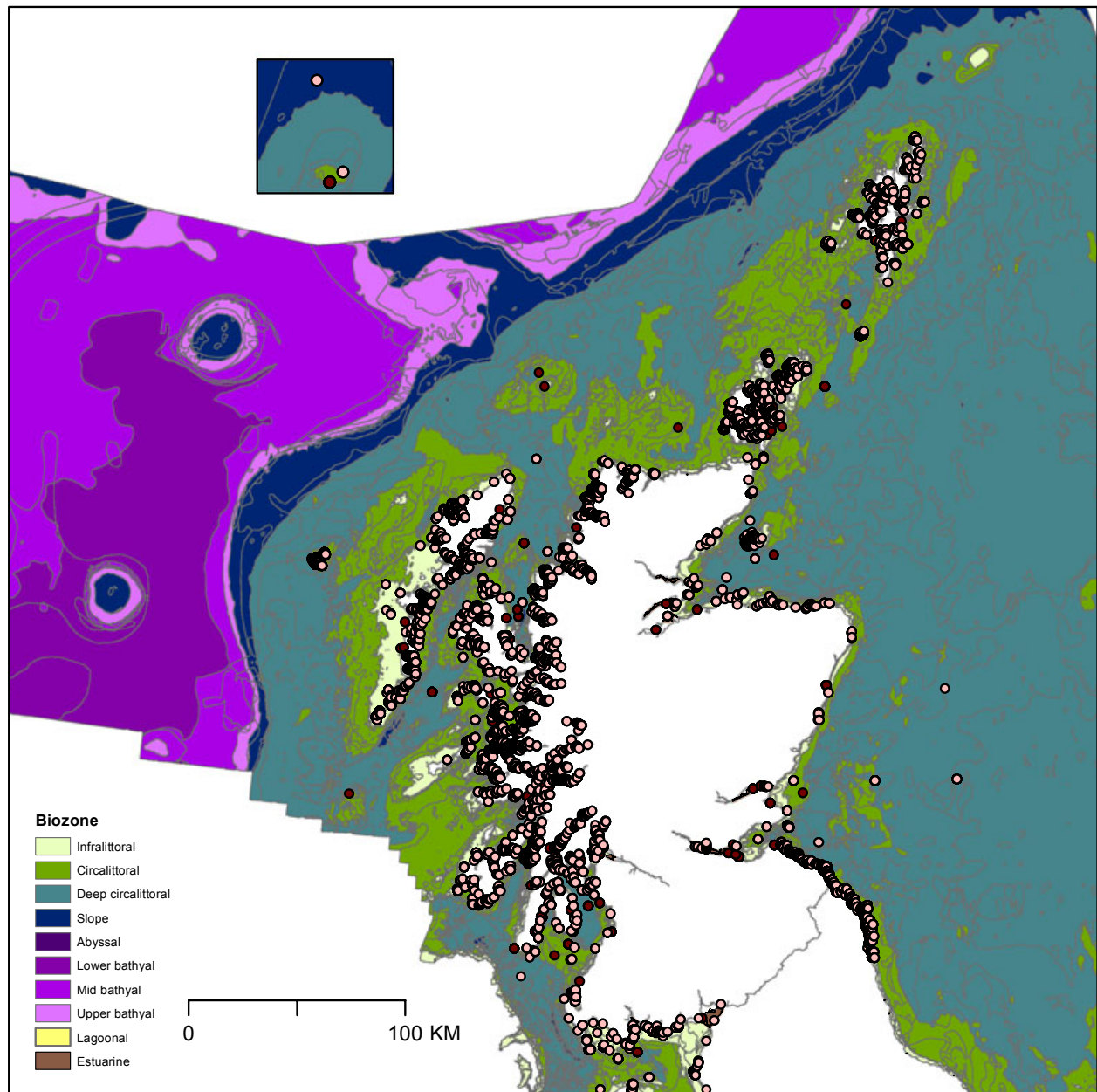
Fig. 5. Distribution of bryozoans in Scotland and the predicted MESH-EUNIS biozones of the Scottish region. Lighter circles represent precise records, whereas darker circles are an approximate location. Inset showing Rockall.

bryozoans reflects underlying differences in the marine environment. Norman (1869) notes that within the British Isles, bryozoan species that are typical of the Mediterranean region can colonise higher latitudes on the west coast than on the east coast. He attributes this to the influence of the Gulf Stream on the west coast, which results in higher temperatures and a more stable temperature regime on the western coast of Scotland (Bennett & Covey 1998a). Temperature has previously been suggested as an important factor in determining the bryozoan

assemblages in the English Channel (Hayward & Ryland 1978), Norway (Ryland 1963) and Hawaii (Soule *et al.* 1988). Many bryozoan species have a narrow temperature tolerance, and variation in temperature impacts growth rates and tolerance to salinity (Dick *et al.* 2006). In addition to temperature differences, water in the east of Scotland can become highly stratified in the summer, with a lack of vertical mixing (Dyer *et al.* 1983). This results in a restricted supply of nutrients to the photic zone, and low primary productivity (McIntyre 1961). It may therefore be

Fig. 6. Distribution of bryozoans in Scotland and predicted MESH-EUNIS energy levels within the Scottish region. Red = high energy; orange = intermediate energy; green = low energy. Lighter circles represent precise records, whereas darker circles are an approximate location. Inset showing Rockall.

expected that little organic material reaches the seabed. In contrast, strong winds on the west coast generally prevent stratification in the summer (Lee 1981) and hence there is a greater availability of phytoplankton to bryozoans. The availability of phytoplankton as a food source is thought to be important in determining bryozoan distribution (Best & Thorpe 1983, 1986; Pratt 2008) and may, in part, be responsible for differences between the west and east coast. Alternatively, differences between the two coasts may not be ecological, but instead represent evolutionary

trends, with Lusitanian species having had insufficient time to colonise the east coast.

The number of species present in the different MESH-EUNIS marine zones gives a further indication of the potential factors influencing bryozoan distribution in Scotland. Bryozoans are typically expected to inhabit intermediate energy zones where there is some degree of shelter (Ryland 2005). Currents and wave exposure will influence bryozoans through impacts on food supply, attachment ability and substrate-mediated effects
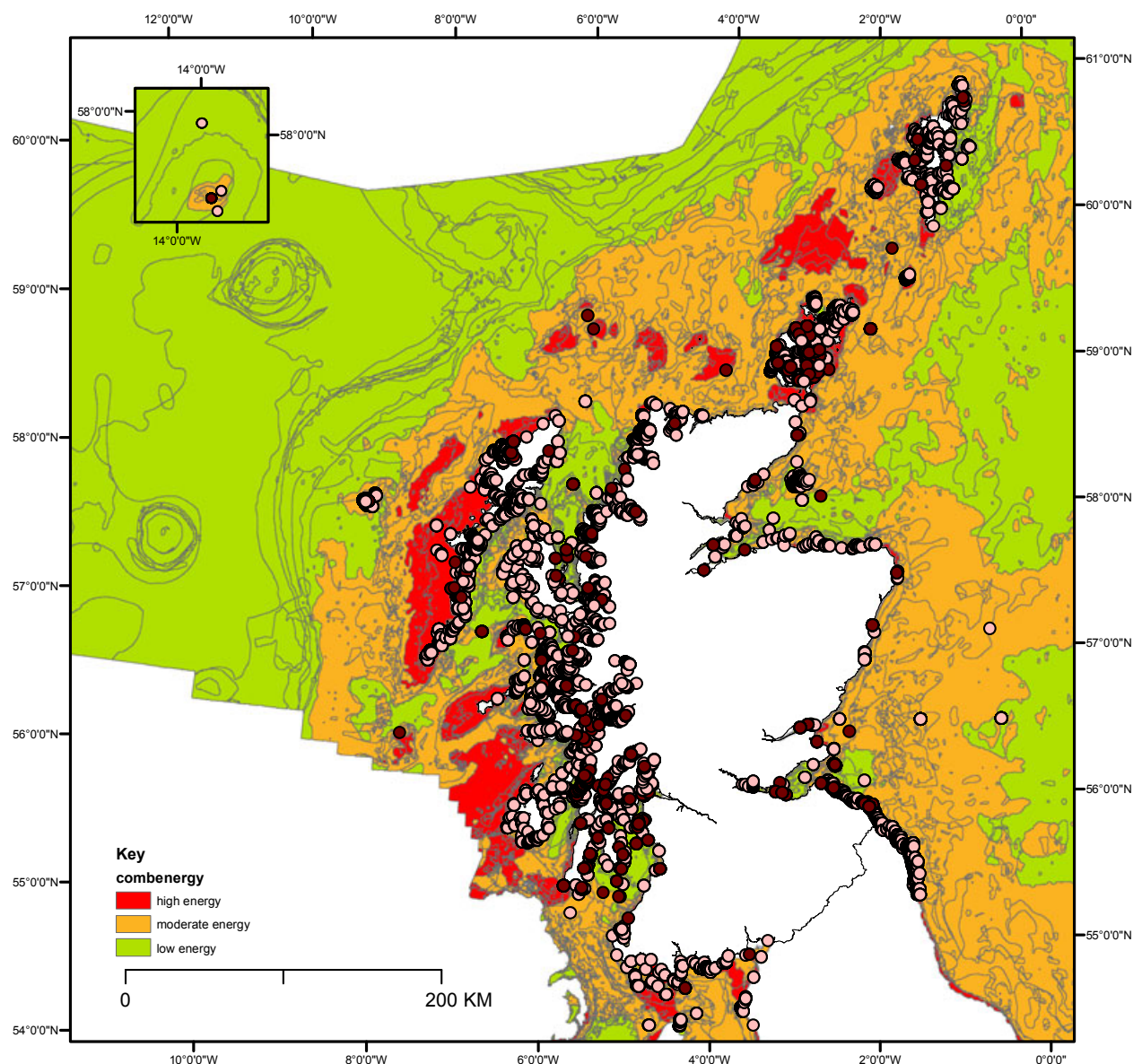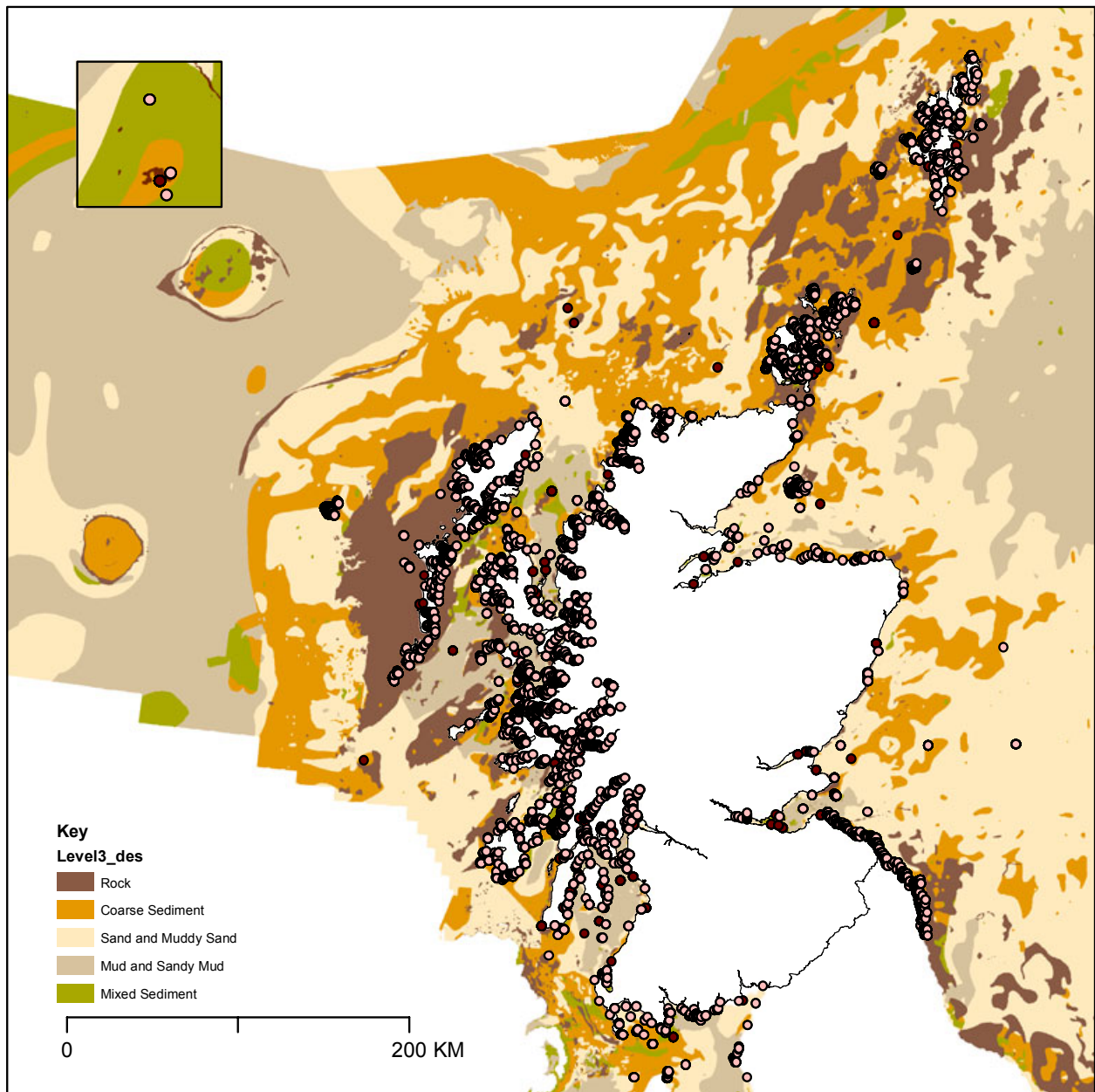
Fig. 7. Distribution of bryozoans in Scotland and predicted MESH-EUNIS substrate type within the Scottish region. Lighter circles represent precise records, whereas darker circles are an approximate location. Inset showing Rockall.

(Cabioch 1968; Dick *et al.* 2006). The greatest number of bryozoan species in Scotland are from high-energy, rather than intermediate energy, environments, and the greatest biodiversity (AvTD) is within low-energy environments. The three energy zones have similar numbers of bryozoan records, such that sampling should have less of an influence on the number of species. However, the energy layer of the MESH-EUNIS model is constructed only from information on tidal currents and excludes any measure of wave exposure. High energy, therefore, represents areas

of strong or very strong tidal currents with unknown exposure. If wave action were to be incorporated, our understanding of the distribution and diversity of bryozoans across the different energy zones might differ.

Currents will also affect the distribution of bryozoans through their influence on substrate availability and heterogeneity. For all marine species, environmental heterogeneity is one of the strongest correlates of species number (Huston 1994), and peaks in bryozoan species number have been found previously to correspond to

Table 2. Average taxonomic distinctness (AvTD), number of bryozoan species (S) and number of records (R) in different MESH-EUNIS zones in Scotland.

| Zone	AvTD	S	R
Biozone			
Infralittoral	62.60	191	13,240
Circalittoral	63.11	98	1179
Deep circalittoral	65.90	74	382
Estuarine	61.82	27	70
Lagoon	65.19	11	34
Slope	–	3	4
Energy zone			
Low energy	64.19	139	4650
Intermediate energy	63.83	122	5130
High energy	62.44	173	5135
Substrate type			
Rock	61.98	193	12,076
Coarse sediment	64.05	76	1259
Mixed Sediment	63.77	89	619
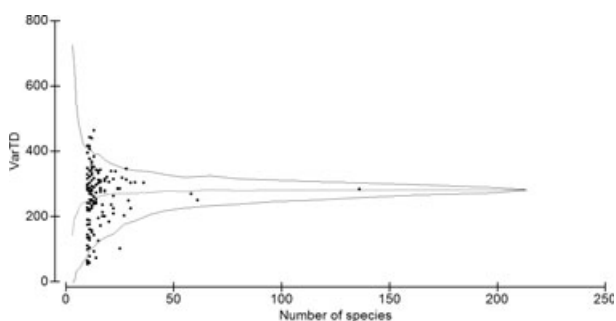
Fig. 8. Departure from the theoretical mean VarTD and 95% confidence funnel of sites in Scotland where >9 species of Bryozoa have been recorded.

increased substratum heterogeneity (Hayward & Ryland 1978; Clarke & Lidgard 2000). Rock, as defined by the MESH-EUNIS model, includes a range of substrates available for bryozoans to colonise, such as rocks and biogenic reefs. Kelp and other species that may be colonised epizooically by Bryozoa will also be available on rocky substrata. In contrast to the results presented here, the greatest number of boreal species has generally been encountered on coarse sediments (Eggleston 1972; Kuklinski *et al.* 2005); however, biodiversity (AvTD, rather than species number) of bryozoans in Scotland was found to be highest on coarse sediments. This further emphasises the need for greater sampling of coarse and mixed sediments in Scotland. The lack of available substratum may be a determining factor influencing low species number on the north coast of Scotland. North of the mainland, there are large areas of sediment and only one sea loch
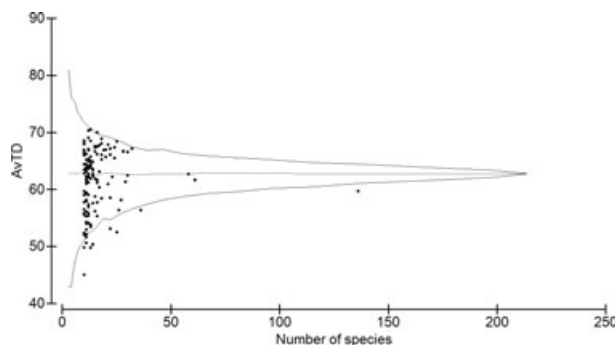
Fig. 9. Departure from the theoretical mean AvTD and 95% confidence funnel of sites in Scotland where >9 species of Bryozoa have been recorded.

(Bennett & Covey 1998b), therefore much of the area is likely to be unsuitable for bryozoan colonisation.

Both the availability and heterogeneity of substratum, and current strength will vary with depth. The MESH-EUNIS biozones GIS layer indicates the depth at which Bryozoa have been recorded. The greatest numbers of species have been recorded from the infralittoral zone; however, this category has more than 10 times the number of records as any other zone. The diversity measured by AvTD is highest in the deep circalittoral.

Overall, it is likely that a variety of physical factors contribute to the observed patterns of bryozoan distribution in Scotland and between different marine habitats. It is necessary to ensure that sufficient sampling has been undertaken to eliminate sampling bias as an explanatory factor for bryozoan occurrence. Gaining a better idea of substrate, temperature and exposure differences between sub-regions and offshore and coastal areas will allow for further explanation of the observed patterns. As seabed maps improve in the future, it will be possible to establish the patterns in bryozoan distribution with greater confidence.

Spatial patterns in bryozoan diversity

The average taxonomic distinctness was calculated for all sites with 10 or more species and from Scottish sub-regions as an index of biodiversity. Depth alone appeared to have little influence on bryozoan diversity. However, the range in diversity values was less variable in deeper waters, which may be due to the lower number of records. Substrate, currents, temperature and food availability vary with depth, and these are factors that are known to influence bryozoan assemblages (Eggleston 1972; Hayward & Ryland 1978; Hughes 2001; Kuklinski *et al.* 2005). The AvTD of bryozoans in New Zealand was found to vary with depth, peaking between 10 and 40 m in areas consistent with high habitat heterogeneity
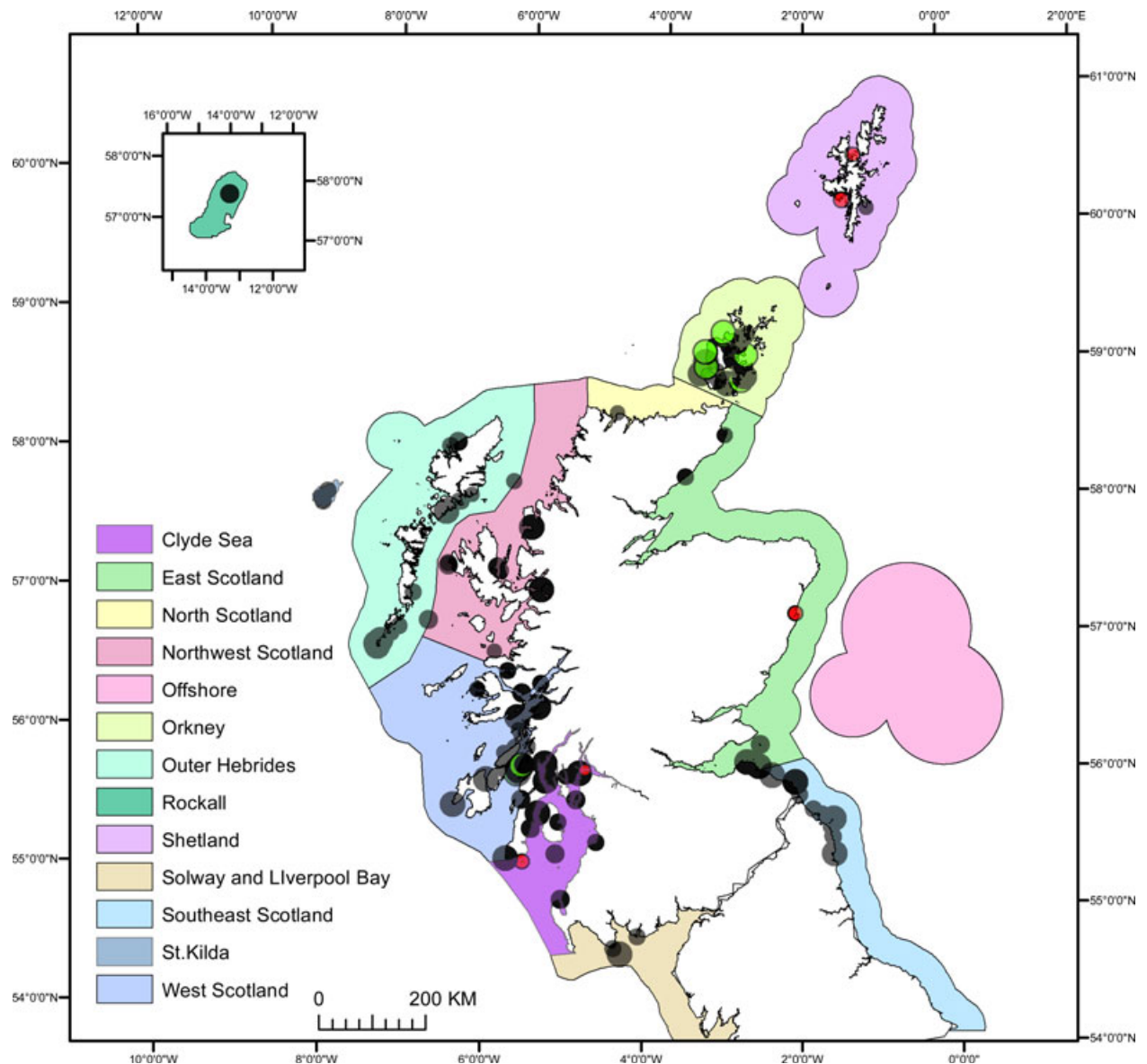
Fig. 10. Distribution of sites with >9 species of Bryozoa recorded in Scotland and associated AvTD values. Circle size is proportional to AvTD. Red circles represent values of AvTD that are significantly lower than the regional mean, and green circles represent those values that are higher than the regional mean.

(Rowden *et al.* 2004). Similarly, Ellingsen *et al.* (2005) concluded that the relationship between AvTD and depth for soft-sediment macrobenthos in the Norwegian continental shelf was due to depth-related differences in the abundance and variation of habitat types. Although it is generally expected that habitat heterogeneity will vary with depth due to reduced light and wave scour, this is not necessarily the case in Scotland. In addition, the diversity within the MESH-EUNIS biozones suggests that habitat heterogeneity is influential for bryozoan diversity. Diversity peaks in the deep circalittoral zone (Table 2), which is defined as starting at the maximum depth that

the seabed is influenced by waves, and extending to 200 m (Coltman *et al.* 2008). This zone will tend to be diverse with a variety of substrates including gravel, shingle, cobbles and shells, typically with a low silt content and reduced wave scour (Connor *et al.* 2004). It is also possible that the lack of a relationship between diversity and depth is the result of the limited depth data available for sampling sites and lack of sampling from deeper water locations.

Bryozoan biodiversity varied across Scotland, with a concentration of high values in Orkney and West Scotland. Both of these regions contain sites in which the
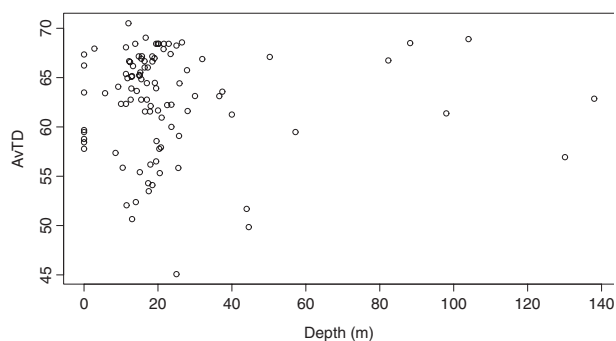
Fig. 11. AvTD in relation to depth for sites with >9 species of Bryozoa in Scotland.

bryozoan fauna has an AvTD significantly higher than the expected regional mean. Lower biodiversity values were widely spread across the region, from Shetland to the Clyde Sea, and were significantly lower than the expected regional mean in Shetland and West Scotland. The concentration of sites with a high diversity in Orkney may reflect the variety of habitats present in this area. As discussed above, habitat heterogeneity is a major factor influencing diversity. Additionally, the stable temperature regime and the availability of sheltered areas with strong tidal currents in Orkney (Bennett & Covey 1998a) are likely to contribute to the high diversity of the area. Hiscock & Breckel (2007) found that the AvTD for benthic assemblages, including species of Bryozoa, was highest in Scottish sea loch environments, followed by island habitats, and similarly attributed this to the variety of habitats that these environments provide. Orkney is made up of 70 islands and has many sea lochs; this is likely to contribute to the higher than expected values of AvTD found for bryozoans in Orkney. Likewise, the West Scotland sub-region has many of these habitat types.

At a regional scale, values of biodiversity of the MNCR sub-regions did not show much variation. The greatest diversity was in the Rockall sub-region, followed by the Clyde Sea and the Outer Hebrides. The lowest values were in North Scotland, which has only one sealoch, and Shetland. Regional patterns for bryozoans were similar to those described by Hiscock & Breckel (2007) for benthic assemblages, who found the highest AvTD at sites within the Northwest Scotland, West Scotland and the Outer Hebrides MNCR regions.

Neither the diversity of individual sites nor the diversity of sub-regions shows any cline with latitude. Latitudinal patterns have generally only been considered in terms of species richness and little work has been done on the relationship between average taxonomic distinctness and latitude. Furthermore, poleward clines in species richness appear to be taxon-specific. For example, Ellingsen et al. (2005) found that the AvTD of annelids, crustacean and

molluscs (combined) decreased with latitude on the Norwegian continental shelf, yet if annelids alone were considered, AvTD increased with latitude. Any interpretation of species gradients is confounded by similar latitudinal gradients in the physical characteristics of the environment (Narayanaswamy et al. 2010). A more detailed study of bryozoans, possibly over a greater latitudinal range, would be necessary to decipher any relationship.

Spatial scale

The role of spatial scale has long been recognised as important when assessing biodiversity, and is a limiting factor when comparing sites of different size. Different processes operate at different spatial scales and the size of an area will influence the degree of environmental heterogeneity for which biodiversity is assessed (Huston 1994). The problem of spatial scale is highlighted in the present study. Individual sites with 10 or more species show the highest biodiversity within Orkney, but when data are combined on a regional scale, Orkney as a whole has one of the lowest biodiversities of the Scottish sub-regions. Deciding on an appropriate scale to assess diversity for conservation purposes is problematic and is often determined by the spatial scale for which data are available. Frequently, species–area curves are used to examine the effect of spatial scale on species richness and can be used to work out the minimum sample area needed to capture species diversity adequately. There has been little consideration of the impact of area on AvTD but it is likely that larger areas will cover a greater variety of habitats and hence support a greater taxonomic range of species.

Temporal patterns in bryozoan diversity

Temporal patterns in the diversity of bryozoans were assessed by calculating the AvTD for each time period. AvTD varied over time, peaking between 1792 and 1849, and then again between 1975 and 2000 (Table 3). AvTD was lowest in the present time period (2000–2010). The greatest diversity (AvTD) was associated with the fewest number of species and records. It is likely that during this early time period, a few conspicuous intertidal bryozoans would have been collected. In the intertidal zone the key members of the bryozoan community most likely to be encountered will represent a very broad phylogenetic range. An example of a typical sample could be the Ctenostome *Flustrellidra hispida*, the Cheilostome *Electra pilosa* and the Cyclostome *Crisia denticulata*. This would give a species richness of 3, which is low; however, the AvTD for this would be high due to each species belonging to a different Order. From 1850 to 1949, sampling effort increased due to the activities of the British
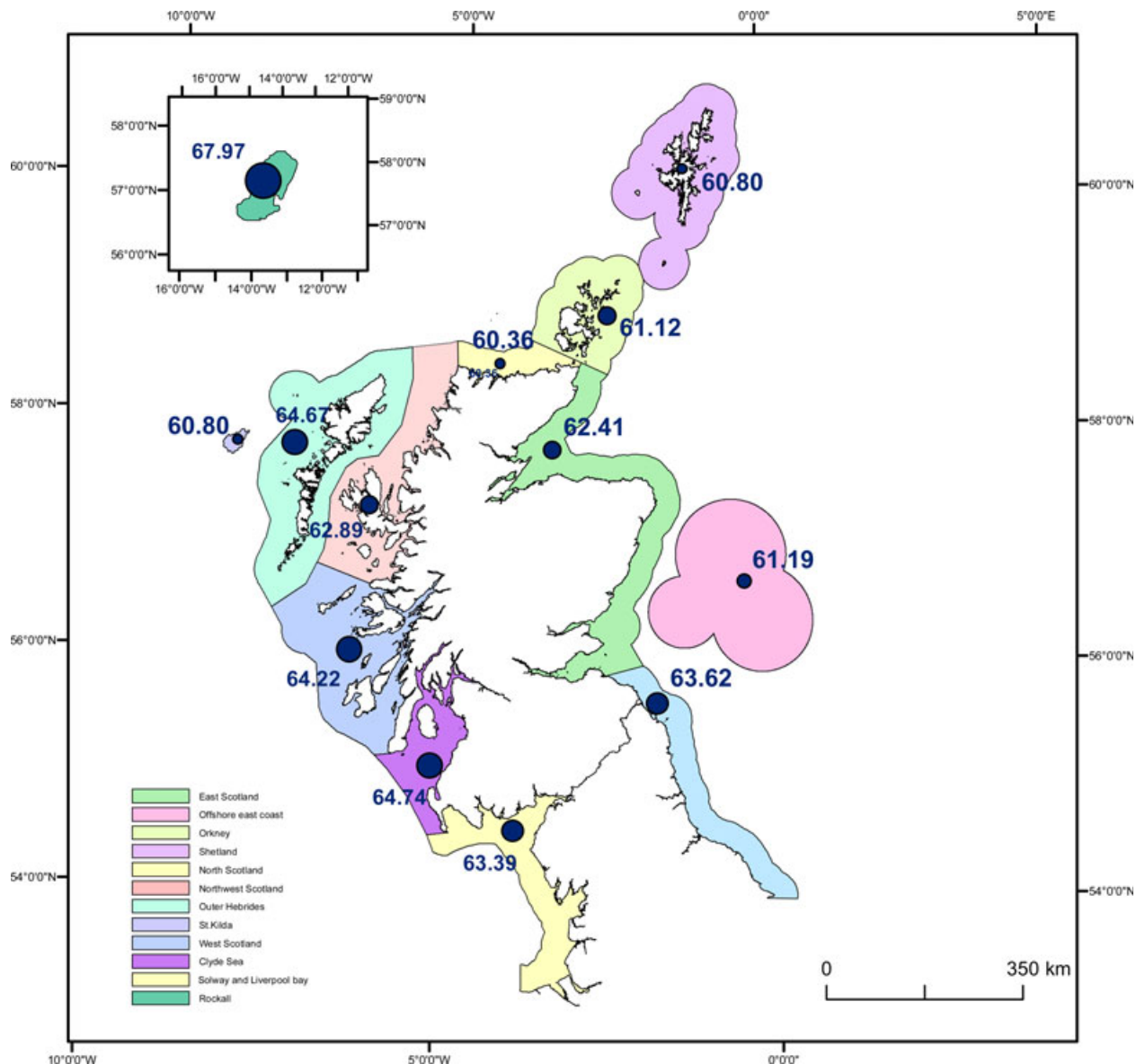
Fig. 12. Biodiversity (as measured by AvTD) of Scottish sub-regions. Circle size is proportional to AvTD value.

Dredging Association. More species would have been collected, increasing the species richness and number of records. Additional species will of necessity belong to one of the three Orders sampled from the intertidal example in the previous time period. It follows then that large increases in the AvTD would not be expected.

With the current data it is not possible to separate genuine temporal changes in Scottish bryozoan diversity from the effects of an uneven spread of records across the time periods and changes in methods of collecting and identifying specimens. Additionally, the geographical range of records in each time period is not equal. This means that the biodiversity for one time period may not

be comparable with that of another time period. Data were insufficient to calculate the AvTD for each time period in each of the Scottish sub-regions, which would have helped to overcome this problem. A more thorough search of historical records, including other museum collections, may yield more data and enable a more robust assessment of diversity through time, in the future.

Implications and recommendations

The data presented here provide baseline information on the distribution and diversity of bryozoans in Scotland,

Table 3. Average taxonomic distinctness (AvTD), number of bryozoan species (S) and number of records (R) in different time periods.

Time period	AvTD	S	R
1792–1849	64.01	40	59
1850–1949	61.17	157	695
1950–1975	61.75	52	74
1975–2000	63.75	142	19,012
2000–2010	59.46	65	2352

which is a vital prerequisite for conservation and environmental monitoring. The identification of sites with a high biodiversity may be used to identify priority sites for protection and conservation.

However, it is important not just to value sites of high diversity. Sites with a low AvTD may represent a small number of closely related species, but those species may be ecologically rare. For example, few species are present in tide-swept faunal communities, yet the species that are able to tolerate such conditions, such as *Alcyonidium diaphanum* and *Flustra foliacea*, may be considered to be valuable. Benedetti-Cecchi (2004) noted that the identity of the species in an assemblage, rather than a diversity index, may be needed to make inferences on ecosystem function. The distribution of bryozoans must, therefore, also be considered in terms of individual species, rather than just the broad-scale patterns.

Taxonomic distinctness, like other indices, does not capture all aspects of diversity. Most notably, AvTD measures based on presence–absence data do not account for the abundance of species within an assemblage. Not only were abundance data not available for many of the bryozoan records, but the quantification of abundance for modular or colonial animals is problematic and varies across studies (Mckinney & Jackson 1989). It is, therefore, likely that policy decisions based on biodiversity assessments will need to make use of a variety of diversity measures, including taxonomic distinctness and species richness, to capture fully all aspects of diversity.

The sensitivity of AvTD, and its application in environmental monitoring, still remains uncertain. Salas *et al.* (2006) found that only total, not average, taxonomic distinctness was satisfactory in determining disturbed situations, and AvTD was less sensitive than other commonly used diversity measures. Furthermore, Heino *et al.* (2005) found that the AvTD varies along natural gradients, thereby reducing its ability to discriminate between the effects of human disturbance and natural variability.

The availability of regional species pool data is a basic requisite for the application of AvTD to biodiversity assessment but it cannot be certain that the regional species pool, here assumed to be bryozoans of the Celtic and North Sea, is truly representative. Comparisons of Scot-

tish fauna to that of other regions are therefore limited. Moreover, a fully resolved phylogenetic framework is not available for bryozoans, and relatedness is based on the somewhat arbitrary Linnaean classification system (Warwick & Clarke 1995). For bryozoans, as with most phyla, certain groups have received more taxonomic attention than others. In particular, the taxonomy of cyclostomes remains unclear (Waeschenbach *et al.* 2009). This results in a truncation of the hierarchy towards the more studied groups. Weighting taxonomic ranks on a linear scale is also somewhat arbitrary, and it must be remembered that the AvTD is an index that provides only a relative measure of biodiversity, for use in comparing areas, rather than providing an absolute measure.

Despite these problems, AvTD is one of only a few indices that make historic, diffusely collected species lists amenable to valid biodiversity analysis. As the historic dataset for Scottish bryozoans represents a significant proportion of the total dataset (~2300 records), and a common aim of biodiversity assessment is to understand temporal changes, it is important that these historical data are included in analysis and not simply ignored because of the limitations of sampling effort. While limitations in taxonomy may limit comparisons between Scotland and other regions, and between Bryozoa and other Phyla, comparisons between sites within Scotland should give a reasonably accurate indication of spatial differences in diversity within Scotland.

The present analysis highlights the need to maintain meticulous records and transparency across institutions. Recent EU and UK legislation aim to make data collection consistent and improve accessibility. In particular, the EU Inspire Directive (2007) focuses on maintaining spatial data and improving transparency across agencies.

Conclusion

In this study we describe how the distribution and diversity of bryozoans varies across Scotland. Sampling effort is largely responsible for the spatial patterns described here; however, indirect evidence from broad-scale habitat data suggests that the patterns may also be determined by a number of ecological factors. Depth, unexpectedly, appears to have little influence on bryozoan diversity. Habitat heterogeneity and substrate availability are likely, in part, to be important factors in determining bryozoan assemblages. To develop more robust explanations for the observed patterns, rather than pure descriptions, it will be necessary to increase sampling effort to those regions (e.g. the north coast) and habitats (e.g. the deep circalittoral) that have been neglected previously. The interpretation of temporal patterns is confounded by the availability of historical data, and a more thorough search

of museum specimens and historical records will be necessary to further investigate temporal changes in bryozoan occurrence. Understanding the factors that influence AvTD has so far been limited by the lack of studies that have used AvTD to assess benthic fauna. Furthermore, there are several caveats when applying AvTD to biodiversity assessments, and it is likely that it will need to be combined with other measures of diversity to provide holistic information on which to base policy decisions.

Acknowledgements

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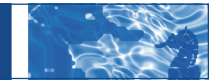
ORIGINAL ARTICLE

Selection of habitat by a marine amphipod

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Abstract

Marine algae are known to provide habitats for a wide range of marine organisms. Populations of marine epiphytal invertebrates are generalists and are less adapted to live in only one macroalga species. However, there are some examples of local adaptation and, in particular, amphipods have shown strong host specificity. *Amphitholina cuniculus*, an amphipod with an alga-burrowing habit, has been mainly observed in *Bifurcaria bifurcata* mats on the intertidal rocky shores of the southern region of the Galician coast (NW Spain; 42º11.27′ N, 8º48.25′ W). We designed a laboratory experiment conducted in June 2009 to analyse the association between the amphipod and the macroalga. In particular, we hypothesised that (i) this species would exhibit different behaviour during the day and at night, and (ii) adults of *A. cuniculus* would prefer *B. bifurcata* as habitat rather than *Fucus vesiculosus* or *Sargassum muticum*. Results supported the hypothesis that adults of *A. cuniculus* preferred *B. bifurcata*, although such preferences did not show the day/night variability predicted. This amphipod might be considered a specialist species, at least on rocky intertidal shores along the Galician coast.

Introduction

The association of small invertebrates with larger sessile organisms such as plants or macroalgae that provide habitat and/or food has been a major topic in ecological and evolutionary studies, in both terrestrial and marine systems. However, patterns of habitat use in terrestrial and marine systems differ. For example, most species of terrestrial herbivorous insects show habitat specialisation (Fox & Morrow 1981), whereas most marine invertebrates are associated with multiple macroalgal families as habitats (Hay & Fenical 1988). Some marine invertebrates, however, are specialised and it has been argued that an understanding of these rare cases may provide insight into the ecology and evolution of feeding and habitat specialisation in general (Poore *et al.* 2000; Sotka 2007).

For small mobile epifauna, seaweeds are a refuge from stressful conditions associated with life on rocky intertidal shores. For example, seaweeds can provide cool and protective canopies and interstices and ameliorate the influence of rapid changes in temperature, desiccation and hydrodynamic forces on intertidal shores (Bates 2009). In addition, seaweeds may also offer shelter from predation and food for some species of invertebrates (Cronin *et al.* 1995; Bates 2009).

Patterns of distribution of mobile epifauna are frequently explained by the behaviour of the species under consideration, or of those with which they interact (Chapman 2000). Preference for different habitats, for example, may occur during site selection and settlement of larvae (Highsmith 1982), during exploratory behaviour of post-larvae and juveniles (Pardo *et al.* 2007), and during the period of adult life (Aikins & Kikuchi 2001). Changes in behaviour may also occur during a daily cycle due to changes in patterns of activity of epifauna. For example, small soft-bodied herbivores tend to move at night when predators are less active (Hay *et al.* 1987; Bell 1991; Brawley 1992). In contrast, large herbivores such as fishes, urchins and some gastropods exhibit high mobility with relatively little probability of being successfully attacked by their predators (Hay *et al.* 1987). In

particular, some amphipods are more active at night than during the day. For example, the filter-feeding amphipod *Ericthonius brasiliensis* lives in domiciles it constructs by curling terminal segments of its host seaweed *Halimeda tuna*, but exclusively at night (Sotka *et al.* 1999). Criteria for epifauna host choice include intrinsic factors of the host such as cell wall components, defensive biochemistry, nutritive value and palatability, toughness and architectural complexity of seaweeds (Hawkins & Hartnoll 1983; Chemello & Milazzo 2002; Van Alstyne & Houser 2003). In addition, habitat selection may be determined by abundance, size and patchiness of habitat (Kumagai 2008).

When animals are associated with a particular habitat, it is often assumed that they have actively 'selected' that habitat. A common problem is the probable or actual misuse of the term 'preference'. It is always necessary to test (and be able to provide evidence to support) the model that behaviour of the animals can indeed account for the observed patterns (see Olabarria *et al.* 2002 and references therein). To avoid confounding preference (an active behaviour) with other causes of greater numbers being found in some habitats, it is also necessary to determine the pattern of occupancy of each type of habitat when available alone (i.e. when there is no choice and therefore no preference). If organisms choose a certain habitat (i.e. show a preference) they should select more of that habitat when presented with a mixture than would be the case when each of the various choices is presented alone. Therefore, experiments to demonstrate preferences are quite complex.

On intertidal shores, amphipods are often among the most dominant macroepifauna on macroalgae and seagrasses (Hay *et al.* 1987; Bell 1991; Aikins & Kikuchi 2001; Bates 2009). Despite generalist habits of most species of this group, some organisms have shown a strong host specificity (Duffy & Hay 1991; Poore *et al.* 2000), and they have been suggested to play a similar role to insects in terrestrial habitats because they are locally abundant, small relative to the host that they use for both habitat and food, and can have large impacts on host assemblage structure (Duffy & Hay 2000). An interesting group of amphipods that has been used as a model in evolutionary approaches and in several studies on ecology and behaviour is the family Ampithoidae (Arrontes 1999; Poore & Steinberg 1999; Cruz-Rivera & Hay 2001; Sotka 2003; Poore & Hill 2006). Previous studies have revealed that ampithoid amphipods differ in host specificity and composition (Poore *et al.* 2008), suggesting that evolutionary history may affect the ability of this family to colonise diverse algal taxa. As an example, the genus *Peramphithoe* rarely uses available macrophytes in the order Dictyotales, and as a consequence, displays a more restricted host range than other genera. However, other species from the

family are very often associated with the host genus *Sargassum* (Poore *et al.* 2008).

Amphitholina cuniculus (Stebbing 1874) is a small species that belongs to the family Ampithoidae and exhibits an alga-burrowing habit (Myers 1974). This species has been found burrowing into the thalli of different macroalgae such as *Alaria esculenta* (Linnaeus) Greville, *Fucus vesiculosus* Linnaeus or *Bifurcaria bifurcata* R. Ross (Myers 1974; Viejo 1999; Gestoso *et al.* 2010). A previous study done on the Galician coast showed that this species was especially abundant in *B. bifurcata*, although it also appeared sporadically associated with *Sargassum muticum* Yendo (Fensholt) (Gestoso *et al.* 2010) and with *F. vesiculosus* (I. Gestoso, personal observation). Due to this differential pattern of distribution, the study suggested that *A. cuniculus* could exhibit a preference for certain habitats.

Based upon these observations we designed a laboratory experiment in which we analysed the association of *A. cuniculus* with the most abundant algae that form mixed stands on low intertidal rocky shores along the Galician coast (Northwest Spain). An important aspect of the experimental design used here was the unconfounding of preference (active behavioural choice) from any other reasons for different occupancies of different types of habitat (e.g. differences in accessibility). Many studies on selection of habitats by different invertebrates have not used an experimental design like that used here, i.e. also determining the pattern of occupancy of each habitat when there is no choice (Durante & Chia 1991; McDonald & Bingham 2010). Here, we tested the hypotheses that (i) this species would exhibit different behaviour during the daily cycle (day *versus* night), and (ii) adults of *A. cuniculus* would prefer *B. bifurcata* as habitat rather than *F. vesiculosus* or *S. muticum*.

Material and Methods

Collection of amphipods and macroalgae

Amphipods and macroalgae (i.e. *Bifurcaria bifurcata*, *Fucus vesiculosus* and *Sargassum muticum*) were collected on a semi-exposed intertidal rocky shore in the southern region of the Galician coast (42º11.27′ N, 8º48.25′ W) during the low tide in June 2009. Macroalgae were carefully removed from the substratum and taken to the laboratory in insulated containers filled with seawater.

More individuals of *B. bifurcata* were collected because amphipods were more abundant in this species. Apical parts of *B. bifurcata* were examined carefully under a light microscope to pick up amphipods. After being removed from the algae, amphipods were kept in small containers filled with seawater until the experiment started (within 2 h). All amphipods used in the experiments were

adults with ~ 4 mm total length (Myers 1974; Lincoln 1979). Individuals of *B. bifurcata* that were used to collect amphipods were discarded and were not used as habitats in the laboratory experiments.

Because amphipods used in the experiments were collected from *B. bifurcata*, there might be a predisposition to be attracted to their original host. Prior to the experiment we conducted a pilot experiment to test whether amphipods collected from *B. bifurcata* were able to burrow indiscriminately in the three selected macroalgae. For that, we put ~ 50 g of each species of algae in two replicated 3-l circular plastic containers filled with seawater. Then we added four amphipods (collected in *B. bifurcata*) in each replicate and, after 12 h, we counted the individuals of *Amphitholina cuniculus* that were able to burrow into each alga. We also filmed the burrowing behaviour in the three species of algae. Results indicated that amphipods burrowed indiscriminately in the three algae ($F_{2,3} = 0.50$, $P = 0.645$). In addition, recorded films showed that amphipods were able to burrow in all algae within 2 h, although they were faster when burrowing in *B. bifurcata* or *F. vesiculosus* than in *S. muticum*.

Set-up of habitats

Macroalgae were kept in transparent 3-l circular plastic containers that were divided into three equal sections (using a plastic template) (Fig. 1), each containing an experimental habitat: B was *Bifurcaria bifurcata*, F was *Fucus vesiculosus* and S was *Sargassum muticum*. Pieces of approximately 10 g wet weight of alga were used as habitats. Containers were filled with filtered seawater and randomly placed on a table. Each experiment was run for 4 h because previous observations indicated that an amphipod could burrow into a thallus in 2 h, and it therefore was not unreasonable to think that they would demonstrate any choice of a habitat within 4 h.

Experimental design

Six individuals of *Amphitholina cuniculus* were placed into one experimental habitat (i.e. one of the three pieces of alga) using a paintbrush. Prior to the experiment, amphipods from 10 individuals of *Bifurcaria bifurcata* were counted and biomass of each alga was determined. Then, the maximum number of individuals per 1 g wet weight of alga was calculated to use similar densities to those found in the field (2.04 ± 0.94 per 1 g wet weight of alga).

The experimental design consisted of six different treatments, three of multiple choice and three others of no choice (see treatments in Fig. 2; *n* = 6 replicates of each treatment). The experiment compared the proportion of individuals of *A. cuniculus* in each of the habitats when presented together (treatments 1–3) or alone (treatments 4–6). At the end of the experiment in each treatment, we calculated the proportion of all amphipods found in each habitat, included the one in which they were initially placed (shown with a circle for each treatment in Fig. 2). Dead or swimming individuals were discarded from analyses. The experiment was run during the day and at night to test the first hypothesis that *A. cuniculus* exhibits different behaviour in a daily cycle. Each experiment was repeated twice (15 June 2009 and 23 June 2009) to check the consistency of results.

Preference for *B. bifurcata* would be reflected by a greater proportion of the amphipods in this habitat at the end of the experiment, compared with what is expected by chance if no preference is expressed. The chance of occurrence in habitats at the end of the experiment is estimated from the treatments where there is no choice (4–6; see Fig. 2). Preference for *B. bifurcata* involves acceptance of the following hypothesis:

Hypothesis 1: nB/N_1, $nB'/N_4 > nS/N_2$, nF/N_3

Where, at the end of the experiment, nB, nB', nS or nF is the number of individuals in the macroalgae where they were initially placed (*B. bifurcata*, *Sargassum muticum* or *Fucus vesiculosus*); N is the total number of individuals recovered from that container at the end of the experiment, $i = 1....6$, indicates the treatment.

The proportion of individuals in *B. bifurcata* (if amphipods really prefer this habitat) should be greater than in the other macroalgae. Thus, in treatments 1 and 4 this proportion will be significantly greater than in treatments 2 and 3 (non-preferred habitats) because in these two cases the amphipods should move to the preferred habitat and, therefore, out of *S. muticum* and *F. vesiculosus*, where they were placed initially (Fig. 2). There are different possibilities for differences among treatments 1–4. For instance, amphipods in treatment 4 may move to random positions because they have other individuals of *B. bifurcata* apart from the initial one where they were placed, resulting in $nB/N_1 > nB'/N_4$. Alternatively, they may not move out of their starting habitat because they would be already in their preferred habitat, resulting in nB/N_1 and nB'/N_4 being similar. In both cases, preference requires a smaller proportion of amphipods in S and F of treatments 2 and 3 than in B and B' of treatments 1 and 4, respectively.

Hypothesis 2: $nS'/N_5 > nS/N_2$

Hypothesis 3: $nF'/N_6 > nF/N_3$

because amphipods are more likely to move from S in treatment 2 and F in treatment 3 into the preferred macroalga (B), than into non-preferred habitats (S'' and S''') in treatment 5 and (F'' and F''') in treatment 6.
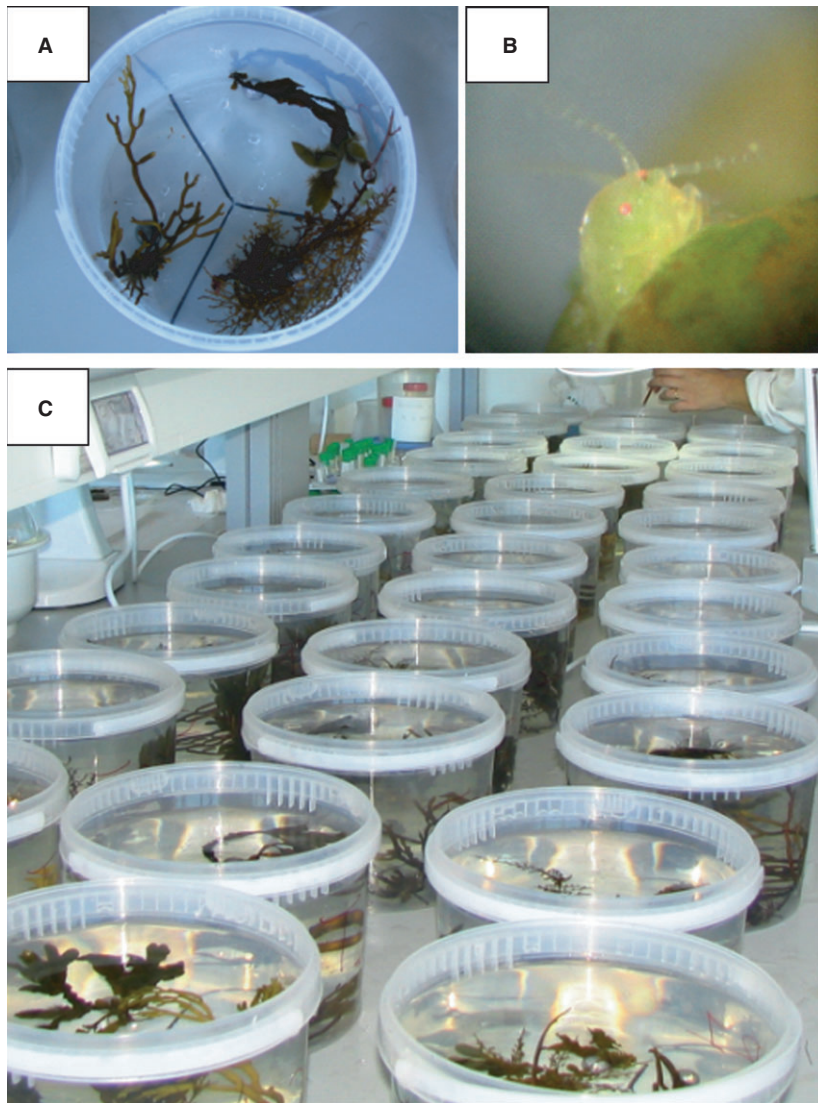
Fig. 1. Montage showing (A) the arrangement of macroalgae in containers, (B) one specimen of *Amphitholina cuniculus* (10 ×) and (C) the random set-up of containers on a table.

Treatments 5 and 6 do not have *B. bifurcata*, so amphipods should not move from initial macroalga, whereas in treatments 2 and 3, individuals have a choice and would move into the preferred *B. bifurcata*.

Analyses of data

To test the hypothesis that *Amphitholina cuniculus* exhibit different behaviour during a daily cycle, data were analysed using three orthogonal analyses of variance (ANOVA). Treatment (six levels, fixed), Time of day (two levels: day *versus* night, fixed), and Trial (two levels, random) were orthogonal factors. The hypotheses of preference were tested by analyses of variance which, when designs are balanced (as in this case), are robust to violations of assumptions of normality and homoscedasticity (Olabarria *et al.* 2002).

Results

A total of 864 individuals of *Amphitholina cuniculus* were placed into different habitats at the beginning of the experiments but only 763 individuals (88%) were recovered in any of the three habitats at the end of experiments (i.e. after 4 h).

The hypothesis that *A. cuniculus* exhibit different preference behaviour during the day than at night was not supported. Although proportions of amphipods varied significantly among treatments, this variation was consistent during the daily cycle (i.e. no significant main effect of Time of day, or significant interaction of Time of day with any other factor; Table 1). In addition, response was consistent in the two trials (i.e. no significant main effect of Trial, or interaction of Trial with any other factor; Table 1).

As Trial and Time of day did not have any effect on the proportions of amphipods found in each habitat, replicates
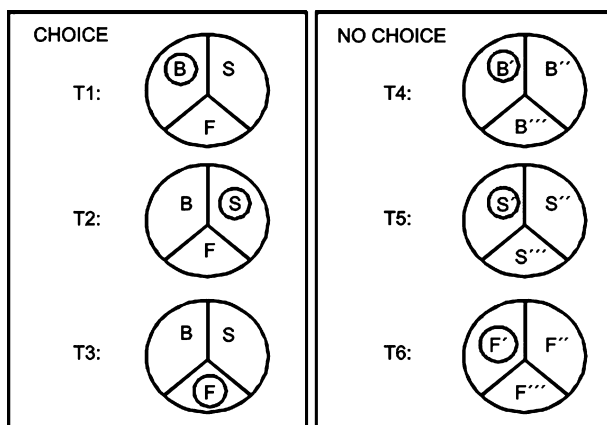
Fig. 2. Experimental design with six treatments ($n = 6$). B is *Bifurcaria bifurcata* habitat, S is *Sargassum muticum* habitat and F is *Fucus vesiculosus* habitat. The circle indicates the place where the individuals of *Amphitholina cuniculus* were placed at the beginning of the experiment.

Table 1. ANOVA analysis of proportions of *Amphitholina cuniculus* in experimental treatments. Time of day (day *versus* night) and treatment (six levels) were fixed factors, and Trial (two levels) was a random factor ($n = 6$).

Source	df	MS	F	P
Trial	1	0.1726	3.72	0.0560
Time of day	1	0.0508	2.19	0.3785
Treatment	5	0.4433	7.00	**0.0261**
Trial × Time of day	1	0.0232	0.50	0.4804
Trial × Treatment	5	0.0633	1.37	0.2420
Time of day × Treatment	5	0.0939	4.06	0.0752
Trial × Time of day × Treatment	5	0.0231	0.50	0.7762
Residual	120	0.0463		
Total	143			

Table 2. ANOVA analyses of proportions of *Amphitholina cuniculus* in experimental treatments. Data from the two experiments (day and night) and two trials were pooled ($n = 24$).

|	df	MS	F	P
Hypothesis 1: nB/N1, nB/N4 > nS/N2, nF/N3 (more in preferred habitat)				
Among treatments	3	0.7169	15.48	**0.0000**
Residual	92	0.0463		
Hypothesis 2: nS/N5 > nS/N2 (fewer in non-preferred habitats where there is choice)				
Among treatments	1	0.4020	6.39	**0.0150**
Residual	46	0.0629		
Hypothesis 3: nF/N6 > nF/N3				
Among treatments	1	0.4134	7.37	**0.0093**
Residual	46	0.0561		

[Correction added after online publication, 22 May 2013: in Hypothesis 2, col. 1, 'no choice' changed to 'choice'.]

of the different trials and different experiments (day and night) were pooled for analyses of preference ($n = 24$).

There were significant differences among the proportions of individuals in different habitats (Table 2). All analyses supported the hypotheses derived from the model in which adults of *A. cuniculus* prefer *Bifurcaria bifurcata*.

To test the second hypothesis of preference (see Material and Methods), *a priori* determined contrasts were used which demonstrated that a greater mean proportion of the individuals were recovered of those initially placed in *B. bifurcata* habitat (B in treatments 1 and 4; Fig. 3A) than in *Sargassum muticum* (S in treatment 2) or *Fucus vesiculosus* habitats (F in treatment 3) (contrast: nB/N_1, nB/N_4 *versus* nS/N_2, nF/N_3; $F_{1,92} = 16.1769$; $P = 0.0001$). This was entirely consistent with the hypothesis. There were also greater proportions of *A. cuniculus* in *B. bifurcata* when choices of preferred habitat were available (treatment 1) than when choices were not available (treatment 4) (con-

trast: nB/N_1 *versus* nB/N_4; $F_{1,92} = 5.8315$; $P = 0.0177$), probably due to the random movement of amphipods. Choice treatments of non-preferred habitats showed similar proportions (treatments 2 and 3) (contrast: nS/N_2 *versus* nF/N_3, $F_{1,92} = 0.3732$; $P = 0.5428$). [Correction added after online publication, 22 May 2013: 'No' deleted from start of preceding sentence.] In addition, there were smaller proportions of amphipods in non-preferred habitats when choices were available (treatments 2 and 3) than in those habitats when choices were not available (treatments 5 and 6) (Table 2, Fig. 3B,C). [Correction added after online publication, 22 May 2013: text '(treatments 5 and 6)' and '(treatments 2 and 3)' transposed in preceding sentence]. These results, therefore, supported hypotheses 2 and 3.

Discussion

The hypothesis that this species exhibits different behaviour during the daily cycle was not supported. In contrast, results confirmed the hypothesis that adults of *Amphitholina cuniculus* show a preference for *Bifurcaria bifurcata* habitat.

Previous studies have found that marine invertebrates may exhibit different behaviour during a daily cycle (Hay *et al.* 1987). In particular, amphipods are more active at night than during the day (e.g. Edgar 1983; Hay *et al.* 1987; Sotka *et al.* 1999). These organisms may constrain their foraging movements to avoid predators because the risk of predation increases when moving between seaweeds. However, our results indicated that *A. cuniculus* did not change patterns of selection of habitat during the daily cycle. In general, individuals were quite active during the day and at night in both trials (I. Gestoso, personal observation). Nevertheless, we have to take into account that experiments under laboratory conditions have certain constraints. For
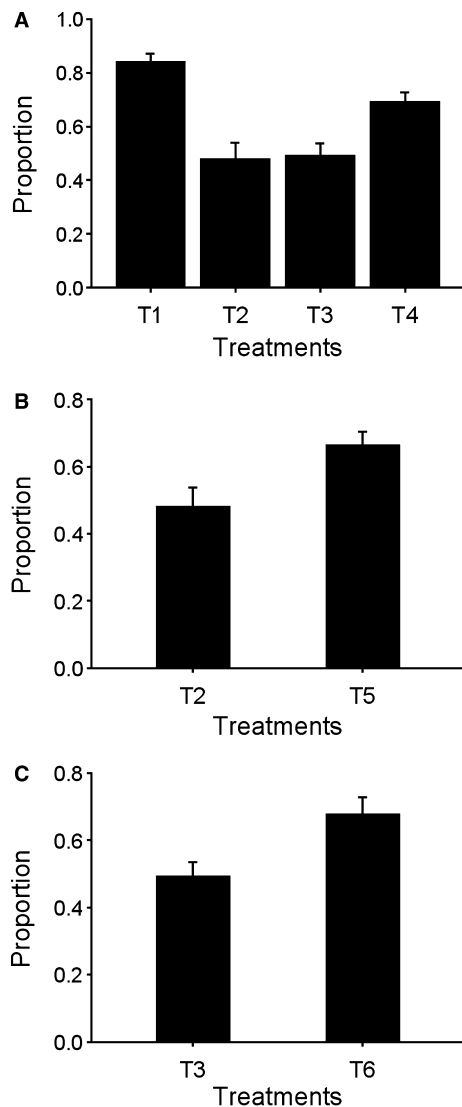
Fig. 3. Mean (+ SE) proportion of *Amphitholina cuniculus* remaining in treatments in the habitats where they were initially placed: (A) proportion of individuals in multiple choice and no choice treatments; (B) proportion of individuals remaining in *Sargassum muticum* habitat in choice (T2) and no choice (T5) treatments; (C) proportion of individuals in *Fucus vesiculosus* habitat in choice (T3) and no choice (T6) treatments.

example, the absence of large predators in the laboratory experiments might have an effect on the behaviour of this species because individuals may change behaviour in response to stimuli from predators (Chapman 2000).

In marine habitats, small herbivorous invertebrates tend to be generalists and very few species are host-plant specialists (Hay & Steinberg 1992; McDonald & Bingham 2010). In addition, most amphipods associated with seaweeds are habitat generalists (Duffy & Hay 1991). However, *A. cuniculus* might be considered a specialist species at least on

rocky intertidal shores along the Galician coast (Gestoso *et al.* 2010). The active behaviour shown by this species in the experiments may explain in part the distribution of this amphipod. For example, after widespread recruitment, adults of *A. cuniculus* could choose the favoured habitats (Underwood & Denley 1984). Alternatively, populations could distribute randomly across available habitats, but differential mortality would lead to a decrease of individuals in unfavourable habitats (Russo 1987).

Several works have pointed out that distributions of amphipods are rarely determined by differential mortality due to predation, active selection of habitat being a more important factor in determining patterns of distribution (Duffy & Hay 1991; Aikins & Kikuchi 2001; Poore 2004). Many factors related to intrinsic characteristics of macroalgae such as morphology, toughness, longevity, nutritional value or content of polyphenols may influence the choices of species (Hawkins & Hartnoll 1983; Chemello & Milazzo 2002; Van Alstyne & Houser 2003; McDonald & Bingham 2010). More complex morphologies may allow amphipods to escape detection or attack by visual predators (Hay 1991) or prevent dislodgement by waves (Sotka 2007). In this particular case, morphological complexity did not seem to play an important role. Although not formally analysed, *Fucus vesiculosus* and *Sargassum muticum* have a more complex morphology than *B. bifurcata*, which was the preferred habitat. *Fucus vesiculosus* and *S. muticum* are more branched and present vesicles, whereas *B. bifurcata* is a less frondose and cylindrical macroalga. Furthermore, algal toughness and stability of habitat (influenced by longevity of algae) have been reported to be more important factors than morphology (McDonald & Bingham 2010). A greater stability of habitat might favour certain species of amphipods, especially in habitats where risk of predation is high or environmental conditions are stressful (Duffy & Hay 1991). The fact that *B. bifurcata* is a perennial alga might explain why the amphipod *A. cuniculus* prefers this species over the pseudoperennial *S. muticum*.

The chemical traits of the host may also affect the susceptibility of amphipods to predation, and predation on small invertebrates may be reduced due to their association with chemically rich hosts that are avoided by omnivorous fish (Poore *et al.* 2000). *Bifurcaria bifurcata* is well known for its capacity to synthesise a wide variety of acrylic diterpenes (Ortalo-Magné *et al.* 2005). *Amphitholina cuniculus* might be able to tolerate these compounds because it supposes a lower risk of being consumed than if it selects more palatable species (Taylor & Steinberg 2005). Nevertheless, within the family Ampithoidae, responses to non-polar metabolites in host algae are very variable, with species showing negative relationships (*Peramphithoe*, *Biancolina*), positive relationships (*Exampithoe*) or no relation (*Ampithoe* spp.) (Poore *et al.* 2008). In addition, the nutritional

value of host seaweeds may influence the selection of host alga (Cruz-Rivera & Hay 2000).

In conclusion, it is very likely that the amphipod should get some benefit from selecting *B. bifurcata* against *F. vesiculosus* and *S. muticum*. For example, the amphipod might have greater fitness (i.e. maximising growth or mate encounter rates), get a good quality food and refuge from abiotic stress and predators, or avoid competition with other species, among other possibilities. Unfortunately, this experiment did not allow us to determine the underlying mechanisms of habitat choice and, therefore, the evolutionary forces that maintain this restricted host choice are unclear.

Preference experiments in the laboratory should be extrapolated to natural systems with caution (Chapman 2000). For example, the laboratory preference assays were conducted on small scales that may not represent the scales on which organisms discriminate among hosts in the field. Therefore, factors such as availability of preferred habitat or proximity of available hosts (Jonsen *et al.* 2001) might influence the distribution of amphipods in the field. Indeed, the spatial arrangement of plants affects strongly patterns of colonisation of marine invertebrates associated with vegetated substrates (Bell *et al.* 2001). Not only colonisation, but also post-colonisation processes (competition, predation, disturbance, immigration/emigration, etc.) may be important in determining patterns of spatial distribution of these organisms (Olabarria 2002, and references therein). Thus, it is essential that laboratory studies be accompanied by field tests of related hypotheses, so that differences between field-based behaviour and laboratory-based behaviour can be measured and the relevance of the laboratory studies sensibly evaluated. We are concerned about these difficulties but tried to make the conditions as realistic as possible, maintaining amphipods in the laboratory along with natural algae prior to the experiment. The experiments were also repeated twice to examine the consistency of patterns. In addition, these experiments were based on observations from a previous field study in which the distribution of mobile epifauna, including this species, was evaluated at different spatio-temporal scales. It would be desirable to carry out some parts of these experiments in the field if logistic problems could be overcome, using the information gained here as a guide. Nevertheless, results suggested that the behaviour of these amphipods may explain in part their distribution in the field.

Acknowledgements

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