

## ORIGINAL ARTICLE

**Recent innovations in marine biology**

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**Abstract**

Modern ecology arose from natural history when Vito Volterra analysed Umberto D'Ancona's time series of Adriatic fisheries, formulating the famous equations describing the linked fluctuations of a predator–prey system. The shift from simple observation to careful sampling design, and hypothesis building and testing, often with manipulative approaches, is probably the most relevant innovation in ecology, leading from descriptive to experimental studies, with the use of powerful analytical tools to extract data (from satellites to molecular analyses) and to treat them, and modelling efforts leading to predictions. However, the historical component, time, is paramount in environmental systems: short-term experiments must cope with the long term if we want to understand change. Chaos theory showed that complex systems are inherently unpredictable: equational, predictive science is only feasible over the short term and for a small number of variables. Ecology is characterized by a high number of variables (*e.g.* species) interacting over wide temporal and spatial scales. The greatest recent conceptual innovation, thus, is to have realized that natural history is important, and that the understanding of complexity calls for humility. This is not a return to the past, because now we can give proper value to statistical approaches aimed at formalizing the description and the understanding of the natural world in a rigorous way. Predictions can only be weak, linked to the identification of the attractors of chaotic systems, and are aimed more at depicting scenarios than at forecasting the future with precision. Ecology was originally split into two branches: autecology (ecology of species) and synecology (ecology of species assemblages, communities, ecosystems). The two approaches are almost synonymous with the two fashionable concepts of today: 'biodiversity' and 'ecosystem functioning'. A great challenge is to put the two together and work at multiple temporal and spatial scales. This requires the identification of all variables (*i.e.* species and their ecology: biodiversity, or autoecology) and of all connections among them and with the physical world (*i.e.* ecosystem functioning, or synecology). Marine ecosystems are the least impacted by human pressures, compared to terrestrial ones, and are thus the best arena to understand the structure and function of the natural world, allowing for comparison between areas with and areas without human impact.

**The Science of Environment**

Ecology has been studied since the dawn of culture. Humans depended on the natural world for survival: knowing about both animals and plants was vital to draw

resources from the environment, and our ancestors had to learn where to get the animals and plants they needed. Our understanding of environmental functioning, however, was aimed at exploitation and did not care about management and conservation. In the Pleistocene,

humans pushed many terrestrial species to extinction simply by hunting them (Alroy 2001), and then, with agriculture (Diamond 2002), they devastated pristine habitats for millennia, leading to environmental catastrophes that resulted in the disappearance of the civilizations that caused them (e.g. Jansen & Scheffer 2004). Only recently have humans started to understand that nature is limited and that exploitation of its resources requires proper management and conservation (Malthus 1798). The response of human populations to environmental devastation, until now, has simply been demographic collapse due to famine, disease, or war. Even if the origin of taboos is linked with a perceived need to preserve some precious and delicate species or habitats (Colding & Folke 1997), non-formalized science rarely attempted to conserve natural resources. This was particularly true for the marine environment, originally presumed to be inexhaustible (Huxley 1884). Only recently, again, have humans perceived that the exploitation of marine populations cannot proceed indefinitely.

The precondition of a proper understanding to perform proper management has led to deeper studies of marine ecosystems, with great conceptual innovations based on a philosophical revolution in marine ecology. Everything started from fisheries.

### Reductionistic Fisheries Science

Modern ecology started with Vito Volterra's models of fisheries (Kingsland 1995; Gatto 2009). However, the famous equations, obtained independently by Lotka, accounted for just two species. It is a common approach, especially in physics, to consider variables in couples, assuming that the rest of the system remains unvaried. Complex systems, thus, are analysed as simplified subsets to reduce their complexity, the subsets then eventually re-assembled to make inferences about the structure and function of the system while considering all its variables. This attitude stems also from the famous Poincaré's 'problem of the three bodies' (Poincaré 1890), postulating that when more than two variables (bodies) are considered, their behaviour becomes inherently unpredictable after just a few interactions. The same conclusion was reached later by chaos theory. In fisheries science, the two variables were the abundance of the target species and the fishing effort of humans. The target species (the prey) was considered as a single item, and its population dynamics were studied by considering the succession of cohorts extrapolated from the measurement of individuals. Humans, with fisheries, are the main source of mortality for the target species. It is true that overfishing is an overwhelming source of mortality for many species, but this scenario is far too simple. Fisheries management resulted in tragic failure (Jackson

*et al.* 2001), partly due to the oversimplification of models and partly due to the lack of responsiveness by both artisanal and industrial fisheries to the advice of fishery scientists. The fish-human approach, thus, required a drastic change in attitude, and the European Union embraced the ecosystem approach, proposed in 1992 by the Rio Convention on Biological Diversity. Humans and fish cannot be extracted from the ecosystem(s) they inhabit. This means that the number of variables is much greater than two. Another innovation, stemming from the unavoidable uncertainty of the sciences of complexity, was the precautionary principle. To prevent is better than to cure: it is always safer to consider the worst case scenario when the available knowledge is not sufficient to perform safe predictions about the effects of our action.

### Fisheries Statistics

Although the modelling of fisheries is not reliable in terms of predicting the future, fisheries statistics are a precious source of information to understand the present by analysing the past. Another great innovation was thus the synthesis of fisheries statistics worldwide to assess the health of fish populations. After all, fisheries is a sort of sampling, and from fisheries data we have a continuous quantitative evaluation of fish populations. Samples, however, should not disturb the investigated system, whereas in the case of fisheries, sampling is highly destructive. The late Ransom Myers, a mathematician, became famous by collecting the scattered data of fisheries yields worldwide and by analysing them to estimate the global conditions of fish populations. His sampling effort was negligible, the samples were there, in the drawers of fisheries scientists. He 'just' retrieved the pieces of the puzzle and put them together. The derived picture was appalling (Myers & Worm 2003). Large fish are quickly disappearing and, as Pauly *et al.* (1998) said, we are 'fishing down marine food webs', as the higher levels of food webs are almost exhausted. In the Pleistocene, human hunters depleted the terrestrial megafauna and drove many species to extinction. Now we are doing the same to the marine megafauna. Our ancestors, however, did not know the consequences of their actions and aimed at proximate advantages, not having a perception of the ultimate disadvantages. Today, we do know what will happen. Maybe our models are not so accurate as to predict with precision when the collapse will take place, but we can be pretty sure that it will take place.

### Compartmentalized Marine Ecology

For many years ecology was a no-man's land. The general principles were drawn from observations on natural sys-

tems, usually carried out at easily reachable places, where manipulations are possible. The intertidal, instead of being what it is, an ecotone, became the paradigm of all environments, and the 'rules' inferred there became generalized. This naturalistic approach had little connection with the branches of marine studies that had some link with human activities. These worked in almost total isolation from the 'naturalistic' branch. Fisheries studies, on the one hand, considered only human-impacted systems, almost divorcing them from the rest of nature, whereas the rest of marine studies considered non-impacted systems only, as if man did not exist. The two fields grew separately, with different scientists publishing in different journals and speaking different languages. Then, studies on pollution became popular, especially after the occurrence of Minamata disease, and heavy metals took their toll in marine science, together with oil and sewage pollution. When humans started to realize their own impact on marine habitats, they started to protect some areas and the era of marine conservation began, with flourishing studies on Marine Protected Areas. The link of without-humans ecology with with-humans ecology is probably one of the greatest revolutions of the environmental sciences. Compartmentalizations began to be broken down.

### Fisheries and Aquaculture

Aquaculture is commonly perceived as a way to save natural fish populations from the overexploitation of fisheries (Anonymous 2003). Looking back on human history, we can see that the passage from hunting and gathering to agriculture did not save terrestrial natural populations but, instead, further impacted on them. Natural environments and populations were drastically modified as sites for agriculture. In the sea, this process is even more acute. On land, in fact, the only animals we rear for feeding purposes are herbivores, whereas in the sea, we mainly rear carnivores, the tigers of Naylor & Burke (2005). What do we feed them with? There are three possible answers: (i) pellets made of smaller fish, coming from natural populations, (ii) pellets of plant food such as soybean, as a substitute for animal proteins, (iii) pellets made with the remnants of terrestrial animals that we use for food. In the first case, the use of fish food to sustain farmed fish represents an unwise way of solving the problem of the overexploitation of marine resources, as the conversion coefficient of this food is low and the impact on nature great (Brunner *et al.* 2009). After having depleted the populations of large fish, we are fishing down the food web to feed captive specimens of those same depleted species. To transform carnivores into herbivores, furthermore, is a forcing of nature that may have

unpredictable effects, as happened on land when we tried to transform herbivores into carnivores (and cannibals). Similar outcomes might result also from the third option. It is very probable that the farming of large carnivores will not solve the problem of food demand due to our numerical increase, as this practice is evidently unsustainable from an environmental point of view, not to speak of the impact of fish farming on the natural environment (Pusceddu *et al.* 2007) and the vulnerability of farmed fish to both disease and predators such as jellyfish (Boero *et al.* 2008a).

### From a Fish to a Jellyfish Ocean

The ecosystem approach can teach us that the standing biomass of a given ecosystem is more or less stable, but that its repartition among the species that make up that ecosystem changes constantly. If we remove biomass from an ecosystem, the absence of a sink (the target species, seen as a resource simply because of its abundance) will channel production towards other species, leading to an overall biomass similar to the pre-impact stage. Quantity is more or less the same, once the carrying capacity of the system is reached, but quality can change drastically. The constant reports of lack of large vertebrates worldwide are counterbalanced by the continual reports of jellyfish blooms worldwide. We are witnessing the shift from a fish to a jellyfish ocean! We catch the large fish? The biomass that was once channelled towards them can move in another direction, for instance jellyfish. If jellyfish prevail, they are a further blow to fish populations, as they prey upon the prey of fish larvae (crustacean plankton) and on the larvae themselves, acting as both competitors and predators of fish (Boero *et al.* 2008a).

### A Ctenophore Lesson

When the alien ctenophore *Mnemiopsis leyidi* reached the Black Sea, the impact of the new arrival on fish populations was more severe than that of industrial fisheries (CIESM 2002b). The collapse of Black Sea fisheries, in fact, did not result from a long history of human pressures, but from the sudden presence of an alien ctenophore! The blooms of gelatinous plankton are an essential variable to assess the causes leading to the viability of fish populations. A similar scenario might occur for cod at Georges Bank, where the enormous numbers of floating colonies of the hydroid *Clytia gracilis* are probably impairing the recruitment of the fish, by feeding on cod larvae and on their crustacean food (Bollens *et al.* 2001).

Jellyfish blooms are not considered in fisheries models! Larval mortality is taken into consideration as a more or less constant toll that the populations have to pay, but it

is becoming obvious that this toll can change from year to year, and if jellyfish are present in great numbers, they cause catastrophic larval mortality! Jellyfish, furthermore, can also cause adult fish mortality. A recent bloom of *Pelagia noctiluca* drove the farmed salmon of Ireland to extinction, showing that cage aquaculture is extremely vulnerable to jellyfish blooms. Before this episode, it was thought that larger fish escaped from jellyfish predation: small fish are vulnerable but, as they grow, their size makes them invulnerable to jellyfish weapons. Evidently this is not the case. Fish escape jellyfish when they shift from planktonic larvae and juveniles to nektonic adults simply because they are faster swimmers, they escape by... escaping. If kept in a cage, they cannot escape from jellyfish and are killed by them. In nature, a jellyfish bloom affects fish populations by impairing their recruitment; with cage mariculture, jellyfish blooms impact on the adults (whereas eggs and larvae are safe in artificial hatcheries and nurseries).

Gelatinous plankters, thus, are not freaks in marine biology and ecology. Their irregular, sudden, and massive presence is the shuffling of the cards of the ecological play, probably preventing the prevalence of a few, extremely successful, nektonic species by reducing their population via predation on their larvae and on their food (Boero *et al.* 2008a).

### Story-telling versus Experimental Approaches and Mathematical Modelling

There was a demise of natural history at the dawn of modern ecology, because of its story-telling approach, lacking precision and aimed more at the description of patterns and processes with verbal models (the stories) than at predictive mathematical modelling based on experimental tests of hypotheses. This led to the reductionistic approach that divided ecology into many sub-ecologies and transformed the discipline into an oxymoron. Reductionistic ecology loses the very spirit of ecology: the identification of the network of intricate interactions leading to the emerging properties of complex systems.

Story-telling and descriptive approaches have been and still are ridiculed by experimentally and mathematically oriented scientists (Gardner *et al.* 2007). This led to the relegation of descriptive ecology to local journals with no impact factor, and into peripheral institutions, with low funding availability and low attractivity for bright new students. Paradoxically, knowing animals and plants was less mandatory for the young generations of ecologists than knowing complex mathematics and statistics. 'Theoretical' approaches were invariably mathematically oriented and aimed at making predictions that were obtained by the

identification of general rules (laws). The quest for ecological laws was great and stemmed from the Popperian approach to the natural sciences. Each rule (law), in fact, is to be universal and thus applicable to all situations. If it fails to explain a situation that should follow its command, then the law is falsified and is to be rejected. The universal laws of ecology and evolution do not exist, and when they operate, they are the laws of physics and chemistry! The 'laws' that predict the development of a community are three (at least) and each time one is valid, the others are falsified. What is the 'law' that predicts the development of a community? There are no universal laws in ecology, the laws are existential: they predict the existence of a given set of events, but do not postulate their universality (Boero *et al.* 2004). *There are no sharks in the bay* is a universal statement that can be only falsified, it cannot be verified, falling into what is demanded by Popperian science, whereas *There are sharks in the bay* is an existential statement that can be only verified but cannot be falsified. Ecology and marine biology are based on existential rules that sometimes are valid and sometimes are not. At the same time, mathematical modelling becomes too rigid and cumbersome to account for such situations, and its precision is so low anyway that there is not much difference between it and verbal modelling. Of course the mathematical make-up has the allure of precision for any attempt at modelling nature, but confidence in such approaches is slowly fading, as we recognize that ecology is a historical discipline and that the mathematical modeling leading to predict future history is just an illusion.

Story-telling, as remarked by Gardner *et al.* (2007), is essential to generate hypotheses which then are to be tested with the most rigorous experiments. It is important, however, to understand whether we are looking for universal or existential statements. Asking for universal solutions in a domain of existential problems leads nowhere.

The 'grand picture' of ecology has still to be depicted, but the various components are slowly emerging, first as single elements. Here are some of the recently discovered ones.

### The Microbial Loop. Or is it a Pathway?

It has been known for a very long time that the bulk of production of the biosphere is based on the activity of the microscopic protists we call phytoplankton. Textbook knowledge teaches that nutrients are taken up by photosynthetic microbes, which in their turn feed herbivorous plankton (mainly crustaceans). These then fuel a food web leading to the largest fish and mammals. The flow of energy thus should go from microbes to metazoa. Very few people considered that the heterotrophic microbes, besides

decomposing bacteria, play an essential role in the turnover of organic matter. Heterotrophic protists such as ciliates and flagellates feed upon bacteria and phytoplankton and represent a powerful sink of marine production. The appreciation of this component led to considering the microbial world as a loop (Azam *et al.* 1983) in which organic matter flows indefinitely, independent of contact with larger organisms. This microscopic world was, indeed, self sufficient (and thus a loop) for billions of years, before multicellular organisms evolved. The source of mortality of bacteria, when no 'higher' organisms were present, was presumably viruses, as still happens in a massive way in the deep sea (Danovaro *et al.* 2008). Since the Cambrian explosion, however, metazoa dominate the seas. Certainly, they depend on microbes for both primary production and decomposition but in doing so, they broke the microbial loop, transforming it into a pathway. As higher metazoans, it is obvious that, while giving proper importance to the basic role of microbial worlds, we are more interested in the parts of the biosphere that are more directly connected with us, taking for granted that the basal part will work, as it did for some billion years! After a period of oblivion, the microbial world has been given the importance it merits.

The study of marine viruses focused on even smaller worlds and is just opening new perspectives, leading to a better appreciation of the structural and functional complexity of marine realms (Danovaro *et al.* 2008).

### Marine Ecology from Molecules to Space

The exploration of the microbial world required a thorough change in the techniques employed. The microscope was not enough. Molecular studies started to reveal unexpected worlds in microbial diversity (*e.g.* Delong 1992) with strong insights also in human health. The discovery of the association of *Vibrio* species with copepods (*e.g.* Rawlings *et al.* 2007) and other chitin-wrapped organisms (Stabili *et al.* 2008), both in the plankton and in the benthos, is suggesting new scenarios for human epidemics. Molecular studies have enhanced taxonomy (see below) and especially biogeography to even a greater extent. The study of both historical ecology and phylogeography is allowing further insights for understanding present-day situations of marine biodiversity (Wares & Cunningham 2001). The use of electronic tracking by satellite, coupled with molecular analyses, is providing precious information on the patterns and processes that lead to the distribution of organisms such as the blue fin tuna (Boustany *et al.* 2008).

### Alien Species

Human activities, from shipping to aquaculture, to the opening of new connections among basins, are widening

the distribution of species, allowing the crossing of previously unsurmountable geographic barriers. The problem of alien, or, better, Non-Indigenous-Species (NIS), and their transport is a key issue in understanding the patterns and processes involved in marine biodiversity (CIESM 2002b). Molecular techniques are crucial to reconstruct the patterns of introduction of NIS, their sites of origin, and the viability of their newly founded populations (*e.g.* McIvor *et al.* 2001).

### What about Pathogens and Parasites?

Parasites, along with pathogens, are extremely important in controlling the populations of the various species, and their study is still at its dawn in all branches of ecology, not only marine ecology (Mattiucci & Nascetti 2008). Pathogens and parasites are among the future frontiers of marine ecology. They might be the equal of predators (after all, parasites are micropredators) in controlling natural populations but this aspect of marine ecology is still far from being incorporated into ecological theory (and practice) (see Rhode 2005, for a comprehensive treatise on marine parasitology).

### Life Cycles and Life Histories

The succession of stages that an organism undergoes throughout its existence makes up its life cycle. The quantities of these stages (from gametes, to zygotes, larval stages, juveniles, reproductive adults) make up its life history. Most of the life cycles of known marine organisms are unknown, and we know even less about their life histories (Giangrande *et al.* 1994). Every stage of life history has its own ecological niche, and the sum of the ecological niches of all stages makes up the ecological niche of the species. These aspects are studied by population dynamics and are very advanced in fisheries studies, even though egg and larval mortalities of fish are a black hole in the knowledge of their population dynamics. We still have to describe a great quantity of unknown species, to properly evaluate marine biodiversity and, for each of them and for the already known ones, we still have to describe the life cycle and reconstruct the life history.

### Looking for Links

Life-cycle dynamics have only been incorporated into ecological studies for a short time. The biology of coastal plankters, for instance, taught us that many species spend the adverse season as resting stages in coastal sediments, and even on the bottom of marine canyons (Boero *et al.* 1996; Marcus & Boero 1998; Della Tommasa *et al.* 2000). Benthic pelagic coupling started as a biogeochemistry



issue, with nutrients flowing from the water column to the bottom and then to the water column again. However, it is evident that this coupling also concerns life cycles and that many so-called holoplankters are the reverse of the commonly perceived meroplankton. Meroplankton, in fact, is the larval component of plankton and, after this stage, it becomes either benthos or nekton. But there are organisms that have a planktonic life as adults and spend their egg, or zygote or larval life in the benthos, as resting stages. These species can be either protists or metazoans. The boundaries between benthos and plankton are no longer clear, and the two domains are too connected to each other to be properly understood by approaches that are too tightly focused. The appreciation of these links might lead to even subtler connections, such as that hypothesized between plankton diversity and the predation of meiobenthos on resting stage banks, giving a keystone role to the meiobenthos (Pati *et al.* 1999). Other unexpected connections might be, for instance, that mollusc overfishing prevents shell deposits on beaches, leading to coastal erosion (see below).

### Putting the Pieces Together

It is evident that fisheries, aquaculture and jellyfish biology are important parts of ecosystems and that, sooner or later, they must be merged so as to have a proper appreciation of the complexity of marine systems (Pauly *et al.* 2008). This exercise is very hard, though, as complexity hinders our comprehension and, furthermore, what has been listed so far is just a small portion of the variables that make up marine ecosystems. In recent years, we discovered that life is expressed in many unexpected ways. The importance of microbes, taken for granted but never explored in detail, led to the recognition of the microbial loop. The exploration of the deep sea led to the discovery of hydrothermal vent communities, virtually independent of sunlight as a source of energy. The exploration of interstitial environments led to the discovery of new phyla and of unexpected connections among marine domains that had previously been regarded as very separate. If the meiobenthos can feed on the resting stages of plankters, then the meiofauna can be a keystone guild controlling the diversity of plankton! A still almost completely neglected aspect of marine biology is the ecological importance of marine parasites. They are studied by parasitologists, but their ecological role is rarely taken into account when dealing with the dynamics of marine populations, contributing to ecosystem functioning. Behavioural ecology, furthermore, is well developed on land but is still at its dawn in the marine world, with the exception of a very few obvious species such as cetaceans. For the overwhelming majority of species whose existence we are

aware of, we know neither the life cycle nor the ecological role. It is obvious that our level of ignorance is still very great.

These aspects, and many others, do not stem from a theoretical approach that is aimed at depicting a grand scenario that is then to be explored in detail, component by component. The approach is purely inductive. It is discovered that hydrothermal vents exist, and then they are explored. It is realized that microbes are important and then they are studied. The general synthesis occurs later, from an almost random assemblage of disconnected pieces.

### Goods and Services

A common, human perception of nature is that everything is there for us to take advantage of. Biodiversity is there to provide us with the 'goods and services' so essential for our well-being. This is still the notion that is usually put forward to convince people that biodiversity is important (Hooper *et al.* 2005). The vision is anthropocentric, and does not consider the 'right' of nature to continue to thrive. As we are part of nature, our impact is natural. We are like the first organisms that started to produce oxygen as a by-product of their metabolism. They made the world less hospitable for the anaerobic biota. Nature knows no compassion. Maybe we could shift from the merely utilitarian approach to biodiversity to a deeper vision, paradoxically sometimes expressed more by religion than by science. As people tend to listen more to religious authorities than to scientists (Wilson 2006), we are running the risk of being surpassed by some religious community in explaining why biodiversity is to be protected.

### Habitats

If biodiversity is usually perceived as 'species' by the lay people, it is commonplace within the scientific community that the other two levels (the genetic and the habitat-ecosystem) are equally important (Gray 1997). A very long time will be required to answer May's (1988) question: How many species are there on Earth? But it may be easier to answer the question: How many habitats are there on Earth (Fraschetti *et al.* 2008)? Once the list is made, we will have to answer the question: Where do they occur? Another great innovation is the introduction of GIS in ecology and biogeography. We can now locate single individuals and follow them in their migrations (Block *et al.* 2001). And we can reconstruct maps of habitat distribution in extreme detail, and monitor the boundaries of these habitats to see whether they are stable, or are growing or regressing. The exploration of the

environment is crucial and there is, also at this stage, a passage from an imprecise and descriptive account of habitat distribution to a precise and experimental way to draw information from the environment so to have repeatable observations.

These approaches are relatively easy for benthic communities, but plankton and nekton also have their habitats in the water column, albeit less definite than benthic ones, within their boundaries. The definition of the boundaries of pelagic habitats is obviously based on current regimes, but these can change, too, and many strange patterns of water movement are constantly being discovered, such as the places where deep-water formation occurs at the surface of the sea (*e.g.* the Northern Adriatic and the Gulf of Lions for the deep Mediterranean waters) (Boero *et al.* 2008b).

## History

Ecology and biology are characterized by never-ending change. All living things are born and eventually die, continents move, new seas are formed and old ones die. History is never the same, even though some patterns may recur. The long-term history of life is evolution, leading, for instance, to the origin of new species. Ecology must cope with a short-term history that, however, is affecting the structure and function of communities by local extinctions and immigrations, by climatic changes and, of course, by our impact. History is ruled by two opposite drivers: constraints and contingencies (Boero & Bonsdorff 2007). Our understanding of how the environment works is based, first of all, on the appreciation of constraints; as these set the 'rules' that allow for the perpetuation of life, they may be the 'general laws' sought by Popperian logic. Contingencies, however, are the expression of the quintessence of history: the beat of the wings of a butterfly that (sometimes) can lead to a hurricane. Chaos theory demonstrates that deterministic systems are ruled by constraints (attractors, in the terminology of the theory) but that the system has a great freedom to change within the limits of these constraints, and this change is often caused by contingencies (the existential side of the way life is regulated).

We are attracted by regularities, by norms and laws. We have thus dedicated great efforts to single out the constraints that rule the world. Now it is time to perceive that rules are broken all the time, and that, sometimes, irregularities rule the world. The best example is the already mentioned shuffling of the cards by massive blooms of gelatinous organisms (Boero *et al.* 2008a), but more and more evidence in this direction is accumulating (Benincà *et al.* 2008; Doak *et al.* 2008).

## Bureaucracy

The way science proceeds is linked to fund availability. Projects are commonly short term, and are focused on specific topics leading to testable results that, usually, should be 'novel' and 'exciting'. Fashionable research topics, usually sustained by scientific lobbies, receive much funding, whereas other topics, not sustained by powerful lobbies, tend to disappear or, worst, are 'invaded' by other lobbies. The worst case is that of taxonomy. Molecular and computerized taxonomy is taking almost all the funding, leaving traditional taxonomy in almost complete poverty (Ebach & Holdrege 2005). It is often the case that the format of research projects is very complicated and the requirements to be fulfilled are so cumbersome that many scientists simply give up applying. Furthermore, results are seldom validated and controls focus on financial aspects only, often with costly financial audits. The outcome of all this is that the successful scientists are the ones good at managing large projects. Devoting most of their time to this, they obviously dedicate less time to research. As scientific validation is a useless optional, at least in some projects, funding policies often hamper scientific progress. Apparently, this behavioural pattern is not pursued in the USA, at least by the National Science Foundation. No wonder the scientific community of the USA is much more productive than the European one! We spend most of our time dealing with bureaucracy: they spend it on science. Our results are often validated by satisfying bureaucratic requirements, whereas theirs are validated by scientific excellence. This explains why the brain drain affecting many European countries is going towards the USA more than anywhere else (Boero 2001).

## Publications

The impact factor frenzy has affected research trends for several decades, determining career and funding opportunities. Some areas of biological research have profited from this situation, others have been damaged by it. The need for scientific scoops in high impact journals is not supportive of long-term research: projects are usually short and are renewed on the basis of the scientific production in the 'best' tribunes (*i.e.* journals with high impact factor). Career and funding opportunities are based on the number of articles and the sum of their impact factors. A nicely conducted ecological experiment, based on an impeccable design and data treatment is usually publishable in good journals, and can be carried out in a relatively short time. A long series of observations, on the other hand, besides requiring more time, is usually labelled as 'descriptive' and has low viability in high impact journals. Paradoxically, however, these data

become invaluable to assess trends of change and reference conditions. Data-mining is becoming a fashionable activity in research projects, as the data readily available in bibliographic repertoires, covering a limited set of journals, are often useless to make an assessment of the history of biota. In this period of global change, the possibility of retrieving data over long time spans is important to make assessments about the state of the environment. If all scientists had followed the predominant trends, we would know much less about the state of the environment. The lack of importance given to 'descriptive' studies is leading to a decreasing quality control (in the form of peer review) of their outcomes. Meta-analyses, based on the so-called grey literature or on unpublished data, or on data published on journals that do not perform a severe control of what they publish, might be conducted with the most beautiful algorithms and rationales, but the old saying of statisticians – 'garbage in, garbage out' – cannot be circumvented by statistical formulations. Very few journals, if any, perform a strict control of the accuracy of the list of species that describes the biodiversity within ecological papers. Many journals do not even provide the list, whereas they publish impeccable treatments of data that might not be as reliable as the reputation of the journal might warrant.

### Trendy Biodiversity Studies

The era of biodiversity started with the Rio de Janeiro Convention on Biological Diversity in 1992. Since then, the presence of the word 'biodiversity' in any project has been a warranty of success. Some calls were aimed at using biodiversity as a tool to understand the environment, other calls at promoting biodiversity research in a direct way, as it is obvious that biodiversity exploration is not complete and, anyway, the distribution of biodiversity changes all the time. In the same period, however, the science of biodiversity identification and description (*i.e.* taxonomy) started to enter into a deep crisis. There has been a great development of molecular taxonomy, with the birth of new journals dedicated just to it, but traditional taxonomy, based on morphology and developmental biology, started to be perceived as obsolete, partly because most of the journals dedicated to it (*e.g.* Museum journals) did not have any impact factor and, thus, publishing in them was not of great help in scientific careers and in applications for research funds. Most of the money dedicated to biodiversity went to molecular taxonomy and to the building of computer-based inventories (*e.g.* Arvanitidis *et al.* 2006), assuming that morphological approaches produced all possible fruits. Taxonomy became (and still is) synonymous with identification. Taxonomic sufficiency, furthermore, implies that the study of higher taxa is sufficient to

appreciate biodiversity. This position is somehow ambiguous. Taxonomic sufficiency might be sufficient to detect impacts, but it does not by definition reflect biodiversity at species level and thus cannot be sufficient to appreciate the core of biodiversity – species (Terlizzi *et al.* 2009). Ecology and taxonomy should go together, as both can profit much from each other (*e.g.* Neto 1992).

### Safe Topics

An ideal trend of research projects should be to propose something that has been already accomplished (at least in part), so to be sure to have results and fulfil the requirements of the project, and then invest the freshly available money into some innovative field, with no risks if the results do not fulfil our expectations. The risk of failure in a daring scientific project (such as the study of immortality in a jellyfish species able to perform ontogeny reversal, or the impact of meiofauna predation as a regulator of plankton diversity) is obviously great. If the results are positive, the scientific impact is great, but if the results are negative, then there is not much justification for the sustained economic effort. Of course, if the results are irrelevant in the evaluation of projects, and bureaucratic requirements are sufficiently met, the risk of its failure being 'discovered' is low. However, a study that does not produce any good results, leads to a lower publication score for the researcher, so diminishing his or her status in the scientific community. It is better to stick to well established procedures and tackle 'safe' topics. No wonder the investigations on the populations of limpets in the intertidal are extremely popular and successful (680 articles in the ISI Web of Science from 1986 to 2008, h-index 38), whereas studies on thaliacea are much less popular (79 papers, h-index 15).

The overall importance of limpet grazing on algal mats is almost irrelevant to ecosystem functioning, whereas the roles of thaliacea might be paramount in determining planktonic primary production. However, the risk of failure of thaliacean studies is great, due to their erratic presence, so it is much safer to stick to limpets, possibly shifting to barnacles (1990 papers, h-index 64), for a change.

### From Knowledge to Management and Conservation

It is evident, from all the above, that we still know little of what should be known to properly evaluate marine biodiversity and its bearing on ecosystem functioning. The exploration phase has still to be accomplished (Bouchet *et al.* 2002), and the elucidation of mechanisms is even more in its infancy. Knowledge of the patterns and processes governing ecosystem functioning is a prerequisite



for management. Of course, we cannot wait to describe all species and to elucidate the relationships of each of them with the rest of the biosphere to start actions aimed at mitigating our evident impact on the natural world. There are some actions that can be taken even with very little knowledge (for instance, discharging nuclear waste in the environment is bad, and we do not need to understand perfectly all the facets of it to decide on its ban). On the other hand, there are very subtle ecological processes (such as the impact of thaliacean grazing on phytoplanktonic primary production, or jellyfish impact on fish populations, see Boero *et al.* 2008a) that might lead to undesirable situations; whereas with proper knowledge, we might avoid non-obvious environmental catastrophes.

### Shellfish Overfishing and Coastal Erosion

Productive seas, such as the Adriatic Sea before the 1980s, sustain enormous populations of bivalve molluscs that are used as a food source by humans. At the end of the summer, the populations of many of these species go through catastrophic mortality and the dead shells are stranded on beaches, forming large masses of calcium carbonate. Wave action, instead of affecting the sand of beaches and causing erosion, impacts on the stranded shells, breaking them down and leading to the formation of biogenic sand grains. In other words, stranded mollusc shells are a natural beach replenishment, year after year.

Studies on the contribution of this process to sediment balances on beaches are rare (Lopez *et al.* 2008). However, it is suggestive that the populations of these molluscs are more and more depleted by overfishing, and shell stranding does not occur anymore, for example, along the Adriatic Sea. The whole Italian coast of the Adriatic, from the Gargano peninsula to the Gulf of Venice, is affected by severe beach erosion and, in the last two decades, it has been 'protected' by an almost continuous line of coastal defences: the great wall of the Adriatic. The causes of beach erosion are manifold, such as the irrational urban development along sandy shores and the barrage of rivers with dams, so preventing sediment input through riverine outflows (CIESM 2002a); railway tracks on the shore, furthermore, led to the perception that any change in coastline is a catastrophe. The overfishing of bivalves, in this complex scenario, ruled by multiple causality, might have been the last straw that broke the camel's back. And maybe it was not a straw!

### The Wheel Re-invented: Biodiversity and Ecosystem Functioning

Some decades ago, ecology was divided into two main branches: autoecology and synecology. Autoecology was

the ecology of species and essentially concerned their biology. According to the organisms studied, autoecologists were either zoologists or botanists. A prerequisite for studying autoecology, in fact, was knowledge of the existence of species and, thus, the exploration of biodiversity. Synecology stemmed from the widely shared concept of the emerging properties of ecology, the whole being more than the sum of the parts. This means that synecology is not simply the sum of the autoecologies of each species. The ecology of species (biodiversity) contributes to the ecology of species assemblages within their physical environment (ecosystem functioning) in a complex way, and it is vain to pretend that knowledge of a species by species ecology might lead to understanding the functioning of the ecosystems. However, the perception of the value of species diversity calls for some justification. Biodiversity is important because it makes ecosystems function, but how? Do we know enough about species roles (the old autoecology) (Piraino *et al.* 2002)? Especially in the sea, we barely know the list of species living at a certain place, not to mention their roles. Lists and roles are better known for species-poor seas, such as the Baltic or the North Sea, whereas they are barely known in species-rich seas such as the Mediterranean Sea. This is why synecology is more advanced in Northern Europe than in Southern Europe: the systems are simpler.

The old paradigms are still valid. The first step is the exploration of biodiversity (how many species are there on Earth?). Then there is their autoecology (what are their life cycles, what are their ecological niches?) and synecology (how do ecosystems work?). In spite of having re-discovered auto- and synecology, the introduction of BEF (Biodiversity and Ecosystem Functioning) has the merit of having merged the two approaches, calling for a timely conceptual unification (Boero *et al.* 2004; Boero & Bonsdorff 2007). Ecology is the science of interactions, compartments are to be broken, reductionism to lead to multiscalar approaches, both in time and space. Ecology is the study of the processes leading to the patterns of distribution and abundance of organisms. Biogeography, faunistics, floristics, macroecology, synecology, biology, autoecology, fisheries science, anthropology, economics and many other branches of the study of nature require a synthetic approach that does not see reductionism as the opposite of holism. All approaches are necessary and none is sufficient. Maybe, with a slightly modified meaning, the old term 'natural history' is the best one to depict the necessary unification of the life sciences.

### Conclusion

The search for general rules and 'laws' governing environmental systems, with the aim of predicting their future

behaviour, is crashing up against the wall of the intrinsic unpredictability of complex systems. We must describe properly and try to understand, singling out the relevant variables and their interactions. This will lead to the proposal of very weak predictions, or scenarios, that will not pretend to predict future history but that will depict the possible shape of the main attractors of chaotic environmental systems. Regularities are extremely important, and we have dedicated most of our efforts to identifying them. Now it is the turn of irregularities. The drivers of change may be extremely subtle: e.g. the absence of stranded molluscs, leading to coastal erosion, or the predation of small jellyfish, leading to the impairment of fish recruitment, or the dredging of harbour sediments, leading to red tides by waking up dinoflagellate cysts buried in the sediments, or the grazing of a salp bloom. All these possible drivers have not been investigated as much as they deserve. It is time to make daring hypotheses on functional links, and it is time to start exploring again, passing from the computer and the laboratory into the field. We abandoned long-term series, and we must revive them: (natural) history can only be understood through archives (containing raw data); libraries (containing the elaboration of data) are not enough (after the available data have been elaborated).

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## ORIGINAL ARTICLE

# Taxonomic sufficiency in two case studies: where does it work better?

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## Keywords

Soft-bottom macrozoobenthos; taxonomic structure; taxonomic sufficiency.

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The authors declare no conflicts of interest.

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## Abstract

In marine macrobenthos studies the identification of organisms at species level is the best entry to ecological and biological information about the animals. An accurate identification requires excellent conservation of the organisms, reliable fauna description, experts and lengthy work in the laboratory. The aim of this work is to test taxonomic sufficiency (TS) in two deliberately selected different case studies to understand whether and how the taxonomic complexity of a benthic assemblage influences the results of TS and where it works better. The first benthic settlement was collected in an area characterized by homogeneous depth and grain size composition (case study A) around an off-shore gas platform, while the second one was collected along a coast-wide transect in an area with human pressure limited to fishing activities (case study B). Univariate and multivariate statistical analysis was used to assess differences in the taxonomic structure of benthic assemblages and to test TS on the two different datasets. TS seems to work in both sites, from species to higher taxonomic levels, and the family taxonomic level appears the best compromise for taxonomic resolution when an accurate identification is not achievable. The application of TS does not indicate a significant difference between the two datasets and appears therefore to be a valid instrument to analyse and describe the structure of benthic settlements in the case of taxonomically complex communities.

## Problem

The importance of soft-bottom macrofauna in environmental and ecological studies arises from several factors: sedimentary habitats cover most of the ocean bottom and constitute the largest single ecosystem on earth in terms of spatial coverage (Snelgrove 1997); most marine species are benthic (Gray 1997) and have the ability to respond significantly to natural and anthropogenic environmental variations (Pearson & Rosenberg 1978; Gray 1981; Gray *et al.* 1990; Warwick & Clarke 1991; Castelli *et al.* 2003). Anthropogenic impact studies on the marine environment often use benthic macrofaunal assemblages as indicators: these organisms respond promptly to environmental disturbances owing to their relatively short lifespans, and

they are small and can be sampled quantitatively (Warwick 1993; Vanderklift *et al.* 1996).

With reference to the role of systematics in assessing pollution effects, Carriker (1976) stressed the importance of accurate identification to unlock the storage and retrieval system of scientific information, given that a species name is the doorway to its literary pedigree and makes information accessible and useful via published aspects of organism biology.

Unfortunately, an accurate identification often requires an excellent conservation of the organisms, the presence of a reliable fauna description, more experts and lengthy work in the laboratory. The easiest way to simplify this process would be to use coarser taxonomic resolutions (e.g. genus instead of species or family instead of genus).



Taxonomic sufficiency (TS) involves identifying taxa to the highest category possible (genus, family, order, *etc.*) without losing statistically significant vigour in assessing impacts (Ellis 1985).

Literature on the application of TS is abundant and is spread over different geographical areas and different kinds of impact studies, such as oil extraction fields (Ols-gard *et al.* 1997), oil spill (Gomez Gesteira *et al.* 2003), heavy metal pollution (Vanderklift *et al.* 1996), and organic enrichment (Castanedo *et al.* 2007), using diverse sampling procedures (Ferraro & Cole 1992), the relationship between taxonomic resolution and spatial scales (Anderson *et al.* 2005) and uses of datasets (Warwick 1988).

Most of these studies (Dauvin *et al.* 2003; Raymond *et al.* 2005) suggest that a lower taxonomic resolution can be sufficient when studying benthic assemblages composition and that determining the family may be satisfactory in many routine monitoring surveys. In highly disturbed areas, it is suggested that the results of multivariate analyses based on higher taxa might more closely reflect gradients of contamination or stress than those based on species data, the latter being more affected by natural environmental variables.

However, not all researchers agree with the use of TS-based approaches and several authors (Dauvin *et al.* 2003; Raymond *et al.* 2005) stress that when studying poorly known environments, it is essential to acquire detailed information before applying a rougher taxonomic resolution; therefore, the use of TS is likely to be the most advantageous if species level baseline studies have already been completed. Terlizzi *et al.* (2003) emphasizes that evidence suggests that family is a sufficient taxonomic level, but underlines that this statement stems from too limited a number of case studies. Relations among TS and sampling procedures, data analyses, spatial scale, habitat features and assemblages structure remain to be assessed and are far from being generalized.

## Objectives Statement

This work intervenes in the debate on TS application as a contribution towards a further understanding of the diverse possibilities for using this instrument in diverse datasets. Our aim is to test TS in two deliberately selected different case studies to understand whether and how the structure and the taxonomic complexity of a benthic assemblage influences the results of taxonomic sufficiency, and where it works better.

The first study concerned animals collected around an off-shore gas platform at a homogeneous depth, where granulometric gradient is absent. This benthic assemblage was characterized by a small number of species and a

simple taxonomic structure. A second dataset referred to a settlement collected along a coast-wide transect with anthropogenic pressure limited to fishing activities (G. Franceschini, personal communication). In this case the benthic assemblage was characterized by a high number of species and a complex taxonomic structure.

## Methods

Case study A was related to the monitoring of an off-shore gas platform located in the Central Adriatic Sea sited at a depth of about 120 m. A radial sampling design of 12 stations positioned at increasing distances from the installation (0, 50, 500, 1000, 2000 m) was developed to evaluate the spatial distribution of benthic assemblages and potential effects induced by the presence of the platform and its influence on them (Trabucco *et al.* 2006). Station A0 was located at the installation, stations A1, A2, A3, A4 at 50 m from it, stations A5, A6, A7, A8 at 500 m from it, station A9 at 1000 m from it, and control stations K2 and K3 were positioned at the upper-current about 2000 m NW of the platforms, on a bottom which presented geo-morphological characteristics similar to the study area.

Case study B was developed to monitor an area along a coast-wide transect in the North Adriatic Sea. The sampling was carried out at 33 stations on a bathymetric gradient from 1 to 29 m depth.

Data analyses were done on data pooled from two samples collected at each station using a Van Veen grab (0.1 m<sup>2</sup>; 25 l) and processed through a sieve (1-mm mesh size). The macrozoobenthic component was sorted and identified to the lowest possible taxonomic level, *i.e.* species level.

Species abundances of Polychaeta, Mollusca, Crustacea and Echinodermata from each dataset were aggregated at genus, family, order, class and phylum levels, following the MARBEF database taxonomic classification (<http://www.marbef.org/data/erms.php>).

At each station, variations in the taxonomic structure of benthic assemblages were analyzed through the difference  $NT_x - NT_{x+1}$  defined as 'loss of information  $\alpha$ ' ( $\alpha$ ).  $NT_x$  expresses the number of taxa identified at the station at a certain taxonomic level  $X$ .  $X$  can range from species to phylum level.  $X + 1$  expresses instead the next higher taxonomic level compared to the one which is taken into consideration as  $X$  (*i.e.* if the  $X$  level is species,  $X + 1$  will be genus). The parameter ' $\alpha$ ' describes the loss of information on the taxonomic structure of a benthic settlement which can be registered at the passage from a lower ( $X$ ) to a higher ( $X + 1$ ) taxonomic level. ' $\alpha$ ' values therefore give general information about the taxonomic heterogeneity-complexity inside each level considered. Higher

values correspond to a major 'loss information  $\alpha$ ' and *vice versa*, while a value equal to zero explains a null 'loss information  $\alpha$ '. In this study the ' $\alpha$ ' was calculated step by step from the lowest to the highest taxonomic level considered (species-genus, genus-family, family-order, order-class, class-phylum).

Statistical analysis was performed using the PRIMER package, version 6 (Clarke & Gorley 2006). Assemblage abundance matrices were produced for each of the six taxonomic levels (species, genus, family, order, class, phylum) and were square-root transformed to reduce the weighting of the most abundant taxa.

The affinities among the stations were established using non-metric Multi-Dimensional Scaling (MDS) calculated with 'zero-adjusted' Bray-Curtis similarity (Bray & Curtis 1957; Clarke *et al.* 2006) using Kruskal's stress Formula 1 (Kruskal & Wish 1978; Clarke & Green 1988). The stress value ( $S$ ) measures the degree of coupling of real between-sample distances and between-sample distances in the ordination: high stress values thus indicate poor reliability, as apparent differences between samples may simply be numerical artefacts (Gomez Gesteira *et al.* 2003). As a rough guide to efficiency,  $S > 0.5$  is probably random,  $0.5 > S > 0.25$  is a poor result,  $0.25 > S > 0.1$  is a satisfactory result and  $S < 0.1$  is a good result (Kruskal 1964). Moreover, hierarchical clustering (group average) on species, genus, family, order, class and phylum taxonomic levels was calculated and visualized in the MDS ordination plots. A one-way ANOSIM test (Clarke & Green 1988) was performed among the groups that stood out at species level analysis at each analyzed taxonomic level. Finally, Spearman's rank correlation coefficient ( $\rho$ ) was calculated between matrices at species level and at higher taxonomic levels.

## Results

### Case study A

Dataset A comprises a total of 287 individuals from the phyla Polychaeta, Mollusca, Crustacea and Echinodermata. Forty-nine taxa were identified at species level.

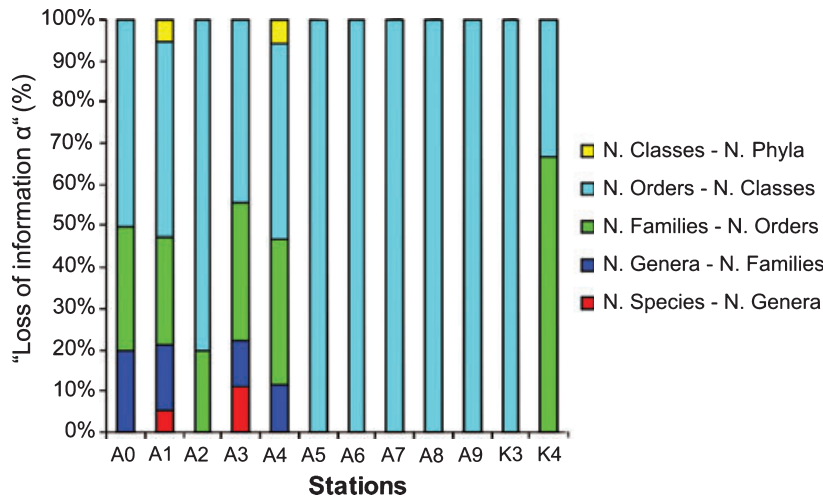
The benthic assemblage shows a different species composition in the stations near the platform (located 0–50 m from it, 'inner group'), in comparison with the stations located at greater distances (500, 1000, 2000 m, 'outer group'). The presence of such differences between the 'inner' and 'outer' group is related to the platform structure, which promotes the presence of a coarser secondary substratum and therefore the occurrence of different species (Trabucco *et al.* 2006).

A low number of species and individuals and a simple taxonomic structure were recorded. Only two genera

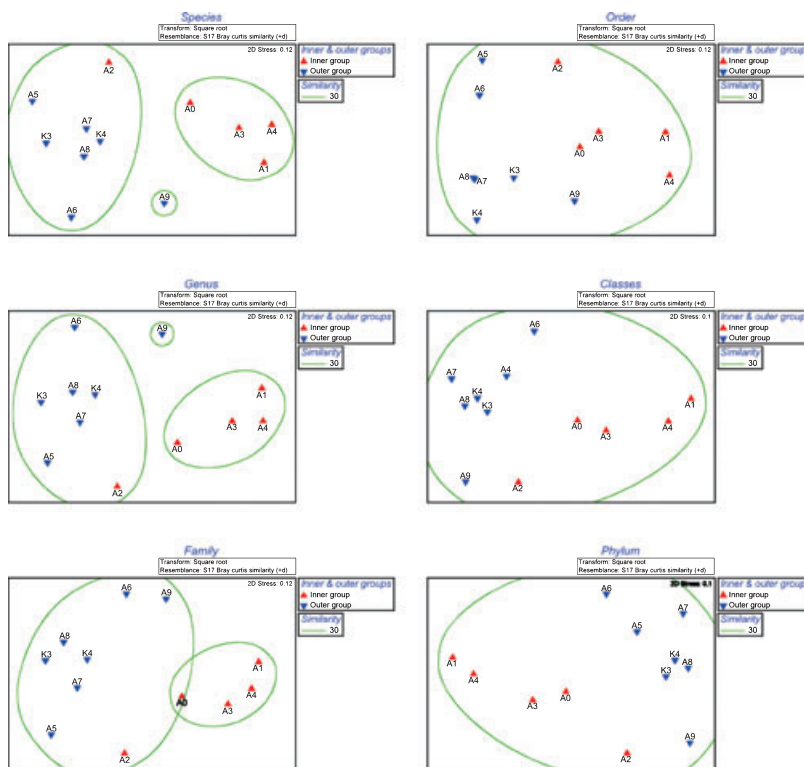
contained two species, the rest contained only one. Similarly, most families contained only one species: families with two species were uncommon and only one family contained four species. ' $\alpha$ ' values confirm that the taxonomic structure of the benthic assemblage is generally very simple, especially in the 'outer group' stations (Fig. 1). In the 'inner group', two stations (A1, A3) show a 'loss of information  $\alpha$ ' descending from species to genus level because there are one or more genera which comprise more than one species. Four stations (A0, A1, A3, A4) show loss of information between genus and family levels, indicating the presence of one or more families with more than one genus. Five stations (A0, A1, A2, A3, A4) show loss at family-order level because of the presence of one or more orders with more than one family. Five stations (A0, A1, A2, A3, A4) show loss at order-class level due to the presence of one or more classes with more than one order. Finally, two stations (A1, A4) showed loss at the class-phylum level, indicating the presence of one or more phyla with more than one class. In general, a 'loss of information  $\alpha$ ' is evident in the 'inner group' if the benthic assemblages are studied at a higher taxonomic level than that of species. In contrast, in the 'outer group' most of the stations (A5, A6, A7, A8, A9, K3) show a 'loss of information  $\alpha$ ' only in the passage class-order level, because of the presence of only a single species for each genus; similarly, all families contained a single genus and all orders contained only a single family, displaying a value equal to zero for most of the levels considered. In this case there is a null 'loss of information  $\alpha$ ' if the benthic assemblage is studied at higher taxonomic level than species level.

Abundance matrices were analyzed from species to phylum level and six different ordination plots are shown (Fig. 2). Two different groups of stations (30% of similarity), corresponding to the 'inner' and 'outer' locations, are distinguished in the analysis performed from species to family level. Station A2 is an exception, given that it is located near the installation (50 m) but linked to the 'outer group'. Station A9 detaches from the 'outer group' at both species and genus levels, underlining its differentiation (Fig. 2). At higher taxonomic levels (order, class, phylum) there is reduction of information and it is no longer possible to identify the two clusters at the same percentage of similarity (Fig. 2). However, the 'inner group' and the 'outer group' remain distant, and the dissimilarity among clusters is significant ( $P < 0.01$ ).

In case study A there is therefore a good correlation between species level and ordination plots at lower taxonomic levels (genus, family;  $P > 0.94$ ), whereas at higher levels (order, class, phylum) the information about the structure of the benthic assemblages decreases ( $P < 0.77$ ).



**Fig. 1.** 'Loss of information  $\alpha$ ' in percentage scale from lower to higher taxonomic levels at the 12 stations placed around the platform in case study A.



**Fig. 2.** nMDS and hierarchical cluster analysis at species, genus, family, order, class and phylum levels for case study A.

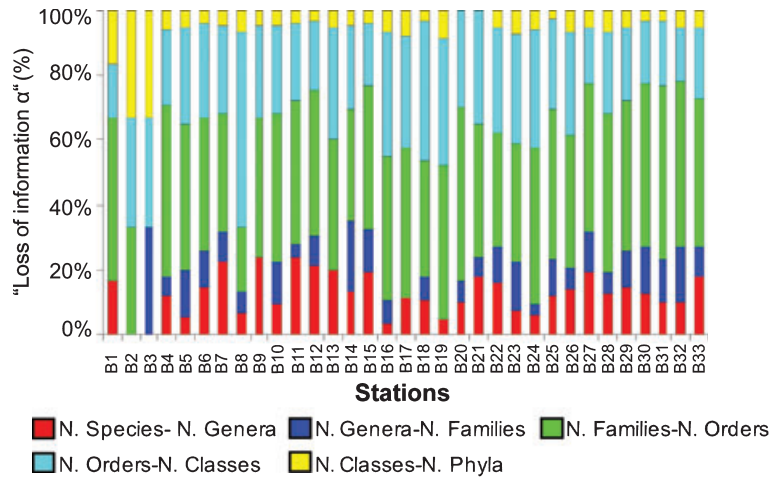
**Case study B**

Dataset B included a total of 12,318 individuals from the phyla Polychaeta, Mollusca, Crustacea and Echinodermata, and 225 taxa were identified at species level.

The study showed the presence of a classic soft-bottom benthic assemblage along a coast-wide transect sited in the North Adriatic Sea. Most of the species collected near the coast were characteristic of the Biocoenose des Sables Fins des Hauts Niveaux, SFHN, and the Biocoenose des Sables Fins Bien Calibrés, SFBC. At increasing depth there

was a group of stations where the presence of several exclusive/characteristic species of different biocoenoses (SFBC, VTC, DC) was recorded. This settlement is defined here as 'transitional stations' (TS). Most of the species collected far from the coast were characteristic of the Biocoenose de la Vase Terrigène Cotière, VTC, and the Biocoenose des Fonds Détritiques Cotière, DC (Vatova 1949; Peres & Picard 1964; Gamulin-Brida 1967; Meinesz *et al.* 1983; Bacci *et al.* 2009).

A high number of species and individuals and a complex taxonomic structure were recorded. It is possible to

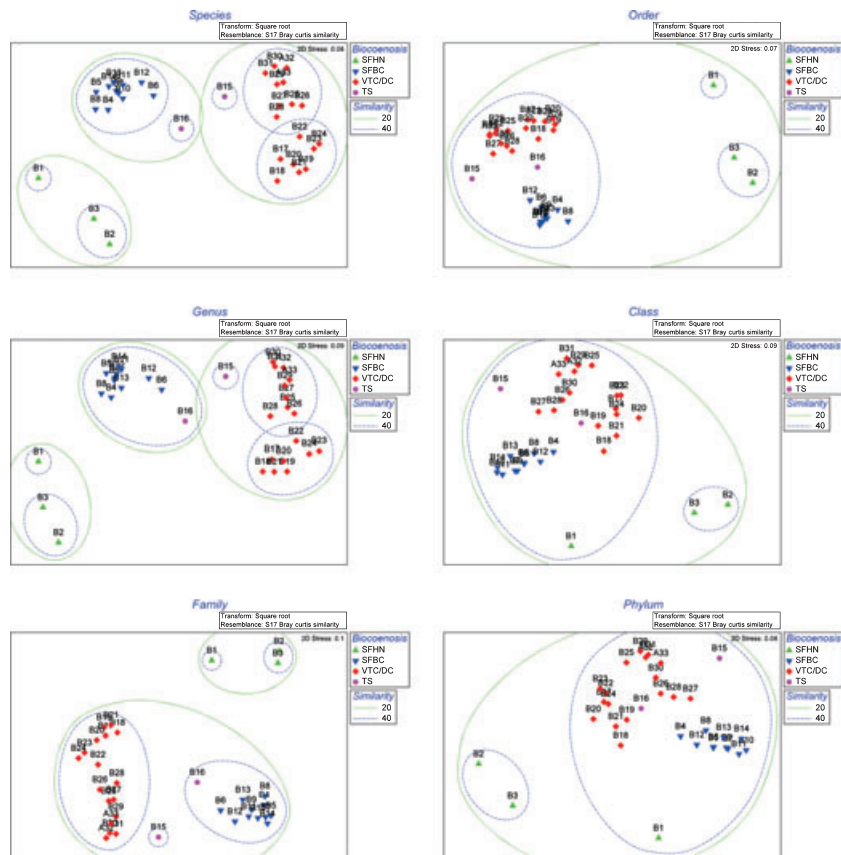


**Fig. 3.** ‘Loss of information  $\alpha'$  in percentage scale from lower to higher taxonomic level at the 33 stations along the coast-wide transect in case study B.

note up to five different species per genus and seven genera per family. This complex taxonomic structure occurred in most of the stations along the transect off-coast as highlighted by ‘ $\alpha'$  values (Fig. 3). ‘Loss of information  $\alpha'$  can be recorded in the passage between all levels considered in the majority of the stations (B4, B5, B6, B7, B8, B10, B11, B12, B14, B15, B16, B18, B22, B23, B24, B25, B26, B27, B28, B29, B30, B31, B32, B33). In fact, most of the benthic assemblages along the entire

coast-wide transect showed the presence of one or more taxa within all taxonomic levels, from genus to phylum.

Abundance matrices have been analyzed from species to phylum level and six different ordination plots are shown (Fig. 4). Different groups of stations (40% of similarity) stand out in the analysis performed from species to family level (Fig. 4). At higher taxonomic levels there is a reduction of information and it is no longer possible to identify the stations near the coast (SFHN and SFBC)



**Fig. 4.** nMDS and hierarchical cluster analysis at species, genus, family, order, class and phylum levels for case study B.

and the stations far off the coast (VTC, DC) at the same percent of similarity (Fig. 4). However, different biocoenoses remain distant, and the dissimilarity among clusters is significant ( $P < 0.01$ ). It is interesting to observe that stations B1, B2 and B3 are grouped together from species to family level at 20% similarity because they actually belong to the same biocoenosis (Fig. 4). Nevertheless, B1 is separate from stations B2 and B3 at a higher similarity (40%); this is due to a quantitative difference caused by the great abundance of some species in this site. A second observation should be made of the VTC/DC stations. At species and genus levels the stations identified as VTC/DC group together with a 20% similarity. The same does not happen at family level, where they group together with a 40% similarity. This difference of information is due to the fact that at lower taxonomic levels the graphs highlight different proportions of species characteristic of VTC and of DC (more abundant in the stations more distant from the coast), which are instead disguised at family level.

In case study B there is a good correlation between species level and ordination plots at lower taxonomic levels (genus, family;  $P > 0.94$ ), whereas at higher levels (order, class, phylum) the information about the structure of benthic assemblages decreases ( $P < 0.87$ ).

## Discussion

The two datasets differ for environmental conditions such as grain-size sediment, bathymetric gradient and human pressure. These diverse conditions contribute to the differentiation of the two benthic assemblages. The benthic assemblage along the off-coast transect appears to have a more complex structure than the benthic assemblage around the off-shore gas platform, due to a considerably higher number of species and individuals and therefore a more complex taxonomic structure.

Taxonomic heterogeneity is well described by 'α' values, which appear higher in case study B (*i.e.* higher loss of information on the taxonomic structure of benthic settlement) because of the general presence of more than one taxon within all taxonomic levels, from genus to phylum.

Nonetheless, taxonomic sufficiency seems to work in both sites, from species to higher taxonomic levels, as shown by ANOSIM. Other statistical analyses such as MDS, hierarchical clustering and Spearman's correlation suggest the family taxonomic level as the best compromise for taxonomic resolution (*i.e.* null reduction of information) when an accurate identification is not possible. MDS results display stress (S) values minus or near to 0.1 in both our cases, justifying a good measure of the degree of coupling of real between-sample distances and between-sample distances in the ordination

(Kruskal 1964; Gomez Gesteira *et al.* 2003) for each taxonomic level considered.

Contrary to expectations, we observed that in case study B there was not a high loss of information about the general structure of the benthic assemblages from species to family taxonomic level, despite the high number of species per genus and high number of genera per family. We could formulate the hypothesis that a high number of species, genera and families makes the benthic assemblage structure stable and diverse up to family level, compensating the loss of information at the passage to the higher taxonomic level.

The application of taxonomic sufficiency does not indicate a significant difference between the two datasets and appears therefore to be a valid instrument to analyze and describe the structure of benthic settlements in the case of taxonomically complex communities as well.

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## ORIGINAL ARTICLE

# Scale-dependent effects of nutrient loads and climatic conditions on benthic and pelagic communities in the Gulf of Finland

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## Keywords

Baltic Sea; benthic invertebrates; climate change; mesozooplankton; nutrient load; spatial scale.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

Eutrophication and climate change are ranked among the most serious threats to the stability of marine ecosystems worldwide. The effects of nutrient loads and climatic conditions vary in direction, magnitude and spatial extent. To date the factors that are behind the scale-specific spatial and temporal variability are poorly known. In this study we assessed how variability in nutrient loads and climatic conditions at local, gulf and regional scales explained the spatial patterns and temporal trends of zooplankton and benthic invertebrates in the Gulf of Finland. In general both local and gulf scale environmental variability had an important effect on benthic invertebrate species and the variability was mainly due to local nutrient loading, gulf scale temperature and salinity patterns. Zooplankton species were equally affected by environmental variability at all spatial scales, and all nutrient load and climatic condition variables contributed to the models. The combination of variables at all spatial scales did not explain the substantially larger proportion in invertebrate variability than variables at any individual scale. This suggests that large-scale pressures such as nutrient loads and change of climatic conditions may define broad patterns of distribution but within these patterns small-scale environmental variability significantly modifies the response of communities to these large-scale pressures.

## Problem

Eutrophication and climate change are ranked among the major threats to the stability of marine coastal environment and can have severe impacts on near-shore biodiversity and functioning (e.g. McGowan *et al.* 1998; Howarth *et al.* 2000; Jackson *et al.* 2001). Nutrient loads may lead to algal blooms, accumulation of organic matter and development of anoxia, and consequently can cause significant changes in ecosystems (Andersen *et al.* 2006; Paerl 2006). The effects of climatic variability on coastal ecosystems are less known due to the mismatch of important scales between climatic conditions and biological

variables. The effects of climatic conditions operate through local weather parameters such as temperature, wind, rain, snow and current patterns, as well as interactions among these (Stenseth *et al.* 2002). Shifts in climatic conditions are known to have profound ecological impacts, altering the patterns of distribution, abundance and diversity of species (Hughes 2000; Lotze *et al.* 2006). Such effects vary largely among regions, reflecting system-specific attributes and direct and indirect responses that act as a filter to modulate the responses to enrichment and climate change (Cloern 2001; Rönnerberg & Bonsdorff 2004; Hewitt & Thrush 2009). As different regions respond differently to the same type of environmental

stress, the areal-specific ecological responses should be described.

Taking this into account, there is no single natural scale at which the effects of nutrient loads and climatic conditions could be studied (Levin 1992; Karlson & Cornell 1998). To identify the most important governing factors one needs to determine the scales where the links between nutrient load and climatic condition variables and biotic patterns are the strongest (Steele & Henderson 1994). Although it is recognized that processes affect ecosystems simultaneously at many spatial scales (Steele & Henderson 1994; Denny *et al.* 2004), to date the relative importance of small- and large-scale processes in the formation of marine communities is little known (*e.g.* Hewitt *et al.* 2007). Large-scale environmental stresses and disturbances (*e.g.* climatically driven changes in seawater temperature, sea level or the intensity of ice scouring) can synchronize population changes over wide geographical areas and define broad patterns of distribution, if they have a direct effect on recruitment or mortality. Within these patterns, smaller-scale processes operate at a lower intensity to modify distributions, abundances and functioning of communities (Kotta & Witman 2009). Recently, it was shown that the degree of interaction between large-scale environmental factors and smaller scale variability was not consistent across sites or species. Knowledge about such variability may affect our ability to predict effects of nutrient loads and changing climatic conditions on coastal communities (Hewitt & Thrush 2009).

In this study we evaluated how nutrient load and climatic condition variables estimated at local (10s km), gulf (100s km) and regional scales (1000s km) contributed to the biomass of zooplankton and benthic invertebrate species in a shallow brackish water ecosystem of the Baltic Sea. Nutrient loads have been an increasing ecological threat in the Baltic Sea for the past 50 years. During this time the load of nutrients has grown four-fold for nitrogen and eight times for phosphorus, leading to an increased production at all trophic levels in the ecosystem (Elmgren 2001; Rönnerberg & Bonsdorff 2004). Although rising temperature has caused major shifts in the community structure in many European water bodies (*e.g.* Connors *et al.* 2002), such temperature-induced shifts have not been observed in the Baltic Sea in recent decades. It is plausible that recent changes in the mean water temperature are not ecologically important as large seasonal variation counteracts the potential effects of recent global warming. On the other hand, the indirect effects of global warming can be important and can potentially affect the structure and function of the Baltic coastal communities.

Mesozooplankton is both passively and actively mobile and capable of moving both vertically and horizontally in

the aquatic environment. Their mobility allows them to transfer materials between different environments and to give mesozooplankton the potential to form strong links between different subsystems (Lundberg & Moberg 2003). Therefore it is expected that the biomasses of mesozooplankton are influenced by large-scale environmental variability rather than small-scale environmental variability. Benthic invertebrates, however, are thought to be relatively stationary, longer lived and temporally less variable than mesozooplankton. However, benthic invertebrates do not behave as a single entity and there exists a large within-group variability among benthic invertebrates. Earlier studies have shown that suspension-feeders are directly linked to pelagic primary productivity (Cloern 1982; Kotta & Møhlenberg 2002) and benthic grazers and deposit-feeders to benthic primary productivity (Granéli & Sundbäck 1985; Orav-Kotta & Kotta 2004; Kotta *et al.* 2006). Thus, it is expected that local variables explain better the distribution of benthic grazers and deposit-feeders and large-scale variables that of benthic suspension-feeders. Besides, mobile benthic species possess the ability to escape direct small-scale physical disturbances or food depletion, whereas non-migrating benthic species are more susceptible to such disturbances and rely completely on local food levels (*e.g.* Tillin *et al.* 2006; Kotta *et al.* 2008). Therefore it is also expected that local variables explain better the distribution of non-migrating benthic species and large-scale variables that of mobile benthic species.

## Study Area

The study was conducted in the Gulf of Finland, Northern Baltic Sea. The average depth of Gulf is 37 m and the maximum depth 123 m. Sand, silt or sandy clay bottoms dominate. The Eastern Gulf of Finland receives fresh water from a huge drainage area and the Western Gulf is a direct continuation of the Baltic Sea proper, therefore the gulf has a permanent east–west gradient of salinity. The salinity range of stations was 2.2–7.3 psu. The area is influenced by diffuse and point source nutrient loads.

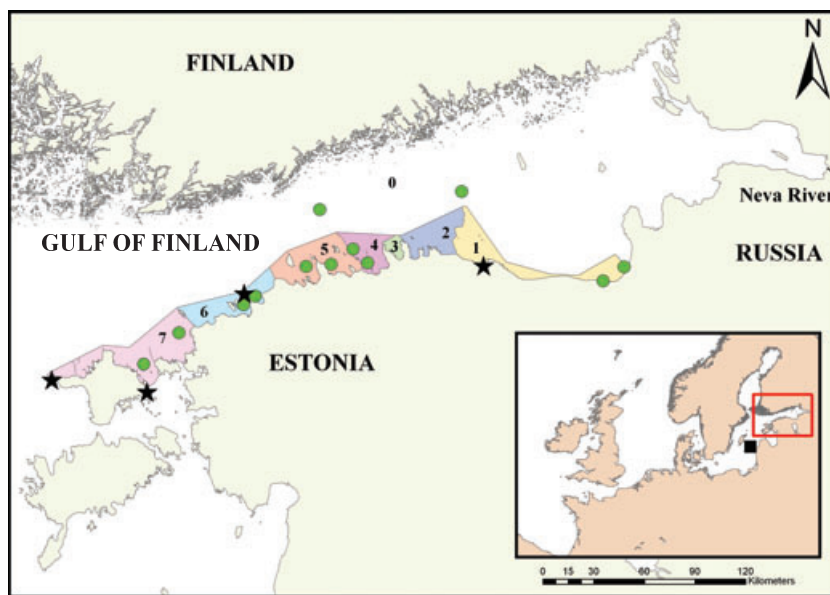
The Water Framework Directive 2000/60/EC (WFD) is the most significant piece of European water legislation that prevents further eutrophication of the ecosystem of the Gulf of Finland. According to the directive the waters of the Gulf of Finland have been divided into water bodies and the assessment of the ecosystem state is made by these basic management units. In our study we evaluated relationships between nutrient loads, climatic conditions and ecosystem variables by each water body to provide a better ecological basis for the WFD classification scheme.

## Material and Methods

Within each water body two stations were sampled between 1996 and 2005 (Fig. 1, Table 1). Zoobenthos samples were collected each year during May using a Van Veen grab (0.1 m<sup>2</sup>). The depth of sampling sites ranged from 8 to 100 m and encompassed coarse sand, medium sand and silt sediments. Grab samples were sieved in the field on 0.25-mm mesh screens. The residues were stored at -20 °C and subsequent sorting, counting and determination of invertebrate species were performed in the laboratory using a stereomicroscope. All species were determined to the species level except for oligochaetes

and insect larvae. The dry weight of species was obtained after drying the individuals at 60 °C for 2 weeks. During sampling we recorded near-bottom oxygen (minimum layer) and depth-integrated salinity values.

Zooplankton was collected at the same stations as used for zoobenthos samples in May and August over 1996–2005. The samples were collected by vertical tows with a Juday closing plankton net (mesh size 90 µm, mouth area 0.1 m<sup>2</sup>). The samples were preserved in 4% formaldehyde solution in seawater. The abundances of zooplankton species were estimated from a number of subsamples according to the methods recommended by HELCOM (1988). Biomasses (wet weights) were calculated using the



**Fig. 1.** Sampling locations (circles), weather stations (asterisks) and water bodies along the Estonian coastline in the Gulf of Finland. Water bodies 1–7 are defined by the EU Water Framework Directive, water body 0 represents the offshore conditions of the Gulf of Finland. Black square on minimap indicates the location of Gotland Basin.

**Table 1.** Characteristics of the studied water bodies (WB0...7) in the Gulf of Finland.

Environmental characteristics	WB0	WB1	WB4	WB5	WB6	WB7
Water renewal time, years	1.1	1.4	0.8	0.4	0.3	0.1
Average depth, m	65	21	52	37	27	13
Mean water flow from rivers, m <sup>3</sup> ·s <sup>-1</sup>	0.0	>400	<5	10...20	10...20	<1
Near-bottom oxygen concentration, ml·l <sup>-1</sup>	4.6	7.7	5.2	8.4	8.7	8.1
Salinity	6.3	4.5	6.4	6.2	6.2	6.3
Sea surface temperature in May	5.9	8.8	6.7	5.8	7.8	9.1
Air temperature in May	9.0	9.5	8.9	9.1	9.1	8.5
Wind speed in May	3.5	3.5	3.5	3.4	3.4	3.1
Nitrogen load from point sources into a water body, t·year <sup>-1</sup>	0.0	434.1	0.1	827.2	6.0	0.0
Phosphorus load from point sources into a water body, t·year <sup>-1</sup>	0.0	8.9	0.0	57.4	1.2	0.0
Riverine nitrogen load into a water body, t·year <sup>-1</sup>	0.0	8941.7	115.0	1739.2	1487.1	0.0
Riverine phosphorus load into a water body, t·year <sup>-1</sup>	0.0	798.4	4.4	32.3	31.7	0.0

biomass factors for different taxonomic groups and developmental stages (Hernroth 1985).

The data on the annual point source and riverine loads of total N and total P to the Gulf of Finland in 1996–2005 was obtained from the Estonian Ministry of Environment and from the MARE homepage (<http://www.mare.su.se/>). The data of annual total N and total P loads and runoff of River Neva was obtained through Baltic-Nest (<http://nest.su.se/nest/>) from NW Administration of Roshydromet (Russia). The loads into six water bodies of the Estonian coast of the Gulf of Finland were used as nutrient load variables at the local scale. In general, the diffuse nutrient loads were the major type of loading in the study area. Depending on the water body the contribution of the diffuse nutrient N loads to the total N loads varied between 68 and 100% and the contribution of the diffuse nutrient P loads to the total P loads between 35 and 100%. The sum of loads due to Estonia, Finland and Russia represented nutrient load variables at the gulf scale. The concentrations of total N and total P in the Central Baltic Sea in winter were used as a proxy of regional nutrient load variables because the plankton has not yet taken up the nutrients. Inorganic nutrients that have accumulated during the winter are assimilated during the following spring bloom (HELCOM 2002).

As a proxy of atmospheric conditions the winter index of the North Atlantic Oscillation was used to relate the global climate pattern to the variation of biological data in the study area (NAO December–March, <http://www.cgd.ucar.edu/cas/jhurrell/nao.stat.winter.html>) (Barnston & Livezey 1987; Ottersen *et al.* 2001). The NAO is an alternation in the pressure difference between the subtropical atmosphere high-pressure zone centred over the Azores and the atmospheric low-pressure zone over Iceland. NAO's connection with the wind, temperature and precipitation fields is strongest during winter. The link between the NAO and sea water temperature may persist over the summer, however, being highly region-dependent and should be assessed for each site separately (*e.g.* Ottersen *et al.* 2001). During the years of high NAO there is a substantial increase in the rainfall and consequently of the fresh-water inflow into the Baltic Sea (Hänninen *et al.* 2000). The increased pressure differences result in higher winter temperatures in Northern Europe (Rogers 1984). As an additional global climatic conditions variable, we used the Baltic Sea Index (BSI), which is the difference of normalized sea level pressures between Oslo in Norway and Szczecin in Poland. The BSI is significantly related to NAO and is used as a regional calibration of the North Atlantic Oscillation index (Lehmann *et al.* 2002). As the local, gulf and regional scale proxies of climatic condition variables we used average wind speed, air and water temperatures, water column salinity and near-

bottom oxygen concentration and water temperatures at the respective scale obtained from the Estonian Hydrometeorological Institute (Table 2).

Multivariate data analyses on abiotic environment and invertebrate communities were performed by the statistical program PRIMER version 6.1.5 (Clarke & Gorley 2006). Invertebrate biomass data were square-root transformed to down-weight the dominant species and increase the contribution of rarer species in the multivariate analysis. 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). Environmental variables were normalized prior to analyses. Non-metric multidimensional scaling analysis (MDS) on square-root transformed data of macrobenthic biomasses was used to quantify the dissimilarities between study areas and invertebrate species. Statistical differences in benthic invertebrate and mesozooplankton communities among water bodies were assessed by the ANOSIM permutation test (Clarke 1993).

BEST analysis (BVSTEP procedure) was used to relate the patterns of environmental variables measured at local, gulf and regional scales to the biomasses of invertebrate species. The analysis shows which environmental variables best predict the observed biotic patterns. A Spearman rank correlation ( $r$ ) was computed between the similarity matrices of environmental data (abiotic variables; Euclidean distance) and different invertebrate species (a zero-adjusted Bray–Curtis distance). A global BEST match permutation test was run to examine the statistical significance of observed relationships between environmental variables and biotic patterns. The separate and additive contribution of nutrient loads and climatic condition variables was assessed in one analysis and the contribution of local, gulf and regional scale variables in another analysis.

## Results

Generally, correlations between the studied abiotic environmental variables were poor ( $P > 0.05$ ). Among nutrient load variables there were significant correlations between total N at 10 m surface layer in Gotland Basin during winter and total P at 10 m surface layer in Gotland Basin during winter (Spearman rank correlation,  $R = 0.47$ ,  $P < 0.05$ ), total N and total P point discharges at local scale ( $R = 0.98$ ,  $P < 0.001$ ), total N point discharge and riverine total P load at local scale ( $R = 0.85$ ,  $P < 0.001$ ) and among climatic condition variables between sea surface temperature predicted by nearest air temperature and sea surface temperature at station during



**Table 2.** The list of the studied abiotic variables with their relation to spatial scale, nutrient loads and climatic conditions.

Variable	Nutrient loads	Climatic conditions	Regional	Gulf	Local
Total N at 10 m surface layer in Gotland Basin during winter	+		+		
Total P at 10 m surface layer in Gotland Basin during winter*	+		+		
Total N at 220 m in Gotland Basin	+		+		
Total P at 220 m in Gotland Basin	+		+		
Nearbottom oxygen concentration in Gotland Basin	+		+		
Total Finnish N load into GoF	+			+	
Total Finnish P load into GoF	+			+	
Average near-bottom oxygen concentration in GoF	+			+	
Total Estonian N load into GoF*	+			+	
Total Estonian P load into GoF	+			+	
Near-bottom oxygen concentration at station during sampling	+				+
Total riverine N load into a water body	+				+
Total riverine P load into a water body	+				+
Total N load from point sources into a water body	+				+
Total P load from point sources into a water body*	+				+
NAO <sub>decmar</sub>		+	+		
BSI		+	+		
Maximum ice cover in the whole Baltic Sea during winter		+	+		
Salinity at 100 in Gotland Basin		+	+		
Sea surface temperature in Gotland Basin in May		+	+		
Average number of days with wind >5 m·s <sup>-1</sup> in all weather stations		+		+	
Average salinity in GoF		+		+	
Average air temperature during May–August in all weather stations		+		+	
Average wind speed during May in all weather stations*		+		+	
Average salinity at station during sampling		+			+
Sea surface temperature at station during sampling		+			+
Average yearly air temperature at nearest weather station		+			+
Average yearly wind speed at nearest weather station		+			+
Sea surface temperature predicted by nearest air temperature*		+			+
Number of days with wind >5 m·s <sup>-1</sup> at nearest weather station*		+			+
Average air temperature during May at nearest weather station*		+			+
Average wind speed during May at nearest weather station*		+			+

An asterisk denotes variables not used in the statistical analyses.

sampling ( $R = 0.52$ ,  $P < 0.05$ ), sea surface temperature predicted by nearest air temperature and average air temperature during May at nearest weather station ( $R = 0.64$ ,  $P < 0.05$ ) and average yearly wind speed at nearest weather station and average wind speed during May at

the nearest weather station ( $R = -0.53$ ,  $P < 0.05$ ). Therefore, total P at 10 m surface layer in Gotland Basin during winter, total Estonian N load into the Gulf of Finland, total P load from point sources into a water body, average wind speed during May in all weather

stations, sea surface temperature predicted by nearest air temperature, number of days with wind  $>5 \text{ m}\cdot\text{s}^{-1}$  at nearest weather station, average air temperature during May at nearest weather station and average wind speed during May at nearest weather station were excluded from the further statistical analysis. The lack of other strong corre-

lations suggested that colinearity was never a problem for the final models.

Altogether, 27 benthic invertebrate and 21 zooplankton taxa were identified in the study area. *Macoma balthica*, *Monoporeia affinis*, *Saduria entomon*, *Acartia* spp., *Eurytemora affinis* and *Synchaeta baltica* were the most fre-

**Table 3.** Average biomass of benthic ( $\text{mg}\cdot\text{dry weight}\cdot\text{m}^{-2}$ ) and pelagic ( $\text{mg}\cdot\text{wet weight}\cdot\text{m}^{-2}$ ) in each water body in May 1996–2005.

Species/taxon	WB 0	WB 1	WB 4	WB 5	WB 6	WB 7
<b>Benthic invertebrates</b>						
<i>Balanus improvisus</i>	0	0	0	0	2	89
<i>Bylgides sarsi</i>	0	0	0	6	0	0
<i>Cerastoderma glaucum</i>	0	0	0	0	0	1584
Chironomidae larvae	0	0	0	0	192	8
<i>Corophium volutator</i>	0	2	0	5	0	118
<i>Gammarus salinus</i>	28	0	0	0	0	15
<i>Halicryptus spinulosus</i>	0	0	59	111	30	16
<i>Hediste diversicolor</i>	0	0	0	0	0	48
<i>Hydrobia ulvae</i>	0	0	0	3	4	23
<i>Hydrobia ventrosa</i>	0	0	0	5	2	0
<i>Idotea chelipes</i>	0	0	0	0	0	0
<i>Jaera albifrons</i>	0	0	0	0	0	0
<i>Macoma balthica</i>	497	16,970	10,127	33,513	35,495	21,491
<i>Manayunkia aestuarina</i>	0	0	0	0	0	0
<i>Monoporeia affinis</i>	48	107	17	94	2	6
<i>Mya arenaria</i>	0	0	0	127	57	10,370
Oligochaeta	0	13	0	0	2	9
<i>Pontoporeia femorata</i>	184	0	5	0	1	0
<i>Potamopyrgus antipodarum</i>	0	6	0	0	26	0
<i>Pygospio elegans</i>	0	0	0	0	0	0
<i>Saduria entomon</i>	887	629	112	501	0	29
<i>Theodoxus fluviatilis</i>	0	18	0	0	0	59
Trichoptera larvae	0	0	0	0	0	1
Total zoobentos	1673	17,745	10,319	34,373	35,818	33,889
<b>Pelagic invertebrates</b>						
<i>Acartia</i> spp.	879	23	361	349	177	211
<i>Balanus improvisus</i> nauplii	0	0	0	0	0	2
<i>Bivalvia</i> larvae	77	976	689	269	15	46
<i>Bosmina maritima</i>	4	15	0	2	1	0
<i>Centropages hamatus</i>	32	0	29	8	2	10
<i>Cercopagis pengoi</i>	0	0	0	0	0	0
Cyclopidae	10	32	2	1	0	0
<i>Eurytemora affinis</i>	699	66	224	43	34	56
<i>Evadne nordmanni</i>	34	13	8	21	24	28
<i>Fritillaria borealis</i>	179	4	185	185	169	69
<i>Keratella cochlearis</i>	0	1	0	0	0	0
<i>Keratella cruciformis</i>	0	0	0	0	0	0
<i>Keratella quadrata</i>	20	19	5	3	0	1
<i>Limnocalanus macrurus</i>	587	106	336	6	6	1
<i>Pleopsis polyphemoides</i>	0	0	0	1	0	15
<i>Podon intermedius</i>	0	0	0	0	1	0
<i>Pseudocalanus elongatus</i>	156	1	310	14	7	13
<i>Synchaeta curvata</i>	0	0	0	0	2	21
<i>Synchaeta monopus</i>	20	2	5	4	43	4
<i>Synchaeta baltica</i>	1992	167	1097	1936	512	407
<i>Temora longicornis</i>	30	1	39	14	4	11
Total zooplankton	6731	1594	4392	4793	1554	1329

quently detected taxa. The total biomass of benthic and pelagic invertebrates in samples ranged from 0 to 188 g-dry weight·m<sup>-2</sup> and from 3 to 62,000 mg-wet weight·m<sup>-2</sup>, respectively (Table 3).

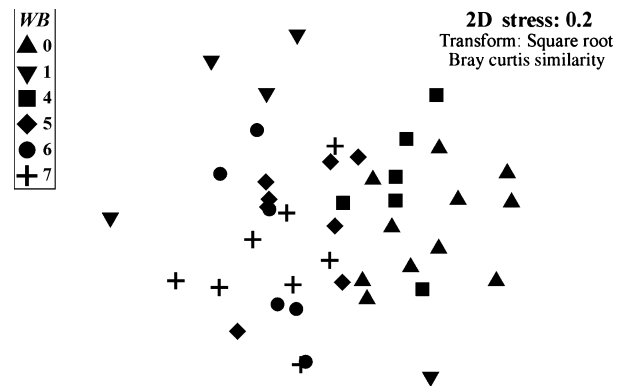
When all biomasses of pelagic and benthic invertebrates were pooled, the ordination of stations reflected the east-west gradients of the Gulf of Finland. ANOSIM analysis confirmed the trend and showed that most water bodies were significantly different in terms of the biomass structure, *i.e.* the studied water bodies behave independently of each other (global  $R = 0.448$ ,  $P < 0.001$ ) (Fig. 2).

Pelagic species had larger spatial and temporal variability of biomasses compared to that of benthic invertebrate species. In terms of spatial and temporal variability patterns the majority of benthic invertebrate species were statistically distinguished from zooplankton species (ANOSIM test,  $P < 0.05$ ). However, mobile benthic species such as *Corophium volutator*, *Pontoporeia femorata*, *M. affinis* and *S. entomon* were statistically dissimilar from zooplankton species (ANOSIM test,  $P > 0.05$ ). Small and abundant rotifers were placed inside the zooplankton cluster but close to the non-migrating benthic species, whereas larger and less dominating copepods were separated from the non-migrating benthic species (Fig. 3).

The relationship between abiotic environment and benthic invertebrate species was strongest at local and gulf scales (depending on the species, Spearman rank correlations varied between  $r = 0.19$  and  $0.38$ ) and weak at regional scale ( $r = 0-0.17$ ). The regional scale variability was significant only for *Halicyptus spinulosus* ( $r = 0.11$ ), *Mya arenaria* ( $r = 0.14$ ) and *M. balthica* ( $r = 0.18$ ). The combination of variables at all spatial scales did not explain the substantially larger proportion of benthic invertebrate variability than variables at any individual scale (difference in  $r_{\text{all scales combined}} - \text{any scale} = 0-0.05$ ) (Fig. 4).

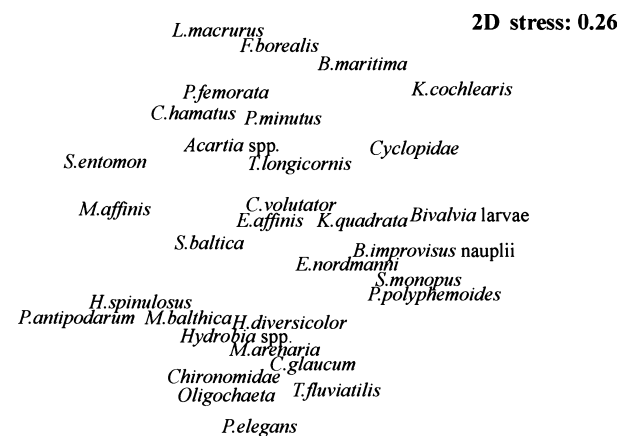
In contrast to benthic invertebrates the relationship between abiotic environment and zooplankton species was often described by abiotic variability at all spatial scales studied (depending on the species Spearman rank correlations varied between  $r = 0.18$  and  $0.42$ ). As an exception, the biomass of bivalve larvae and *Pleopsis polyphemoides* in May was only described by environmental variability at local scale ( $r = 0.18$  and  $r = 0.19$ ) (Fig. 5).

Among benthic invertebrates *P. femorata*, *H. spinulosus*, *Hydrobia* spp., Oligochaeta and Chironomidae larvae were described only by nutrient load variables ( $r = 0.18-0.56$ ) and *S. entomon* and *Hediste diversicolor* only by climatic condition variables ( $r = 0.17-0.37$ ). Among mesozooplankton, *P. polyphemoides* and bivalve larvae were described only by nutrient load variables ( $r = 0.17-0.18$ ) and *Bosmina maritima* and *Keratella quadrata* by climatic



**Fig. 2.** Similarity of water bodies according to the benthic and pelagic invertebrate communities. Pooled samples collected within each water body and each year during the late spring (May) were used for this ordination.

condition variables in May ( $r = 0.23-0.27$ ). *Pleopsis polyphemoides* was explained by nutrient load variables ( $r = 0.56$ ) and *S. baltica* and Cyclopidae by climatic condition variables in August, respectively ( $r = 0.33-0.37$ ). All other benthic and zooplankton species were related to both climatic conditions and nutrient load variables ( $r = 0.17-0.63$ ) (Fig. 6). In the biomass models of zooplankton species the contribution of nutrient load variables increased almost linearly with the contribution of climatic condition variables (Fig. 7). For some dominant benthic invertebrate species such as *M. affinis*, *Potamopyrgus antipodarum* and *Theodoxus fluviatilis* the links between environmental variability and biotic patterns were not statistically significant. For mesozooplankton the models for *Balanus improvisus* larvae, Cyclopidae and *Cercopagis pengoi* were not statistically significant in May



**Fig. 3.** Ordination of taxonomic groups; pooled samples collected within each water body and each year during the late spring (May) were used.

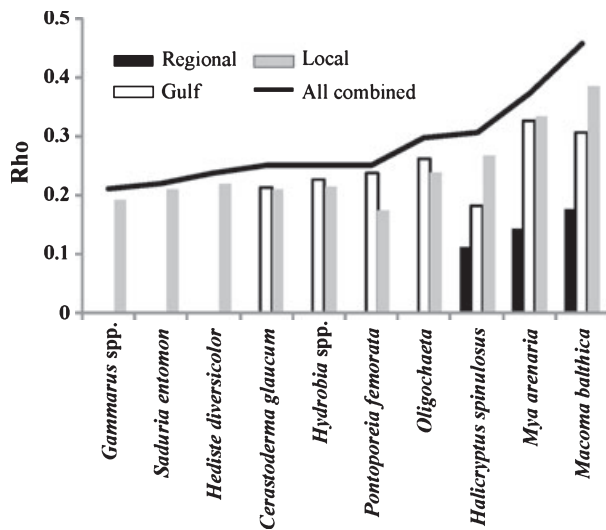


Fig. 4. Separate and combined effects (Rho, BVSTEP) of abiotic environmental variables at different spatial scales on benthic invertebrate species. Only significant relationships are shown.

and the models for *Balanus improvisus* larvae, *Limnocalanus macrurus* were not significant in August.

## Discussion

The main findings of the study are that (i) the effect of local and gulf scale environmental variability was important on benthic invertebrate communities and (ii) the variability was mainly due to local nutrient loading, gulf scale temperature and salinity patterns. In addition, we found that (iii) zooplankton species were equally affected by environmental variability at all spatial scales and that (iv) all nutrient loads and climatic condition variables contributed to the models of zooplankton species.

This suggests that large-scale pressures such as nutrient loads and change of climatic conditions may define broad patterns of distribution but that within these patterns, small-scale environmental variability significantly modifies the response of communities to these large-scale pressures. As such, this confirms the recent findings of Hewitt & Thrush (2009) on the nature of scale-dependent interactions between climatic condition variables and benthic invertebrate patterns, supports the multiscale theory that assumes interactions between processes operating over different scales (e.g. Wu *et al.* 2000), and can be used to predict location-dependent responses of the studied broad-scale factor in the Gulf of Finland. Our study also suggests that the consistency of effects of broad-scale factors likely depends on the degree of the small-scale heterogeneity of habitat (models included those local variables that are known to have large variability) and the develop-

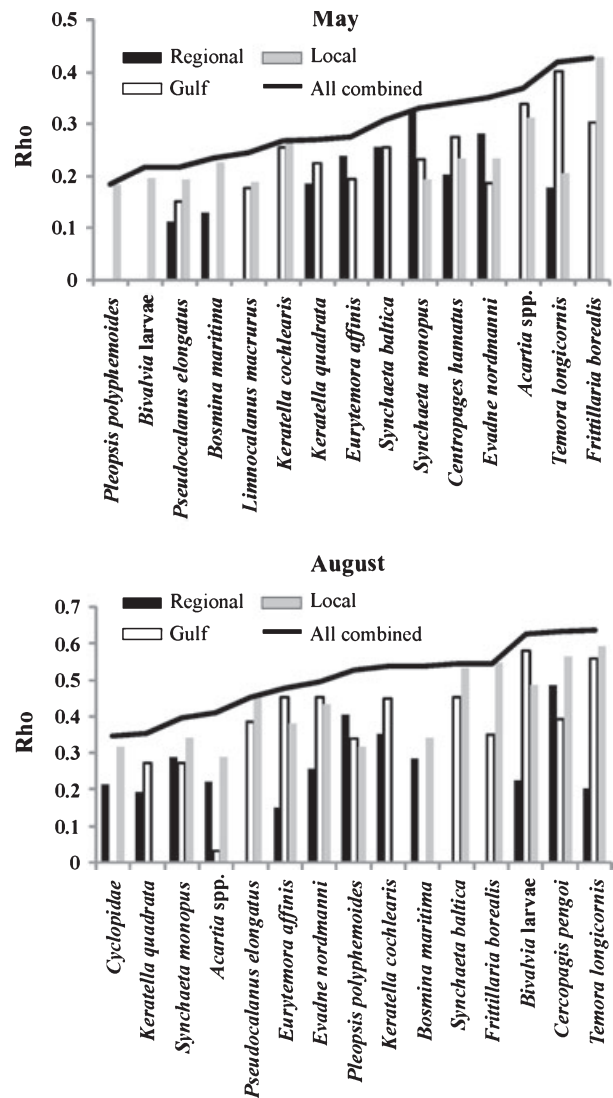


Fig. 5. Separate and combined effects (Rho, BVSTEP) of abiotic environmental variables at different spatial scales on zooplankton species. Only significant relationships are shown.

mental characteristics of species (pelagic *versus* benthic species, larval development *versus* direct development) (Kotta & Witman 2009). Our results show a clear difference between how benthic invertebrates and mesozooplankton responded to changes in nutrient load and climatic condition variables. Namely, the predictive power of the benthic invertebrate model was highest using a mixture of local and gulf scale variables. In contrast, for the mesozooplankton model, all studies scales were statistically significant.

Increasing nutrient loads are known to lead to higher abundances and biomasses of benthic invertebrates, but too high concentrations are known to cause hypoxia and disappearance of the species (Posey *et al.* 1999; Kotta

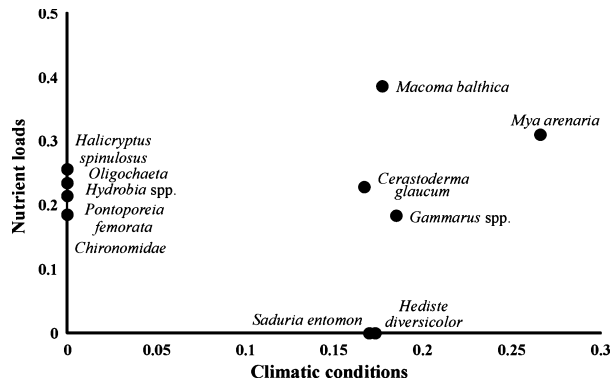


Fig. 6. Relationship (Rho, BVSTEP) between nutrient loads, climatic condition variables and benthic invertebrate species. Only significant relationships are shown.

et al. 2000, 2007; Karlson et al. 2002). Among benthic invertebrates, *Pontoporeia femorata*, Oligochaeta, *Hydrobia* spp., *Halicryptus spinulosus*, and Chironomidae larvae were only related to nutrient load variables. The former two species are severely decimated at low oxygen levels and the strong inverse relationship between nutrient load variables and invertebrates may refer to the negative consequences of hypoxia to the named species. On the other hand, *Hydrobia* spp. prefer elevated nutrient loads and tolerate moderate hypoxia. The latter two taxa are the typical inhabitants of severe organic enrichment and hypoxic conditions and the positive relationship between nutrient load variables and biomasses indicates the facilitative effect of nutrient loading on the species (Kotta & Orav 2001; Lauringson & Kotta 2006).

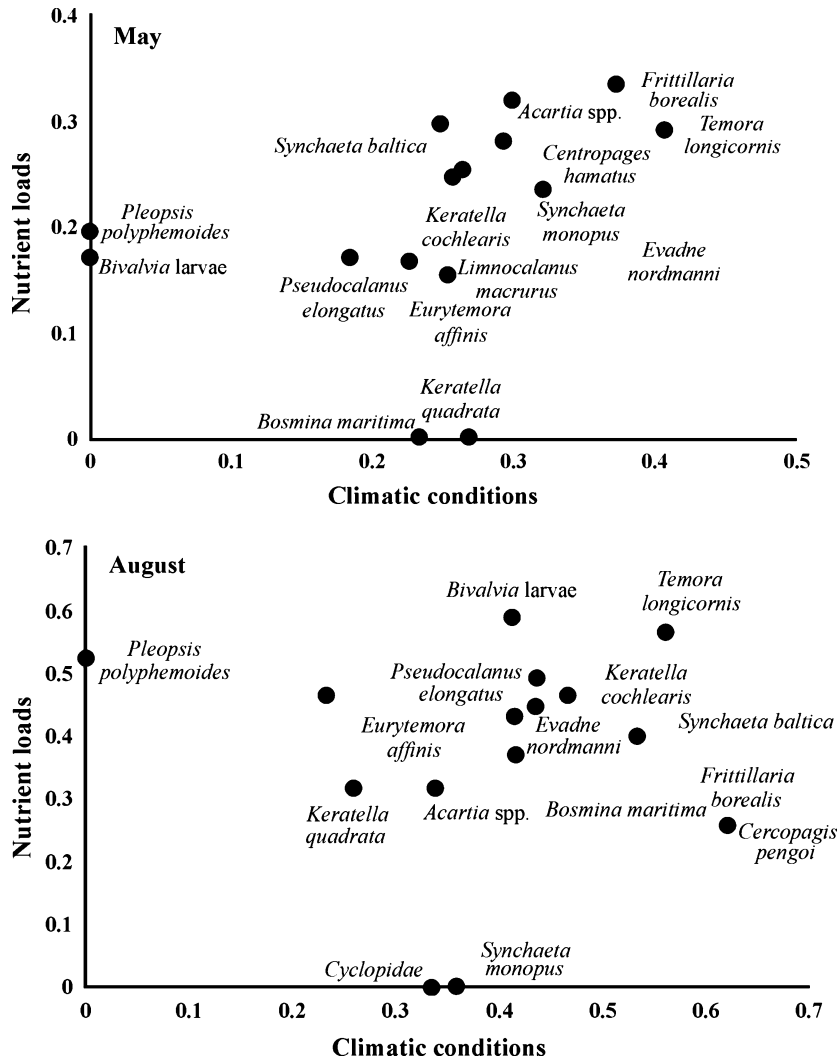


Fig. 7. Relationship (Rho, BVSTEP) between nutrient loads, climatic condition variables and zooplankton species. Only significant relationships are shown.



We are not aware of any studies reporting clear evidence of the links between nutrient load variables and zooplankton communities in the Baltic and North Sea areas (e.g. Colijn *et al.* 2002). There is some indication that the density of adult *Temora longicornis* increases with eutrophication level (Fransz *et al.* 1992). Besides, nutrient loading is known to correlate with mesozooplankton communities in the Gulf of Finland (Pöllumäe & Kotta 2007). However, the latter study did not take into account other abiotic factors (e.g. weather patterns, long-term hydrology) that may be behind this relationship. In fresh-water ecosystems, nutrient loading is known to raise the biomass and change the species composition of zooplankton (Ostoji 2000; Kangur *et al.* 2002; Straile & Geller 1998). In this respect our result on the significant interactions between nutrient load variables and zooplankton communities in the brackish Gulf of Finland should be treated as exceptional. Our study not only reports zooplankton total biomass but also takes into account the community composition. Total biomass, as solely reported in many other studies, may not capture the links between nutrient load variables and the responses of separate zooplankton species.

Change of climatic conditions is known to cause the massive blooms of benthic invertebrates (Lawrence 1975), replacement of key species (Southward *et al.* 1995) and other major shifts in community structure (Connors *et al.* 2002). Among other effects benthic communities are exposed to severe winter storms and reduced ice scour under rapidly changing climate (Gutt 2001; Strasser *et al.* 2001). We are not aware of studies reporting the effects of climatic conditions on the distribution of benthic species in the Baltic Sea.

In our study the distribution of *Saduria entomon* and *Hediste diversicolor* was only related to climatic condition variables. Similarly, the distribution of *Macoma balthica*, *Cerastoderma glaucum*, *Mya arenaria* and *Gammarus salinus* also had a large component of climatic condition variability. In contrast, the distribution of these species was previously thought to be largely regulated by trophic status of the Baltic Sea (e.g. Kotta *et al.* 2007). At the same time the population dynamics of the bivalves is strongly related to seawater temperatures in Northwestern European estuaries where a series of mild winters results in low bivalve recruit densities and small adult stocks (Philippart *et al.* 2003). In the North Sea area, however, low temperatures strongly affect *Cerastoderma edule* but cause no increased mortality in *M. arenaria* or *M. balthica* (Strasser *et al.* 2001). It is likely that changes in the mean water temperature of the Baltic Sea are not very important for benthic invertebrates as large seasonal variation counteracts the potential effects of climatic condition change on water

temperature and the indirect effects of climatic conditions change such as increased wave action, decreased ice scrape, reduced photosynthetic light intensity (cloudiness) and diminished salinity are more important and potentially affect benthic invertebrates. Practically all our models demonstrated the strong links between salinity and biomass patterns of benthic invertebrates referring to salinity limitation. Most invertebrate species of marine and fresh-water origin live near to their distribution limit in the Gulf of Finland. Therefore reduction in salinity (associated to recent mild winters) has important consequences for these species. As an exception, *S. entomon* is a glacial relict and temperature and ice conditions determined the observed pattern of the species (Leonardsson 1986), whereas the effect of salinity was not significant.

Earlier studies have clearly demonstrated the links between climatic condition variables and zooplankton communities in the Baltic Sea area (Hinrichsen *et al.* 2007) and established the functional relationships between temperature, salinity, species composition and biomass of zooplankton (Ojaveer *et al.* 1998; Vuorinen *et al.* 1998; Möllmann *et al.* 2000). Piontkovski *et al.* (2006) demonstrated that the effect of climatic condition variables on zooplankton community depended on geomorphology of the basin; pelagic communities in small basins responded faster to climatic condition change than those in large basins. In our study we observed significant relationships between environmental variability and zooplankton communities at all scales. Thus, differences in geomorphology of the studied water bodies do not explain the observed patterns of zooplankton communities. More likely, the spatial distribution of zooplankton reflects the east–west gradient in the water circulation patterns of the Gulf of Finland shown by the statistical significance of salinity and spring-time temperature in the models of zooplankton species.

To conclude, our study demonstrated that nutrient loads and climatic condition variables largely explained the observed patterns in benthic and pelagic invertebrate communities. The mobility of organisms determined the relative contribution of small- and large-scale environmental variability to the biomass patterns of invertebrates. Knowledge on the correlation scales between environmental and biotic patterns can provide an insight into how processes generate these patterns. The prevalence of the key processes, however, is further complicated to an unknown extent by regional scale variability. We believe that together with the increase in studies on relationships between nutrient loads, climatic condition variables and biotic patterns at multiple spatial scales and in different regions, meta-analyses (e.g. Gurevitch *et al.* 2001) can tackle this problem.

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## ORIGINAL ARTICLE

# A multidisciplinary study of the Cape Peloro brackish area (Messina, Italy): characterisation of trophic conditions, microbial abundances and activities

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## Keywords

Microbial activities; C/N; carbon cycle; particulate organic carbon (POC); transitional ecosystem.

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## Conflicts of interest

The authors have declared no conflicts of interest.

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## Abstract

In the framework of the VECTOR DIVCOST Project, a 2-year investigation was started in 2006, with the aim of testing the sensibility of microbial parameters to environmental changes and of assessing whether they can provide information about functional changes in the carbon cycle. The investigation was performed in the surface waters of two small brackish ponds (Ganzirri and Faro), located in the Cape Peloro transitional area (Sicily, Italy). The seasonal changes in both the microbial compartment [bacterioplankton, vibrios, exoenzymatic hydrolysis of proteins and polysaccharides, bacterial secondary production (HBP) and community respiration] and the trophic state of suspended matter [total suspended matter (TSM), particulate organic carbon (POC), particulate organic nitrogen (PON), C/N] were analysed in relation to the hydrological characteristics [temperature, salinity, oxygen, fluorescence, NH<sub>4</sub>, NO<sub>2</sub>, NO<sub>3</sub>, PO<sub>4</sub>]. Despite marked differences in the nutritional input and the diversification in both carbon budget and trophic level, the two ponds show similar trends in many of the investigated factors, hardly influenced by seasonal variations. Temporally coupled trends were observed for some parameters (enzyme activities, vibrios abundances, respiratory activity), whereas others (POC, PON, heterotrophic bacterial production, bacterioplankton) showed a seasonal shift between the two lakes. The different behaviour found for the some biotic parameters suggests that their response to environmental conditions may be modulated differently between the two lakes, which, despite their spatial proximity and reciprocal connection, do not always show contemporaneous functional processes.

## Problem

Transitional areas are among the most geochemically and biologically active of the biosphere and play an important role in the global biogeochemical cycles. These ecosystems are characterised by typical hydrological and geomorphologic features, such as shallow depth, confined circulation, weak hydro-dynamism, marked space–time variations in salinity and temperature, *etc.* Moreover they are usually affected by nutrient enrichment from terrestrial runoff, which, concomitantly with extreme climatic conditions,

such as rapid temperature increase, high pressure, lack of wind, *etc.*, may lead to occasional and sometimes dramatic anoxia crises.

The rapid changes in the environmental features which characterise these areas, make them particularly suitable for ecological investigations, concerning the study of the ecosystem functioning overall.

Notwithstanding their peculiarities and biodiversity, to date there has been little research concerning microbial community and biogeochemical processes in these areas. In this context, a 2-year investigation was started in



2006, in the framework of the VECTOR DIVCOST Project. This study was devoted to the analysis of the microbial community, in term of abundances (bacterioplankton, vibrios) and activities (ectoenzymatic hydrolysis of proteins and polysaccharides, bacterial secondary production and community respiration), which was performed contextually with suspended matter quality [total suspended matter (TSM), particulate organic carbon (POC), particulate organic nitrogen (PON), C/N], and physical and chemical parameters (temperature, dissolved oxygen, fluorescence,  $\text{NH}_4$ ,  $\text{NO}_2$ ,  $\text{NO}_3$ ,  $\text{PO}_4$ ).

The aim of this study was to test the sensibility of microbial parameters to the climatic changes and assess whether they can provide information about the functional changes in the carbon cycle, in order to hypothesize their possible utilization as environmental indices. Moreover, our study may contribute to knowledge of all the factors involved in microbial biogeochemical activities and their reciprocal interactions, also with the goal to provide a helpful tool for planning proper management strategies for the transitional ecosystems.

The carbon flux in aquatic environments is ruled by the microorganisms (Hoppe 1991) that provide, through the production, decomposition, and respiration processes,

turnover of organic matter, regulating the carbon flux through the trophic web (Azam *et al.* 1983; Cho & Azam 1988; Chrost 1990; Fuhrman 1992; Azam *et al.* 1993).

Many studies show that the elemental composition of particulate organic matter changes in relation to the different incidences of autotrophic and heterotrophic biomasses and detritus, reflecting the trophic status of the ecosystem (Fabiano *et al.* 1999; Ferrari *et al.* 2003; Bates *et al.* 2005). Integrated analyses of the microbial compartment, together with particulate organic matter stoichiometry, may provide ecological information on the efficiency of microbial metabolism involved in the transformation of organic matter.

### Study Area

The Cape Peloro (Messina, Italy) transitional area is located in the north-easternmost part of Sicily, between the Tyrrhenian and the Ionian Sea. It is constituted of two brackish neighbouring basins denominated Lake Ganzirri and Lake Faro, with noticeable differences in geomorphologic, hydrographic and trophic features (Fig. 1).

Lake Ganzirri is a brackish coastal pond; it covers a 34-ha area (maximum depth: 7 m; water volume:  $106 \text{ m}^3$ ). Its

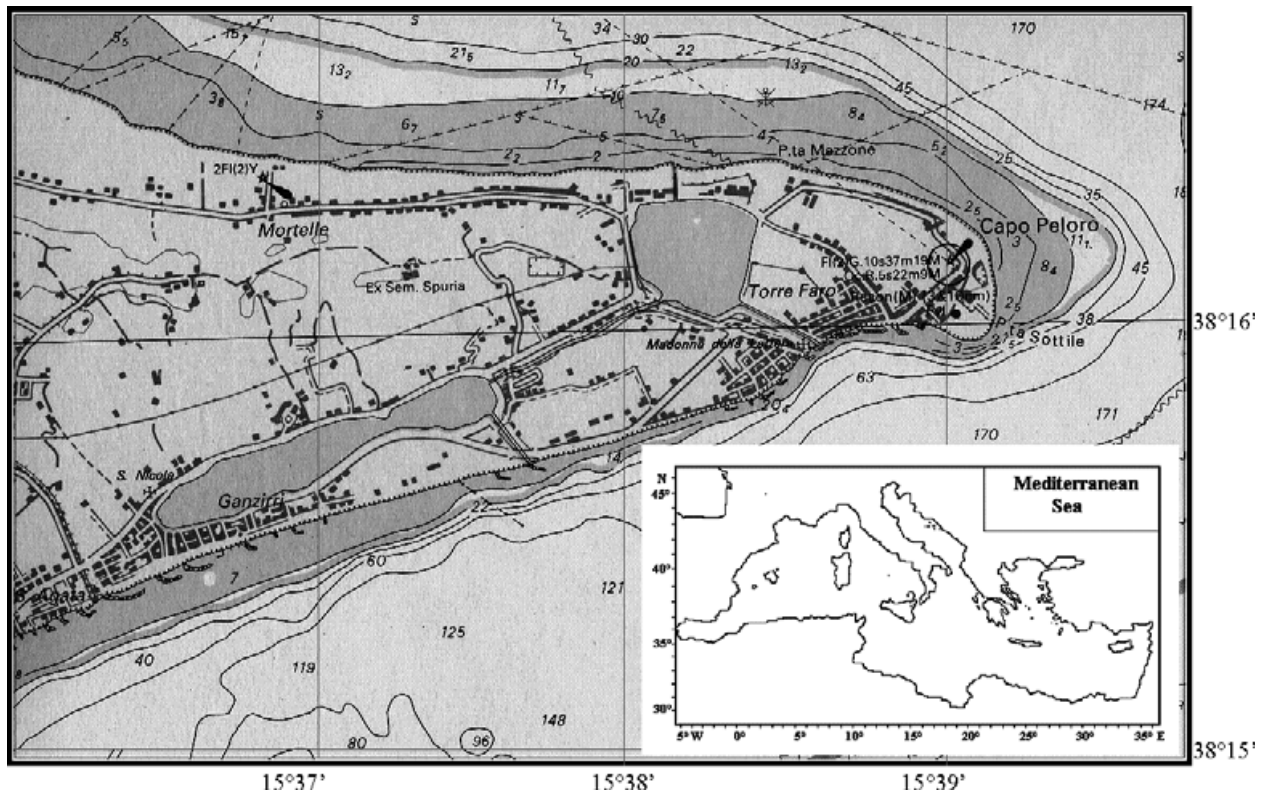


Fig. 1. Map of the study site (Cape Peloro transitional ecosystem).

length and width are 1670 m and 282 m, respectively. It communicates with the Straits of Messina and Lake Faro by means of narrow shallow channels (Vanucci *et al.* 2005). Lake Ganzirri is colonised by macroalgae and frequently suffers dystrophic crisis. Sometimes the blooms spread over the lake, causing temporary dystrophic events and marked reduction of the dissolved oxygen (Giacobbe *et al.* 1996).

Lake Faro is a small and relatively deep meromictic coastal pond. It covers a 26-ha area and has a nearly circular shape. Its depth in the central part reaches 28 m. It communicates with the Tyrrhenian Sea and with the Straits of Messina through artificial shallow channels. Lake Faro, with its particular shape, represents a rare example of a meromictic basin and is an interesting subject of study (Acosta Pomar *et al.* 1988; Brugnano *et al.* 2006; Saccà *et al.* 2008). It is characterised by massive development of coloured phototrophy sulphur bacteria capable of carrying out photosynthesis even in the absence of oxygen at depth (Trüper and Genovese 1968).

## Material and Methods

Water samples were seasonally collected from the surface layer in a central station of Lake Faro and of Lake Ganzirri, for a 2-year period starting in September 2006.

Temperature, salinity, fluorescence and oxygen measurements were taken using an oceanographic multiparametric sensor (SBE 19 Plus).

For the dissolved oxygen analysis, water samples were fixed immediately after collection, and then analysed using Winkler's method (Carpenter 1965).

Samples for nutrient determinations ( $\text{NH}_4$ ,  $\text{NO}_2$ ,  $\text{NO}_3$ ,  $\text{PO}_4$ ) were filtered using GF/F glass-fibre filters and kept frozen ( $-20^\circ\text{C}$ ). Analytical determinations were performed according to Strickland & Parsons (1972), and  $\text{NH}_4$  was measured according to Aminot & Chaussepied's method (1983). All nutrient concentrations were determined using a Varian Mod. Cary 50 spectrophotometer.

Total suspended matter (TSM) was evaluated by a gravimetric method using a Mettler AT261 electronic microbalance (accuracy  $\pm 1.0\ \mu\text{g}$ ). Particle material was collected by filtering variable volumes of water on pre-combusted ( $480^\circ\text{C}$  for 4 h) pre-weighted glass fibre filters (Whatman GF/F), which was then oven-dried at  $60^\circ\text{C}$  for 24 h.

For estimation of particulate organic carbon and nitrogen (POC and PON), 500-ml water samples were concentrated on precombusted Whatman GF/F glass-fibre filters and processed at  $980^\circ\text{C}$  in a Perkin-Elmer CHN-Autoanalyzer 2400, using acetanilide as standard (Iseki *et al.* 1987).

Bacterioplankton (BA) abundance was determined using DAPI (Porter and Porter & Feig 1980) and image analysis (Zeiss AXIOPLAN 2 Imaging microscope). Bacte-

rioplankton biomass (BB) was estimated by cell counting and volumetric measurements according to La Ferla *et al.* (2004).

Vibrios abundance was determined on TCBS agar added with 1.5% of NaCl and incubated at  $35^\circ\text{C}$  for 24 h (Zaccone *et al.* 1992).

Microbial ectoenzymatic activity measurements were performed to estimate the potential activity rates of leucine aminopeptidase (LAP) and  $\beta$ -glucosidase ( $\beta$ -GLU), two enzymes involved respectively in protein and polysaccharide decomposition mediated by the microbial community. The enzymatic assay relies on the hydrolysis of specific fluorogenic substrates, L-leucine-7 amido-4-methyl coumarin hydrochloride (Leu-MCA) and 4-methylumbelliferyl-  $\beta$ -D-glucoside, respectively a derivative of methylcoumarin (MCA) and a derivative of methylumbelliferone (MUF), following the method reported by Caruso *et al.* (2005). Increasing amounts (from 20 to 400  $\mu\text{mol}$ ) of substrates were added to 10-ml subvolumes of water and spectrofluorometer measurements were performed at the initial time and after incubation at '*in situ*' temperature for 2 h. Through calibration with the standard curves obtained with known amounts of MCA and MUF, LAP and  $\beta$ -GLU values were expressed in term of maximum velocity of hydrolysis ( $V_{\text{max}}$ , in nmol of substrate hydrolysed per litre and per hour,  $\text{nm}\cdot\text{h}^{-1}$ ) and converted into nanograms of C mobilised assuming that 1 nmol of substrate hydrolysed released 72 ng of C.

Net heterotrophic bacterial production (HBP) (Ducklow & Carlson 1992) was estimated from the rate of [ $^3\text{H}$ ] leucine incorporation using the micro centrifugation method according to Smith & Azam (1992). Triplicate 1.0-ml samples and two blanks were incubated in the dark, for 1 h at *in situ*  $\pm 1^\circ\text{C}$  temperatures with L-[4,5 $^3\text{H}$ ]leucine (Amersham Biosciences UK Limited) (25 nM final concentration). HBP was calculated according to Kirchman (1993) using leucine isotopic dilution factor (ID) *in situ* determined according to Pollard & Moriarty (1984). Bacterial turnover rate (BTR) was defined as the amount of days necessary to produce one bacterial standing stock BB ( $\text{BTR} = \text{BB}\ \text{l}^{-1}\cdot\text{HBP}\ \text{l}^{-1}\cdot\text{day}^{-1}$ ).

The respiration rates and the consequent metabolic production of  $\text{CO}_2$  (R) were measured by the study of the electron transport system activity (ETS); the assay is based on the conversion of tetrazolium salt in formazan (Packard & Williams 1981). The results are reported as  $V_{\text{max}}$  and have been converted into C by using a respiratory quotient of 1.

## Results

The obtained values of biotic and abiotic parameters are reported in Fig. 2.

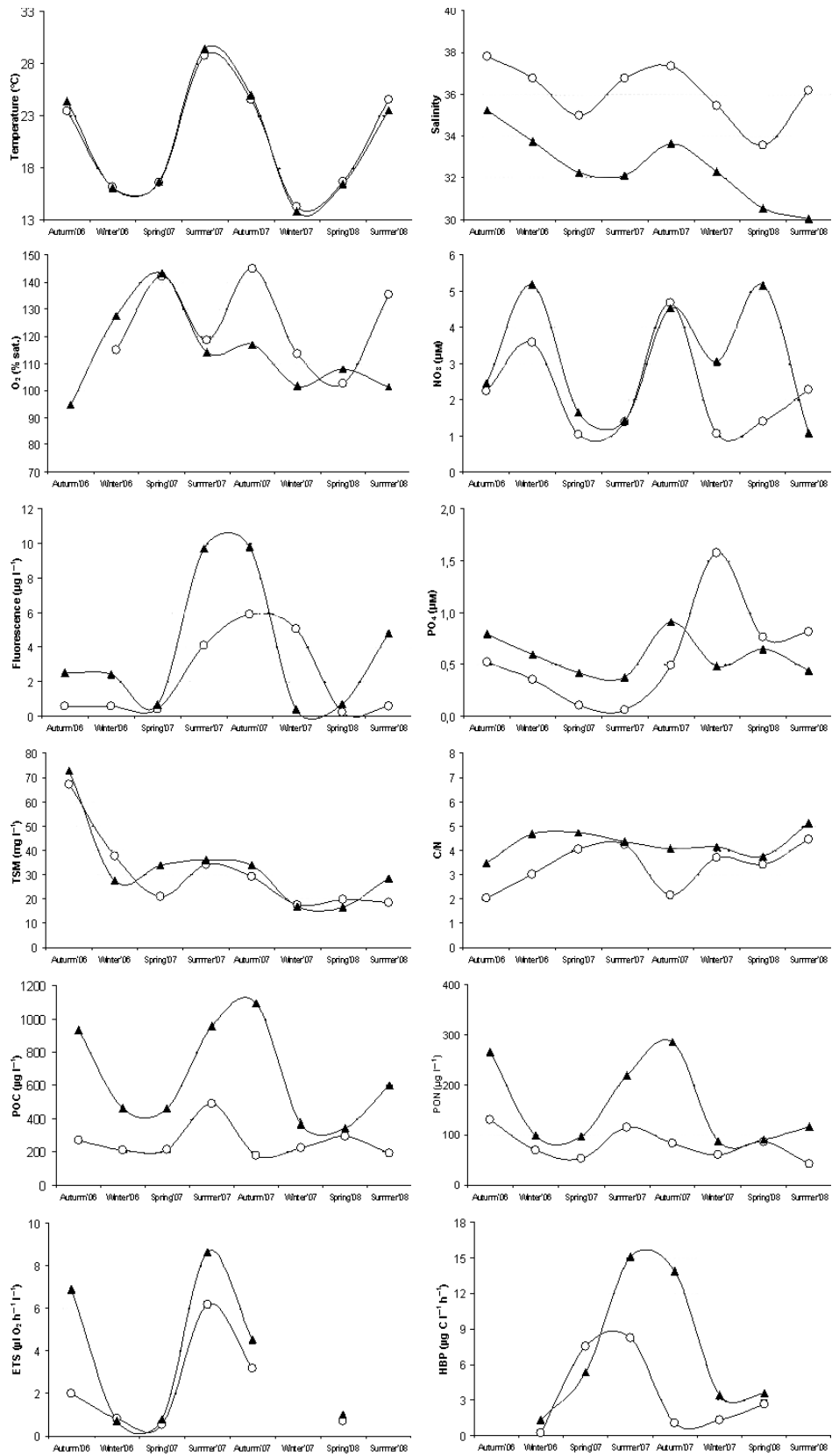


Fig. 2. Seasonal patterns of investigated parameters. ▲, Lake Ganzirri; ○, Lake Faro.

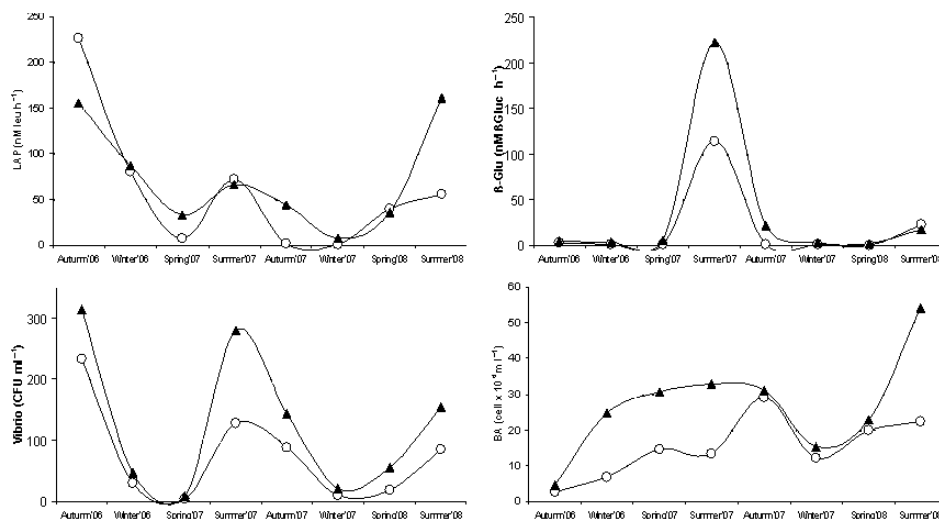


Fig. 2. (Continued.)

Temperature values ranged from 13.80 to 29.43 °C and from 14.23 to 28.79 °C in Lake Ganzirri and Lake Faro, respectively, showing typical seasonal trends in both ponds.

Salinity values were higher in Lake Faro than in Lake Ganzirri, ranging from 33.56 to 37.83 and from 30.56 to 35.24, respectively. The seasonal trend is characterized by lower values in spring in both ponds.

In both lakes, during the investigated period, dissolved oxygen expressed in terms of saturation values were above the 100% level, with maximum values in spring (145%) and minimum values in summer (118%).

Lake Ganzirri showed concentrations of NH<sub>4</sub>, NO<sub>2</sub>, NO<sub>3</sub> and PO<sub>4</sub> (average values 1.61, 0.19, 3.54 and 0.58 μM, respectively) similar to those found in Lake Faro (average values 1.71, 0.22, 3.08 and 0.57 μM, respectively). During all investigated periods the nitrate incidence was never lower than 1.0 μM in Lake Ganzirri and 1.5 μM in Lake Faro. The ammonia (data not shown) increased in the autumn and winter periods (>2.4 μM) in both the lakes and phosphates were lower during the spring–summer period.

The highest TSM content (90.20 mg·l<sup>-1</sup>) was found in Lake Ganzirri in September 2006. In general, lower values were recorded in the winter–spring period and higher values in the summer–autumn period in both ponds.

Higher POC incidences were recorded in Lake Ganzirri (range 273.00–1245.50 μg C·l<sup>-1</sup>) than in Lake Faro (range: 179.49–491.18 μg C·l<sup>-1</sup>); similarly were found for PON, which was significantly correlated with POC in both investigated lakes (Ganzirri,  $r = 0.95$ ,  $P < 0.01$ ; Faro,  $r = 0.58$ ,  $P < 0.01$ ). The seasonal distribution in Lake Ganzirri was characterised by higher values in the warm

period (summer–autumn), and lower values in the cold period.

The C/N ratio, ranging between 3.48 and 4.73, indicated a significant predominance of the heterotrophic biomasses in the particulate matter. Highly significant correlations were in fact found between POC and ETS ( $r = 0.67$ ,  $P < 0.01$ ), as well as between POC and HBP ( $r = 0.66$ ,  $P < 0.01$ ), and vibrios abundance ( $r = 0.507$ ,  $P < 0.01$ ).

There was a very wide range of BA ( $0.46$ – $5.42 \times 10^7$  cells·ml<sup>-1</sup> in Lake Ganzirri and  $0.26$ – $2.91 \times 10^7$  cells·ml<sup>-1</sup> in Lake Faro) with no apparent seasonal pattern. Substantial differences were observed between Lake Ganzirri and Lake Faro; in the latter, the values registered were always lower. In Lake Ganzirri the prokaryotic biomass, modulated by both cell abundances and biovolumes, was in the range 74.28–1341.49 μg C·l<sup>-1</sup>, with the highest value in summer 2008.

Vibrios abundance showed the highest peaks in summer and a coincident trend in both lakes during 2006 and 2007. A comparable peak was found in autumn 2006, when high fish mortality occurred in Lake Ganzirri. The minimum values were observed in winter and spring 2007, coinciding with a temperature decrease. In general, mean values were lower in Faro than in Lake Ganzirri, as observed for other parameter trends (Fig. 2).

Enzyme activity levels showed high spatial and seasonal variability, although similar activity patterns were found in both the studied lakes. On the whole, higher enzyme activity rates were measured in Ganzirri compared to Lake Faro (Fig. 2), both for LAP (ranging from 7.78 to 161.44 nm·h<sup>-1</sup> and from 1.059 to 226.76 nm·h<sup>-1</sup>, respectively) and β-GLU (ranging from 1.49 to 222.4 nm·h<sup>-1</sup> and from 0.42 to 114 nm·h<sup>-1</sup>, respectively). High LAP



activity rates were measured during autumn 2006; another peak of proteolytic activity, although slightly lower, was also observed in summer 2007, followed by a decline in winter months. During the successive spring–summer period (2008) LAP increased again. During the warmest months, in Lake Ganzirri, LAP values were, on average, twice those detected during the coldest months, whereas in Lake Faro they were on average three times higher than those observed during the coldest months. The observed trends in LAP activity data reflected those of temperature values and total suspended matter content, although statistical relationships calculated between LAP activity values and these parameters did not reach the level of significance (Fig. 2).

Low rates of  $\beta$ -GLU activity (Fig. 2) were always detected during the autumn–spring period, with values lower than 2.61 and 4.60  $\text{nm}\cdot\text{h}^{-1}$  in Lake Faro and Lake Ganzirri, respectively; the highest activity rates were detected during both summer samplings. In Lake Ganzirri, these peaks in  $\beta$ -GLU activity were associated with the highest levels of BA. During the warmest months,  $\beta$ -GLU values in Lake Ganzirri were on average 20 times higher than those measured during the coldest months, whereas in Lake Faro, the values were 50 times higher than those detected during the coldest months.

The calculation of the LAP/ $\beta$ -GLU ratio provided some information on the quality of the hydrolysed organic matter. In Lake Faro, the LAP/ $\beta$ -GLU ratio showed values lower than 0.75 from spring 2007 to winter 2007, suggesting during this period a greater importance of polysaccharides within the organic matter. Conversely, during autumn–winter 2006 and in spring 2008, the most labile protein fraction of the organic matter was prevalent, as suggested by values of LAP/ $\beta$ -GLU ratio ranging from 6.26 to 7.78.

HBP seasonal values in Lake Ganzirri varied from 0.64  $\mu\text{gC}\cdot\text{l}^{-1}\cdot\text{h}^{-1}$  in winter 2006 to 21.30  $\mu\text{gC}\cdot\text{l}^{-1}\cdot\text{h}^{-1}$  in summer 2007, higher than those usually reported for coastal lagoons. In Lake Faro, HBP values were about one order of magnitude lower than in Lake Ganzirri.

Respiratory activity (ETS) determined in the two ponds ranged from 0.53 to 6.18  $\mu\text{l O}_2\cdot\text{h}^{-1}\cdot\text{l}^{-1}$  in Lake Faro and from 0.73 to 8.65  $\mu\text{l O}_2\cdot\text{h}^{-1}\cdot\text{l}^{-1}$  in Lake Ganzirri. In the two analysed ponds the seasonal trends were similar, with lower values in winter and spring, and higher values in summer and autumn. The levels of ETS activity in Lake Ganzirri were generally higher than in Lake Faro.

## Discussion

From the overall data, seasonal variability was generally high for all the examined parameters; however, as

reported in previous studies, strong fluctuations in both abiotic and biotic parameters are typical for transitional areas (Caruso *et al.* 2005, 2006).

Nitrogen and phosphate levels recorded in Lake Ganzirri and Lake Faro fell within a range of values usually found in other studied transitional ecosystems (Leonardi *et al.* 2005); they were not limiting elements for phytoplankton production, as suggested by the overproduction of  $\text{O}_2$ , which always exceeded the 100% saturation level.

Despite marked differences in the nutritional input and the diversification in both carbon budget and trophic level, ranging from oligotrophy (Faro) to mesotrophy (Ganzirri) (Caruso *et al.* 2006), the two ponds show similar trends in many of the investigated factors, hardly influenced by the seasonal variations.

During warm periods there were increases in both microbial metabolism and vibrios abundance, as well as in POC and PON contents. Positive correlations were observed between temperature and microbial activities (HBP,  $\beta$ -GLU, ETS), and particulate organic matter (POC, PON) in both the lakes. Vibrios abundance was significantly correlated with temperature in both lakes ( $r = 0.86$  in Faro and  $r = 0.56$  in Ganzirri,  $P < 0.01$ ), indicating a direct stimulation of warm season on bacterial growth (Zaccone *et al.* 1992; Crisafi 1998). The correlations observed throughout the study among LAP,  $\beta$ -GLU and vibrios abundance (Ganzirri  $r = 0.78$ ,  $P < 0.01$  and  $0.35$ ,  $P < 0.05$ , respectively; Faro:  $r = 0.59$  and  $0.79$ ,  $P < 0.01$ , respectively), underline the role of vibrios in organic matter cycling; similarly, in Lake Ganzirri, BA was positively related to  $\beta$ -GLU ( $0.34$ ,  $P < 0.05$ ) and HBP ( $0.66$ ,  $P < 0.01$ ).

Microbial metabolism was particularly intense in Lake Ganzirri, in relation to the availability of both autochthonous and allochthonous nutritional sources. As microbial ectoenzyme activity is specifically, functionally and biochemically tightly coupled with the presence of high-molecular-weight substrates that are predominating constituents of organic matter, the activity of some enzymes may respond to their concentrations and thus may depend on the trophic conditions of lakes (Chrost & Siuda 2006). Our LAP and  $\beta$ -GLU activity values confirmed this assumption. Both the enzyme activities were significantly, inversely, related to oxygen content in Lake Ganzirri ( $r = -0.40$  and  $-0.41$ ,  $P < 0.05$ , for LAP and  $\beta$ -GLU, respectively) but only LAP in Lake Faro ( $r = -0.43$ ,  $P < 0.05$ ), confirming that the oxygen was consumed by the active microbial community living in their waters.

C/N values, always lower than 5, suggested the predominance of heterotrophic biomasses in this ecosystem, as confirmed by the significant correlation found between POC and ETS, HBP, and vibrios abundance.



Respiratory activity appeared a key parameter linking the different ecosystemic components (autotrophic and heterotrophic compartments as well as the pool of organic matter). In Lake Ganzirri, R was positively correlated with the fluorescence, vibrios density, the microbial ectoenzymatic activities, HBP and the pool of matter (TSM, POC, PON).

The seasonal range of POC and PON was comparable with that reported by Bertoni *et al.* (2004), but markedly higher than those recorded in other Sicilian transitional areas (Sarà *et al.* 1999; Leonardi *et al.* 2000, 2006).

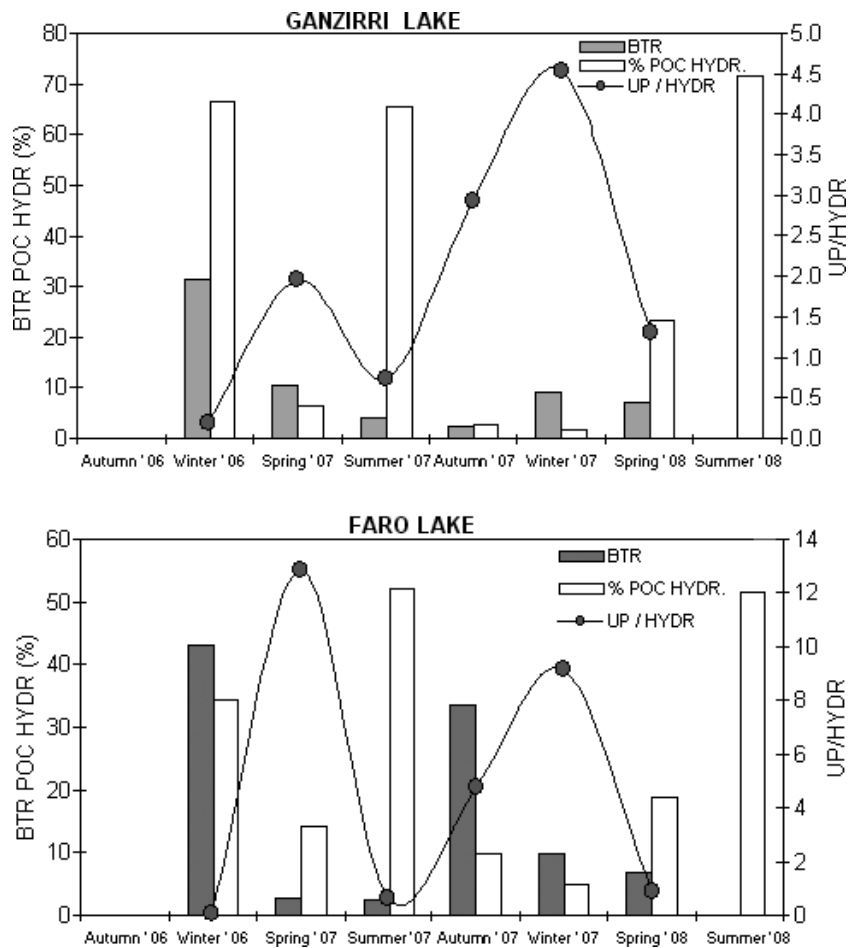
The enzymatic values recorded in our study fell within a range similar to other coastal ecosystems as well as to lagoons and estuarine waters (Hoppe *et al.* 2002; Williams & Jochem 2006), but they were higher than those detected in marine environments (Caruso & Zaccone 2000; La Ferla *et al.* 2001; Zaccone & Caruso 2002). LAP activity provided not only a C but also an N source to sustain microbial metabolism.

In both ponds the ETS data comprised the range of values already determined in the Mediterranean Sea. In

winter and summer, the values were comparable to those from pelagic areas (La Ferla *et al.* 1996), whereas in summer and autumn they were comparable with those from estuarine environments (La Ferla *et al.* 1996, 2001).

BA values are comparable to those reported by Araújo & Leal Godinho (2008) in a tropical fluvial-lagunar system ( $2.7\text{--}5.1 \text{ cells} \times 10^7 \text{ ml}^{-1}$ ) and lower than those reported by Gocke *et al.* (2004) in a study in the coastal lagoon of the Colombia ( $0.6\text{--}9.1 \text{ cells} \times 10^7 \text{ ml}^{-1}$ ). BB values were similar to those found in a coastal lagoon of the Colombia by Gocke *et al.* (2004) (ranging from 77 to  $1542 \mu\text{gC}\cdot\text{l}^{-1}$ ) and also to those reported in coastal lakes of Southeastern Brazil by Farjalla *et al.* (2001) (ranging between 480 and  $1432 \mu\text{gC}\cdot\text{l}^{-1}$ ). Our BB values were higher than those measured in a tropical lake by Erikson *et al.* (1999), between 240 and  $930 \mu\text{gC}\cdot\text{l}^{-1}$ .

An estimate of C flux through the microbial community was performed. Heterotrophic bacteria derive their carbon and energy sources from biologically available dissolved and particulate organic carbon sources. Figure 3 shows the percentage of POC potentially mobilised *per*



**Fig. 3.** Uptake to Hydrolysis (UP/HYDR) ratio, Bacterial Turnover rates (BTR), percentage of mobilised POC by hydrolysis (% POC/HYDR), calculated in Lake Ganzirri and Lake Faro.

day by enzyme hydrolysis (LAP+ $\beta$ -GLU) (%POC-HYDR). High percentages of POC were hydrolysed during summer 2007 and 2008, due to the high activity rates measured during those periods (Ganzirri: 65.44–71.41% of POC; Faro: 51.43–52.09% of POC); however, high percentages of POC were also hydrolysed in both the lakes (66.66% and 34.34% in Ganzirri and Faro, respectively) during winter 2006, due to the low amount of POC available in the presence of still enhanced enzyme activity levels. Analysing the amounts of C potentially released by LAP+  $\beta$ -GLU activities (HYDR, hydrolysis) and incorporated into BB (UP, uptake), together with bacterial turnover rates (BTR), a different behaviour of Lake Ganzirri and Lake Faro was observed. In the studied environments, the bacterial C uptake exceeded the amount of monomeric C produced by hydrolysis, namely the C uptake: the C hydrolysis ratio was greater than 1 (Lake Ganzirri: spring 2007, autumn 2007, winter 2008, spring 2008; Lake Faro: spring 2007, autumn 2007, winter 2008), indicating that considerable amounts of dissolved monomers, other than those produced by enzymatic hydrolysis (*i.e.* exudates), flowed into BB. Coinciding with these periods of imbalance between HYDR and UP, lower BTR were observed. A marked reduction of BTR from 30–40 days to 2–3 days coupled with small changes in BB would suggest the development of active bacterial control mechanisms, such as viral lysis and/or grazing processes. Consequently, important amounts of bacterial DOC can flow to the surrounding environments, supplying, within the microbial loop, an additional source of biologically available carbon for bacterial cell production.

## Conclusion

This study provides evidence of the importance of a multidisciplinary approach to understand ecosystem microbial processes in the transitional ecosystems, underlying the active role of microbes and the significance of heterotrophic processes, such as decomposition and bacterial production, in carbon turnover. Integrated investigation of microbial activities together with particulate organic matter stoichiometry provided ecological information about the functional changes in the carbon cycle in the Cape Peloro ecosystem, which may be useful indices of the actual trophic conditions, as already asserted by Chrost & Siuda (2006). The determination of organic matter decomposition allowed quantification of the amounts of C potentially flowing through the microbial community, while the enzymatic assays led us to define to what extent the microbial community is actively involved and efficient in organic matter processing.

In particular, our study demonstrated that microbial production, enzymatic degradation, and oxidation of

organic matter in the studied area varied with seasons, as well as vibrios abundance.

Temporally coupled trends were observed for some parameters (enzyme activity, vibrios abundance, respiratory activity), while others (POC, PON, HBP) varied with a seasonal shift between the two lakes. Although the activity levels were generally lower in Lake Faro than in Lake Ganzirri, the similarities found in the enzyme patterns led us to suppose that microbial metabolism is driven by similar environmental forcing factors (such as temperature and trophic availability). Conversely, the seasonal shift found for some biotic parameters suggests that their response to environmental conditions may be modulated differently between the two lakes, which, despite their spatial proximity and reciprocal connection, do not always show contemporaneous functional processes; further, more detailed, studies are indicated.

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## ORIGINAL ARTICLE

# Comparison of benthic and pelagic suspension feeding in shallow water habitats of the Northeastern Baltic Sea

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## Keywords

Baltic Sea; *Dreissena polymorpha*; eutrophication; grazing; *Mytilus trossulus*; phytoplankton; suspension feeders; zooplankton.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

Combining field experiments with the biomass distribution data of dominant suspension feeders we compared the benthic and pelagic suspension feeding rates in shallow non-tidal brackish water coastal habitats. We found that pelagic grazing exceeded benthic grazing in almost all cases, on average from 14 to 4819 times depending on the site. Benthic grazing rates were related to site-specific environmental parameters and showed no relationship with water chlorophyll *a* (Chl *a*) content, whereas pelagic grazing rates varied both spatially and temporally and were related to water Chl *a* content. Our results indicate that in several shallow coastal habitats, pelagic suspension feeding substantially exceeds benthic suspension feeding. This suggests that pelagic recycling is higher than the amounts of energy redirected from pelagic to benthic food webs by benthic suspension feeders. These results increase our knowledge of the energy flows in coastal ecosystems.

## Problem

Suspension feeding mode is a highly important part of ecosystem processes in marine and limnic systems. Suspension-feeding organisms can be divided into two major groups: benthic and pelagic suspension feeders. The majority of benthic suspension feeders (BSF) are relatively long-lived organisms with low mobility, characterized by high spatial variability but temporal stability (Kotta *et al.* 2005). Conversely, most pelagic suspension feeders (PSF), being short-lived and able to quickly spread over long distances, are characterized by high temporal variability and spatial stability (Lundberg & Moberg 2003).

Although benthic and pelagic suspension feeders exploit the same food, they have quite different roles in food webs. Whereas PSF recycle a large share of the nutrients in the water column (Viitasalo *et al.* 1999), making them again quickly available to phytoplankton and facili-

tating phytoplankton growth (Jackson 1980), this is not always the case for BSF, which are often shown to redirect a large share of nutrients from the pelagic to the benthic system (Reusch *et al.* 1994; Norkko *et al.* 2001; Norling & Kautsky 2007). BSF have therefore been considered a natural eutrophication control, especially in turbid, nutrient-replete systems (Cloern 1982; Officer *et al.* 1982; Kautsky & Evans 1987). In the marine environment, pelagic food webs are believed to prevail in open-sea areas, whereas benthic–pelagic coupling is generally more important in coastal sea areas and estuaries due to the short vertical distance between benthic and pelagic systems in shallow areas (Kautsky & Evans 1987; Kotta *et al.* 2008). The Baltic Sea represents a large and diverse estuarine habitat and BSF populations are widespread throughout the coastal range due to the absence of predators in brackish-water conditions. Therefore, BSF are believed to have an important role in the energy flows of the Baltic





**Fig. 1.** The study area. Audrurand – eutrophic exposed, Kõiguste – non-eutrophic exposed, Kuressaare – eutrophic sheltered and Rame – non-eutrophic sheltered site.

Sea (Kautsky & Evans 1987; Kautsky 1995). Locally, dense populations of BSF are capable of clearing the overlying water column 20 times daily and BSF patchiness is considered to process about 90% of the phytoplankton stock in the coastal range of the Baltic Sea (Kotta & Møhlenberg 2002; Kotta *et al.* 2005). In such habitats PSF, namely mesozooplankton, are scarce and they are considered to have a marginal role in the energy flows (Kotta *et al.* 2008). PSF in marine and estuarine environments have seldom been studied as a functional group and their role in and impact on ecosystems are mainly speculative (Bushek & Allen 2005). A number of studies have been published separately on both benthic and pelagic grazing for a wide range of water-bodies. Despite the fact that these two feeding modes are exploiting the same food, comparative studies that experimentally evaluate benthic and pelagic grazing are rare and are almost absent for estuarine ecosystems. The subject is essential to improve our understanding about the roles that different functional groups play in coastal ecosystem functioning.

The aim of our study is to compare the grazing rates of benthic and pelagic suspension-feeding modes in a temperate non-tidal brackish coastal sea. We tested whether benthic grazing on phytoplankton prevails over pelagic grazing in different shallow water habitats and how local environmental conditions modify the grazing rates. Our experimental observations covered the main growing seasons of phytoplankton as well as small hydrodynamic, eutrophication and salinity gradients naturally occurring in the area. We assumed that (i) grazing by BSF is higher than grazing by PSF in shallow coastal areas

and (ii) different environmental variables modify the relative contribution of benthic and pelagic grazing.

### Study Area

The study area is a non-tidal, brackish-water ecosystem located in the Northeastern Baltic Sea (Fig. 1). We chose four study sites in the Gulf of Riga covering local gradients in exposure, eutrophication and salinity: Audrurand represents an exposed eutrophic site, Kuressaare a sheltered eutrophic site, Kõiguste an exposed mesotrophic site, and Rame a sheltered mesotrophic site. The sites have flat-bottom topography. A mixture of pebbles, sand and silt characterizes the studied sites with a higher proportion of silty sediments in eutrophic sites. All sites were located away from large riverine loads or upwelling areas. Salinity varied between 1.5 and 5, temperature was 12–28 °C, water velocity 0–20 cm·s<sup>-1</sup>, and water chlorophyll *a* (Chl *a*) content 3.6–56 mg·m<sup>-3</sup> (Table 1).

### Material and Methods

Benthic and pelagic grazing was experimentally evaluated at 1 m depth at each study site in spring, summer and autumn 2003. The two dominant benthic suspension feeders *Mytilus trossulus* Gould and *Dreissena polymorpha* (Pallas) were collected from the Audrurand and Kõiguste sites and allowed to adapt to the ambient conditions for 12 h before the start of the experiment. Three mussels were placed on a net with a mesh size of 1 cm. The mesh was glued on the top of a solid funnel (diameter 8 cm,

**Table 1.** Environmental factors, biomasses of different ecosystem elements and pelagic primary production by different sites and seasons.

Site/season	T	S	Velocity	Chl <i>a</i>	PP	MesoZP	MeroP	MicroZP	Mussels	
									<i>Mytilus</i>	<i>Dreissena</i>
Audrurand										
spring	14.7	3.8	10.1 ± 26.3	18 ± 1	0.45 ± 0.10	13 ± 10	2 ± 1	1411 ± 170	71 ± 112	14571 ± 8889
summer	28.1	4.2	0.0 ± 0.0	21 ± 16	*0.00	219 ± 201	28 ± 12	574 ± 120	107 ± 161	20000 ± 8346
autumn	14.3	4.6	2.7 ± 3.0	56 ± 16	0.80 ± 0.42	26 ± 22	501 ± 102	112 ± 98	107 ± 151	21786 ± 8415
Kuressaare										
spring	13.8	2.0	2.3 ± 2.3	21 ± 5	0.21 ± 0.18	39 ± 33	69 ± 34	2731 ± 73	54 ± 10	0
summer	22.7	1.5	4.3 ± 10.7	8 ± 0	0.19 ± 0.08	11 ± 7	0	3107 ± 65	71 ± 21	0
autumn	11.9	4.1	1.1 ± 0.7	44 ± 0	0.49 ± 0.30	13 ± 6	0	2317 ± 103	89 ± 14	0
Kõiguste										
spring	14.7	3.8	0.0 ± 0.0	4 ± 1	0.09 ± 0.06	5 ± 2	0	333 ± 153	3250 ± 1234	0
summer	25.6	4.5	6.7 ± 14.4	4 ± 1	0.09 ± 0.08	24 ± 21	0	899 ± 147	3964 ± 2499	0
autumn	13.2	4.7	20.4 ± 76.3	14 ± 9	0.25 ± 0.16	19 ± 17	0	88 ± 75	4286 ± 3865	0
Rame										
spring	14.5	2.5	2.9 ± 2.6	9 ± 1	0.13 ± 0.12	42 ± 33	0	2730 ± 104	3975 ± 3294	0
summer	27.2	4.9	1.0 ± 0.3	11 ± 6	0.44 ± 0.28	139 ± 100	3 ± 1	1323 ± 131	4500 ± 1898	0
autumn	12.8	4.7	0.0 ± 0.0	5 ± 1	0.12 ± 0.09	15 ± 11	0	1795 ± 125	5500 ± 3482	0

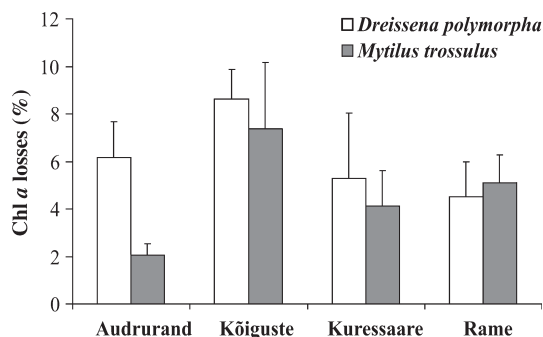
T = temperature (°C); S = salinity (psu); Velocity = water current speed (cm·s<sup>-1</sup>); Chl *a* = water chlorophyll *a* (mg·m<sup>-3</sup>); PP = phytoplankton primary production (mg Chl *a* h<sup>-1</sup>·m<sup>-3</sup>); MesoZP = mesozooplankton biomass (mg wet weight m<sup>-3</sup>); MeroP = meroplankton biomass (mg wet weight m<sup>-3</sup>); MicroZP = microzooplankton biomass (mg wet weight m<sup>-3</sup>); Mussels = biomass of *Mytilus trossulus* and *Dreissena polymorpha* (mg dry weight m<sup>-2</sup>).

'±' SE values; '\*' phytoplankton decomposition exceeded primary production values.

height 5 cm) allowing biodeposits to sediment into the collecting vial below. The unit was located on the sediment surface, and was similar in size to the dimensions of boulders in the study area (Kotta & Møhlenberg 2002). As the mussels were deployed on the top of the unit and there were no structures separating the mussels and the ambient water, it is likely that the bivalves were exposed to the same amount of phytoplankton as measured in the ambient water column. Each incubation lasted 4 h. In every season we performed at least five incubations with three concurrent replicates. Water temperature and salinity were monitored at the beginning and at the end of each experiment using CTD profiling. Salinity was measured using the Practical Salinity Scale. Current velocity was measured every second using a calibrated electromagnetic current recorder connected to a data logger (Compact-EM data recorder by Alec Electronics). After deployment, the shell lengths of mussels were recorded, and the sedimented material from the vials was sorted under a dissecting microscope. Faeces were collected with a pipette and filtered on Whatman CF/F filters within 4 h of retrieval. Filters were 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*. Pseudofaeces production was uncommon at studied sites and times. During deploy-

ment, water for Chl *a* measurement was sampled every 4 h by a diver at near-bottom layer, 25 cm from the cages. On each occasion, 2 liters of water was sampled three times and filtered on Whatman CF/F filters within 4 h of retrieval. Further processing of samples followed the procedures described above.

To estimate loss of Chl *a* in the digestive tract, separate experiments were carried out on land. Mussels were incubated in 5-liter buckets filled with natural seawater for 4 h. In each bucket, five mussels were used; buckets without experimental animals served as controls. At the end of incubation, biodeposits were collected from the buckets by careful pipetting and water samples for Chl *a* were taken. The content of Chl *a* and Pha *a* was estimated in biodeposits and water samples as described above. The loss of Chl *a* during gut passage was estimated as the ratio of the loss of Chl *a* in water to biodeposit production, taking into account algal growth and sedimentation in the control bucket. Functional relations between biodeposition and environmental variables were determined after correction for loss of Chl *a* during gut passage. The loss of Chl *a* during digestion by BSF did not exceed 20% and was usually estimated at less than 10% (Fig. 2). Grazing by individuals of different sizes ( $G_l$ ) was scaled by shell length, *i.e.*  $G_l = G_{20} \times l^2/20^2$ , where  $G_{20}$  is the grazing rate of 20 mm individuals and  $l$  the shell length (Kjørboe & Møhlenberg 1981). Biodeposition rate of mussels was expressed as the amount of Chl *a* and phaeopigments digested by a 20-mm



**Fig. 2.** Estimates of loss of Chl *a* in the digestive tract of bivalves at different sites.

bivalve in 1 h, adjusted for Chl *a* loss in the digestive tract (Lauringson *et al.* 2007).

Pelagic suspension feeding was evaluated in 15-liter plastic bags filled with seawater consisting of natural phytoplankton and zooplankton communities. Control bags were filled with water sieved through a 55- $\mu$ m net to remove most of the zooplankton and were kept in transparent (photosynthetic production experiments) or dark plastic bags (dark respiration experiments). Chl *a* samples were taken at the beginning of the experiment and after 2 and 5 h of deployment. Plastic bags were suspended in the upper 0.5 m of the water column and incubated simultaneously with benthic grazing experiments. At the end of the experiment all water was filtered through Whatman CF/F filters and processed in the same way as other water samples described above. Changes in Chl *a* over the experiment in the transparent control bag were used as a proxy of net photosynthetic production of phytoplankton. Changes in Chl *a* in the dark control bag gave us an estimate of the decomposition of phytoplankton. Pelagic grazing was assessed as the amount of Chl *a* removed in the transparent plastic bag containing zooplankton, adjusted for Chl *a* production and losses in the control experiment.

Filtration and extraction of water samples for Chl *a* measurement were carried out within 1 h after sampling. The samples were filtered onto Whatman GF/F filters. Chl *a* and Pha *a* were measured as noted above. In every site and season, additional water samples were taken to determine the species composition of phytoplankton. These samples were preserved in acid Lugol solution and counted under an inverted microscope. Wet weight of different species was calculated from cell geometry (HEL-COM 1988). We were not able to determine phytoplankton species composition in autumn samples due to the very high turbidity of these samples.

To determine the time-scale of the fluctuations in food abundance for suspension feeders, water Chl *a* content

was recorded every second using the calibrated chlorophyll/turbidity recorder connected to a data logger (Compact-CLW data recorder by Alec Electronics) at Kõiguste site during the ice-free seasons of 2003. The temporal patterns of phytoplankton are known to be similar in all four locations studied, although there are differences in the peak values due to their different eutrophication levels (Kotta *et al.* 2008).

During experiments the size structure and biomass of benthic grazers were quantified from the shoreline down to 3 m depth. Samples were collected by a diver at 0.1, 1, 2, and 3 m depth using 20  $\times$  20 cm frames placed randomly on the sea floor. Three replicates were taken at each depth. All mussels within the frame were collected and their length was measured to the nearest 0.1 mm using a Vernier caliper. Animals were dried with shells at 60  $^{\circ}$ C for 48 h and their dry weight (DW) determined.

Mesozooplankton was sampled by a 90- $\mu$ m net from the upper 1 m of the water column. Samples were preserved in 4% formaldehyde solution and counted subsequently under binocular microscope following the standardized procedure of HELCOM ([http://www.helcom.fi/groups/monas/CombineManual/PartC/en\\_GB/main/#c4](http://www.helcom.fi/groups/monas/CombineManual/PartC/en_GB/main/#c4), HELCOM 1988). Biomasses (wet weights) were calculated using the biomass factors for different taxonomic groups and developmental stages (Hernroth 1985). Microzooplankton samples were taken from the surface water and preserved in acid Lugol solution. Samples were concentrated before counting in 50-ml tube, animals were counted under an inverted microscope and their wet weights (WW) were calculated by formulas based on the volume of animals.

Community grazing rates were expressed as the average amount of Chl *a* removed from the water column above 1-m<sup>2</sup> bottom area in the depth range of 0.1–3 m. This range was chosen as the density of BSF quickly decreases at greater depths in the studied sites. The biodeposition rates of BSF were multiplied by the average biomasses at a site in the same season and these values were used as average estimates of benthic grazing in the area. For pelagic grazing, experimental results were extrapolated to the whole water column. Pelagic grazing was assumed to be homogeneous both vertically and horizontally due to an intense wind-induced mixing of the water column.

Grazing rates of benthic and pelagic suspension feeders at the studied sites were compared by factorial ANOVA and ANCOVA. Residual analysis was performed to test the assumptions for parametric tests. Relationships between grazing and environmental variables were tested by factorial ANOVA and linear multiple regression (StatSoft, Inc. 2007). Community data analyses were performed using the statistical program PRIMER version 6.1.5 (Clarke & Gorley 2006). The spatial and temporal

variability of species composition and biomass structure of pelagic suspension feeders was analysed by ANOSIM and contributions of different species to the variability were assessed by SIMPER (Clarke 1993).

## Results

Water salinity, temperature, velocity and Chl *a* content did not differ statistically among the studied sites and seasons (2-way ANOVA,  $P > 0.05$ ). The Chl *a* measurements with data logger at the Kõiguste site showed mostly stable Chl *a* concentrations until the autumn, when we observed a pronounced peak in September (Fig. 3). Phytoplankton communities were generally dominated by diatoms and dinoflagellates (Fig. 4). Benthic suspension feeders had significantly different species composition and biomasses at different sites, whereas seasonal variability was not important (2-way ANOVA, for site  $F_{3,41} = 6.52$ ,  $P = 0.001$ , for season  $P > 0.05$ ). The biomass of BSF was related to salinity, temperature and water Chl *a* content (multiple regression,  $R = 0.54$ ,  $F_{3,40} = 5.59$ ,  $P = 0.003$ ) but not to phytoplankton production ( $P > 0.05$ ). All sites were characterized by the marine bivalve *Mytilus trossulus* except Audrurand, where the brackish water bivalve *Dreissena polymorpha* prevailed over *M. trossulus*. The biomass of bivalves was about 10 times higher at Audrurand than at the other sites (Table 1). *Mytilus trossulus* had the highest biomasses at 3 m depth except for Kõiguste Bay, where the species had a maximum biomass at 2 m depth. The biomass of *D. polymorpha* had a clear maximum at 3 m depth (Fig. 5). The biomass of *M. trossulus* was positively related to water velocity and salinity and negatively related to water Chl *a* content (multiple regression,  $R = 0.63$ ,  $F_{3,40} = 8.53$ ,  $P < 0.001$ ). The biomass of *D. polymorpha* was positively related to both water temperature and Chl *a* content (multiple regression,  $R = 0.55$ ,  $F_{2,41} = 8.90$ ,  $P < 0.001$ ). Microzooplankton bio-

mass was higher at the sheltered Rame and Kuressaare sites (ANOVA,  $F_{3,20} = 36.4$ ,  $P < 0.0001$ ), while total mesozooplankton biomass was higher at the Audrurand site (ANOVA,  $F_{3,23} = 7.16$ ,  $P < 0.001$ ) (Table 1, Fig. 6). The biomass of microzooplankton was negatively related to water salinity (regression analysis,  $R = 0.54$ ,  $F_{1,22} = 9.14$ ,  $P = 0.006$ ). The biomass of meroplankton was positively related to water Chl *a* content (regression analysis,  $R = 0.75$ ,  $F_{4,22} = 7.22$ ,  $P < 0.001$ ) and the biomass of other mesozooplankton was positively related to water temperature and salinity (multiple regression,  $R = 0.81$ ,  $F_{4,22} = 10.24$ ,  $P < 0.0001$ ). The species composition of microzooplankton was similar at all studied sites but differed significantly between different seasons (ANOSIM,  $r_{\text{spring/summer}} = 0.89$ ,  $P = 0.029$ ,  $r_{\text{spring/autumn}} = 0.72$ ,

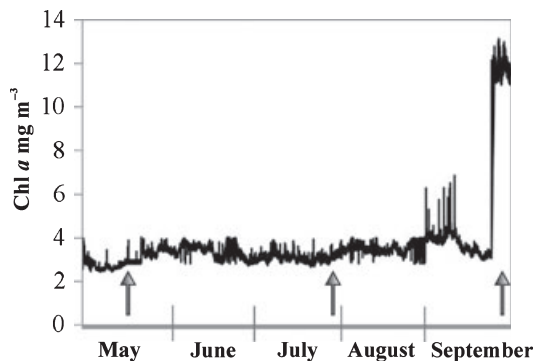


Fig. 3. Logger recordings for water Chl *a* content at the Kõiguste site in 2003. Arrows indicate the dates of experiments.

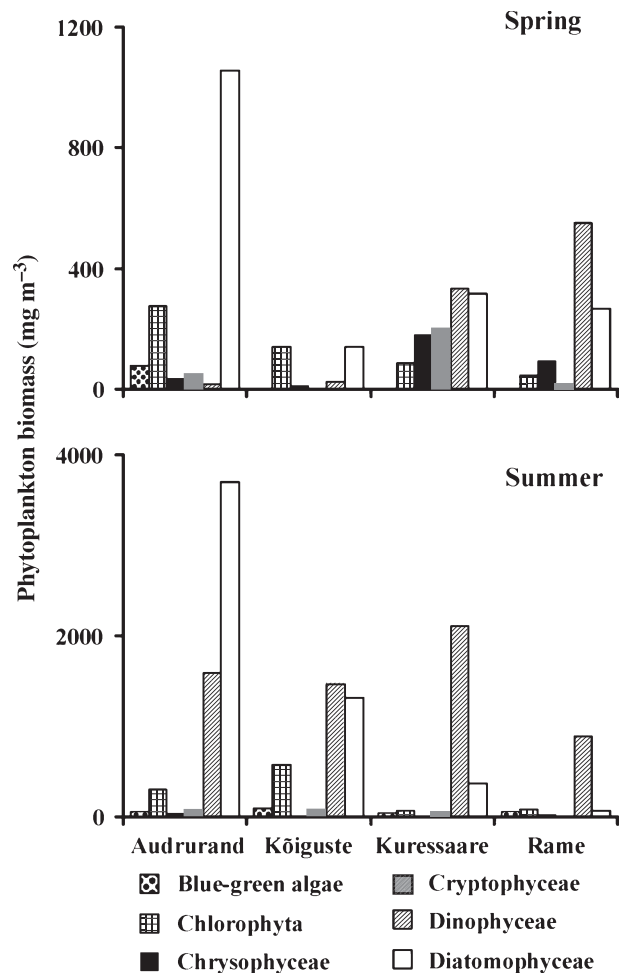


Fig. 4. Biomasses (wet weights) of phytoplankton taxa at study sites at the time of experiments. Autumn values are excluded due to methodological difficulties in processing (highly turbid samples). Audrurand – eutrophic exposed, Kõiguste – non-eutrophic exposed, Kuressaare – eutrophic sheltered and Rame – non-eutrophic sheltered site.

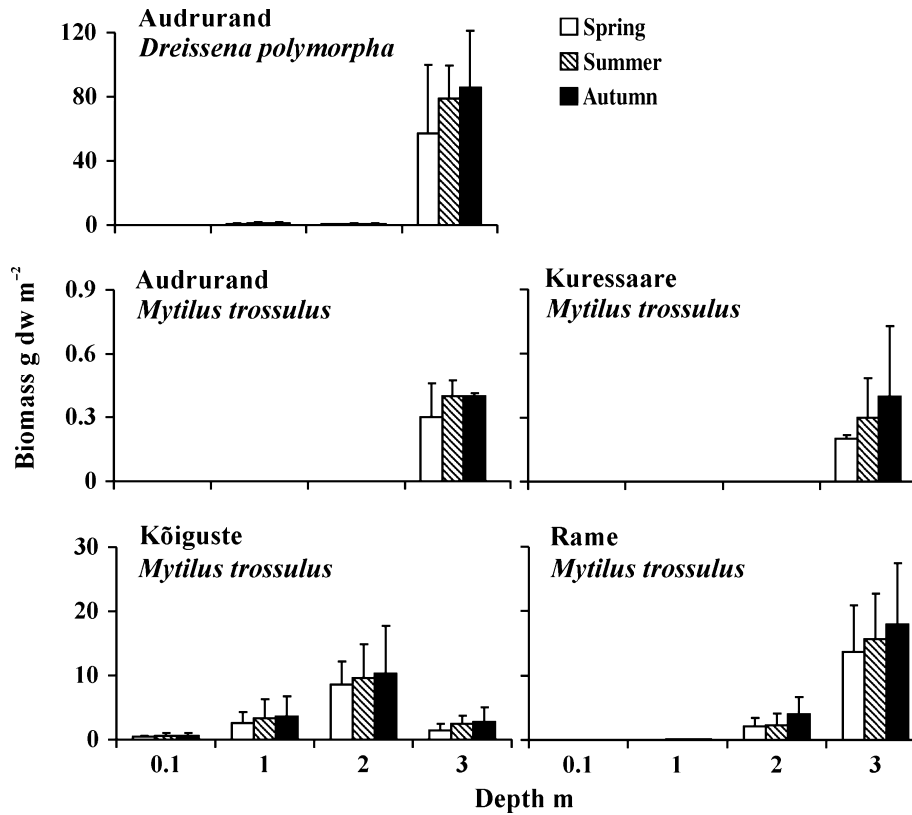


Fig. 5. Biomasses (dry weights with shells) of benthic suspension feeder species at different depths and seasons. Vertical bars indicate SE values. Audrurand – eutrophic exposed, Kõiguste – non-eutrophic exposed, Kuressaare – eutrophic sheltered and Rame – non-eutrophic sheltered site.

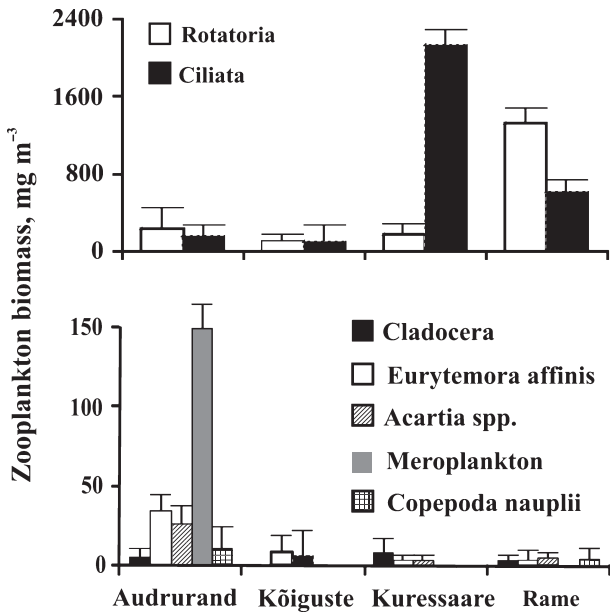


Fig. 6. Biomasses (wet weights) of meso- and microzooplankton taxa at study sites. Vertical bars indicate SE values. Audrurand – eutrophic exposed, Kõiguste – non-eutrophic exposed, Kuressaare – eutrophic sheltered and Rame – non-eutrophic sheltered site.

$P = 0.008$  and  $r_{\text{summer/autumn}} = 0.58$ ,  $P = 0.003$ ). *Notholca* sp., *Strobilidium* sp. and *Vorticella* sp. were the most typical species in spring, *Keratella quadrata* (Müller), *Strobilidium* sp., *Synchaeta baltica* Ehrenberg and *Keratella cochlearis* (Gosse) in summer and *Strobilidium* sp. and *Strombidium* sp. in autumn (PRIMER, SIMPER, contribution of a single species to the similarity of sample group >10%). The species composition of mesozooplankton was similar among study sites and seasons (PRIMER, ANOSIM,  $P > 0.05$ ).

Pelagic suspension feeders had significantly higher grazing rates than benthic suspension feeders and both site and season affected the grazing rates (ANOVA, Table 2, Fig. 7). Pelagic grazing exceeded benthic grazing  $13.7 \pm 8.4$  times at Audrurand,  $39.8 \pm 17.3$  times at Kõiguste,  $56.2 \pm 22.7$  times at Rame and  $4818 \pm 1617$  times at Kuressaare. Differences in water Chl *a* content and, to a lesser extent, in phytoplankton production significantly explained the observed effects of site and season (ANCOVA, Table 2).

Benthic suspension feeder *D. polymorpha* had significantly higher grazing rates than *M. trossulus* at Audrurand where two mussel species co-existed (2-way ANOVA with



**Table 2.** Differences in grazing rates between benthic and pelagic suspension feeders. Grazing rate – the amount of Chl *a* removed from the water column from 0.1–3 m depth  $\text{h}^{-1}$ .

	df	F	P
ANOVA			
Site	3	54.19	<0.0001
Season	2	42.52	<0.0001
Suspension feeder group	1	293.26	<0.0001
Site $\times$ season	6	42.87	<0.0001
Site $\times$ suspension feeder group	3	46.67	<0.0001
Season $\times$ suspension feeder group	2	49.68	<0.0001
Site $\times$ season $\times$ suspension feeder group	6	40.68	<0.0001
ANCOVA			
Temperature			ns
Salinity			ns
Water velocity			ns
Water Chl <i>a</i>	1	36.48	<0.0001
Phytoplankton production	1	9.29	0.003
Suspension feeder group	1	25.84	<0.0001
df error for ANOVA	65		
df error for ANCOVA	73		

season as an additional factor  $F_{1,12} = 8.44$ ,  $P = 0.01$ ). Different grazing rates resulted from different population sizes of these species at Audrurand. Biodeposition rates for 20-mm individual mussels were similar for both species (ANOVA,  $P > 0.05$ ).

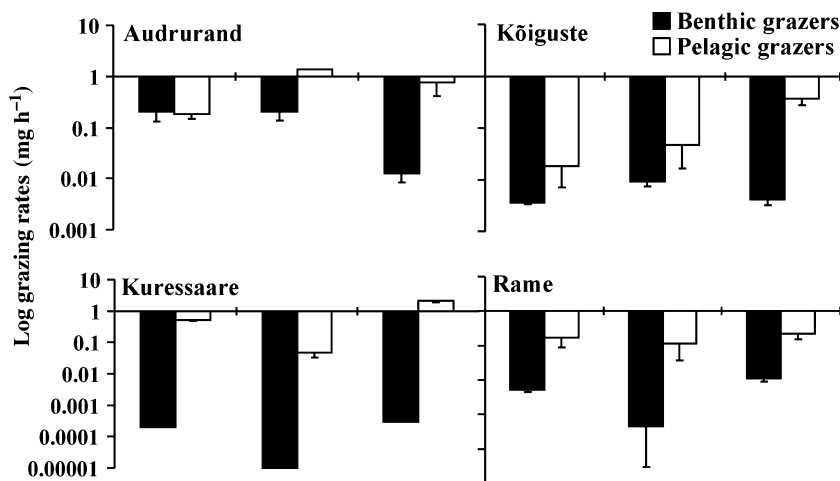
When suspension feeder groups were analysed separately, it appeared that for benthic suspension feeders, only site modulated the grazing rates (2-way ANOVA,  $F_{3,32} \text{ site} = 12.68$ ,  $P < 0.0001$ ). The grazing rate of BSF was not related to any of the measured environmental

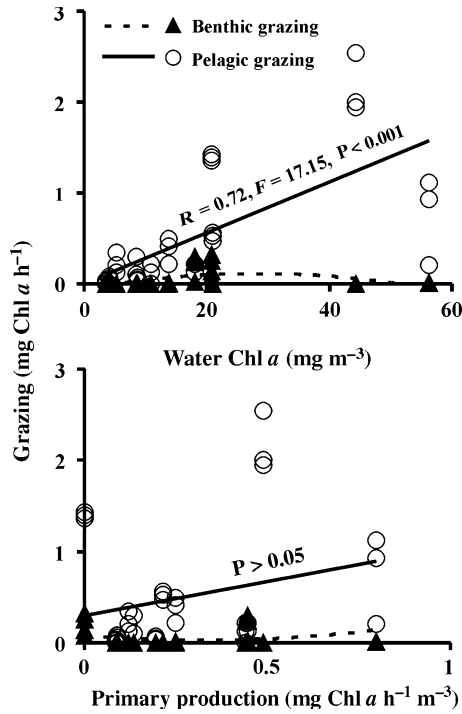
factors (multiple regression,  $P > 0.05$ , Fig. 8); however, there was a high scatter between sites (Fig. 9). For pelagic suspension feeders, site, season and their interactions affected the grazing rates (2-way ANOVA,  $F_{3,24} \text{ site} = 42.76$ ,  $F_{2,24} \text{ season} = 41.28$  and  $F_{6,24} \text{ site} \times \text{season} = 35.16$ ,  $P < 0.0001$ ). When all sites were pooled together, the grazing rate of pelagic suspension feeders was positively related to water Chl *a* content but not to phytoplankton production (regression analysis,  $R = 0.72$ ,  $F_{2,33} = 17.15$ ,  $P < 0.001$ , Fig. 8).

## Discussion

The results show that benthic suspension feeders removed much smaller amounts of phytoplankton biomass than pelagic suspension feeders did in our study area. Several studies have indicated the high potential importance of benthic suspension feeding worldwide (e.g. Cloern 1982; MacIsaac *et al.* 1992) and likewise in the archipelago areas of the Northern Baltic Sea spatially close to our study area (Kautsky & Evans 1987; Kotta *et al.* 2005). Restricted food availability due to the benthic concentration boundary layer has been generally shown to limit the feeding of BSF (Fréchette *et al.* 1989; O'Riordan *et al.* 1995; Ackerman *et al.* 2001). Steep slopes can induce turbulent mixing in bottom boundary layer (Slinn & Riley 1996), reducing near-bottom food limitation for BSF. Indeed, it has been shown that at steeper slopes, there is no food limitation for the benthic communities (Kotta *et al.* 2007). The steep slopes characteristic of the rocky habitats of the Northern Baltic archipelago areas may therefore account for the capacity of these areas to host noticeably larger biomasses of benthic suspension feeders than those recorded in our study area. BSF-driven energy fluxes also play an important role in large river mouths in various

**Fig. 7.** Grazing by benthic and pelagic suspension feeders at studied sites and times. Grazing is estimated as the average amount of Chl *a* removed from the water column above 1-m<sup>2</sup> bottom area in the depth range 0.1–3 m. Vertical bars indicate SE values. Audrurand – eutrophic exposed, Kõiguste – non-eutrophic exposed, Kuressaare – eutrophic sheltered and Rame – non-eutrophic sheltered site.

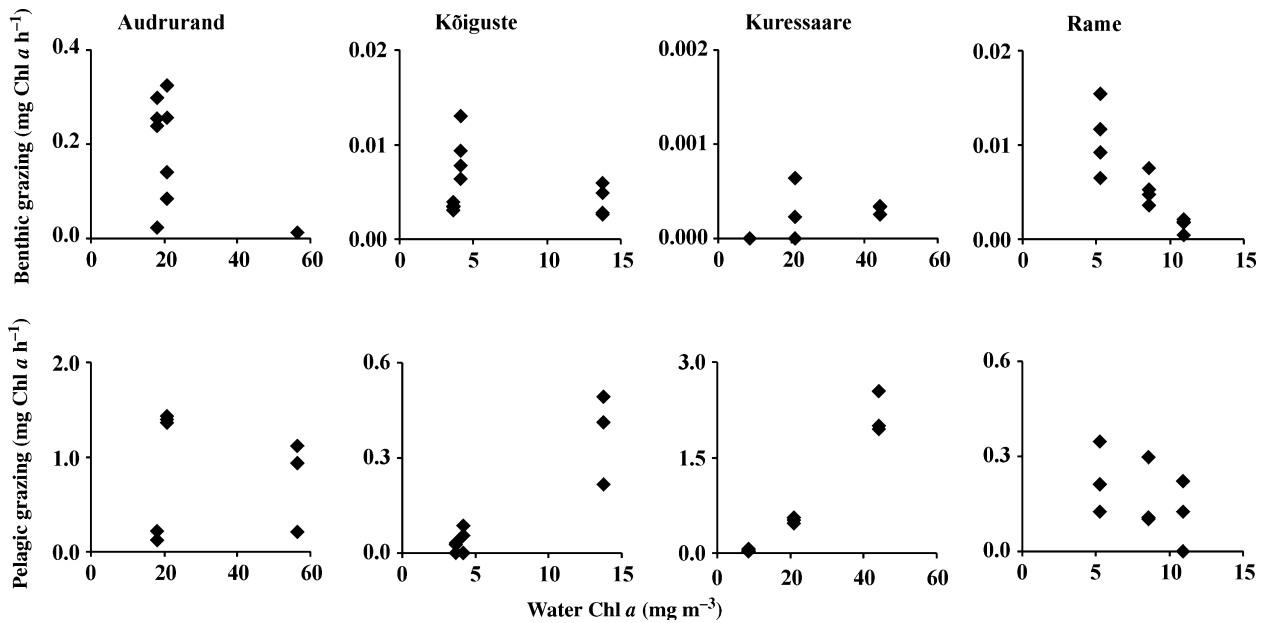




**Fig. 8.** Benthic and pelagic grazing rates related to water Chl *a* content and phytoplankton primary production in the study area. Grazing is estimated as the average amount of Chl *a* removed from the water column above 1-m<sup>2</sup> bottom area in the depth range of 0.1–3 m.

parts of the Baltic Sea where low salinity allows *Dreissena polymorpha* to establish (Orlova *et al.* 2004; Kotta *et al.* 2005; Daunys *et al.* 2006) and constant nutrient inflow helps to sustain much higher biomasses of mussels than in adjacent sea areas (Kotta *et al.* 2008). In contrast to these observations, our results indicate that in flat coastal habitats away from large rivers and upwelling areas, benthic suspension feeders play only a minor role in food-web dynamics, and pelagic processes strongly dominate over benthic–pelagic coupling. Such habitats are widespread and typical for the Northeastern Baltic Sea and likewise in coastal areas of South Africa, Arctic, and Antarctic seas, to name just a few (Gutt 2001; Bownes & McQuaid 2006; Kotta *et al.* 2008).

We found that site location was the best predictor of the grazing rates of the BSF, while season and water Chl *a* content were the best predictors for pelagic grazing rates. Site-specific characteristics can influence the balance between pelagic and benthic grazing. Exposure is connected to long-term patterns of water velocity. Benthic grazers are often faced with a limited food supply (Wildish & Kristmanson 1984), which can be extremely harsh at sheltered sites. BSF are also sensitive to oxygen deficiency, which may become crucial in very sheltered sites (Riisgård & Poulsen 1981; Møller & Riisgård 2007). Westerbom & Jattu (2006) hypothesized that higher sedimentation may restrict recruitment of BSF at less exposed



**Fig. 9.** The site-specific scatter of benthic and pelagic grazing related to water Chl *a* content. Grazing is estimated as the average amount of Chl *a* removed from the water column above 1-m<sup>2</sup> bottom area in the depth range of 0.1–3 m. Audrurand – eutrophic exposed, Kõiguste – non-eutrophic exposed, Kuressaare – eutrophic sheltered and Rame – non-eutrophic sheltered site.

localities, which is reflected in population sizes. On the other hand, high water velocities at the exposed Audru-rand site suspend lots of clay particles in the water column that may inhibit pelagic grazers (Levine *et al.* 2005). The different relationships between water Chl *a* content and benthic and pelagic grazing likely indicate the ability of pelagic grazers to adjust their grazing rates to the dynamics of phytoplankton. PSF have been shown to increase their abundance quickly in response to high food levels (e.g. Martin 1970; Banse 1994). BSF, on the other hand, are not capable of following short-term dynamics of phytoplankton as they can only modulate their feeding activity (Fréchette & Bourget 1987) but cannot effectively multiply or reduce their abundance and biomass in response to phytoplankton fluctuations. Conversely, very high phytoplankton concentrations have been shown to inhibit mussel feeding (Clausen & Riisgård 1996) and may induce oxygen depletion in near-bottom water (Cloern 2001). Our results may hint that complex environmental variables such as eutrophication and exposure which describe systems at longer time scales are appropriate descriptors of benthic grazing, whereas single environmental variables that reflect processes at short time scales can effectively describe the dynamics of pelagic grazing. On the other hand, the biomass and grazing rate of pelagic grazers showed both high temporal and spatial variability in our study. However, as benthic and pelagic grazing are concurrent processes, ecosystem functioning traits such as the prevalence of benthic–pelagic or pelagic–pelagic links in the system may depend on the values of both integrated and single environmental parameters affecting either the benthic or the pelagic component of the system. To estimate benthic grazing rates in coastal sea areas, a low frequency high spatial resolution sampling regime seems to be appropriate, while for pelagic grazing rates both high time and space resolutions may be necessary. To follow the long-term changes in benthic–pelagic coupling the temporal resolution required for the mapping of benthic grazing may be even less than seasonal resolution in our study area. Data from *in situ* loggers (see Material and Methods section) indicated a stochastic, presumably wind-induced weekly or monthly variance in water Chl *a* content. Pelagic grazing was strongly related to water Chl *a* content in the present study, therefore the observations on pelagic grazing should match the scale of variability in Chl *a* concentration, *i.e.* they should be performed on weekly basis.

Usually, studies drawing system-wide conclusions have been focused on either benthic or pelagic suspension feeders, and the role of one group is investigated separately, paying no special attention to the potential role of the other group. Among the few exceptions are the Great Lakes where the recent invasions of *D. polymorpha* and

*Dreissena bugensis* resulted in questions about the potential re-arrangement of pelagic food webs due to an addition of an almost completely new function to the ecosystem. This stimulated a small cascade of studies addressing the potential food competition between zooplankton and dreissenids in some lakes (Bridgeman *et al.* 1995; Horgan & Mills 1999; Noonburg *et al.* 2003; Conroy *et al.* 2005). However, the conclusions from these studies have been controversial for different and even the same localities (Wu & Culver 1991; MacIsaac *et al.* 1992) and the actual role of Dreissenids in ecosystem functioning remains under debate.

While the above-mentioned studies concentrated on ecosystems in non-equilibrium state, the problem remains similarly unresolved for more mature communities. For example, BSF, light limitation, tidal mixing, and low residence time are all considered possible causes of low phytoplankton biomass in San Francisco Bay (Cloern 2001). In the majority of food-web studies, food consumption pathways are addressed rather than competitive or more complicated interactions (Paine 1994). Indeed, BSF are able to consume their pelagic competitors in addition to other seston (MacIsaac *et al.* 1991; Wong *et al.* 2003; Prins & Escaravage 2005), but the scope of other interactions between these feeding modes seems equally relevant in ecosystem functioning. The majority of research focusing on benthic–pelagic coupling tends to deal with a simplification considering solely the benthic component and its pelagic prey extracted from the real system consisting of a much more complex web of interactions. We might consider that benthic and pelagic suspension feeders are actually consuming the same stock of food at the same time and phytoplankton of shallow water bodies has to deal simultaneously with both benthic and pelagic grazing pressures.

The variability in ecosystem functioning pathways is highly dependent on the energy flow characteristics of the system. Møller & Riisgård (2007) found that BSF were indirectly influenced by predation on pelagic grazers in an eutrophied Danish fjord as algal blooms induced oxygen deficiency in near-bottom water that severely depressed BSF. A study on another trophic cascade reveals traits in the opposite direction in San Francisco Bay, which has been characterized by substantial BSF-mediated energy flows and where increased predation on benthic grazers caused enhanced phytoplankton blooms (Cloern *et al.* 2007). Our results indicate a bigger share of energy flows through PSF, and therefore trophic interactions generating stronger effects may likely operate through pelagic food-webs in our study area.

As BSF recycle nutrients from phytoplankton and facilitate benthic vegetation (Reusch *et al.* 1994; Kotta *et al.* 2006), they are assumed to drive an ecosystem from the

dominance of pelagic to benthic primary production. In such cases, a sustained population of mussels countervailing an abundant phytoplankton stock seems to be an unstable state of an ecosystem. Indeed, a few monitored episodes of an introduction of benthic grazers have shown to result in dramatic and abrupt shifts of ecosystems to a new “steady” state. There is some recent evidence for such unbalancing effects of benthic suspension feeders from a Danish estuary (Petersen *et al.* 2008), San Francisco Bay (Alpine & Cloern 1992) and the Great Lakes (Roberts 1990). Nevertheless, most such examples represent quite “new” equilibria, which seem to be stable in given conditions and chosen time scale but may well prove to be highly dynamic at longer time scales. Our study area represents a typical coastal area of the North-eastern Baltic Sea with low water velocities compared to tidal seas, diffuse nutrient load, lack of upwelling, or nutrient loads from point sources. No shifts in the distribution of BSF have recently taken place (Kotta *et al.* 2008) and the area can probably be characterized as an ecosystem in a stable state (Scheffer *et al.* 2001). *Dreissena polymorpha*, although an invasive species, has already a history of invasion for at least 150 years in the study area (see references in Lauringson *et al.* 2007). In our study area the populations of BSF were dense enough to periodically consume phytoplankton equal to PSF only at an eutrophic exposed site with high water velocities constantly supplying near-bottom layers with food-rich water. It must be noted, however, that the distribution of BSF was spatially limited and BSF showed low feeding rates at high Chl *a* concentrations. Therefore BSF were unlikely to influence phytoplankton dynamics in a system-wide scale or cause any shifts in ecosystem functioning.

The issue of benthic control over pelagic system in estuarine and freshwater habitats has been discussed quite often in recent decades and the probability of such a control has usually been assumed to be high (Cloern 1982; Officer *et al.* 1982; Reeders & Bij de Vaate 1990; Alpine & Cloern 1992; Prins & Escaravage 2005). Although our study is not focussed on the control issues but rather discusses the flows of energy in the coastal ecosystem, the results still suggest factors that may be important in controlling phytoplankton abundance. Phytoplankton is a temporally highly variable food source and therefore challenges its consumers to overcome big fluctuations in food supply. Pelagic suspension feeders have evolved a good strategy to gain maximum profit from that food source, both temporally by mimicking the ephemeral life strategy of their prey and spatially by inhabiting the same habitat. Benthic suspension feeders, on the other hand, are faced with certain difficulties in both spatial and temporal terms. Removing large quantities of phytoplankton would demand (i) access to the whole water column and (ii) surviving the clear-water phases between

phytoplankton blooms with high biomasses to benefit from the blooming phase of phytoplankton. There are clear Chl *a* gradients measured above mussel beds, indicating possible food limitation due to Chl *a* depletion in the near-bottom water layer (Fréchette *et al.* 1989; Dolmer 2000; Ackerman *et al.* 2001) as the water column is not stirred up evenly. Therefore mussels commonly experience suboptimal food conditions in the field and cannot use their maximum filtration potential to remove phytoplankton from the whole water column. Unfortunately, the majority of studies on the grazing potential of benthic suspension feeders have been performed in the laboratory. As the actual grazing potential in the field depends on the availability and refiltration rates of food-rich water, extrapolation from laboratory studies can easily lead to an overestimation of the real consumption. Bigger size also gives BSF an advantage over PSF as it enables BSF to survive clear-water phases and start feeding immediately at the beginning of the bloom. However, the threshold of starvation may depend on the species characteristics, the condition of individuals before the clear-water phase, and the ability of individuals to use additional food sources besides phytoplankton. Several authors have shown that benthic suspension feeders can utilize benthic food sources suspended by waves or tidal currents (Machas *et al.* 2003; Garton *et al.* 2005; Sara 2006). There is a possible scenario for BSF population to use benthic microalgae and detritus during clear-water phases and therefore to be able to maintain sufficient biomass necessary to profit from peak phytoplankton productions. However, in cases of excess food supply, benthic suspension feeders have been shown to reduce their filtration rates (Clausen & Riisgård 1996) and there is a rising evidence that mussels are unable to fully utilize phytoplankton blooms (Kotta & Møhlenberg 2002; Caraco *et al.* 2006). In our study, mussels reached the highest biomasses in an exposed eutrophic area, indicating that food supply was the limiting factor for mussel distribution. However, grazing by the mussel population did not increase with Chl *a* levels as did pelagic grazing, and was low at high Chl *a* values. We suggest that the periods of low phytoplankton availability limited the grazing potential of the mussel population even at the exposed eutrophic area. It is reasonable to argue that the density of mussel populations at our study sites reflects the optima to survive clear-water phases rather than to utilize fully the peak phases of phytoplankton.

## Conclusions

The populations of BSF grazed considerably less phytoplankton than the populations of PSF in the flat coastal habitats of the Northeastern Baltic Sea. The grazing rates of BSF were related to site-specific environmental parameters and showed no relationship with water Chl *a*

content, whereas pelagic grazing rates showed both spatial and temporal variability and were related to water Chl *a* content. Our results may indicate that in stable-state non-tidal coastal habitats with no steep bottom slopes or direct riverine inputs, BSF-driven energy flows play only a minor role in food-web dynamics compared to pelagic processes.

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## ORIGINAL ARTICLE

# Effects of the suspension feeding mussel *Mytilus trossulus* on a brackish water macroalgal and associated invertebrate community

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## Keywords

Baltic Sea; benthic pelagic coupling; biotic interactions; mutualistic.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

The importance of suspension-feeding mussels is particularly apparent in benthic communities; however, the role of this feeding strategy on the development of macroalgal and associated invertebrate communities is in general poorly known. The effect of suspension-feeding mussels *Mytilus trossulus* on benthic communities was studied in an *in situ* factorial field experiment in the Northern Baltic Sea over one ice-free season. The experiment was performed under different regimes of wave exposure (low and moderate) and on different sedimentary habitats (soft bottom with high organic content, soft bottom with low organic content, and hard bottom). In general the presence of mussels was associated with increased biomass of filamentous algae, herbivores and deposit feeders and decreased biomass of charophytes. The effect of *M. trossulus* interacted with the effect of exposure and substrate. Stronger responses were observed in moderately exposed than in sheltered areas. The presence of *M. trossulus* affected charophytes and deposit feeders on sand with low content of organic matter and filamentous algae on pebbles but not on other substrate types. The magnitude of the effects varied between months. The results suggest that (i) even in dynamic coastal systems the biodeposits and excretions of mussels are at least partly assimilated locally and are not flushed away to the open sea, (ii) the accumulation of faecal material induced elevated growth of deposit feeders, (iii) mussels enhanced the growth of ephemeral macroalgae and reduced the growth of perennial macroalgae, and (iv) together with increasing benthic primary production, mussels indirectly increase the production of herbivores.

## Problem

Suspension-feeding mussels link water column productivity to the benthos by removing pelagic organisms, increasing rates of particle deposition and promoting nutrient recycling (Cloern 1982; Kotta & Møhlenberg 2002; Lauringson *et al.* 2007). Part of the deposited faeces and pseudofaeces are consumed by deposit feeders (Zhou *et al.* 2006) and the remaining part decomposes, thereby

increasing the availability of inorganic nutrients that may be channelled into benthic and pelagic production (Reusch *et al.* 1994; Marinelli & Williams 2003; Giles & Pilditch 2006). Besides notable biodeposition, bivalves excrete ammonia and thus may directly influence nutrient levels in seawater (Dame *et al.* 1991; Bracken 2004; Bracken & Nielsen 2004). Despite the importance of studies quantifying the effects of suspension feeders on macroalgal and associated invertebrate communities, such studies are

scarce (Reusch *et al.* 1994; Peterson & Heck 1999; Cummings *et al.* 2001) and often based on modelling approaches (e.g. Fulton *et al.* 2004; Dowd 2005).

It is generally known that increased sedimentation of organic matter leads to an increase in benthic algal production (Nilsson *et al.* 1991). It has been experimentally demonstrated that benthic algae take advantage of sediment nutrients through diagenesis (Cercó & Seitzinger 1997) as the algae can readily assimilate nutrients diffusing across the sediment surface (Asmus 1986). Consequently, macroalgae inhabiting sediments that are rich in organic matter can take advantage of remineralization and use porewater nutrients in periods of low nutrient levels in seawater. Thus, the growth of macroalgae is expected to be higher on sediments rich in organic matter than on sediments poor in organic matter. The presence of benthic suspension feeders may alter this relationship, as they are expected to reduce this nutrient limitation through considerable excretion of ammonia and biodeposition of organic matter and thereby increase algal growth.

The rate of biodeposition is dependent upon mussel density and availability of food (e.g. phytoplankton; Dame & Dankers 1988). The availability of food in turn depends on the concentration of phytoplankton and exchange of water. It is known that mussels can deplete the overlying water of algae (Fréchette *et al.* 1989; Peterson & Black 1991; Muschenheim & Newell 1992) and thus, food limitation may occur. Under food limitation, rising flow velocity (exposure) to a certain threshold value increases food supply and biodeposition rates. The added biodeposits, if not flushed away, may support the development of macrophytes and associated invertebrates. However, if mussels are not food limited then the mutualistic effects of mussels on adjacent biota are expected to be stronger in sheltered areas where the transport of biodeposits to deeper sea is lower than in exposed areas.

To date, strong links between suspension feeders, deposit feeders and macrophytes have been demonstrated in dense mussel beds, for example in the context of mussel farms and harvesting (Valentine & Heck 1993; Peterson & Heck 1999; Bologna & Heck 2002; Crawford *et al.* 2003; Airoidi *et al.* 2005). There exist a few *in situ* studies on evenly dispersed and less dense populations of suspension feeders (Norkko *et al.* 2001; Kotta *et al.* 2005), although such communities are widespread and typical of the coastal sea of South Africa, Baltic, Arctic and Antarctic Seas, to name just a few (Gutt 2001; Bownes & McQuaid 2006; Kotta *et al.* 2008). It is expected that the response of adjacent biota to the presence of suspension feeders is context-dependent. At high mussel densities notable biodeposition may deplete oxygen and result in hypoxic conditions, dominance of ephemeral algae and

opportunistic invertebrates. At low densities, however, the addition of mussels through a moderate fertilizing effect may foster the diversity of macrophyte assemblages and associated invertebrates (Bracken & Nielsen 2004).

In this study we experimentally evaluated the effect of the suspension-feeding mussel *Mytilus trossulus* Gould on macroalgal and associated invertebrate communities that occur in sediments differing in organic content and presence of rock and at different exposure regimes in the Northern Baltic Sea during one ice-free season. Our hypotheses were as follows:

- 1 Mussels enhance the growth of macrovegetation and increase the biomass of herbivores and deposit-feeders.
- 2 Increasing the degree of exposure may either amplify or weaken the effects.
- 3 The effects are stronger on organisms that occur in sediments poor in organic matter than on sediments rich in organic matter.

### Study Area

The effects of the suspension feeder *Mytilus trossulus* on macroalgal communities and associated invertebrates were experimentally studied in Kõiguste Bay (58°22.10' N 22°58.69' E) in the Northern Baltic Sea during June–September 2004. The study area receives fresh water from a huge drainage area and therefore has reduced salinity of 5.0–6.5 psu. In general, the bottom relief of the area is quite flat, sloping gently towards deeper areas. The prevailing substrate types of the bay are sandy clay mixed with pebbles, gravel or boulders. The bottom depths of Kõiguste Bay are typically between 1 and 4 m. The area is moderately eutrophied (Kotta *et al.* 2008). The benthic vegetation is well developed and extensive proliferation of ephemeral macroalgae has been reported in the area in recent years. At the later stages of annual succession the drift algal mats may form in shallow areas and these mats provide macroalgae and invertebrates additional nutrient source (Lauringson & Kotta 2006). *Mytilus trossulus* dominates among suspension feeders with abundances and biomasses at 500 ind·m<sup>-2</sup> and 40 g·dw·m<sup>-2</sup>, respectively. The species inhabits hard substrata, but after establishing byssal connections among mussels, individuals may also form dense clumps in sedimentary habitats. As a result of the low salinity, mussel growth is reduced compared to fully marine areas and mussel length therefore rarely exceeds 2 cm (Kotta & Møhlenberg 2002).

### Material and Methods

The experiment ran for 118 days from 3 June to 28 September 2004. The following factors and levels were included in the experimental design: exposure (sheltered

or moderately exposed site), mussels (presence or absence of *Mytilus trossulus*) and substrate (soft bottom with high organic content, soft bottom with low organic content, hard bottom). Each treatment was replicated three times. Experimental design included 144 plastic buckets (11 cm diameter, 10 cm deep) that were placed on the sea floor with the tops open.

Sand was extracted from nearby sea floor at eutrophied and clean sites representing soft bottom with high and low organic content, respectively. Prior to the experiment the sediment was sieved through a 1-mm mesh and dried in the sun for several days to ensure that it was free of living benthic invertebrates. To obtain similar starting conditions, sediment was homogenized before use. After homogenization, soft bottom with high and low organic content contained 0.025 and 0.008 % of organic matter, respectively. The buckets were filled up with sand and were seeded with three 10-cm-long shoots of *Chara aspera* Willdenow cut from plants shortly after sampling. This charophyte species is a dominant member of the phytobenthos in the shallower parts of the study area. Other buckets were filled with pebbles (average diameter 7 cm) that were large enough to be retained in the bucket. The pebbles were collected from the seashore and were devoid of vegetation.

*Mytilus trossulus* were collected from Kõiguste Bay. The experimental individuals were similar in size at the beginning of the experiment. The average length of *M. trossulus* deployed in the experiment was 15 mm. Three individuals of *M. trossulus* were added to each bucket corresponding to a density of 315 ind·m<sup>-2</sup> which is comparable to natural densities at Kõiguste Bay (Lauringson & Kotta 2006; Kotta *et al.* 2008).

The buckets were placed on the sea floor at 1.5 m depth. Half of the buckets were placed in the sheltered and another half in the moderately exposed part of the bay. Simplified Wave Model method was used to calculate the wave exposure for mean wind conditions represented by the 10-year period between 1 January 1997 and 31 December 2006 (Isæus 2004). A nested-grids technique was used to ensure long-distance effects on the local wave exposure regime, and the resulting grids had a resolution of 25 m. The borderline between low and moderate exposure was set at 100,000. At both sites current velocity was measured at 1-s intervals using a calibrated electromagnetic current recorder connected to a data logger (Compact-EM data recorder by Alec Electronics). This instrument also records data on water temperature. The flows were significantly different between exposed and sheltered areas ( $3 \pm \text{SE } 2 \text{ cm}\cdot\text{s}^{-1}$  in the sheltered area and  $11 \pm \text{SE } 3 \text{ cm}\cdot\text{s}^{-1}$  in the moderately exposed area). Temperature did not vary between the two sites and was estimated at 15 °C in June and September and 20 °C in July and August.

The buckets were sampled monthly from June to September. All bucket contents were sieved through a 0.25-mm mesh and placed into plastic bags and frozen prior to laboratory analysis. In the laboratory all samples were sorted under a binocular microscope (20–40× magnification). All individuals were identified to the species level except for oligochaetes, chironomids, and juveniles of gammarid amphipods. Individuals of all taxa were weighed. Prior to weighing, animals and plants were dried at 60 °C for 2 weeks.

The effects of mussels, exposure and substrate on the biomass of seeded and recolonized macroalgal and invertebrate groups were assessed by a repeated measures factorial ANOVA. Using a standard ANOVA in our case is not appropriate because it fails to account for the correlation between the repeated measures, and the data therefore violate the ANOVA assumption of independence. Post-hoc Bonferroni test was used to determine which treatment levels were statistically different from each other in different months (StatSoft, Inc. 2007).

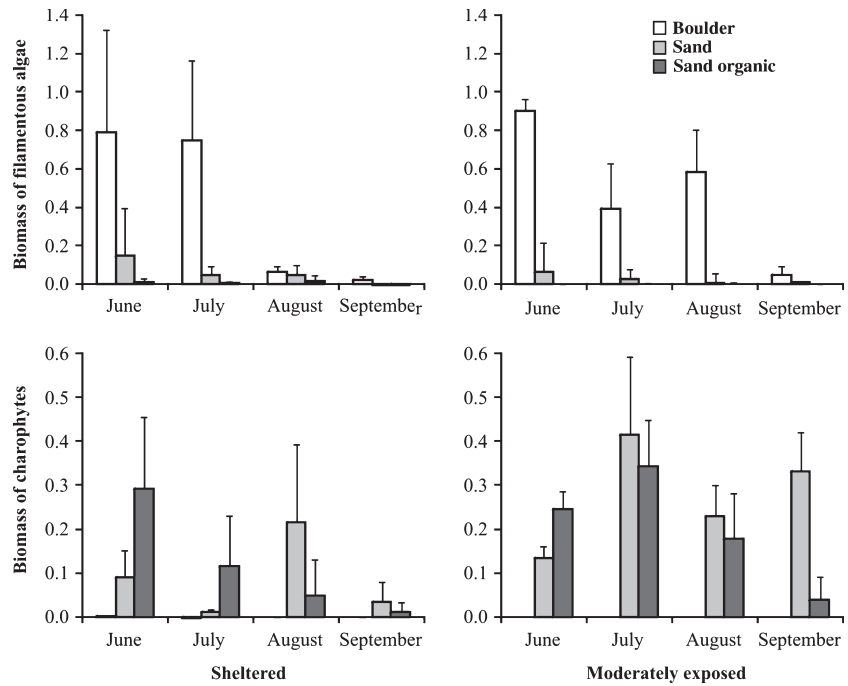
## Results

In the absence of *Mytilus trossulus* the biomass of filamentous algae was highest in June and declined thereafter. The biomass of charophytes was relatively stable over the course of the experiment. Substratum composition affected the dominance structure of filamentous algae. Pebbles were characterized by higher biomasses of *Ulva intestinalis* L. and *Pilayella littoralis* (L.) Kjellman compared to other substrate types. Exposure affected the biomass level of charophytes, with higher biomass associated with the moderately exposed area (Fig. 1).

The biomass of herbivores increased over the course of experiment. Substrate type had consistent effects on herbivores. The biomass of gammarid amphipods, *Theodoxus fluviatilis* (L.) and *Hydrobia ulvae* (Pennant) was significantly higher on pebbles compared to sediments with low and high content of organic matter. The biomass of deposit feeders also increased significantly over time but the changes were less marked. Substrate type had consistent effects on deposit feeders. The biomass of *Macoma balthica* (L.) and Chironomidae larvae was highest in hard bottoms, intermediate in soft bottoms with high content of organic matter, and lowest in soft bottoms with low content of organic matter (Fig. 2).

The repeated measures ANOVA analyses indicated mostly consistent community responses to the experimental treatments. The presence of *M. trossulus* had significant effects on filamentous algae, charophytes, herbivores and deposit feeders. The direction of the effect varied among months and organism groups. The presence of *M. trossulus* resulted in elevated biomasses of filamentous algae and





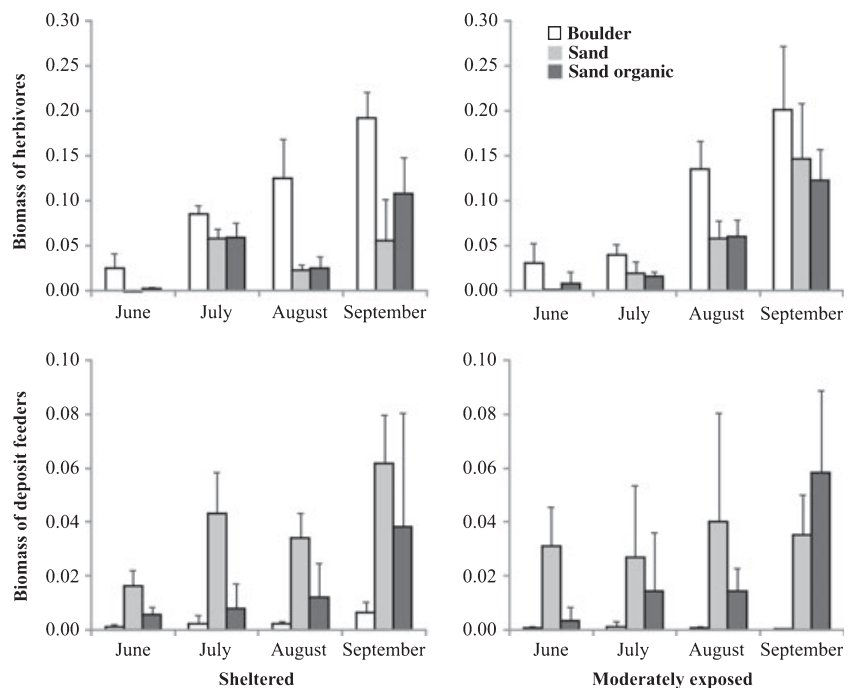
**Fig. 1.** Seasonal changes in the biomass of filamentous algae and charophytes (g dry weight, mean per bucket  $\pm$  SD, N = 3) at different substrate and exposure levels in the absence of *Mytilus trossulus*.

deposit feeders at the initial stages of algal succession (June, July) and in diminished biomass of charophytes at the later stages of algal succession (August, September). The positive effect of *M. trossulus* on herbivores was significant from July to September (Table 1, Fig. 3).

The interactive effect of *M. trossulus* and exposure was only significant for macroalgae and not for benthic inverte-

brates. Stronger responses in macroalgal growth were observed in moderately exposed than in sheltered areas. The direction and timing of responses corresponded to the separate effect of *M. trossulus* on the respective organisms (Fig. 4).

Interactions between substratum and *M. trossulus* were significant for all organism groups except herbivores. The



**Fig. 2.** Seasonal changes in the biomass of herbivores and deposit feeders (g dry weight, mean per bucket  $\pm$  SD, N = 3) at different substrate and exposure levels in the absence of *Mytilus trossulus*.

**Table 1.** Repeated measures factorial ANOVA analyses on the separate and combined effects of *Mytilus trossulus*, exposure and substrate type on filamentous algae, charophytes, herbivores and deposit feeders. n.s refers to non-significant effect and × stands for interaction.

Model/Factor	F	Effect df	Error df	P
<b>Filamentous algae</b>				
Exposure	4.40	4	21	0.010
<i>Mytilus</i>	3.94	4	21	0.015
Substrate	16.79	8	42	0.000
Exposure × <i>Mytilus</i>	12.94	4	21	0.000
Exposure × Substrate	3.60	8	42	0.003
<i>Mytilus</i> × Substrate	2.29	8	42	0.040
Exposure × <i>Mytilus</i> × Substrate	8.50	8	42	0.000
<b>Charophytes</b>				
Exposure	12.19	4	13	0.000
<i>Mytilus</i>	5.73	4	13	0.007
Substrate	13.63	4	13	0.000
Exposure × <i>Mytilus</i>	3.76	4	13	0.030
Exposure × Substrate	2.41	4	13	n.s.
<i>Mytilus</i> × Substrate	7.30	4	13	0.003
Exposure × <i>Mytilus</i> × Substrate	5.22	4	13	0.010
<b>Herbivores</b>				
Exposure	7.77	4	21	0.001
<i>Mytilus</i>	3.21	4	21	0.033
Substrate	6.45	8	42	0.000
Exposure × <i>Mytilus</i>	0.62	4	21	n.s.
Exposure × Substrate	0.85	8	42	n.s.
<i>Mytilus</i> × Substrate	1.08	8	42	n.s.
Exposure × <i>Mytilus</i> × Substrate	0.63	8	42	n.s.
<b>Deposit feeders</b>				
Exposure	1.93	4	21	n.s.
<i>Mytilus</i>	6.07	4	21	0.002
Substrate	26.70	8	42	0.000
Exposure × <i>Mytilus</i>	2.33	4	21	n.s.
Exposure × Substrate	2.15	8	42	n.s.
<i>Mytilus</i> × Substrate	3.25	8	42	0.006
Exposure × <i>Mytilus</i> × Substrate	1.17	8	42	n.s.

presence of *M. trossulus* increased the biomass of deposit feeders and decreased the biomass of charophytes on sand with low content of organic matter but not on other substrate types. The presence of *M. trossulus* also resulted in the elevated biomass of filamentous algae on pebbles but not on sandy substrate. There were no differences in timing of the combined and separate effects of mussels (Fig. 5).

## Discussion

Our study showed that the presence of mussels was associated with an increase in the biomass of ephemeral macroalgae (hypothesis 1). This increase suggests that mussels provide an important local-scale nutrient source for macroalgae inhabiting the shallow water benthic ecosystems

of the Baltic Sea. The provisioning of additional habitat (shells for attachment) was not considered a viable explanation due to their small sizes (1.5–2 cm) and lack of epiphytes on bivalves at the end of the experiment.

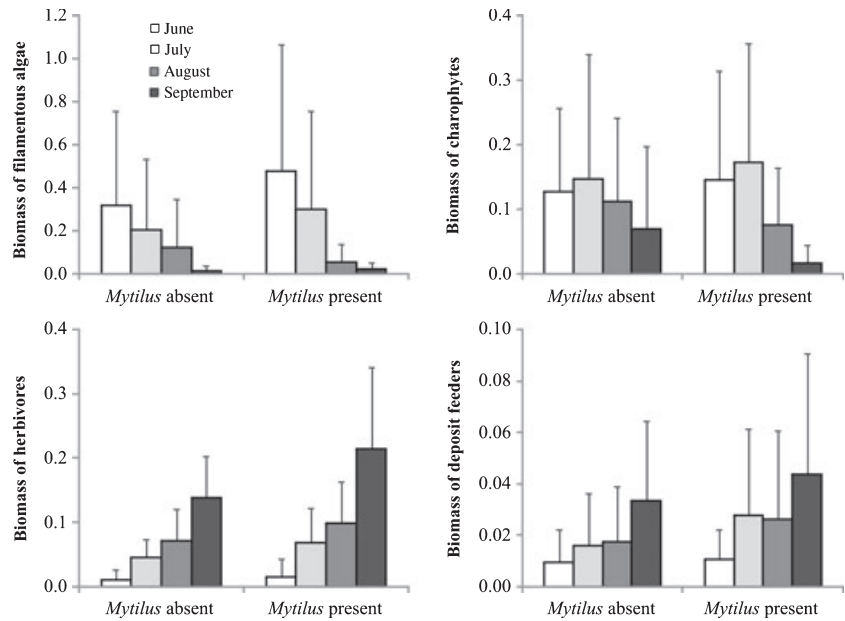
The addition of nutrients may be either direct (through nitrogenous excretory products) or indirect (through biodeposition and subsequent remineralization) (Kautsky & Wallentinus 1980; Dame *et al.* 1991; Bracken & Nielsen 2004; Kotta *et al.* 2006a). It has been shown earlier that nitrogen and organic content is higher in biodeposits than in naturally sedimenting material (Kautsky & Evans 1987). In either scenario it is surprising that even a few mussels can significantly modify soft- and hard-bottom habitats in this dynamic ecosystem.

The mussels significantly increased macroalgal growth at the initial stage of annual succession. It is plausible that *Mytilus trossulus* accelerates the growth of macroalgae through the entire productive season. However, at the later stages of annual succession the effect is likely counteracted by heavy grazing pressure by mesoherbivores. This hypothesis is supported by a significantly higher biomass of grazers in *M. trossulus* treatments in August and September compared to other treatments.

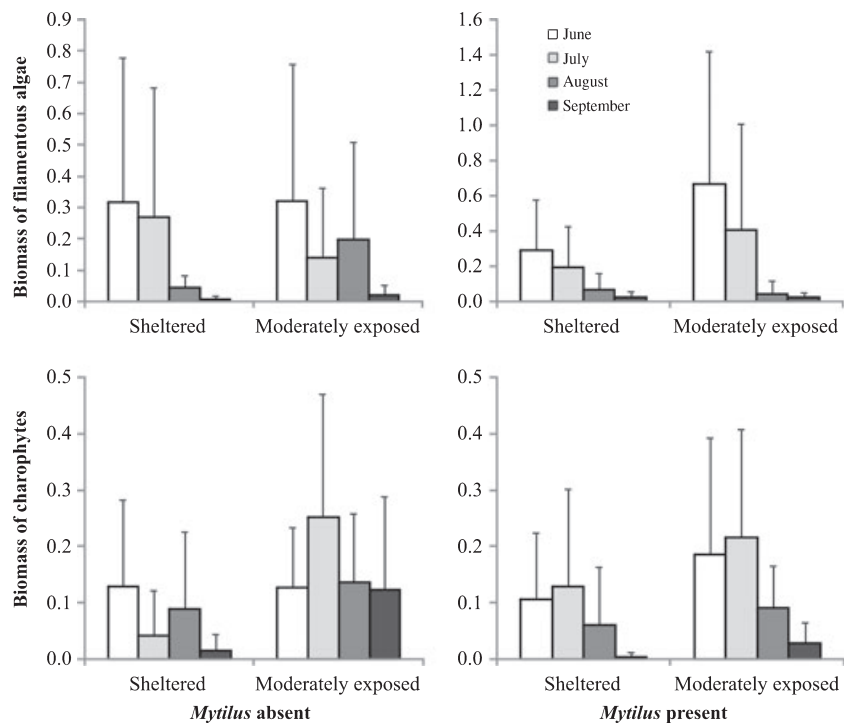
The biomasses of aquatic plants and mesoherbivores are positively correlated in many water bodies as plants provide mesoherbivores with habitat and food resources (Huntly 1991; Lawton 1994; Kotta & Orav 2001; Orav-Kotta & Kotta 2004). Animals respond more strongly to the amount of available resource than to the diversity of plants that provide it (Parker *et al.* 2001; Kotta *et al.* 2006b) and this assertion may explain the consistent effect of *M. trossulus* on herbivores (hypothesis 1), especially on gammarid amphipods, even though the macroalgal assemblages varied among buckets. In our study area gammarid amphipods are capable of foraging different types of food; however, when the filamentous brown alga *P. littoralis* is present it seems to be the most important dietary component of the studied gammarids (Orav-Kotta *et al.* 2009). In the current study *M. trossulus* consistently enhanced the growth of *Pilayella littoralis* over other macrophyte species and this may explain the stronger response of gammarid amphipods compared to other herbivores.

Deposit feeders are often food limited in the temperate coastal marine ecosystems (Levinton & Stewart 1988; Ólafsson & Elmgren 1997; Kotta *et al.* 2001). As seen in the present study, biodeposition by suspension-feeding mussels may reduce or even release deposit feeders from food limitation (hypothesis 1). Our results agree with earlier circumstantial and experimental evidence that the accumulation of faecal material induces elevated settlement and growth of deposit feeders (Posey 1986; Dittmann 1990; Snelgrove & Butman 1994). Strong effects of

**Fig. 3.** Seasonal changes in the separate effects of *Mytilus trossulus* on the biomasses of filamentous algae, charophytes, herbivores and deposit feeders (g dry weight, mean per bucket  $\pm$  SD, N = 18).



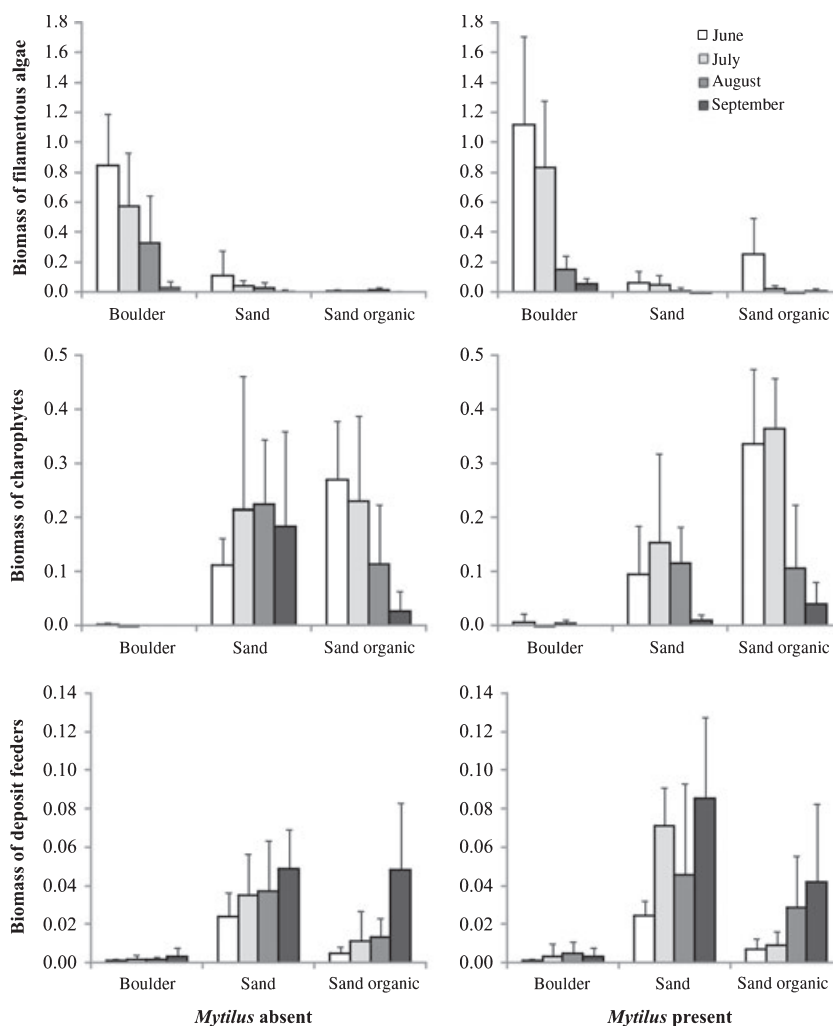
**Fig. 4.** Seasonal changes in the combined effects of *Mytilus trossulus* and exposure on the biomasses of filamentous algae and charophytes (g dry weight, mean per bucket  $\pm$  SD, N = 9).



mussels on deposit feeders were observed in the early stages of annual succession. At the later stages of annual succession, drift algae and their decomposition products provide an additional source of fresh detritus for deposit feeders (Kotta *et al.* 2008) and may explain weak effects of mussels in this period.

The interaction of exposure and *M. trossulus* affected the biomass structure of filamentous algae (hypothesis 2).

Except for phytoplankton blooms, the suspension-feeding bivalves are often limited by the availability of suspended particulate matter (Incze *et al.* 1981; Fr chet te *et al.* 1989; Smaal *et al.* 2001) and increasing current velocity can reduce this limitation (Walne 1972; Fr chet te & Bourget 1985; Wildish *et al.* 1992). Our study demonstrated that suspension-feeding mussels had stronger positive effects on filamentous algae in moderately exposed areas than in



**Fig. 5.** Seasonal changes in the combined effects of *Mytilus trossulus* and substrate type on the biomasses of filamentous algae, charophytes and deposit feeders (g dry weight, mean per bucket  $\pm$  SD, N = 6).

sheltered areas. This difference suggests that mussels were food limited because biodeposition activity was higher in exposed areas with high food availability compared to sheltered sites with low food availability (Kotta *et al.* 2005).

We predicted that the effect of *M. trossulus* would be stronger on sediments poor in organic matter compared to sediments rich in organic matter (hypothesis 3). A significant combined effect of substrate and *M. trossulus* was observed only for deposit feeders. Besides, the presence of *M. trossulus* decreased the growth of charophytes inhabiting sand with low content of organic matter, whereas such an effect was not observed on other substrate types. The apparent lack of interactive effect of substrate and mussels on macroalgae indicates that sediment nutrients were generally not available to algae, and factors, *e.g.* those affecting remineralization and the diffusion, advective

tion or turbulent mixing of porewater nutrients, limited the transport of sediment nutrients to the water column.

The presence of mussels resulted in the decline of charophytes at the later stages of annual succession. As shown earlier, mussels increase nutrient loads, leading to elevated productivity of ephemeral macroalgae. When covered by filamentous algae, charophyte photosynthetic activity presumably decreases and the charophytes become very attractive to herbivores (Kotta *et al.* 2004). Heavy grazing pressure on charophytes is expected given that there was significantly higher grazer biomass in *M. trossulus* treatments.

To conclude, our experiment demonstrated that *M. trossulus* had significant effects on macroalgae and associated invertebrates, especially during early stages of annual succession. Although benthic communities in the Baltic Sea are considered to be primarily physically

controlled (e.g. Herkül *et al.* 2006), our study indicates that physical and biological factors interactively affect benthic community composition at small spatial scales.

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## ORIGINAL ARTICLE

# ***Spartina maritima* (cordgrass) rhizosediment extracellular enzymatic activity and its role in organic matter decomposition processes and metal speciation**

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## Keywords

Extracellular enzymatic activity; metal speciation; salt marsh; sediment.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

Seasonal monitoring was carried out to investigate the influence of extracellular enzymatic activity (EEA) on metal speciation and organic matter cycling in the rhizosediment of *Spartina maritima*. Heavy metal speciation was achieved by the Tessier scheme, and showed a similar pattern of variation of the organic-bound fraction, indicating a decomposition process in progress. Both humic acid and organic matter showed the same seasonal pattern. The basal respiration of the rhizosediments also presented a similar seasonal pattern, indicating a microbial degradation of organic matter. The high organic-bound fraction found in the summer gradually decreased towards the winter. This decrease was found to be related to the increase of activity of peroxidase,  $\beta$ -N-acetylglucosaminidase and protease. Also the activity of sulphatase was found to be related to the depletion on the exchangeable fraction, probably due to sulphide formation and consequent mobilization. The results show an interaction between several microbial activities, affecting metal speciation.

## Problem

Salt marshes located in estuaries frequently receive large inputs of nutrients (Tobias *et al.* 2001), as well as particulate and dissolved organic matter. This high nutrient input makes salt marshes one of the most productive ecosystems of the planet. This high biomass production has as a consequence large necromass generation due to litter senescence (Caçador *et al.* 2009). In highly industrialized estuaries, along with this nutrient input there is also a large input of heavy metals, which will accumulate in salt marsh sediments (Doyle & Otte 1997). These high inputs make salt marshes key zones for the biogeochemistry of the estuary, but also for metal cycling (Weis & Weis 2004). The microbial decomposer communities of salt marsh sediments play an essential role in these cycling processes by decomposing the organic matter, as well as other large complex molecules that reach to the sediments, into more bioavailable forms (Ravit *et al.* 2003).

As verified previously, salt marsh sediments are often very organic (Richert *et al.* 2000), providing large amounts of substrates for the proliferation of microbial decomposers. The generation of large amounts of below-ground necromass and the organic compounds exuded by living plants (Duarte *et al.* 2007) are the major contributors to the organic content of the rhizosediments. The large periods of submersion to which these sediments are subjected, lead to a low oxygenation of the sediments, with adverse effects on plant growth (Richert *et al.* 2000). Some salt marsh plants have the ability to pump oxygen from the atmosphere into the root zone, oxidizing the sediments and consequently promoting aerobic microbial activity (Ludemann *et al.* 2000). This kind of plant–microflora interaction is very variable, depending not only on the plant species but also on season (Wilczek *et al.* 2005; Yang *et al.* 2007). To process the organic matter, microbes produce extracellular enzymes which degrade these large complex molecules into smaller ones, which

are easily up taken by the microbes. These enzymes are divided into two major groups: oxidoreductases, which degrade large molecules by redox reactions, and hydrolases, which decompose complex molecules by breaking them down. These enzymes are very important in the ecosystem overall due to the large amount of necromass generated, maintaining an ecological balance. Several extracellular enzymes, such as proteases, phenol oxidases, peroxidases and  $\beta$ -*N*-acetylglucosaminidases, are very important in this process as they contribute to the breakdown of organic compounds (Kang *et al.* 2005; Oyekola & Pletschke 2006; Acosta-Martínez *et al.* 2007). During the early stages of decomposition (Brüchert & Pratt 1996 in Passier *et al.* 1999) it was observed that sulphur-linked reactions also play an important role in the decomposition process in salt marsh sediments, either by sulphidization reactions of the organic matter (Passier *et al.* 1999) or by leaching of labile organic sulphur present in tissues. Several studies have shown that plant litter occurs in three different stages, independently of the species (Valiela *et al.* 1985; Wilson *et al.* 1986; Benner *et al.* 1991). Initially, there is a rapid leaching process, consisting in a fast loss of the most soluble fractions of the plant material. A second stage consisting in microbial decomposition is slower and ends in a refractory phase (third stage), where the decomposition rate is almost null (Zawislanski *et al.* 2001). During this decaying process, the metals associated with the decomposing matter stay bound to the more resistant fraction of the organic matter, remaining in the nearby sediment. Other factors such as oxygen in the rhizosphere, the redox state, temperature and the microbial community also influence this rate of decomposition (Pereira *et al.* 2007).

Heavy metal inputs reach salt marshes through tidal flooding and are retained in the sediments in various forms, depending on the bonds they establish with the sediment components (Tessier 1979). There is a large variability in this process, depending not only on the sediment characteristics but also on external factors (hydrodynamics, weather, seasonal variations and plant coverage), as seen in previous studies (Reboreda & Caçador 2007; Duarte *et al.* 2008). The literature provides evidence that microbial interactions with metals greatly influence their speciation (Gadd 2001, 2004; Tabak *et al.* 2005; Duarte *et al.* 2008). These transformations include metal precipitation reactions by metallic sulphides and redox reactions, causing changes on the metal species and its associations (Hullebusch *et al.* 2005).

Heavy metal speciation is very variable, depending both on salt marsh localization and physical–chemical characteristics, and on plant coverage (Reboreda & Caçador 2007, 2008). Although there was evidence of the influence of extracellular enzymes on metal speciation in the upper

marsh colonized by *Halimione portulacoides* (Duarte *et al.* 2008), the sediment and hydrological conditions in the lower marsh mainly colonized by *S. maritima* are very different. In this paper the authors compare microbial speciation mechanisms in *S. maritima* with those previously verified in *H. portulacoides*, in order to understand if they are still valid in this environment.

## Material and Methods

### Site description and sampling

Rosário (38°40'N, 9°01'W) is a mature salt marsh (Valiela *et al.* 2000) located in the southern part of the Tagus estuary, in the vicinity of various urbanized and industrialized zones. The upper marsh is mainly colonized by *H. portulacoides* (Chenopodiaceae) and *Sarcocornia fruticosa* (Chenopodiaceae) and undergoes short submersion episodes during high tide. Between April 2006 and January 2007, four samplings were done: April (Spring), July (Summer), October (Autumn) and January (Winter). For each sampling, five sediment cores (50 cm depth) were taken in pure stands of *S. maritima*. The stands were located along the marsh always with a minimum distance of 10 m from each stand. All the collections were made during low tide. The cores were transported in refrigerated bags to the laboratory, where the sediment was sliced. According to previous studies (Reboreda & Caçador 2008) the depth between 5 and 10 cm proved to have high extracellular enzymatic activity (EEA) and it was used for analysis. These sediment samples surrounding the rhizosphere of *S. maritima* are referred hereafter as the rhizosediment.

### Sediment parameters

Redox potential (Eh) and pH values were measured in the fresh, selected segment using a HANNA pH/mV (HI 9025). Calibration of redox potential measurements was done using a standard redox solution (Crison, Eh = 468 ± 5 mV at 25 °C). The pH calibration was performed using buffer solutions of pH 4 and pH 7. Organic matter was determined by the loss on ignition (LOI) method by burning 1 g of sediment at 600 °C for 2 h. Humic acids were extracted and quantified according to Adani *et al.* (2006) with some modifications. Dried and sieved sediment (5 g) was extracted by adding 25 ml of a solution containing 0.1 M NaOH and 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>. The extraction was carried out in an end-to-end shaker for 24 h at 65 °C. After extraction the samples were centrifuged at 45,880 g for 20 min at 4 °C. The supernatant was totally recovered and distilled water was added to the solid residue, which was re-suspended and centrifuged again. This operation was

repeated until the supernatant was clear. The supernatant solutions were combined and acidified with 50% sulphuric acid to pH <1.5 to precipitate the humic acids. These were separated by centrifugation as described above, the supernatant completely evaporated at 60 °C until constant weight, and the humic acids weighed. Phenolic content in the sediment samples was determined according to Folin & Ciocalteu (1927) modified by Waterman & Molle (1994). Briefly, 5 g of fresh sediment was mixed with 50 ml distilled water and 10 ml of this slurry was centrifuged at 653 g for 2 min at 10 °C. The supernatant (0.5 ml) was treated with 2.5 ml of Folin-Ciocalteu's phenol reagent (0.2 N) and 2.5 ml alkali reagent, and left to stand for 2 h. After this period the absorbance was read in a Shimadzu UV-1603 spectrophotometer at 760 nm and compared with a calibration curve made with galic acid. Phenolic content was expressed as galic acid (GA) equivalents per gram sediment fresh weight.

#### Respiratory activity and extracellular enzyme activity in the rhizosediment

Sediment basal respiration was determined by the NaOH-trap method (Isermeyer 1952). Briefly, 5 g of rhizosediment was placed in a tube and adjusted with distilled water to 55% of its water-holding capacity (WHC). This container was placed inside a jar containing 0.05 M NaOH, sealed air-tight and placed in the dark at room temperature for 2 days. After this period the carbon dioxide involved and trapped in the NaOH-trap was determined by titration with 0.05 M HCl. An excess of water was percolated to a portion of the sediment samples and the volume stored in the sample was used for calculation of the WHC.

All enzymatic determinations were carried out with colorimetric methods and the absorbances were read on a TECAN Absorbance Microplate Reader (SPECTRA Rainbow). The utilization of this plate reader system allowed three readings of the same replicate to be made. Phenol oxidase, peroxidase,  $\beta$ -*N*-acetylglucosaminidase and sulphatase were assayed according to Ravit *et al.* (2003) with a modification in the incubation temperature and without dilution of the supernatant. Briefly, 75 ml of sodium acetate buffer (pH 5) was added to 5 g of fresh sediment, and mixed for 1 min to obtain the sediment slurry. The substrates (5 mM) used were *p*-nitrophenyl-*N*-acetyl- $\beta$ -*D*-glucosaminide and *p*-nitrophenyl-sulphate, respectively for  $\beta$ -*N*-acetylglucosaminidase and arylsulphatase. Two ml of each substrate was added to 2 ml of slurry and incubated at 30 °C with gentle agitation for 60 min (sulphatase) and 2 h ( $\beta$ -*N*-acetylglucosaminidase). After incubation, samples were centrifuged at 653 g for 15 min at 4 °C and 0.2 ml of 1 N NaOH was added to stop the reaction and reveal the *p*-nitrophenol (pNP) formed. Absorbance of the

supernatant was read at 410 nm. The absorbance was compared with a calibration curve for pNP and the activity was expressed as  $\mu$ g of pNP released per gram sediment dry weight per hour. Phenol oxidase and peroxidase were assayed using 5 mM *L*-3,4-dihydroxyphenylalanine (*L*-DOPA) as substrate. Two ml was added to 2 ml of slurry (adding 0.1 ml of 0.3% H<sub>2</sub>O<sub>2</sub> for peroxidase assay) and were incubated for 60 min for both enzymes. After incubation, samples were centrifuged at 653 g for 15 min at 4 °C. Absorbance of supernatant was read at 460 nm and the absorbance of phenol oxidase was subtracted from the absorbance of total peroxidase to obtain the real value for peroxidase activity alone. The absorbance was compared with a calibration curve for *L*-DOPA and the activity expressed as  $\mu$ M *L*-DOPA oxidized per gram sediment dry weight per hour. Protease activity was assayed according to Ladd *et al.* (1976). Briefly, 1 g of fresh sediment was incubated with 5 ml of Tris (Tris hydroxymethyl-aminomethane) buffer (0.05 M, pH 8.1) and a 2% (w/v) casein solution, for 2 h at 50 °C. After incubation, the reaction was stopped with 1 ml trichloroacetic acid 17.5% (w/v) and centrifuged at 14,690 g for 15 min at 4 °C. For photometric analysis, 1 ml of supernatant was added to 1 ml of Folin-Ciocalteu's phenol reagent (0.2 N) and 2.5 ml alkali reagent, and left to stand for 90 min. The absorbance was measured at 700 nm and compared with the calibration curve for tyrosine. Activity was expressed as microgram tyrosine equivalents per gram sediment dry weight per hour.

#### Metal sequential extraction and elemental analysis

To determine chemical fractioning of Cu, Cd, Cr, Ni, Zn, Co and Pb on sediments, a sequential extraction procedure was used. For metal determinations all labware was soaked for 2 days in HCl (10%) and rinsed with distilled water to avoid contamination. The metal sequential extraction scheme adopted in this study was that described by Tessier (1979) and modified by Hullebusch *et al.* (2005). One gram of air-dried sediment was sequentially extracted by adding 1 M ammonium acetate (exchangeable/available fraction, corresponding to the most labile fraction of the metal weakly bound to sediment constituents), 0.6 M acetic acid (carbonate-bound fraction, more susceptible to changes in pH), 30% hydrogen peroxide (organic-bound fraction, comprising living organisms, detritus, peptidic molecules and coatings) and aqua regia (residual fraction, mainly primary and secondary minerals containing metals in their crystal structure). Between all steps of the procedure the sediment was centrifuged at 204 g for 10 min at 4 °C and the supernatant filtered using Whatman No. 42 filters (2.5  $\mu$ m pore diameter) and stored at 4 °C until analysis. This procedure



proved to be adequate for this kind of sediment, as described in Duarte *et al.* (2008). A total digestion was made with aqua regia in a Teflon reactor at 110 °C for 3 h to evaluate the efficiency of the sequential procedure. All efficiencies were between 95 and 110%. Concentrations of Cu, Cd, Cr, Ni, Zn, Co and Pb were determined by Flame Atomic Absorption Spectrometry (SpectraAA 50; Varian) or Graphite Furnace ASS (932 plus; GBC). The accuracy of the results was checked by processing reference material CRM 145 and CRM 146.

### Statistics

Statistical analysis was performed using STATISTICA Software version 7.0 from StatSoft Inc. Due to the lack of normality and homogeneity of the environmental values obtained, the significance of the results was evaluated using Kruskal–Wallis non-parametric tests.

## Results

### Sediment characteristics

Organic matter content (LOI) and humic acid content in the rhizosediment of *S. maritima* (Table 1) showed a marked seasonal pattern ( $P < 0.03$ ). The pattern between these two organic components was very similar, with a decrease from spring to summer followed by an increase to autumn. In winter an accentuated decrease was registered, compared with the values verified in the previous seasons. pH values were very constant, showing no significant seasonal pattern ( $P > 0.05$ ). There was only a slight decrease in summer, this being the lowest value verified. There was no significant seasonal pattern for the redox state of the collected sediments. There was a great variability of Eh values in the collected replicates, as shown by the standard error. Although not significant ( $P > 0.05$ ), a progressive increase in the phenolic content was seen towards the winter season.

### Metal content and speciation

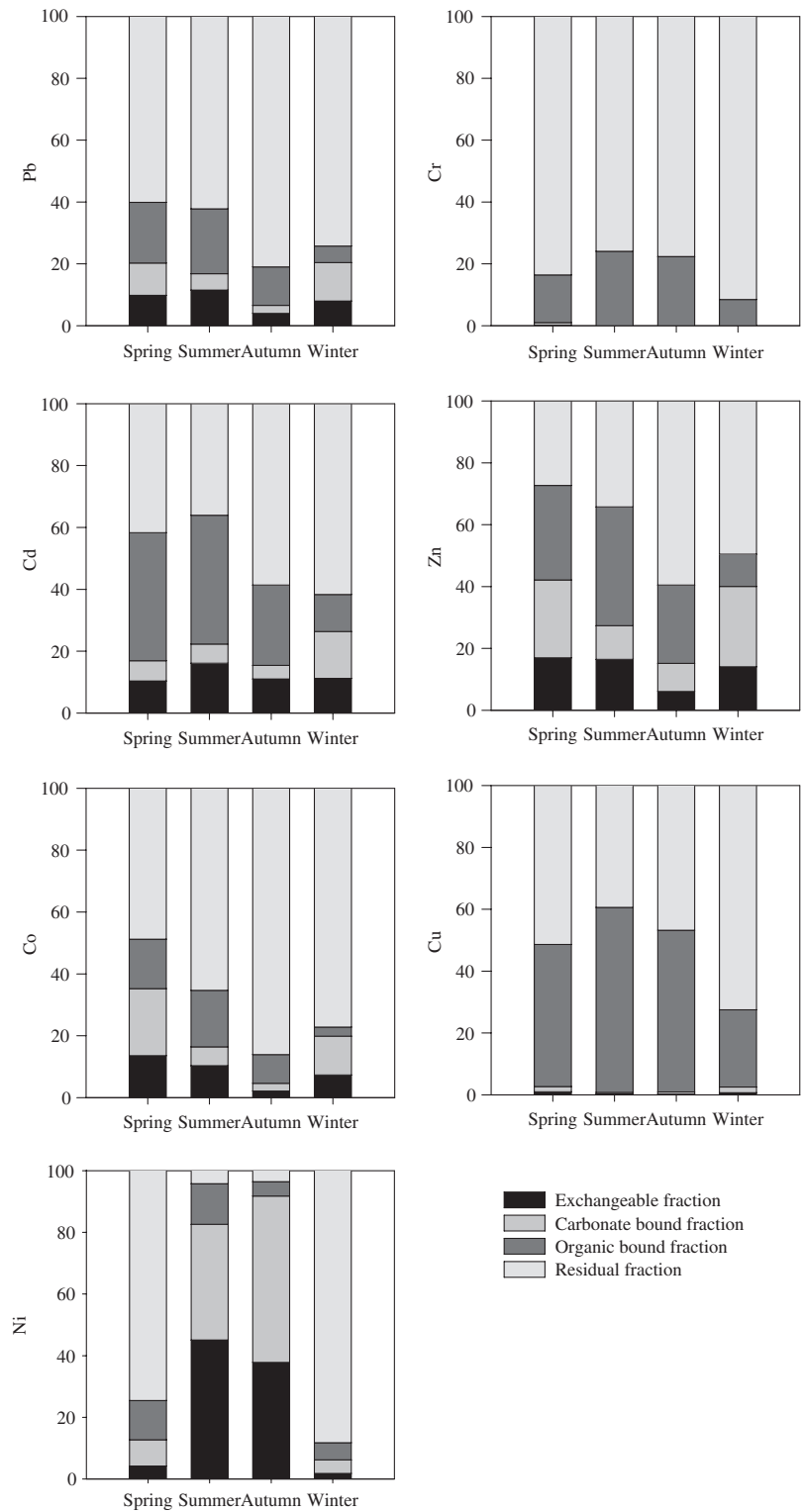
Observing the metal speciation results (Fig. 1) it is possible to see a large variation in the organic-bound fraction of metals, being more significant in Cu, Cr and Ni ( $P < 0.03$

for Cu and  $P < 0.05$  for Cr and Ni). The residual fractions of Co, Cu, Cr and Pb also showed a marked seasonal pattern ( $P < 0.02$  for Co and Cr, and  $P < 0.03$  Cu and Pb). Only Ni showed a marked seasonal pattern in all the fractions except for the residual fraction. All metals evaluated exhibited the same pattern of total and partial concentrations, increasing from spring to summer, corresponding to an increase in all fractions. In autumn and winter a progressive decrease in the concentrations of all fractions was observed similar to those verified in summer, except for Cr and Pb, where the concentrations remained above the summer values. The major fluctuations in the fractions were observed in the residual and organic-bound fractions for all the analyzed metals. All the concentrations found are in agreement with those previously in other studies. Cadmium is often the less abundant metal in salt marsh sediments (Caçador *et al.* 2000). Although it is found mostly in the organic-bound fraction and residual fraction, it was also possible to observe that about 10–15% of the metal is in the more labile fractions (exchangeable and carbonate-bound fractions), with a slight increase of these fractions in winter. Cobalt and Pb exhibit similar behaviour, with an increase of the residual fraction from spring to autumn and consequent depletion of the remaining fractions. In winter both metals showed an increase in the available and carbonate-bound fraction, although the total amount of metal did not increase. Cu and Cr were only found in considerable amounts in the organic and residual fractions, and in different proportions (Fig. 1). Both elements showed an increase of the organic-bound fraction in summer and autumn, with a depletion in spring and winter. Zinc fluctuations from spring to autumn are mostly due to depletions in the more labile fractions and increase of the residual fraction. An increase of the exchangeable and carbonate-bound fractions of zinc was found in winter, proportional to the values verified in spring. Observing Ni distribution in fractions it was possible to verify a strong seasonal pattern in the rhizosediment of *S. maritima* ( $P < 0.03$  for the available and  $P < 0.05$  for carbonate-bound and organic-bound fractions). During summer and autumn the carbonate-bound and available fractions prevailed, whilst in spring and winter these fractions almost disappeared, with a very significant increase in residual fractions.

**Table 1.** Sediment characteristics during the study period (mean value  $\pm$  SE).

	spring	summer	autumn	winter
Eh (mV)	$-12.09 \pm 5.84$	$51.73 \pm 14.50$	$10.65 \pm 2.81$	$-9.43 \pm 10.34$
pH	$7.21 \pm 0.31$	$6.12 \pm 0.73$	$6.60 \pm 0.41$	$7.13 \pm 0.56$
LOI (%)	$22.96 \pm 0.58$	$19.64 \pm 0.75$	$23.42 \pm 0.48$	$18.07 \pm 1.21$
Humic acids (g HA·g <sup>-1</sup> DW)	$34.90 \pm 6.57$	$19.50 \pm 0.64$	$25.10 \pm 2.68$	$8.70 \pm 0.30$
Phenolics (mEq GA·g <sup>-1</sup> DW)	$22.22 \pm 2.37$	$292.46 \pm 30.33$	$11.59 \pm 0.19$	$1457.02 \pm 82.02$



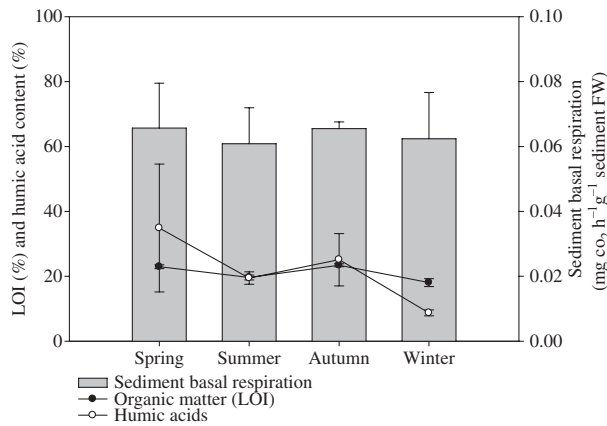


**Fig. 1.** Metal speciation in the rhizosediments of *S. maritima* throughout the study period.

### Sediment basal respiration and EEA

Basal respiration in the rhizosediment of *S. maritima* (Fig. 2) did not show a significant seasonal pattern

( $P > 0.05$ ), although a depletion from spring to summer and an increase to autumn could be observed. A decrease could be verified in winter. The EEA of several extracellular enzymes was also assessed (Fig. 3). The pattern of



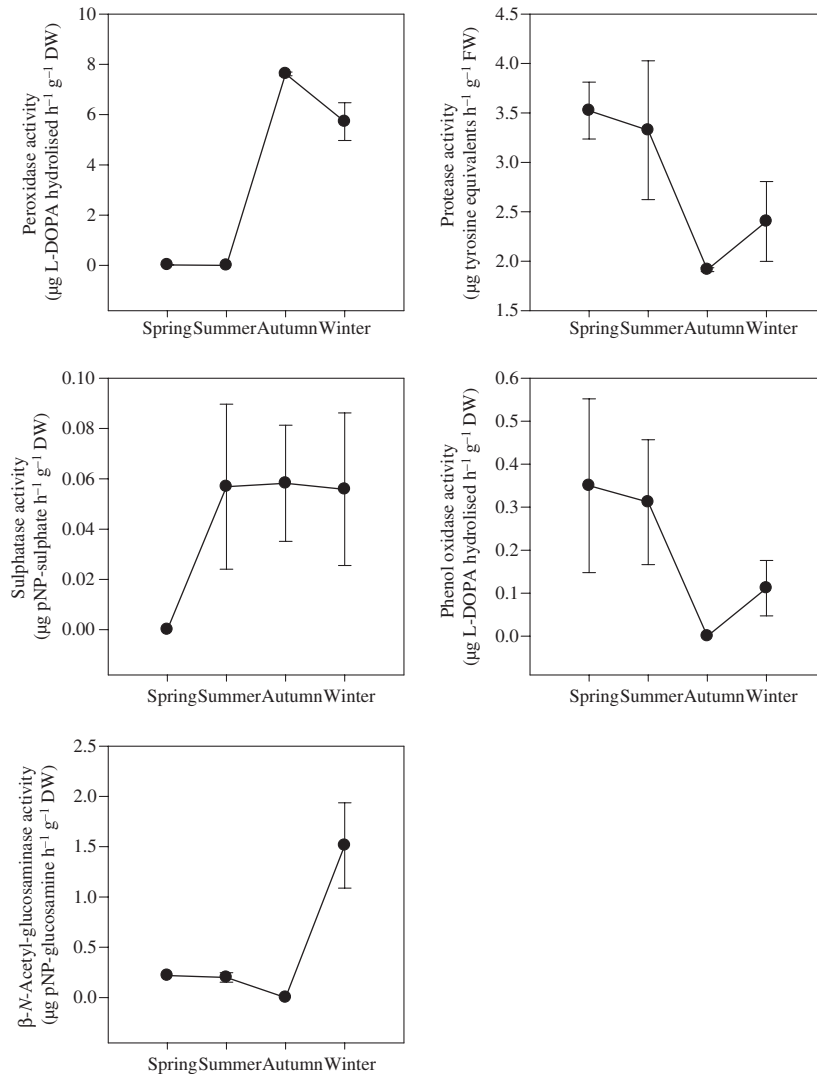
**Fig. 2.** Comparison between organic matter content (LOI), humic acids and sediment basal respiration.

seasonality observed in all the analyzed EEA was statistically significant only for peroxidase ( $P < 0.02$ ). Peroxi-

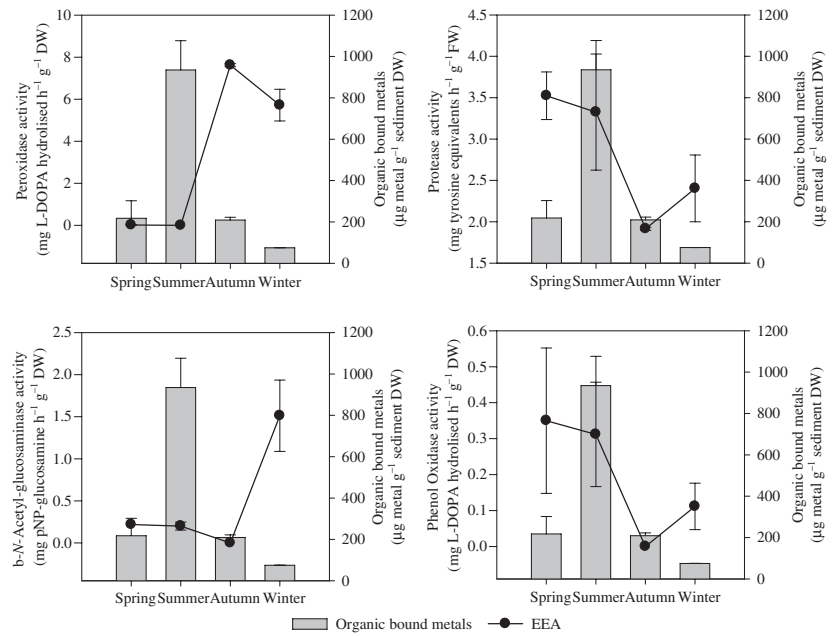
dase and phenol oxidase showed a contrasting behaviour. Whereas phenol oxidase was very active in spring and summer, peroxidase activity was only detected in autumn and winter. Protease activity was always detected in the studied periods, being highest in the warm seasons and slightly low in the colder seasons. The activity of  $\beta$ -N-acetylglucosaminidase was rather low from spring to autumn, followed by a great increase in winter. The activity for sulphatase was also assessed. In this case the enzyme did not show any detectable activity in spring, but during the rest of the year activity was very high and constant in the following seasons.

**Discussion**

Comparing the values of organic matter content, humic acid concentration and basal respiration it is possible to observe similar patterns in all these parameters. This indi-

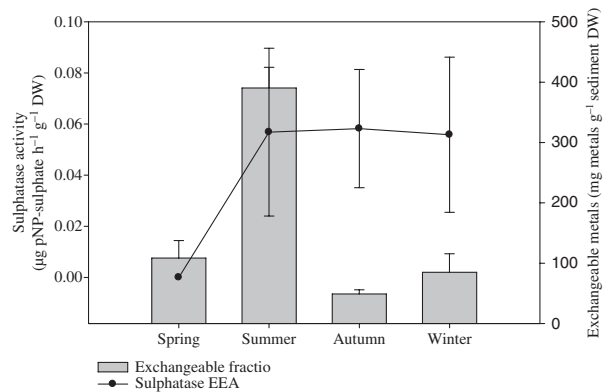


**Fig. 3.** Extracellular enzymatic activities assessed for the rhizosediment of *S. maritima* throughout the study period (SE bars represented).



**Fig. 4.** Influence of extracellular enzymatic activities of protease, peroxidase, phenol oxidase and  $\beta$ -N-acetylglucosaminidase (line) on total organic-bound metals (bars).

cates that the microbial activity is dependent on the organic matter present in the sediment. This is important in particular for the respiratory activity, directly related to the degradation of organic substrates, which are the important ligands of heavy metals, affecting metal speciation, as has already been seen for the upper marsh sediments (Duarte *et al.* 2008). Considering the organic-bound fraction of all metals and organic matter and humic acid content in the sediment, the patterns exhibited by these parameters showed no similarity. This indicates that the peak of metals observed in summer was not due to an increase of organic matter free to establish bonds with metals, but to a beginning of the decomposition process. In this first step of decomposition the dominant process is the leakage of the more labile compounds, such as the low molecular weight (LMW) organic molecules. This is the case for the organic acids, as has been observed in previous studies that established chelator-metal complexes (Mucha *et al.* 2005; Duarte *et al.* 2007). With this leakage of LMW molecules the organic-bound fraction of metals in the rhizosediments increases, as can be seen from our results. As previously referred to, several enzymes are involved in the degradation and breakdown of organic matter. These enzymes have different patterns of activity throughout the seasons, leading to a differential degradation of the organic components of the sediment in different periods of the year. Taking these facts into consideration, the decomposition process will be discussed, in this case, from summer to winter. Carrying over data from enzymatic activities and from metal speciation analysis, it is possible to observe a common pattern (Figs 4 and 5). The values detected in spring are probably



**Fig. 5.** Influence of extracellular enzymatic activities of sulphatase (line) on total exchangeable metals (bars).

the end of decomposition from a previous cycle, and so will not be considered for discussion purposes. Extracellular peroxidase is known to be produced mostly by ligninolytic fungi to degrade plant litter (Johnsen & Jacobsen 2008) and catalyzes the degradation of ligninocellulosic litter in the presence of hydrogen peroxide. The degradation of lignin produces phenolic substances of LMW. The major activity of this enzyme was detected in autumn and winter, after the first step of decomposition, indicating the beginning of a second phase of decomposition. The observed decrease in the organic-bound fraction of metals in the seasons where peroxidase activity was high, indicated that probably the lignin-like compounds which were being degraded are molecules that were bound to the metals. With the decomposition of these molecules the metals are released from the organic-bound fraction.

Together with this depletion it was possible to observe an increase in the phenolic content of the rhizosediments, with the same pattern verified for peroxidase activity, supporting lignin degradation, as already pointed out. This accumulation of phenolic substances is also due to the low activity of phenol oxidase in winter. A great increase of activity of  $\beta$ -N-acetylglucosaminidase in winter was also assessed, coincident with the high depletion of organic-bound fraction of metals. Together with the high peroxidasic activity, this glucosamine polymer degradation contributes to the degradation of large polymers, typically found in teguments. The degradation renders proteins more accessible for protease degradation, as indicated by the small recovery of activity verified for protease in winter. All these three EEA contribute to a strong decrease of heavy metals in the organic-bound fraction, releasing them to the surrounding environment. Previous studies (Hullebusch *et al.* 2005) point out that high sulphatase activity, and consequent formation of sulphates by this enzyme into sulphides, can lead to its conversion into sulphides by sulphate-reducing bacteria (SRB). Sulphides can chemically reduce metals into a stable form for extended periods of time (Tabak *et al.* 2005), decreasing therefore the metal concentrations in the exchangeable fraction of this extraction scheme. This was also observed in this study. From summer to winter the organic matter degradation released heavy metals from the organic-bound fraction, and these processes would be expected to lead also to an increase of the heavy metals in the exchangeable fraction. This was not verified, probably due to the high activity of sulphatase and consequent sulphide generation and metal precipitation, as shown by the decrease of the exchangeable fractions. All these findings are in agreement with those described for the rhizosediment colonized by *H. portulacoides* (Duarte *et al.* 2008), pointing to a similar process independent of the plant coverage. Although the patterns of activity, absolute values of EEA and speciation are very different from those verified for *H. portulacoides* rhizosediment, the inherent mechanism seems to be the same. This supports the important role of microbial processes not only in the sediment biogeochemistry but also in metal speciation processes. This is a very important process for consideration within the entire ecosystem.

## Conclusion

Organic matter is an important and effective sink of heavy metals in the sediments, providing a large source of strong ligands for these elements. All the processes of breakdown or modification that affect sediment organic matter will consequently affect the bonded metals, changing their speciation. It is very important to take these bio-

chemical processes into consideration. Changing metal speciation means that metal availability and mobility are also altered, influencing their effect on the ecosystem community. These processes should also be taken in account when considering bioremediation processes. Stimulating or inhibiting some extracellular enzymes can lead, for example, to the leakage of metals, diminishing their concentration in the sediment.

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## ORIGINAL ARTICLE

# Effects of eelgrass (*Zostera marina*) canopy removal and sediment addition on sediment characteristics and benthic communities in the Northern Baltic Sea

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## Keywords

Baltic Sea; benthic community; disturbance; oxygen flux; seagrass; sedimentation; *Zostera marina*.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

The eelgrass *Zostera marina* is a key structural and functional species across the European coastline. The separate and interactive effects of eelgrass canopy removal and sediment addition on the sediment characteristics and the structure of benthic communities were studied in a factorial field experiment in the Northern Baltic Sea in July–August 2006. The removal of eelgrass canopy temporarily increased the sediment oxygen consumption, reduced the content of fine particles (<100 µm) and organic matter in the sediment, and increased the share of sand fraction (250–500 µm). Sediment addition increased the content of fine particles (<100 µm) and reduced the share of sand fraction (250–1000 µm). The effects were strongest in the presence of eelgrass canopy. Benthic invertebrates and macroalgae were affected by eelgrass canopy removal but not by sediment addition. The removal of eelgrass canopy significantly decreased benthic species richness and invertebrate and macroalgal densities. To conclude, our experiment demonstrates that *Z. marina* defines the patterns of benthic macroalgae and invertebrates but has moderate effects on sediment structure and metabolism in the Northern Baltic Sea.

## Problem

Seagrasses have an important role in the coastal ecosystems worldwide. Seagrasses are known to stabilize the seabed in which they grow, reduce the resuspension of sediments, influence sediment deposition and composition (Terrados & Duarte 2000; Gacia *et al.* 2003; Bos *et al.* 2007; Hasegawa *et al.* 2008; Hendriks *et al.* 2008), release oxygen into the sediment (Enríquez *et al.* 2001; Frederiksen & Glud 2006), and provide habitat and food for a great variety of plant and animal species (Rasmussen 1973; Orth *et al.* 1984; Orth 1992; Mattila *et al.* 1999; Heck *et al.* 2003).

The eelgrass *Zostera marina* is a key structural and functional species across the European coastline. *Zostera marina* grows on a wide range of soft and mixed sediments, and their roots are, in many cases, growing in anoxic environments. To maintain oxygen in their root systems, seagrasses have adapted an O<sub>2</sub> transport system

from leaves to roots (Pedersen *et al.* 1998; Frederiksen & Glud 2006). Oxygen loss from the roots into the sediment may occur, providing an oxidative protection against phytotoxins such as Fe<sup>2+</sup>, Mn<sup>2+</sup> and sulfides (Penhale & Wetzel 1983; Frederiksen & Glud 2006). The release of oxygen by the roots may indirectly accelerate the decomposition of sediment organic matter and facilitate the development of benthic infauna. On the other hand, seagrasses may increase the content of organic matter and fine particles in sediment by increasing sedimentation through a reduction of current velocity (Fonseca & Fisher 1986), direct trapping and binding of particles (Hendriks *et al.* 2008) and enhancing sediment accretion (Bos *et al.* 2007). Lower current velocity also leads to increased settlement of macroalgae, pelagic larvae and juveniles of benthic invertebrates and thus fosters the diversity and density of plant and animal species within the seagrass canopy (Reusch 1998; Bologna *et al.* 2005).

In recent decades the abundance and spatial extent of seagrasses around the world has been declining and this loss has been mainly associated to elevated anthropogenic eutrophication and intensified sediment dynamics (Duarte 2002; Orth *et al.* 2006; Cabaco *et al.* 2008b). Anthropogenic eutrophication is known to reduce light levels for seagrasses, leading to the removal of the seagrass canopy (Short *et al.* 1995; Longstaff *et al.* 1999; Duarte 2002; Hauxwell *et al.* 2003; Cardoso *et al.* 2004; Cabaço *et al.* 2008a). Similar effects have been observed with increasing grazing pressures (Wressnig & Booth 2008). Recent shifts in climate conditions have resulted in elevated winter temperatures and reduced extent and duration of ice cover in Northern Europe (Jaagus 2006). Due to the continuous decrease of the ice cover the winter storms are likely to have increasing ecological impacts on shallow water seagrass communities. Such storms involve frequent burial of seagrasses under sediment and/or removal of the seagrass canopy. As a consequence of canopy disappearance the important functions provided by seagrasses are likely lost in coastal ecosystems.

There are many observational studies comparing benthic communities among vegetated and unvegetated seagrass habitats (*e.g.* Boström & Bonsdorff 1997; Turner *et al.* 1999; Nagelkerken & van der Velde 2004; Nakamura & Sano 2005). Without experimenting, however, these studies are unable to distinguish the effects of seagrasses and provide circumstantial evidence on the difference between vegetated and unvegetated habitats. In fact, these differences can be due to factors other than seagrasses. There exist a few experimental studies that describe the structure of seagrass communities but there are virtually no experimental studies that measure a function in relation to the characteristics of seagrass habitat (Davis & Fourqurean 2001; Pereg-Gerk *et al.* 2002). To date the manipulative studies on seagrasses have been carried out in subtropical or tropical ecosystems, but not in temperate ecosystems.

In this study we experimentally evaluated the separate and interactive effects of *Z. marina* canopy removal and sand addition on sediment characteristics and structure of benthic communities. Our hypotheses were as follows: (i) eelgrass decreases oxygen flux through the sediment–water interface, (ii) eelgrass increases the content of organic matter and fine particles in sediment, (iii) removal of eelgrass reduces benthic biodiversity and (iv) sand addition weakens the effects of eelgrass.

## Material and Methods

### Study area

Experiments were performed near Prangli Island (59.610°N, 25.004°E), Gulf of Finland, Northern Baltic

Sea. The average salinity in the area is 6 psu. Sand and gravel dominate, but stand-alone stones are also found in shallow waters. As the Baltic Sea is a nontidal system, the experimental area is constantly submerged and strongly influenced by currents and wave action. The study area has naturally very sorted sediments, *i.e.* medium to coarse sand with very low content of fine sediment fractions and organic matter. The average biomass of the above-ground parts of *Zostera marina* in the experimental area was  $58.0 \pm 11.2$  g dw m<sup>-2</sup>.

### Experimental design

The *in situ* factorial field experiment was carried out from 2 July until 28 August 2006. The 50 × 50 cm experimental plots were placed in a natural *Z. marina* bed at 4 m depth. The experimental design included two manipulative treatments: canopy removal and sediment addition. The canopy removal treatment had two levels: presence and absence of eelgrass canopy. The treatment without eelgrass canopy was established by manually removing all above-ground parts of eelgrass prior to experiment. Additionally, new shoots were systematically removed in each sampling occasion. We did not attempt to remove roots as this would disrupt too much sediment structure and associated infauna and would not happen in nature. The disappearance of canopy, however, is a very likely event at elevated eutrophication (low light levels) and/or grazing pressure. The treatment of sediment addition also had two levels: sediment was either added or not added. The treatment was established by adding a 2-cm layer of natural seabed sediment to the experimental plots. Prior to the experiment the sediment was sun-dried for several days to ensure that it was free of living benthic invertebrates. It was also visually checked that the sediment was devoid of living animals. This 2 × 2 factorial design was replicated three times, resulting in a total of 12 experimental plots.

### Sampling and processing

Oxygen flux through sediment–water interface, sediment granulometry and sediment organic matter content was measured four times: immediately after setting up the experimental plots on 2 July and then on 15 July, and 7 and 28 August. Samples for benthic invertebrates and macrophytes were collected only at the end of the experiment (*i.e.* on 28 August). Transparent acrylic cores (3.6 cm inner diameter, 27 cm length) with rubber stoppers for both ends were used to measure oxygen flux and to sample sediment for granulometric and organic content analyses. Oxygen flux through the sediment–water interface was studied in light. The cores were inserted

into sediment to a depth of 10 cm and the upper ends of the cores were closed with rubber stoppers. As seagrass cover was always below 100% we could avoid plant leaves in the cores. Thus, the design allowed a direct comparison of oxygen flux between treatments without having to correct for the relative amount of seagrass leaves. One core was inserted into each experimental plot. Oxygen concentrations in the cores were measured polarographically before and after a 2.5-h incubation using a WTW OXI 92 oxygen meter. The cores were taken out from the sediment after incubation, retaining the sediment inside the core for further analyses of the content of organic matter and sediment granulometry. The sediment samples were packed into plastic jars and kept in deep freezer ( $-18^{\circ}\text{C}$ ). In the laboratory the sediment samples were melted, homogenized and subsamples for the analyses of organic matter content and granulometry were taken from each sample. The organic matter content was measured as a percentage loss of weight on ignition ( $500^{\circ}\text{C}$ , 3 h) of dry sediment ( $60^{\circ}\text{C}$ , 7 days). For granulometric analysis, dry sediment ( $60^{\circ}\text{C}$ , 7 days) was sieved through a set of sieves of decreasing mesh size (2000, 1000, 500, 250, 100,  $63\ \mu\text{m}$ ) stacked vertically and placed in a vibratory sieve shaker. The sediment retained in each sieve and the sediment penetrating the  $63\text{-}\mu\text{m}$  sieve were weighed (precision of 0.001 g). A core of 10.3 cm diameter was used for macrobenthos sampling. One sample was taken from each plot. Benthos samples were sieved in the field on 0.25-mm mesh screens. The samples were stored in a deep freezer ( $-18^{\circ}\text{C}$ ). In each sample, all macroscopic plants and animals were picked out and identified to the species level except for juvenile gammarid amphipods, chironomid larvae and oligochaetes. Dry biomass ( $60^{\circ}\text{C}$  for 2 weeks) of each plant taxon and dry biomass and abundance of each animal taxon were estimated (precision of 0.0001 g).

### Statistical methods

Two-way factorial ANOVA (StatSoft Inc. 2007) with canopy removal and sediment addition as factors was used to assess differences in oxygen flux, organic matter content of sediment, total abundance, biomass and species richness of macrobenthos separately by each sampling date. Pooled data of sediment granulometry (mass percentage of each grain size group) from sampling dates 2–4 were analysed using two-way factorial ANOVA with the eelgrass canopy removal and sediment addition as factors. PERMANOVA (Anderson *et al.* 2008) was used to test for differences in the abundance and biomass structure of benthic invertebrates and in the biomass structure of benthic macrophytes among the studied treatments. Data was fourth root-transformed prior to running PERMANOVA

to down weight the dominant species and increase the contribution of rarer species in the multivariate analysis (Anderson *et al.* 2008). Non-metric multidimensional scaling (MDS; Clarke & Warwick 2001) on fourth root-transformed data was used to visualize differences in the structure of benthos.

## Results

### Sediment characteristics

Oxygen measurements showed that all incubated sediments consumed oxygen (Table 1). The oxygen consumption varied between 28 and  $198\ \text{mg m}^{-2}\ \text{h}^{-1}$  with an average of  $87\ \text{mg m}^{-2}\ \text{h}^{-1}$ . The plots without eelgrass canopy consumed significantly more oxygen ( $81\ \text{mg m}^{-2}\ \text{h}^{-1}$ ) than those with eelgrass canopy ( $51\ \text{mg m}^{-2}\ \text{h}^{-1}$ ) but significant differences were observed only in the third sampling (Tables 1 and 2). The effects of sediment addition and interaction between canopy removal and sediment addition were not statistically significant (Table 2).

Similarly to the oxygen flux, the content of organic matter in sediment significantly differed between plots with eelgrass canopy (0.57%) and without eelgrass canopy (0.46%) on the third sampling occasion. No other significant differences were found (Table 3).

As compared to the plots without eelgrass canopy, the plots with eelgrass canopy were characterized by a significantly higher proportion of fine particles ( $<63\ \mu\text{m}$ : 1.29% *versus* 1.80%;  $63\text{--}100\ \mu\text{m}$ : 11.64% *versus* 14.15%) and lower proportion of sand fraction ( $250\text{--}500\ \mu\text{m}$ : 9.07% *versus* 7.05%) (Table 4). The proportion of fine particles ( $<100\ \mu\text{m}$ ) was higher on the plots with added sediment (15.10%) than on the control plots (13.77%). The share of sand fraction ( $250\text{--}1000\ \mu\text{m}$ ) was lower on the plots with added sediment (11.73%) than on the control plots (13.83%). These effects were strongest in the presence of eelgrass canopy.

### Benthic community

A total of 17 invertebrate taxa and seven macrophyte species were identified in benthos samples. PERMANOVA tests revealed that there were significant differences in the abundance and biomass structure of zoobenthos and biomass structure of phytobenthos between plots with and without eelgrass canopy (Table 5, Fig. 1). Sediment addition and the interactive effects of canopy removal and sediment addition were not statistically significant. The plots with removed canopy were mainly characterized by a significantly lower density of *Gammarus* spp., *Idotea balthica*, *Corophium volutator*, and *Hydrobia ulvae* (see

**Table 1.** Mean values and standard errors (SE) on oxygen flux through sediment–water interface, content of organic matter in sediment and sediment grain size at different treatment levels.

Date	Treatment		Oxygen flux (mg m <sup>-2</sup> h <sup>-1</sup> ± SE)	Organic content (% ± SE)	Sediment grain size (% ± SE)						
	Canopy	Sediment			<63 μm	63–100 μm	100–250 μm	250–500 μm	500–1000 μm	1000–2000 μm	>2000 μm
07/02	removed	not added	83.1 ± 29.4	0.63 ± 0.03	1.29 ± 0.09	12.88 ± 0.75	59.13 ± 1.96	9.58 ± 0.65	4.52 ± 0.25	2.85 ± 0.05	9.74 ± 2.57
07/02	removed	added	137.7 ± 14.3	0.6 ± 0.05	1.44 ± 0.14	12.69 ± 1.21	52.53 ± 0.67	8.16 ± 0.58	4.62 ± 0.34	3.13 ± 0.23	17.43 ± 0.72
07/02	not removed	not added	123.7 ± 38.8	0.54 ± 0.01	1.25 ± 0.29	11.64 ± 0.56	49.86 ± 0.73	9.03 ± 0.49	6.24 ± 0.74	3.27 ± 0.22	18.71 ± 2.18
07/02	not removed	added	111.7 ± 23.1	0.72 ± 0.08	1.73 ± 0.14	12.94 ± 1.28	49.25 ± 3.67	6.73 ± 0.95	4.37 ± 0.36	2.88 ± 0.3	22.1 ± 5.73
07/15	removed	not added	94.3 ± 8.2	0.6 ± 0.04	1.26 ± 0.09	11.99 ± 0.7	55.12 ± 1.6	10.38 ± 0.23	5.28 ± 0.11	2.72 ± 0.33	13.26 ± 2
07/15	removed	added	86.7 ± 25.3	0.59 ± 0.14	1.31 ± 0.25	11.62 ± 2.12	48.48 ± 5.53	8.03 ± 0.55	4.58 ± 0.35	2.87 ± 0.48	23.11 ± 8.11
07/15	not removed	not added	102.2 ± 8	0.6 ± 0.02	1.64 ± 0.28	13.33 ± 0.36	52.86 ± 1.39	8.33 ± 1.07	5.44 ± 0.59	3.49 ± 0.46	14.92 ± 1.54
07/15	not removed	added	51.4 ± 15.8	0.58 ± 0.03	1.81 ± 0.12	13.23 ± 0.64	50.61 ± 2.42	6.32 ± 0.37	4.04 ± 0.51	3 ± 0.65	20.99 ± 4.49
08/07	removed	not added	94.1 ± 20.1	0.43 ± 0.02	1.21 ± 0.06	11.69 ± 0.58	50.17 ± 3.53	8.51 ± 0.8	3.95 ± 0.35	2.4 ± 0.47	22.07 ± 5.24
08/07	removed	added	68.3 ± 7.9	0.49 ± 0.06	1.3 ± 0.15	11.44 ± 1.06	49.22 ± 4.18	8.81 ± 0.57	5.1 ± 0.36	3.88 ± 0.98	20.24 ± 4.53
08/07	not removed	not added	50.4 ± 8.2	0.55 ± 0.06	1.73 ± 0.22	13.91 ± 0.73	54.63 ± 1.48	8.84 ± 0.5	5.8 ± 0.82	3.37 ± 0.55	11.72 ± 3.55
08/07	not removed	added	51.6 ± 1.6	0.6 ± 0.04	1.89 ± 0.04	15.14 ± 0.4	53.54 ± 2.4	5.39 ± 0.28	3.13 ± 0.16	2.12 ± 0.09	18.8 ± 2.45
08/28	removed	not added	74.2 ± 9.4	0.47 ± 0.03	1.17 ± 0.1	10.75 ± 0.71	52.26 ± 2	9.08 ± 1.06	5.21 ± 1.08	3.26 ± 0.37	18.27 ± 1.2
08/28	removed	added	53.6 ± 5.7	0.85 ± 0.42	1.46 ± 0.04	12.35 ± 0.38	50.93 ± 3.01	9.59 ± 0.75	5.71 ± 0.4	3.37 ± 0.38	16.6 ± 3.47
08/28	not removed	not added	86.8 ± 27.1	0.57 ± 0.1	1.64 ± 0.12	13.04 ± 0.54	52.14 ± 2.37	7.96 ± 0.5	5.06 ± 1.15	3.42 ± 0.52	16.74 ± 0.87
08/28	not removed	added	105.1 ± 27.3	0.61 ± 0.01	2.1 ± 0.1	16.25 ± 1.03	55.36 ± 4.37	5.48 ± 0.34	3.36 ± 0.21	2.17 ± 0.04	15.28 ± 5.07

**Table 2.** Two-way ANOVA of sediment oxygen flux by each sampling date. Significant P-values are indicated in bold.

Date	Canopy removal		Sediment addition		Canopy × Sediment	
	F	P	F	P	F	P
2/7/2006	0.0686	0.8000	0.5839	0.4667	1.4280	0.2663
15/7/2006	0.7226	0.4200	2.3751	0.1618	2.6152	0.1445
7/8/2006	6.8324	<b>0.0309</b>	1.1309	0.3186	1.3623	0.2768
28/8/2006	2.5713	0.1475	0.0034	0.9549	0.9515	0.3579

**Table 3.** Two-way ANOVA of sediment organic matter content by each sampling date. Significant P-values are indicated in bold.

Date	Canopy removal		Sediment addition		Canopy × Sediment	
	F	P	F	P	F	P
2/7/2006	0.1132	0.7452	2.4684	0.1548	4.7696	0.0605
15/7/2006	0.0032	0.9559	0.3158	0.5895	0.7834	0.4019
7/8/2006	5.5276	<b>0.0466</b>	1.4174	0.2680	0.0337	0.8590
28/8/2006	0.1115	0.7470	0.9956	0.3476	0.6194	0.4540

**Table 4.** Two-way ANOVA of sediment grain size groups (mass percentage data) from sampling dates 2–4. Significant P-values are indicated in bold.

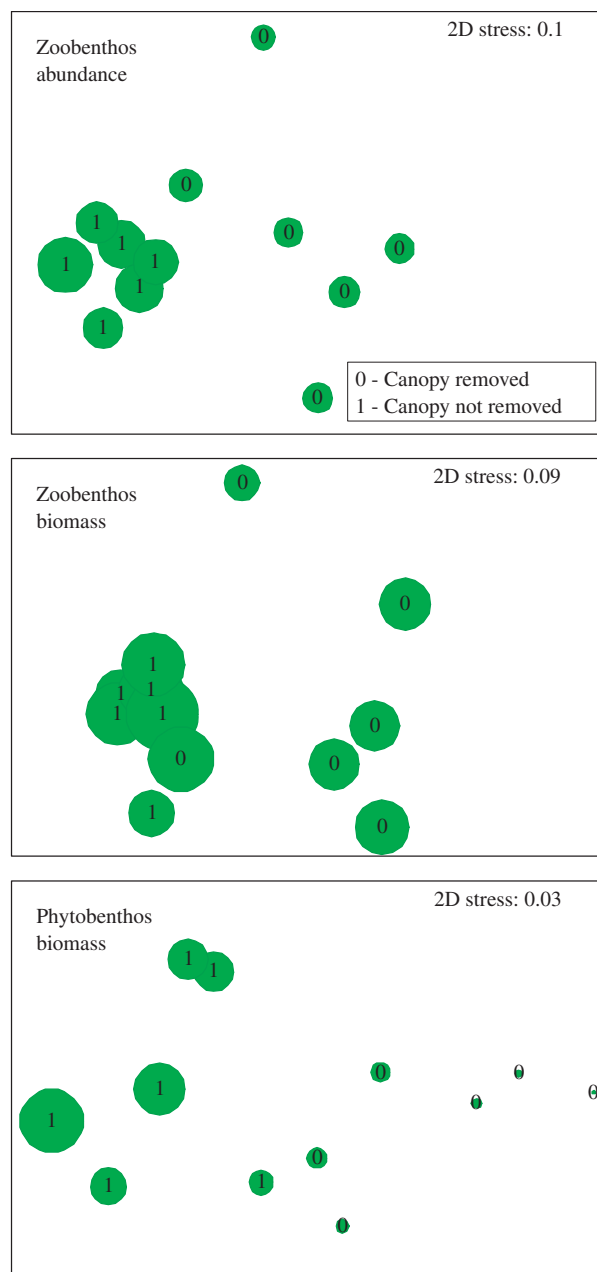
Grain size	Canopy removal		Sediment addition		Canopy × Sediment	
	F	P	F	P	F	P
<63 $\mu\text{m}$	41.0441	<b>&lt;0.0001</b>	7.3704	<b>0.0106</b>	0.3673	0.5487
63–100 $\mu\text{m}$	24.2301	<b>&lt;0.0001</b>	4.7397	<b>0.0370</b>	0.4376	0.5130
100–250 $\mu\text{m}$	1.6209	0.2121	0.0422	0.8386	0.0332	0.8566
250–500 $\mu\text{m}$	27.1953	<b>&lt;0.0001</b>	11.0409	<b>0.0022</b>	12.5157	<b>0.0013</b>
500–1000 $\mu\text{m}$	2.1394	0.1533	5.7887	<b>0.0221</b>	10.2794	<b>0.0030</b>
1000–2000 $\mu\text{m}$	0.2830	0.5984	1.8631	0.1818	4.5245	<b>0.0412</b>
>2000 $\mu\text{m}$	1.2335	0.2750	0.4481	0.5080	1.1002	0.3021

**Table 5.** PERMANOVA of benthic community structure at the end of the experiment. Significant P-values are indicated in bold.

	Canopy removal		Sediment addition		Canopy × Sediment	
	F	P	F	P	F	P
Zoobenthos abundance	6.5193	<b>0.0026</b>	0.7209	0.5350	1.3926	0.2763
Zoobenthos biomass	4.7689	<b>0.0137</b>	0.8505	0.4669	1.3710	0.2753
Phytobenthos biomass	12.3710	<b>0.0016</b>	0.6548	0.5733	1.2348	0.3113

Fig. 2 for details). The removal of eelgrass decreased the biomass of all phytobenthic species but none of these differences was statistically significant.

Removal of the canopy significantly decreased the total abundance and species number of zoobenthos and total biomass and species richness of phytobenthos (excluding

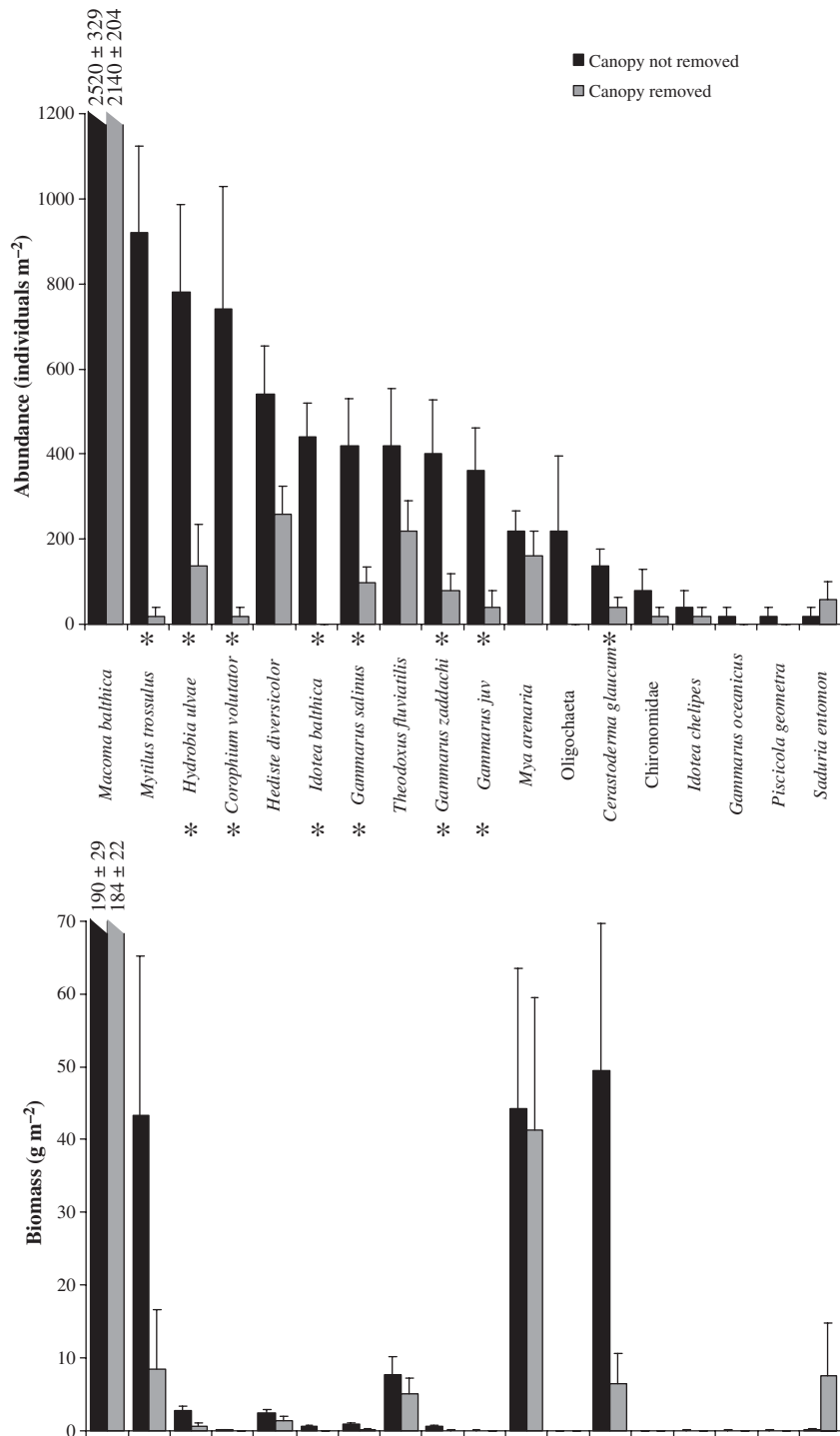
**Fig. 1.** MDS ordination of zoobenthos abundance and biomass structure and phytobenthos biomass structure. The number shows the factor level and the relative size of the bubbles indicates the total abundance or biomass of benthic organisms in a sample.

*Z. marina*) (Table 6). The sediment addition did not significantly affect the biomass of *Z. marina*.

## Discussion

In this study we showed that (i) eelgrass canopy had moderate effects on sediment structure and metabolism, and (ii) benthic invertebrates and macroalgae were





**Fig. 2.** Macrozoobenthic community abundances (number of individuals m<sup>-2</sup> ± SE; upper graph) and biomasses (g m<sup>-2</sup> ± SE; lower graph) of the experimental plots with the canopy of *Zostera marina* removed and the canopy of *Zostera marina* not removed. Asterisk denotes significant differences in ANOVA analyses (P < 0.05).

affected by eelgrass canopy removal but not by sediment addition. *Zostera marina* decreased the oxygen flux through the sediment–water interface on the third sam-

pling. During the third sampling higher seawater temperatures were measured compared to other sampling periods and this may explain the significant differences in

**Table 6.** Two-way ANOVA of benthic community characteristics at the end of the experiment. Significant P-values are shown in bold.

	Canopy removal		Sediment addition		Canopy × Sediment	
	F	P	F	P	F	P
Total abundance of zoobenthos	30.3035	<b>0.0006</b>	0.2586	0.6248	0.4697	0.5125
Total biomass of zoobenthos	2.7208	0.1377	1.5953	0.2421	2.8320	0.1309
Number of zoobenthos species	29.4694	<b>0.0006</b>	0.0816	0.7824	0.3265	0.5834
Total biomass of phytobenthos	12.3267	<b>0.0080</b>	0.7171	0.4217	0.9384	0.3611
Number of phytobenthos species	9.0000	<b>0.0171</b>	0.0000	1.0000	0.0000	1.0000

oxygen demand. The relatively low contribution of eelgrass to the oxygen flux agrees with previous results of Pedersen *et al.* (1998) and Frederiksen & Glud (2006) who found that *Cymodocea rotundata* contributed only 1% and *Z. marina* 12% of the total oxygen flux.

Similarly to the oxygen flux, eelgrass canopy had a moderate effect on the content of organic matter in sediment. The content of organic matter was higher on plots with eelgrass canopy compared to plots where the canopy was removed. A similar facilitative effect of seagrass on sedimentation was documented for different seagrass species (Agawin & Duarte 2002; Peterson *et al.* 2004; Hendriks *et al.* 2008). We also found that eelgrass significantly increased the sedimentation of fine particles (grain size <100 µm). This result was in accordance with the findings of an intertidal *Z. marina* bed (Bos *et al.* 2007), highlighting the role of eelgrass in the increase of water transparency by trapping fine suspended material.

In our study both univariate and multivariate community measures showed that the eelgrass canopy significantly promoted diversity and increased density of benthic macroalgae and invertebrates. Such a facilitating effect has been reported earlier for seagrass species in the Baltic Sea and elsewhere (Reusch & Chapman 1995; Bostrom & Bonsdorff 1997, 2000; Turner *et al.* 1999; Alfaro 2006). The losses due to the removal of eelgrass canopy can be attributed to the loss of habitat complexity and food. Eelgrass is known to be an important substratum for filamentous algae on soft sediments (Borum 1985) and the removal of eelgrass canopy reduces the biomass of macroalgae and, consequently, causes the decrease in the density of herbivores such as gammarid amphipods and *Idotea balthica*. The removal of eelgrass canopy significantly decreased the abundance of *Mytilus trossulus* in our study. *Zostera marina* is known to facilitate the settle-

ment of mussel larvae and also to provide a more stable habitat for adults (Reusch & Chapman 1995; Bologna *et al.* 2005). The removal of eelgrass canopy also decreased the densities of infaunal deposit feeders *Hediste diversicolor* and *Corophium volutator*, which can be attributed to the lower sedimentation rate of suspended matter and algal debris.

Sand addition had moderate effects on the sediment granulometry but no effects on oxygen flux through sediment–water interface and seagrass community structure. Similarly, no consistent interactive effects between seagrass canopy removal and sediment addition were found. The lack of significant effects might be attributed to naturally strong wave disturbance in our study area, *i.e.* the seagrass community is likely adapted to high sediment mobility.

To conclude, our experiment demonstrates that *Z. marina* defines the patterns of benthic macroalgae and invertebrates but has moderate effects on sediment granulometry, organic matter content and oxygen flux through the sediment–water interface. However, intensified sediment dynamics such as the burial of seagrasses under sand has no clear effects on the structure and functioning of seagrass communities in the Northern Baltic Sea. The results are also in agreement with earlier observational studies that compared the structure of benthic communities in vegetated and unvegetated seagrass habitats. All this suggests that *Z. marina* may survive moderate physical disturbances and counteract the effect of eutrophication by increasing sedimentation of fine particles, adding oxygen to the sediment, and providing habitat for many infaunal and epifaunal species.

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## ORIGINAL ARTICLE

**Altering intertidal sediment topography: effects on biodiversity and ecosystem functioning**

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**Keywords**

Dee Estuary; functional traits; intertidal; nutrients; porewater.

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E-mail: diane.jones@liv.ac.uk**Conflicts of interest**

The authors declare no conflicts of interest.

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**Abstract**

Sedimentary marine systems are often highly productive and perform important nutrient regeneration functions as they efficiently decompose organic material. In recent years the role of habitat effects and of species composition in ecosystem functioning has become of interest. Estuarine environments are frequently subject to considerable anthropogenic pressures whilst supporting a variety of habitats ranging from well sorted soft muds through biogenically stable sediments to highly mobile coarse sands. There is therefore considerable spatial complexity in habitat type and faunal composition. This study set out to observe the effects of altering the topographical habitat features of an estuarine mudflat on a range of porewater nutrient concentrations ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$  and SiO) collected from four depths (3, 5, 9, and 12 cm) and on faunal composition. Two treatments (Shelled Nets and Net Controls) were used to alter the topography from simple mud to a mussel shell crumble and were compared to un-manipulated Control areas. Sediment granulometry and organic matter content analyses alongside biological traits analysis of the fauna were also conducted.

Differences were observed in porewater nutrient concentrations between the Control and both netted treatments at 5 cm depth only; the species diversity and abundance were also different in the netted treatments compared to the Controls, although no difference between the two manipulated treatments were observed. The changes in faunal composition were attributed solely to the altered topography and the observed nutrient changes were attributed to the faunal alteration rather than the topographical manipulation.

**Introduction**

Recent concern over the alteration and degradation of the environment has led to speculation about the consequences for the provision of ecosystem services (*i.e.* ecological processes with a value to humanity such as climate regulation) and those ecological functions (*e.g.* nutrient regeneration) that directly or indirectly underpin these services (*e.g.* see Bolger 2001; Naeem 2002; Giller *et al.* 2004; Hooper *et al.* 2005 and references therein; Stachowicz *et al.* 2007). Ecosystem functions result from the biological, chemical and physical processes carried out by the species inhabiting an area (Naeem *et al.* 1994) and

they are determined by the life habits of these species. Life habits (also known as functional traits) can range from mode of locomotion, trophic group to the type of reproductive strategy exhibited.

Several theories have been proposed to explain the relationship between biodiversity and ecosystem functioning (Ehrlich & Ehrlich 1981; Lawton *et al.* 1993; Mooney *et al.* 1995; Naeem 2002). The redundant species hypothesis recognizes that species contribute to a number of 'ecological functions' and different species contribute different mixes of functions, overlapping and giving the system some redundancy. The idiosyncratic hypothesis argues that as individual species roles are varied and complex,



the effects on ecosystem functions of the loss of any one species are unpredictable (Naeem 2002; Solan *et al.* 2004). Therefore consideration must be given to the multiple roles of each species to understand their relationship with ecosystem functioning.

Several studies have found that reduced species diversity impaired the ability of an ecosystem to provide certain services (Naeem *et al.* 1994; Emmerson *et al.* 2001) and that changes in functional diversity have a greater impact on ecosystem services than changes in species diversity, although both have an effect (Tilman *et al.* 1997). More recently, research has focused on the composition of functional traits in an assemblage and how these traits affect ecosystem functions and services. Studies have been concerned with the definition of traits and their effects on the environment (Bremner *et al.* 2003; Welsh 2003; Wolanski *et al.* 2004), how the composition of traits within an area alters after a disturbance and the subsequent effects on ecosystem functioning and delivery of services (Tillin *et al.* 2006; De Juan *et al.* 2007; Savage *et al.* 2007) and the effect of different species classified with the same functional trait(s) on an ecosystem function, *e.g.* whether two 'bioturbators' affect nutrient cycling in the same way (Matisoff & Wang 1998; François *et al.* 1999; Michaud *et al.* 2005, 2006). The latter studies have found that species classified by their functional traits as biodiffusers, but inhabiting different depths in the sediment, affect the depth penetration of oxygenated water differently, thus affecting nutrient cycling in different ways (*e.g.* Michaud *et al.* 2006; Karlson 2007), indicating that species identity may be just as important as the traits they represent.

Within the marine environment most studies have been conducted using intertidal estuarine species (*e.g.* Banta *et al.* 1999; Christensen *et al.* 2000; Emmerson *et al.* 2001) as the species assemblages are typically small (10–20 species), the fauna have been relatively well studied compared to many sub-tidal species and they are usually present in large numbers over a small spatial range, allowing sufficient specimens to be collected for laboratory studies.

The effects of nutrient regeneration and flux within intertidal areas due to diffusive and advective porewater flows, along with changes to oxygen depth penetration, have been studied with regard to both biological and physical factors (Huettel & Gust 1992; Aller & Aller 1998; Kristensen 2000; Kuwae *et al.* 2003, 2006; Mermillod-Blondin *et al.* 2004; Billerbeck *et al.* 2006). Biological factors such as polychaete tubes, burrowing and bio-irrigation by infauna affect nutrient regeneration by altering porewater flow (Huettel & Webster 2001) and the sediment surface area available for oxygen exchange (Mortimer *et al.* 1999; Aller 2001). Physical factors investigated have

included observing the effects of varying topographical features on porewater movement and oxygen penetration (Ziebis *et al.* 1996; Huettel & Webster 2001; Billerbeck *et al.* 2006). It was found that singular protrusions as little as 700  $\mu\text{m}$  from the sediment surface can cause increased advective porewater flow at overlying water flows of  $3 \text{ cm s}^{-1}$ , pulling porewater from deep within to the surface sediments (Huettel & Gust 1992) and increasing oxygen penetration twofold in muddy, low permeability sediments (Ziebis *et al.* 1996).

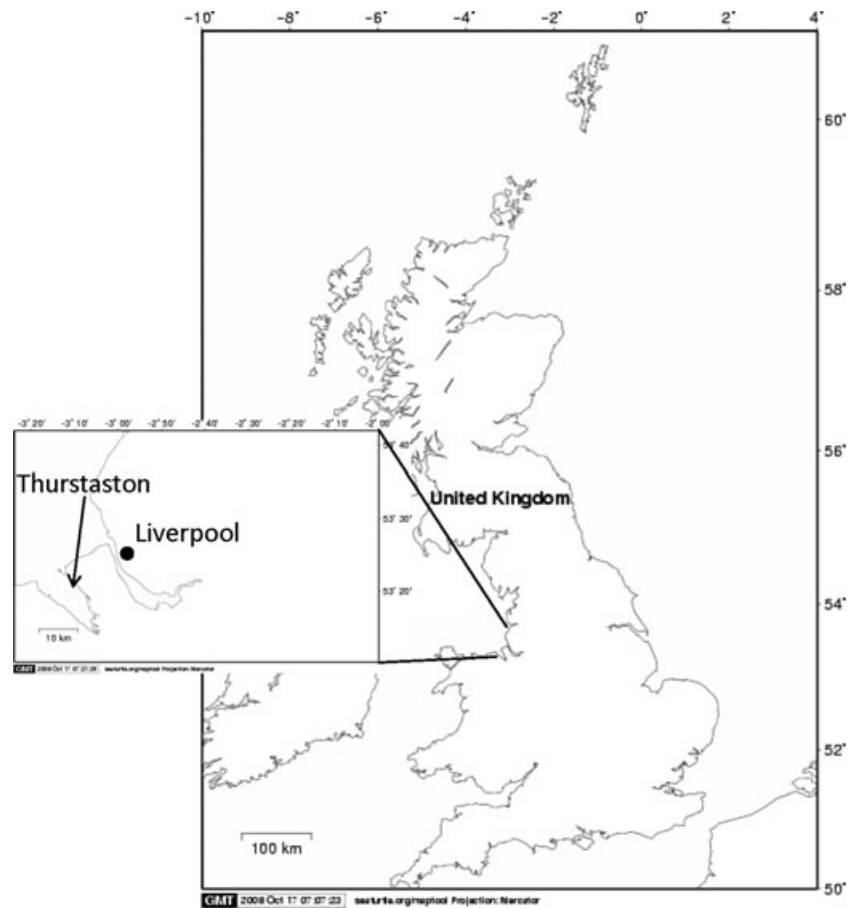
The effects of large species that are also habitat modifiers (*e.g.* *Cerastoderma edule* Linnaeus) have been investigated with regard to nutrient flux and impact on faunal communities (Rossi *et al.* 2008; Cesar & Frid 2009), as have the effects of mussel aggregations and macroalgal growth on infauna (*e.g.* Günther 1996; Raffaelli 2000; Jones & Pinn 2006). The influence of substratum heterogeneity on epibenthic community structure (Bourget *et al.* 1994) and the effects of polychaete tubes on infaunal communities and overlying water flow have been studied (Luckenbach 1986; Friedrichs *et al.* 2000; Callaway 2003). However, the combined effects of topographical variation and subsequent alterations to species composition on nutrient regeneration have not been. As the ecological functions of an area are dependent on the species found within it (Hooper *et al.* 2005), it may be the case that the composition of functional traits and the delivery of ecosystem functions will vary as the species assemblage does.

Due to the nature of estuarine environments a variety of habitats ranging from well sorted soft muds through biogenically stable sediments to highly mobile coarse sands can exist in close proximity to each other. The sedimentary characteristics and the changing effects of tidal flow on these habitats can vary on a small spatial scale, potentially affecting the faunal assemblages present within an area. The present study aims to alter the physical features of an estuarine mudflat area to observe the effects on faunal assemblages, biological trait composition and nutrient regeneration in the sediment to answer the questions:

- 1 Do different micro-habitats in intertidal mudflats contain significantly different faunal assemblages and, if they do, are the trait compositions of the assemblages different?
- 2 If the traits present are different between the two assemblages does this affect nutrient regeneration?

## Method

On the Dee Estuary, at Tinkers Dell Steps ( $53^{\circ}20'16''\text{N}$ ,  $03^{\circ}08'31''\text{W}$ ) (Fig. 1), patches of poorly mixed shell debris and muddy sand, termed 'crumble', occur adjacent to areas of firm, muddy sand. The 'muddy sand', had little



**Fig. 1.** Geographical location of Thurstaston (53°20'16"N, 03°08'31"W) study site in relation to Liverpool, North-West England. Map created using MAPTOOL, <http://www.seaturtle.org>.

surface relief, without obvious stones or shell debris consisting of an average 50% sand (63–150  $\mu\text{m}$ ), 40% silt (<63  $\mu\text{m}$ ) content and little coarse material ( $\sim 1\% \geq 1000 \mu\text{m}$ ). In contrast the crumble sediment consisted of 25% sand, 36% silt content with a larger percentage of coarse material ( $\sim 16\% \geq 1000 \mu\text{m}$ ) comprising stones (mostly encrusted with barnacles), shell debris (mostly mussel shells) and byssus threads from live mussels, creating a rough surface. The faunal assemblage of the muddy sand was characterized by a large abundance of small crustaceans (*Corophium* sp.), spionid polychaetes and species which show a preference for sandy sediments over silty ones (*i.e.* *Owenia fusiformis* Delle Chiaje). The crumble fauna, in contrast, contained surface dwelling bivalves, their associated epifauna, a higher abundance of oligochaetes and several opportunistic deposit-feeding taxa (*i.e.* *Capitella* sp. Fabricius). Organic matter content, determined by loss on ignition, for the two sediments averaged 1% for the muddy sand and 2% for the crumble. These two areas of differing topography provided a 'natural' experimental site in which to compare the influence of boundary layer topography on benthic assemblage composition and ecosystem functioning. Nutrient concentra-

tions ( $\text{mg L}^{-1}$ ) were used as a proxy of nutrient regeneration in this study, as it would be expected that concentrations of nutrients in the sediment would increase as regeneration increased. The investigation was carried out from 15 August 2006 until 15 November 2006.

To isolate the influence of the physical environment on ecosystem functioning, the muddy sand surface was manipulated to mimic the crumble using PVC mesh nets in  $1 \times 1 \text{ m}$  plots. Five nets were produced composed of two layers of mesh, a 50 mm mesh bottom layer and a 20 mm mesh top layer. The 20 mm mesh was cut into  $25 \times 25 \text{ cm}$  panels and empty mussel shells were attached to this using an epoxy resin. Sixteen of these panels (giving  $228 \pm 3.38 \text{ m}^{-2}$  mussel shell density) were attached to the larger 50 mm mesh using cable ties to create an imitation of the crumble surface. Five  $1 \times 1 \text{ m}$  nets were also created in the same manner but without attached shells, to control for any netting effect. On each net, nine holes were cut in the 50 mm mesh, at the intersections of the smaller mesh panels, to allow for subsequent porewater sampling. These were randomly allocated to one of two groups of four; group one was used on the 1st, 3rd and

5th sampling occasion and group two on the 2nd, 4th and 6th, leaving one hole spare for sediment sampling at the conclusion of the study. On 15 August 2006, 15 plots were marked out and each was randomly assigned one of the three treatments [five shelled nets (treatment), five blank nets (net control) and five sediment plots (control)]. The nets were secured in place with 30 cm plastic pegs at each corner.

Using a modified Poreextractor (see Nayar *et al.* 2006 for operational details) sampling of sediment porewaters was conducted, the shallowest depth attainable without surface water contamination being 3 cm. Porewater sampling began on 29 August 2006, 2 weeks after initial set-up, when samples from four depths (3, 5, 9 and 12 cm) were obtained for nutrient analysis. On each sampling occasion a Poreextractor (one for each depth) was inserted into the sediment through one of the pre-cut holes in the mesh. Sampling of porewater was repeated on a fortnightly basis during the neap tide cycle and the samplers were deployed ~3 h after high tide (when the site first became exposed).

The Poreextractors were set according to the method described in Nayar *et al.* (2006), a vacuum was applied using a syringe and the membrane pierced using a plastic rod, after which the device was left in the sediment for 1 h to allow the flow of porewater into the sampling chamber. Once this time had elapsed a plastic pipette was pushed through the membrane and water from the sampling chamber was collected using a 20 ml syringe. For each sample a minimum extraction of 5 ml of water from the sediment was attempted; where more water was available the syringes were filled to capacity. The syringes were placed in cool-boxes and kept in the cold and dark for later filtration and analysis; samples were always returned to the laboratory and filtered within 4 h of collection.

Water samples were filtered using 25  $\mu\text{m}$  Millipore syringe filters and analysed using standard colorimetric methods for ammonia ( $\text{NH}_4^+$ ), total N (nitrate/nitrite), silicate ( $\text{SiO}_2$ ) and phosphate ( $\text{PO}_4^{3-}$ ) on a Bran+Lubbe AA3 continuous flow Autoanalyser. A minimum 1 ml of water (after filtration) was required for high-range nutrient analysis by the Autoanalyser, and 5 ml was required for low-range analysis. For separate nitrate/nitrite analyses, twice this amount (*i.e.* 2 or 10 ml) was required, as the Autoanalyser consisted of a combined nitrate/nitrite channel and separate analyses would be required to obtain data for each. Samples for ammonia analysis were occasionally diluted 1:2 as the levels detected were over-range for the colorimetric method utilized by the Autoanalyser (4 mg  $\text{L}^{-1}$  maximum detectable limit).

Porewater collection was completed on 15 November 2006 and the nets were collected from the shore on 29th November 2006, at which time core samples for

faunal analysis and sediment granulometry were also obtained.

Three sediment samples per plot were obtained from the area underneath the netting using a 0.01  $\text{m}^2$  diameter corer for faunal analysis. The samples were returned to the laboratory where they were washed through a 0.5 mm sieve and fixed in 4% buffered formaldehyde solution. Fauna were extracted and enumerated by taxa, generally species, although some problematic groups were not identified to species level.

The nets were placed in plastic bags on site and returned to the laboratory where accumulated surface sediment was washed off, collected, sieved and preserved using the same method as for the infauna samples. Any fauna that had attached to the netting were collected at this time and added to the overall surface net sample. After allowing the fauna to be fixed the samples were again washed through a series of sieves. Any fauna larger than 20 mm (*e.g.* *Cerastoderma edule*) were retained for identification as this was the minimum mesh size of the experimental nets. Those fauna recognized as surface dwellers (*i.e.* littorinids) or that had been attached to the netting were also retained.

Sediment samples were collected on 29 November for particle size analysis (PSA) and loss on ignition (LOI) using a small (3 cm i.d., 9 cm length) core. The samples were frozen on return to the laboratory for later analysis. When thawed the samples were wet weighed, dried at 60 °C for 24 h and re-weighed to ascertain the water content. Following the method described in Eleftheriou & McIntyre (2005), PSA and LOI were carried out on the dried sediment. For LOI approximately 7 g ( $\pm 1.27$ ) of the dried sediment was homogenized using a pestle and mortar and placed in a pre-weighed crucible before being weighed again. The crucibles were then fired in a muffle furnace at 450 °C for 5 h after which time they were re-weighed. The remainder of the dried sediment was weighed and then passed through a series of graduated sieves (63  $\mu\text{m}$ –2000 mm) on a sieve shaker for 15 min and each fraction weighed for the PSA.

#### Data analysis

Faunal data were collated and the average abundance per experimental plot (core sample data and net surface data) was calculated for final analysis, thus avoiding pseudo-replication (Hurlbert 1984). Bray–Curtis similarity was calculated on square root-transformed abundance data in the statistical package PRIMER (v6). This reduces the influence of the dominant taxa and allows variation in the rarer taxa to influence the pattern. Following examination of the resultant cluster analysis, ANOSIM and SIMPER tests were also carried out.

Biological traits analysis (BTA) was carried out on the combined faunal data from the core and net surface samples using five biological traits that relate to processes affecting nutrient regeneration. Although the inclusion of more traits would provide additional information on ecosystem functioning (Bremner *et al.* 2006), the traits used were chosen as potential factors relating to nutrient regeneration effects of the fauna. Three of these represented behaviour (bioturbation type, feeding type and depth found in sediment) and the remaining two represented life history (longevity and body size). The five traits were further sub-divided into several modalities to better represent the spread of variation for each, e.g., bioturbation was separated into biodiffuser, gallery-diffuser, regenerator, upward-conveyor, downward-conveyor and bio-irrigator (Table 1).

Fuzzy coding (Chevenet *et al.* 1994), with a scoring range of 0–3, was used to code individual taxa for the degree to which they exhibited the different modalities of each trait. No affinity for a trait was coded as 0 and complete affinity as 3, e.g. *Corophium volutator* Pallas can be a deposit-feeder but also actively suspension-feed, and so were coded 2 (Deposit-feeder) and 1 (Suspension-feeder) and 0 for the remaining modalities within the feeding type trait. Information for each of the biological traits was gathered from the literature where there was a direct reference and was also based on autecology of the taxa and descriptions of particular traits, e.g. *Pygospio elegans* Claparède is a tube-dwelling head-up deposit-feeder, and from the descriptions of bioturbation categories in François *et al.* (1997) this species can be classed as a downward-conveyor (2), due to its feeding method of moving sediment from the surface to deeper areas, and as a biodiffuser (1), as it will cause some passive diffusion of oxygen along its tube-length. Taxa that were not identifiable to genus were not included in the BTA.

Trait modality affinity scores per taxon were multiplied by the abundance of each taxon for every sample and subsequently summed to provide an overall value for each trait modality per sample. The trait by sample table was analysed using nMDS in the statistical package PRIMER (v6). Various transformations were performed on the data (including no transformation) to compare patterns on the nMDS ordinations that were plotted. SIMPER analysis was also carried out on the data to observe the effects of the individual traits.

The nutrient data collection followed a repeated measures design and was analysed using a linear mixed model (LMM) in the statistical package SPSS (v15). LMM were used as some data were missing, the data collected at different times were not independent of each other and the Hyunh-Feldt assumption of sphericity was unlikely to be met (Rowell & Walters 1976). Additionally, although data

**Table 1.** Biological trait variables and modalities used to describe fauna identified in the study.

trait	no.	modality
bioturbation	1	none
	2	biodiffuser fauna which move sediment in a random manner over short distances causing diffusive mixing (François <i>et al.</i> 1997)
	3	gallery diffuser biodiffusion and active, intermittent irrigation of tubes in sediment-containing gallery systems (e.g. those created by <i>Hediste diversicolor</i> ) (François <i>et al.</i> 2002)
	4	regenerator fauna which dig in the sediment, transferring material from depth to the surface (Gardner <i>et al.</i> 1987)
	5	upward-conveyor head-down oriented fauna which cause active movement of sediment from depth to the surface (François <i>et al.</i> 1997)
	6	downward-conveyor Head-up oriented fauna which cause active movement of sediment from the surface to depth through their gut (François <i>et al.</i> 1997)
	7	bio-irrigator fauna which actively irrigate burrows by drawing down surface water past their gills
feeding Mode	1	deposit-feeder
	2	suspension: active
	3	suspension: passive
	4	opportunist/scavenger
	5	grazer
	6	predator
depth	1	surface
	2	0–3 cm
	3	3–8 cm
	4	8–15 cm
	5	15–25 cm
body size (mm)	1	≤5
	2	5 < >10
	3	10 < >20
	4	20 < >0
	5	40 < >80
	6	80 < >160
longevity	1	≤1 year
	2	1–2 years
	3	3–5 years
	4	6–10 years
	5	>10 years

were collected every 2 weeks, the collection dates were not exactly 14 days apart due to tidal variations and therefore the temporal spacing was not equal; LMM can accommodate this inequality (Wang & Goonewardene 2004). Of the 16 covariance structures available in LMM

in SPSS (v15.0) only the seven appropriate to a repeated measures design were retained (see Wang & Goonewardene 2004 for further information). These covariance structures were examined for the best fit using the Bayesian information criteria (BIC) where smaller is better, *i.e.* the covariance structure which returned the smallest BIC was used to analyse the data. Data for each nutrient at each depth were analysed separately using the appropriate covariance structure. Maximum likelihood (ML) estimation was used to run the model, as the analysis contained the fixed effects of treatment, which restricted maximum likelihood (REML) estimation is unable to handle (Diggle 2008; Garson).

As LMM does not carry out contrast analysis to separate out differences between individual treatments, the data were coded to indicate the presence (+) and/or absence (–) of shell and net. This method allowed any statistical differences between the treatments to be apparent and made the assumption that the combination of ‘Shell’ and ‘Net’ (*i.e.* Shelled Net treatment) did not alter any effects that ‘Net’ alone may have had.

## Results

### Environment

Comparison of the sand fraction ( $>63 \mu\text{m}$ , arcsin-transformed) from the experimental plots showed a significant difference between the three treatments (one-way ANOVA  $F = 16.26$ ,  $P = 0.001$ ), with both netted treatments differing from Controls. Overall there was a decrease in the proportion of sand present in the Shelled Net and Net Control treatments and an increase in the proportion of larger sediment particles (Fig. 2), indicating an alteration towards a coarser overall sediment composition in both the netted treatments.

One-way ANOVA of the sand fraction from the crumble and muddy sand (long-term monitoring data) showed no difference between the crumble and either netted treatment, indicating an alteration mimicking that of the crumble. Nor was any difference seen between the Control and muddy sand, indicating the Controls were good representations of the wider muddy habitat. Loss on ignition (LOI) data were not significantly different between the three experimental treatments or in comparison with the LOI data from the long-term monitoring.

### Fauna

A total of 29 taxa were identified from the experimental core samples. Of these, five were found only in Shelled Net treatments (*Streblospio shrubsolii* Buchanan, *Capitella* sp., *Manayunkia aesturina* Bourne, *Paranais littoralis* Mul-

ler and *Elminius modestus* Darwin) and two were found only in Control treatments (*Corophium arenarium* Crawford and *Arenicola marina* Linnaeus). Of the taxa found in the Shelled Net, *Capitella* sp., *M. aesturina* and *P. littoralis* are opportunistic, deposit-feeding annelids not previously recorded from the muddy sand sediment (D. Jones, unpublished data). However, all three taxa have previously been identified from crumble samples, indicating a shift in the taxonomic composition of the Shelled Net treatments towards that of the natural crumble over the 12-week study period.

One-way ANOVA performed on Shannon–Weiner diversity indices indicated that the netted treatments were significantly different to the Controls ( $F = 4.77$ ,  $P = 0.032$ ). Pair-wise comparisons from ANOSIM analysis showed significant differences between the Control/Net Control and the Control/Shelled Net treatments ( $R = 0.881$ ,  $0.548$ , respectively,  $P = 0.8\%$  for both), and nMDS ordination (Fig. 3A), plotted from Bray–Curtis similarities, clearly shows a grouping of the Net Control separate from the Control treatment group. The Shelled Net treatments are scattered to the right of the plot, away from the Controls but around the Net Controls, with no significant difference observed between the two netted treatments.

The treatment effects on the eight taxa most common in muddy sand and crumble (as indicated by long-term monitoring data) were further analysed using Kruskal–Wallis tests (Table 2). Four had significantly different abundances (*Pygospio elegans*,  $H = 9.11$ ,  $P = 0.011$ ; *Eteone longa* agg Fabricius,  $H = 9.54$ ,  $P = 0.008$ ; *Heterochaeta costata* Claparède,  $H = 7.16$ ,  $P = 0.028$ ; *Corophium volutator*,  $H = 10.38$ ,  $P = 0.008$ ); examination of Kruskal–Wallis ranks indicate that the differences observed were between the net treatments and the Controls in every case. The remaining four (*Hydrobia ulvae* Pennant, *Macoma balthica* Linnaeus, *Hediste diversicolor* Müller and Nematoda) were not significantly different. Abundances of the polychaetes *P. elegans* and *E. longa* agg, and the amphipod *C. volutator* were higher in the Control treatment compared to the two net treatments, whereas abundance of the tubificid oligochaete *H. costata* were lower in the Control.

SIMPER analysis showed that differences between the netted treatments and the Controls were attributable to six taxa (5% or greater contribution to dissimilarity), *P. elegans*, *C. volutator*, Nematoda, *H. ulvae*, Collembola and *E. longa* agg. The cumulative percentage difference for these taxa was ~60%, indicating that abundances of these six taxa were having a significant effect on the taxonomic differences observed between the treatments, with *P. elegans* making the greatest contribution (Table 3A,B).



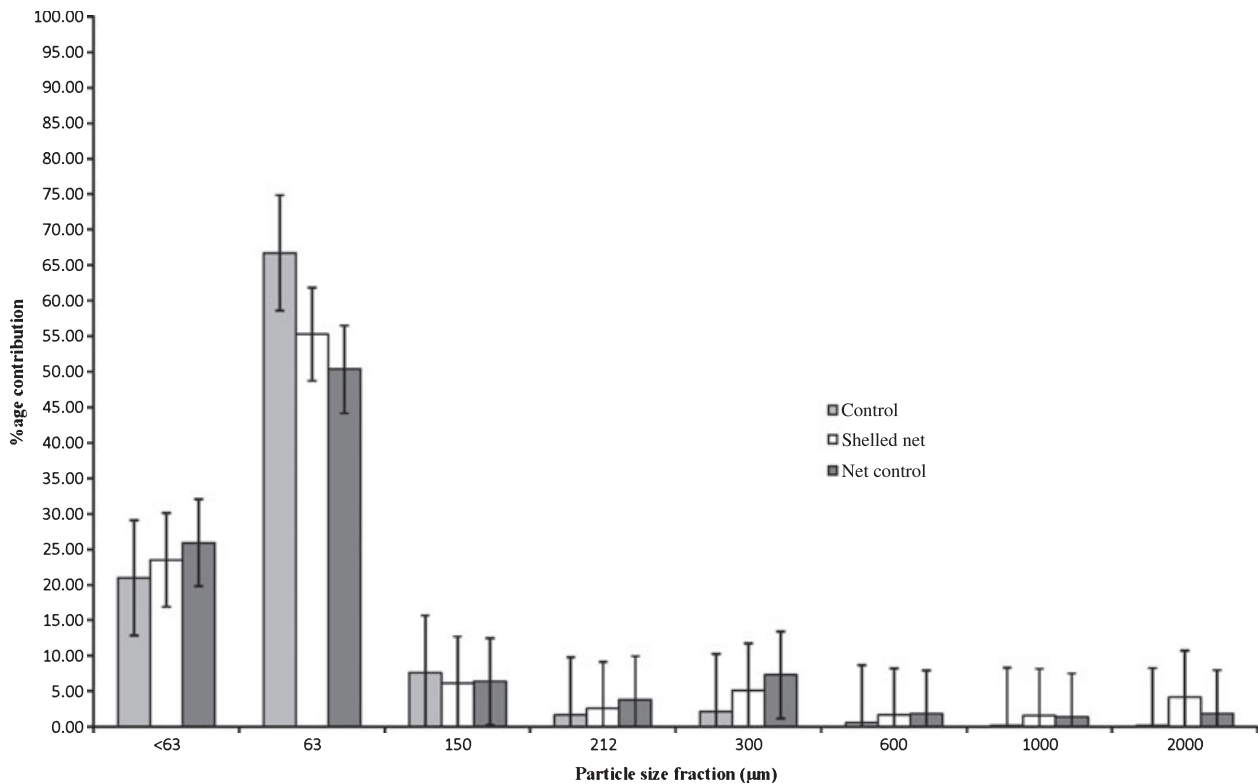


Fig. 2. Sediment particle size analysis for each treatment showing the percentage contribution of sediment to each size fraction.

Nine taxa were identified from the net surface samples. The common mussel *Mytilus edulis* Linnaeus established small aggregations on all net surfaces, although no significant difference between the Net Control and Shelled Net treatments was found for their abundance, or for the abundance of the associated barnacle *Elminius modestus*. Overall abundance of *M. edulis* (combined core sample and net surface) was significantly different between the netted treatments and Controls (Kruskal–Wallis,  $H = 11.33$ ,  $P = 0.003$ ), although no difference was observed for the core data alone. The gastropod *Littorina littorea* Linnaeus was also present in both netted treatments, although no significant difference in abundance was found. This species was not seen in the experimental cores but had been previously identified from the crumble samples.

Overall, several opportunistic taxa common in the crumble appeared only in the Shelled Net treatments and the overall abundance of oligochaete species in the netted treatments increased. There was a decline in the tube-dwelling polychaete *P. elegans* in the netted treatments, with an associated decline of its predator, *E. longa* agg. There was a decline in abundance of the amphipod genus *Corophium*, with *C. volutator* decreasing in number whilst *C. arenarium* disappeared altogether from the netted treatments. Small aggregations of *M. edulis* also appeared on the netted treatments, increasing the overall surface

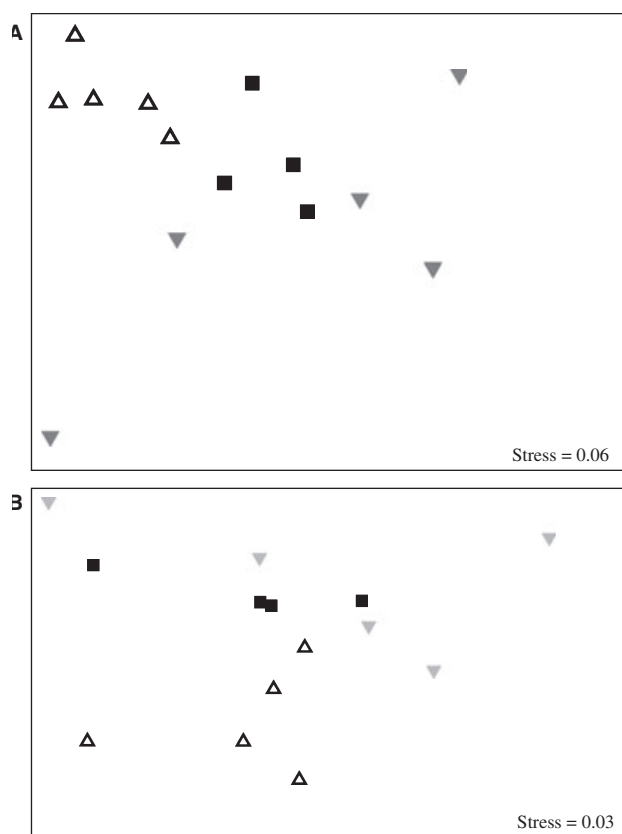
area of the sediment, and providing settlement areas for the barnacle *E. modestus*.

#### Biological traits analysis (BTA)

Of the 29 trait modalities used in the analysis, two ('upward-conveyor' bioturbator modality and '15–25 cm' habitat depth) were not represented in the Net Control, one ('15–25 cm' habitat depth) was not present in the Shelled Net and one ('grazer' feeding modality) was not represented in the Control. *Arenicola marina* and *Capitella* sp. were the only two species to represent the two trait modalities lost from the Net Control. The lack of *A. marina* in the Shelled Net treatment also explains the loss of the habitat depth modality '15–25 cm'.

The bioturbator trait modalities 'None', 'gallery-diffuser' and 'regenerator' were represented to a greater extent in both netted treatments than in the Control. The same was observed for the 'opportunist/scavenger' feeding modality, 'surface' and '8–15 cm' habitat modalities, and the '3–5 years' longevity. The 'deposit-feeder', '≤5 mm' body size modality and '1–2 years' longevity were more highly represented in the Shelled Net treatment than in either Net Control or Control.

The nMDS ordination (Fig. 3B) showed a similar pattern of groupings to that seen in the faunal abundance



**Fig. 3.** MDS ordinations of A) faunal assemblages and B) biological trait distribution for the combined core sample and net surface sample faunal assemblages. (Δ) Control, (■) Net Control, (▼) Shelled Net.

**Table 2.** Kruskal–Wallis analysis from core sample data for the eight most abundant taxa as indicated from long-term monitoring data.

taxon	rank			H	P-value
	control	shelled Net	net control		
Nematoda	10.60	6.00	5.50	4.30	0.116
<i>Tubificoides benedii</i>	6.60	6.80	9.50	1.29	0.526
<i>Hydrobia ulvae</i>	5.3	9.6	7.6	2.65	0.248
<i>Macoma balthica</i>	10.00	6.00	6.30	2.79	0.266
<i>Pygospio elegans</i>	12.00	5.20	4.80	9.11	<b>0.011</b>
<i>Eteone longa</i> agg	12.00	4.20	6.00	9.54	<b>0.008</b>
<i>Heterochaeta costata</i>	4.30	11.20	6.90	7.16	<b>0.028</b>
<i>Corophium volutator</i>	12.00	6.20	3.50	10.38	<b>0.006</b>

Significant p values in **bold**.

ordination (Fig. 3A). Control treatments formed a distinct group, with Net Control forming a second grouping and the Shelled Net treatment scattered over the ordination, whilst being closer to the Net Control than the Control treatments. The ordination was plotted using several transformations and no deviation from the pattern of clustering was observed, indicating the overall groupings

**Table 3.** (A,B) SIMPER analysis showing faunal percentage contribution to average dissimilarity\* between treatments from core sample data. \*Control & Shelled net = 40% dissimilarity; Control & Net Control = 29% dissimilarity; Net Control & Shelled Net = 30% dissimilarity.

taxon	contrib%
(A) Control & Shelled Net	
<i>Pygospio elegans</i>	14.49
Nematoda	13.66
Collembola	11.94
<i>Hydrobia ulvae</i>	10.25
<i>Corophium volutator</i>	9.44
<i>Eteone longa</i> agg	5.29
total	65.07
(B) Control & Net Control	
<i>Pygospio elegans</i>	21.55
<i>Corophium volutator</i>	14.7
Nematoda	12.01
<i>Hydrobia ulvae</i>	8.69
<i>Eteone longa</i> agg	6.1
total	63.05

were stable. ANOSIM carried out on the data showed a significant difference between the Control and both netted treatments (R = 2.92, P = 1.6% for Shelled Net; R = 0.356, P = 4.8% for Net Control) and no significant difference between the netted treatments.

Similarity of percentages (SIMPER) analysis showed differences between the treatments were attributable to 10 of the trait modalities (5% or greater contribution to dissimilarity) (Table 4A,B). Of these, five were common to all comparisons (deposit-feeder, surface dwellers, ‘none’ for bioturbation, 1–2 year longevity and <5 mm body size), three were observed in a single comparison (downward conveyor bioturbation modality, 10–20 mm body size and <1 year longevity in the Control/Net Control comparison) and two were observed in two of the three comparisons (0–3 cm depth in the comparison of Control with both net treatments; biodiffuser in the comparison of Shelled Net to both Control and Net Control treatments).

*Control and Shelled Net comparison*

Seven trait modalities contributed 5% or greater to the dissimilarity between Shelled Net and Control treatments (Table 4A) with Deposit-feeding (DF) being common to many of the taxa identified, therefore a degree of redundancy could be expected. The abundance of DF taxa in the Shelled Net decreased overall compared to the control, indicating a decrease in this trait modality.

The same was true of the ≤5 mm body size and shallow depth (0–3 cm) habit modalities; abundance of the taxa exhibiting these traits (both *Corophium* species, *Pygospio elegans*, *Capitella* sp., *Manayunkia aesturina*, *Para-*

**Table 4.** (A,B) SIMPER analysis showing trait percentage contribution to average dissimilarity\* between treatments. Fauna data from core samples and net surface samples were combined for use in this comparison. \*Control & Shelled net = 34% dissimilarity; Control & Net Control = 25% dissimilarity; Net Control & Shelled Net = 27% dissimilarity.

trait	contrib%
<b>(A) Control &amp; Shelled Net</b>	
deposit-feeder	12.23
surface	11.59
none	11.56
1–2 years	11.37
≤5 mm	7.96
0–3 cm	7.08
biodiffuser	5.94
total	67.73
<b>(B) Control &amp; Net Control</b>	
0–3 cm	10.61
1–2 years	9.83
surface	9.62
none	9.6
deposit-feeder	8.97
10< >0 mm	6.58
≤5 mm	6.28
downward-conveyor	5.83
≤1 year	~5
total	72.32

*nais littoralis* and *Streblospio shrubsolii*) was greater in the Control treatment than the Shelled Net. The change in surface dwelling and 'None' for bioturbation can be attributed to the increased abundance of *Mytilus edulis* and *Carcinus maenas* juv Linnaeus in the Shelled Net treatments along with the appearance of *Littorina littorea*, *Eliminius modestus* and *P. littoralis*. A lower abundance of Biodiffuser (BD) taxa (*Corophium arenarium*, *Corophium volutator*, *P. elegans*, *Eteone longa* agg) present in the Shelled Net treatment compared to the Control indicates an overall decrease in this bioturbation trait modality.

#### Control and Net Control comparison

Nine trait modalities contributed 5% or more of the dissimilarity between Net Control and Control treatments, six of which (Deposit-feeders, 0–3 cm depth habit, Surface dwellers, 1–2 years longevity, no bioturbation and ≤5 mm body size) were the same as found in the Shelled Net and Control comparison. Deposit feeders decreased in abundance in the Net Control and no additional species (*i.e.* *Paranais littoralis*, *Streblospio shrubsolii*) were present to add to their overall abundance, indicating a lack of redundancy for this trait in the Net Control treatment. The trait modalities surface dweller, 'none' for bioturbation and ≤5 mm body size showed the same pattern of change as in the Shelled Net comparison and were attributable to the same taxa.

Three other trait modalities (bioturbation modality downward-conveyor, ≤1 year longevity and 10–20 mm body size) contributed 5% or more to the dissimilarity between the Net Control and Control treatments. The only taxa exhibiting the downward-conveyor trait in the two treatments was *Pygospio elegans*, which decreased in abundance in the Net Control compared to the Control. This decrease in the downward-conveyor modality is solely attributable to this species, indicating a lack of redundancy with respect to this trait modality.

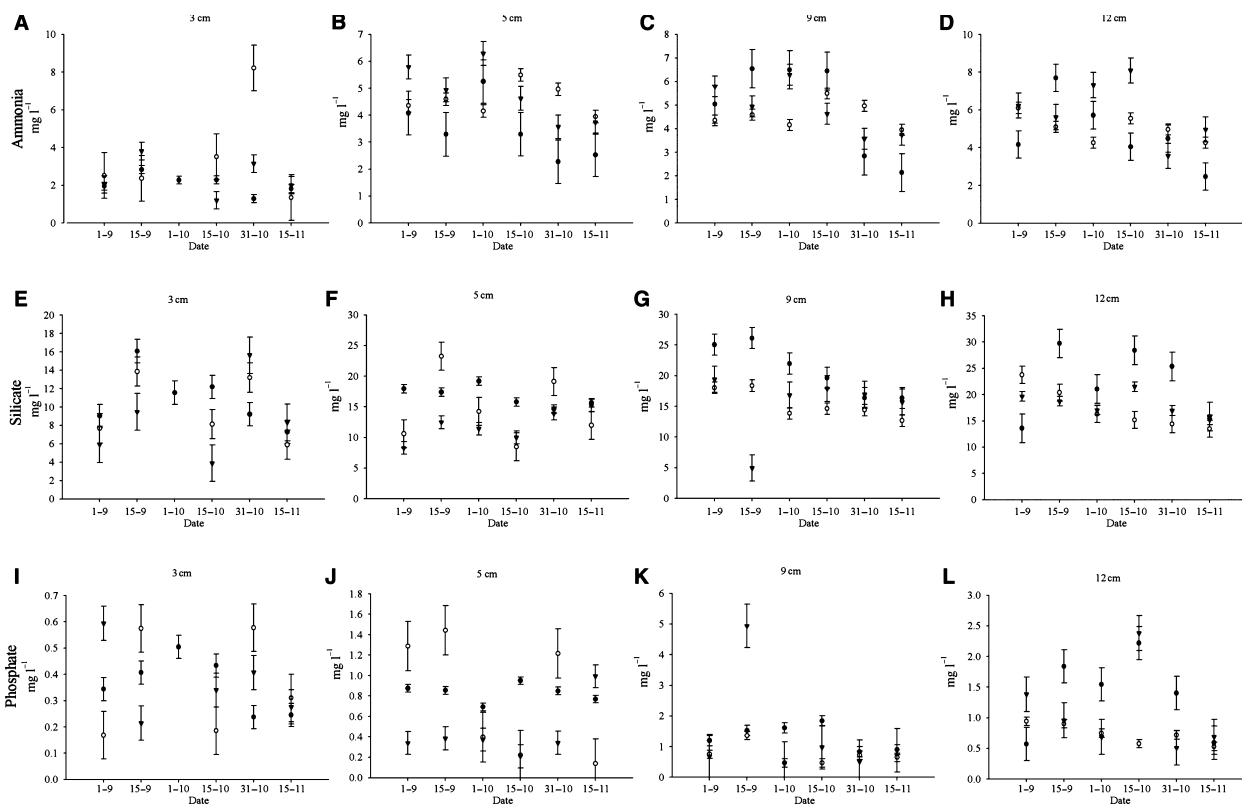
Four taxa exhibit the ≤1 year longevity (*Tubificoides benedii* Udekem, *Heterochaeta costata*, *Corophium volutator* and *Corophium arenarium*). There was an overall decrease in this trait modality from Control to Net Control treatments attributable to the lower *Corophium* abundance observed in the Net Control. *Tubificoides benedii* abundance was similar between the two treatments, therefore the representation of this trait modality by this species would have remained similar. The increase in *H. costata* was not as great as the decrease in *Corophium* and may not have compensated for this loss with regard to this trait modality. The 10–20 mm body size was attributable to several taxa common in both Control and Net Control treatments and there was an overall decrease in the combined abundance of those taxa, and thus a decrease in this trait modality.

Overall there were more surface dwelling, and no bioturbating or medium life span (1–2 years) taxa in the Net Control and Shelled Net treatments, and fewer deposit-feeding, biodiffuser, shallow depth habit (0–3 cm), short-lived (<1 year) and small (<5 mm) taxa than in the Control treatments.

#### Nutrients

Nutrient levels (mg L<sup>-1</sup>) over time were plotted for each depth separately (Fig. 4). Missing data points are due to a lack of water being collected in the porewater device, no sample being available or anomalous data being returned from the analysis, *i.e.* a negative value. Total N (nitrate/nitrite) was below the detection limit of the Autoanalyser whenever sufficient water could be collected and so is not considered further.

An initial increase in silicate and ammonia levels was observed at the 9 and 12 cm depths (also 5 cm for ammonia) and a decrease was noted starting at the 4th sampling time. Levels of ammonia in both netted treatments were generally greater than in Controls at 3 and 5 cm depths. At 9 and 12 cm depth the ammonia levels were highly variable over time and differences between the treatments were not obvious. Phosphate levels over time were more variable but slight increases are seen in the 3, 9 and 12 cm depth charts (Fig. 4I,K,L) for Controls. Both Shelled Net and Net



**Fig. 4.** Nutrient levels ( $\text{mg L}^{-1}$ ) over time for ammonia (A–D), silicate (E–H) and phosphate (I–L), plotted according to collection depth. • Control; ▼ Net Control; ○ Shelled Net.

Control phosphate levels at 12 cm decreased over time. Phosphate levels were generally lower in Net Control compared to Control at 5 cm depth ( $0.2\text{--}0.7 \text{ mg L}^{-1}$ ) and at 12 cm depth phosphate levels in both netted treatments were lower than in the Controls ( $\approx 1 \text{ mg L}^{-1}$ ). Silicate levels were generally lower in the netted treatments at 3 and 5 cm depths by  $4\text{--}10 \text{ mg L}^{-1}$  compared to the Control and a steady decrease in silicate levels over time was observed at 9 and 12 cm depths.

Linear mixed model (LMM) analysis of the nutrient data was carried out for each depth separately over time using the appropriate covariance structure (Table 5A,B); Auto-regressive-1 (AR1) was used for all analyses apart from 12 cm Silicate (Toeplitz) and 12 cm Phosphate (AR1-heterogeneous). At 3 cm depth Time was the only significant factor for both ammonia and silicate and no significant difference was found for phosphate. ‘Net’ was a significant factor for all three nutrients at 5 cm depth, as was Time for silicate and phosphate. Also, the interaction of ‘Shell\*Time’ was significant for phosphate at this depth. At 9 cm depth only ammonia was significantly different (factor ‘Time’ and ‘Net\*Time’). At 12 cm depth the interaction ‘Net\*Time’ was significant for all three

nutrients and Time was significant on its own for silicate. Overall there was a treatment effect for all nutrients at 12 cm depth. Effects observed at other depths were mostly due to variation over time.

### Discussion

This study set out to examine the extent to which the biotic changes that accompany habitat change affect ecosystem functioning. Changes to the topography were expected to alter the overlying water flow dynamics and affect upwelling of nutrient-rich porewaters from deep sediment layers, causing an increase in nutrient concentrations in the near-surface sediments. (Booij *et al.* 1991; Huettel & Gust 1992; Vitousek & Hooper 1993; D’Andrea *et al.* 2002) Further alterations to the habitat caused by accretion of sediment and deposition of detrital material were also expected, affecting nutrient concentrations and faunal composition (*e.g.* McLachlan 1996; Rossi & Underwood 2002; Bishop & Kelaher 2007). Although significant changes to the species diversity and abundance were observed, there were no significant alterations to nutrient

**Table 5.** (A–D) Mixed Model analysis results of nutrient levels mg L<sup>-1</sup> per depth for each nutrient analysed.

	ammonia		phosphate		silicate	
	F	P	F	P	F	P
(A) 3 cm depth						
Shell	1.594	0.215	0.001	0.981	0.217	0.646
Net	0.206	0.653	0.001	0.972	1.515	0.234
Time	3.097	<b>0.020</b>	1.142	0.359	3.538	<b>0.012</b>
Shell*Time	2.439	0.070	1.666	0.182	0.756	0.562
Net*Time	0.375	0.824	1.101	0.375	1.926	0.131
(B) 5 cm depth						
Shell	0.000	0.988	2.571	0.122	3.224	0.081
Net	5.875	<b>0.023</b>	4.646	<b>0.042</b>	11.099	<b>0.002</b>
Time	1.209	0.317	3.687	<b>0.007</b>	3.038	<b>0.017</b>
Shell*Time	1.317	0.272	3.569	<b>0.009</b>	1.998	0.094
Net*Time	0.795	0.559	0.773	0.574	1.077	0.383
(C) 9 cm depth						
Shell	2.176	0.154	0.740	0.789	1.394	0.248
Net	0.824	0.373	0.498	0.488	2.087	0.160
Time	5.151	<b>0.001</b>	0.836	0.531	2.213	0.069
Shell*Time	1.777	0.133	0.238	0.944	0.117	0.988
Net*Time	2.706	<b>0.029</b>	0.692	0.632	0.878	0.502
(D) 12 cm depth						
Shell	1.703	0.203	2.372	0.137	0.775	0.394
Net	3.205	0.086	0.680	0.419	2.569	0.133
Time	2.073	0.083	2.496	0.054	24.957	≤ <b>0.001</b>
Shell*Time	1.469	0.216	0.999	0.436	0.804	0.555
Net*Time	3.170	<b>0.015</b>	4.485	<b>0.005</b>	24.020	≤ <b>0.001</b>

Significant p values in **bold**.

concentrations in either the near-surface or deeper (>9 cm) sediments sampled. The net treatments caused a significant change in the faunal composition and nutrient concentrations at 5 cm depth compared to the Control and both treatments showed an increased accretion of sediment, further altering the habitat by raising the sediment surface above that of the surrounding area and by altering the particle grain size composition. This indicates that the influence of the netting on faunal composition was similar regardless of additional 'roughness' due to the mussel shell debris and it may therefore be the subsequent alteration in species composition that influenced observed nutrient concentrations rather than the altered topography.

The observed biotic shift in the faunal composition reduced the abundance of individuals that actively irrigated burrows (*Corophium volutator* and *Corophium arenarium*) and species inhabiting permanent, or semi-permanent, tubes within the sediment (*Pygospio elegans*). The lower abundances of these bioturbators would have reduced the surface area available for solute exchange (Kristensen 2000; Pearson 2001; Volkenborn *et al.* 2007) and had an effect on microbial community composition in the near-surface sediments that had utilized the

burrows and tubes as oxygenated habitats (Marinelli *et al.* 2002; Mermillod-Blondin *et al.* 2004). As such, an increase in nutrient levels in the near-surface sediments may have been expected, as less oxidation of reduced substances from deeper layers was carried out. This was not the case, suggesting that (i) the nutrients were utilized either by microbial or by micro-phytobenthos communities, (ii) some redundancy with regard to the traits represented by these three species was present in the system.

The burrowing and movement of benthic macrofauna can impact on the sediment structure by causing compaction of sediment grains and increasing rigidity of the sediment layers via tube construction (Jones & Jago 1993; Mermillod-Blondin & Rosenberg 2006; Guillén *et al.* 2008). The oligochaetes present in the study area increased in abundance in the manipulated sediment moving nutrient-rich yet oxygen-poor material towards the surface and resulting in lower levels of nutrients than would have been observed if oxygen had not been available.

The altered species composition in the net treatment was accompanied by a shift in the range of biological traits exhibited by the fauna. This provides further, indirect, evidence of a change in ecological functioning being mediated by the biotic response. Alterations in the bioturbatory modes exhibited in the different treatments may have been enhanced by changes in the horizontal water flow and contributed to differences in the transport of oxygen, nutrients and particles from the sediment–water interface to areas below the redox zone (Biles *et al.* 2003). The combination of a shift in the depth at which the majority of burrowing fauna were residing and the changes to bioturbation, alongside the potential alterations to the sediment caused by the biotic shift, may have accounted for the significant differences observed in nutrient levels at 5 cm depth.

The analysis of the traits did not show a simple relationship with the altered nutrient dynamics. This suggests that whilst BTA may offer a means of predicting/quantifying ecological functioning (Usseglio-Polatera *et al.* 2000; Bremner *et al.* 2003), it relies on the selection of traits used. Bremner *et al.* (2006) have shown that the number of traits used can alter the ability of the analysis to describe the relationships between assemblages. They concluded that the greater the number of traits utilized in an analysis, the clearer any relationships between the traits and faunal assemblages will appear. Although the traits chosen in this study (bioturbatory mode, feeding mode, habitat depth, body size and longevity) were those considered to have an influence on nutrient regeneration, it may have been the case that more traits would have provided a clearer picture. However, the time constraints inherent with collecting trait-based data for any species assemblage force a compromise, causing those traits con-



sidered to be of importance to the ecosystem function of interest to be favoured over those that are not (Bremner *et al.* 2006).

The influence of functional diversity and composition on productivity and resource use has been well documented in terrestrial ecosystems (Hooper & Vitousek 1997; Tilman *et al.* 1997), concluding that alterations to functional group composition can have greater effects on ecosystem functions than functional diversity can (Hooper & Vitousek 1997). Also, changes to habitats via disturbance, species invasions or nutrient enrichment affect ecosystem functions (Tilman *et al.* 1997) and the loss or addition of various traits can have varying impacts on different ecosystem functions (Naeem *et al.* 1994; Tilman *et al.* 1997; Heemsbergen *et al.* 2004; Hooper *et al.* 2005; Rossi *et al.* 2008). The alteration of the topography in this study did cause a significant change to the faunal diversity and the biological traits represented. In particular, the 5 cm data indicated that the microbial and geochemical environment had been altered. However, the relationship of the traits to alterations in nutrient regeneration was less clear.

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## ORIGINAL ARTICLE

**Relict sand dredging for beach nourishment in the central Tyrrhenian Sea (Italy): effects on benthic assemblages**

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E-mail: barbara.laporta@isprambiente.it**Conflicts of interest**

All authors declare no conflicts of interest.

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**Abstract**

The aim of this study is to analyse the effects in space and time of relict sand-dredging activities on macrobenthic assemblages, in an area situated offshore Montalto di Castro (central Tyrrhenian Sea, Italy), and to analyse the recolonisation processes of macrobenthos in the dredged areas. The area in question is characterised by relict sand deposits (Holocene paleo-beaches), used for beach nourishment along the Latium coast. The effects of sand extraction on benthic assemblages were investigated before, during and after three dredging operations. The sites analysed are located within the dredged areas (inside stations) and in neighbouring, not dredged, areas (outside stations). The results showed that the impact of sand extraction was confined to the dredged stations and to the areas in proximity to the dredged areas. During dredging activities, the structure of benthic assemblages within the impacted stations was characterised by low species richness and diversity. Both the direct removal of sediment and the re-suspension and consequent deposition of fine sediment affected benthic assemblages of the impacted stations. A few months after the dredgings, a recolonisation process was still observed at all the impacted stations. A gradual recolonisation process was observed at those stations affected by only one dredging, whereas a different recolonisation was observed at those stations affected by two dredgings over time. This study suggests that differences of re-colonisation processes of benthic assemblages are related to the intensity of dredging operations in terms of dredging frequency.

To combat coastal erosion along the Italian coasts, the local governments and the environmental protection agencies of several regions have planned nourishment operations exploiting relict sand deposits, within the framework of the European project INTERREG IIIC BEACHMED-e (<http://www.beachmed.eu>).

Relict sands are non-diagnosed sedimentary deposits situated along the continental shelf in a state of disequilibrium with the present sedimentary dynamics. The removal of such sediments, occurring offshore at high depths, does not affect the wave motion regime and, therefore, coastal dynamics. The relict sand extraction is performed through the use of suction trailers or anchor dredges. A common consequence of trailer dredging is

the development of shallow furrows 1–3 m in width and sometimes up to 5 m in depth (Desprez 2000). Anchor dredging leads to the formation of deep, cup-shaped depressions, typically up to 8–10 m deep (Boyd & Rees 2003). Both dredging methods can result in significant environmental alterations, which may take place on both physical and biological levels. The main physical effects involve variations in morphological and bathymetric features, modifications of superficial sediment characteristics, and an increase in water turbidity caused by the re-suspension of fine sediment in the water column during dredging activities. Concerning the biological effects, both dredging methods cause severe disturbances in macrozoobenthos assemblages in terms of the direct effect on



sediment removal and the indirect effect associated with the deposition of suspended sediment caused by sand extraction (Desprez 2000; Sardà *et al.* 2000; Boyd & Rees 2003; Szymelfenig *et al.* 2006; Simonini *et al.* 2007). Nevertheless, the type of dredge employed, as well as the nature of the receiving environment, can potentially influence the spatial scale of impact on the benthic fauna, in terms of both direct and indirect effects caused by sand extraction (Boyd & Rees 2003). Boyd & Rees (2003), Newell *et al.* (2004), Robinson *et al.* (2005) and, more recently, Cooper *et al.* (2007) have shown that the impact on benthic assemblages is also related to the process of repeated dredgings within the dredged site. Robinson *et al.* (2005) and Cooper *et al.* (2007) also highlighted that benthic recolonisation processes in repeatedly dredged areas are particularly difficult to predict, because of both the different benthic responses to the intensity of dredging operations in terms of dredging frequency and the variations in environmental characteristics.

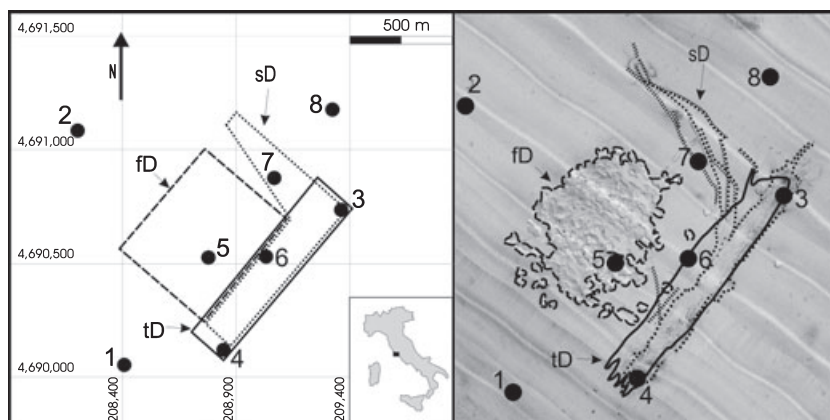
Between July 2004 and September 2005, three relict sand-dredging activities were performed in an area offshore Montalto di Castro (Lazio, Italy) in the central Tyrrhenian Sea, with the final aim of nourishing various beaches along the Lazio coasts. This area was characterised by the presence of relict sand deposits that were covered by a muddy layer of recent deposition, with a thickness that varies between a few centimetres and a few metres (Chiocci & La Monica 1999). For these operations, ISPRA, formerly ICRAM (Central Institute for Marine Research), carried out an environmental impact study related to marine relict sand extraction for beach nourishment, funded by the Regione Lazio local authority. This monitoring program has provided an opportunity to collect useful information for the evaluation of the consequences of sand extraction over a relatively short time period in an offshore area that until now has been poorly investigated. In particular, in this study we analysed: (i) the effects of relict sand-dredging activities on the

macrobenthos assemblages; (ii) the recolonisation processes of macrobenthos in the dredged areas; (iii) the effects over time of repeated dredging activities on macrobenthos assemblages.

## Material and Methods

The study area was located 3.5 nautical miles offshore from Montalto di Castro (Lazio, Italy) in the central Tyrrhenian Sea, on the continental shelf at 50 m of water depth.

The relict sand-dredging activities in this area took place in three different periods, July 2004 (first dredging), June 2005 (second dredging), and September 2005 (third dredging). Over this period, three changes in the boundaries of the extraction areas were reported (Fig. 1). For the first dredging, an anchor dredge was used, whereas for the second and third dredging a trailer dredge was used. The monitoring surveys were carried out from May 2004 to October 2006, before, during and after the dredging activities, as indicated in Nicoletti *et al.* (2006) (Table 1). The sampling plan provided five stations (named stations 1, 2, 3, 4 and 5), one of which was located inside the dredged area in order to monitor the first dredging. The second and the third dredging activities were carried out in proximity (N-NE) to the first area dredged. Three stations (6, 7 and 8) were added to the sampling plan to monitor these dredgings, as shown in Fig. 1. Macrobenthos sampling was carried out using a Van Veen grab with a surface of 0.1 m<sup>2</sup>. Two replicates were collected at each station. Samples were sieved through a 1-mm mesh and the retained material was preserved in 4% CaCO<sub>3</sub> buffered formalin in seawater. For each station, samples of superficial sediments were collected through a box-corer to determine grain size distribution. Superficial sediments were classified according to Shepard (1954). The collected organisms were counted and classified to the lowest possible taxonomic level. In



**Fig. 1.** On the left, the location of relict sand-extraction areas with a map of sampling stations (black point) is represented; on the right, side scan sonar reliefs of the dredged areas (fD = first dredged area; sD = second dredged area; tD = third dredged area) is reported.



**Table 1.** Sand-dredging characteristics and sampling plan of the three dredged areas.

	First dredged area	Second dredged area	Third dredged area
Volume sand extracted (m <sup>3</sup> )	600,000	150,000	700,000
Water depth (m)	50	50	50
Type of dredge	Anchor dredge	Trailer dredge	Trailer dredge
Dredging period	July 2004	June 2005	September 2005
Sampling stations			
Inside the dredged area	5	6,7	3, 4, 6
Outside the dredged area	1, 2, 3, 4	1, 2, 3, 4, 5, 8	1, 2, 5, 7, 8
Surveys			
May 2004 – before dredging (B)	√	–	–
July 2004 – during first dredging (fD)	√	–	–
September 2004 – 2 months after dredging (A2)	√	–	–
April 2005 – 9 months after dredging (A9)	√	–	–
August 2005 – 14 months after dredging (A14)	√	√	√
September 2005 – during third dredging (tD)	√	√	√
May 2006 – 22 months after dredging (A22)	√	√	√
October 2006 – 27 months after dredging (A27)	√	√	√

particular, Polychaeta, Crustacea, Mollusca and Echinodermata were analysed. The main ecological indices (abundance, number of species, Margalef species richness and Shannon–Wiener diversity) were calculated. Multivariate analysis was performed with abundance data to analyse the benthic assemblage variation patterns in terms of species composition and numerically dominant species. The output from the non-metric multidimensional scaling (nMDS) ordination model of the Bray–Curtis similarity matrix was obtained for each station and sampling period. Univariate and multivariate analyses were performed using the software package PRIMER v. 6.1.5 (Clarke & Gorley 2001).

## Results

During the study period, 4553 individuals belonging to 191 species were collected (Table 2). Polychaetes were the most abundant taxon (3371 individuals and 103 species), followed by crustaceans (626 individuals and 48 species), echinoderms (328 individuals and 10 species), and molluscs (228 individuals and 30 species). The most abundant species were the polychaetes *Paralacydonia paradoxa*, *Glycera unicornis*, *Paraprionospio pinnata*, *Metasychis gotoi*, the tanaid *Tuberapseudes echinatus*, and the ophiuroid *Amphiura chiajei*. In general, benthic assemblages were characteristic of muddy bottoms. The species composition did not show considerable variations over time. Only a few taxa showed variation over time; these were the opportunistic species *Corbula gibba* and *Terebellides stroemi*, and the sabulicolous polychaetes *Streblosoma bairdi*, *Nephtys hombergi* and *Diplocirrus glaucus*. These latter species were mainly found at the dredged stations.

The univariate analysis showed that the first dredging caused a drastic reduction of the ecological indices exclusively at station 5 located within the dredged area. Stations 1, 2, 3 and 4, located outside the first dredged area, seemed not to have been affected by dredging. Fourteen months after the end of the first dredging activity, impacted station 5 showed an increase in the ecological parameters. During the second dredging, no surveys for the macrobenthos monitorings were carried out. Nevertheless, the monitoring survey carried out 2 months after the second extraction, showed that only station 6 was characterised by extremely low indices values. During the third dredging, all stations except 1, 2 and 8, which were located outside the dredged area, showed a drastic decrease of the ecological indices (Fig. 2 and Table 3).

In general, data relating to the two monitoring surveys carried out after the end of the third dredging highlighted that all the impacted stations showed an increase in the ecological indices. Stations 5 and 6 were characterised by a particularly strong increase in these values (Fig. 2, Table 3), mainly due to the high abundance of a few opportunistic species (e.g. *C. gibba* and *T. stroemi*) and to the presence and abundance of previously absent species that colonised the impacted substrata (e.g. *S. bairdi*, *N. hombergi*, *D. glaucus*).

The nMDS ordination plot of data relating to each station and to each sampling period shows an overlapping of samples (Fig. 3). Station distribution confirms the homogeneity of the benthic assemblage observed over time. Station 5, which was affected during the first and the third dredging, segregated on the left side of the plot. Furthermore, on the right side we find stations 5 and 6 analysed during the last two monitoring surveys and

**Table 2.** Species collected during the study period.

## Mollusca

*Pseudotorinia architae* (O.G. Costa, 1839)  
*Calliostoma (Ampullotrochus) grammatum* (Von Born, 1778)  
*Turritella communis* Risso, 1826  
*Hyalia vitrea* (Montagu, 1803)  
*Calyptrea chinensis* (Linnaeus, 1758)  
*Polinices macilentus* (Philippi, 1844)  
*Polinices nitida* (Donovan, 1804)  
*Eulima glabra* (Da Costa, 1778)  
*Nassarius (Gussonea) cfr. comicus* (Olivi, 1792)  
*Nucula nucleus* (Linnaeus, 1758)  
*Nucula sulcata* (Bronn, 1831)  
*Saccella commutata* (Philippi, 1844)  
*Thyasira biplicata* (Philippi, 1836)  
*Glans aculeata* (Poli, 1795)  
*Astarte sulcata* (Da Costa, 1778)  
*Plagiocardium papillosum* (Poli, 1795)  
*Lutraria* sp.  
*Phaxas adriaticus* (Coen, 1933)  
*Tellina donacina* Linnaeus 1758  
*Tellina serrata* Brocchi, 1814  
*Gari fervensis* (Gmelin, 1791)  
*Abra alba* (Wood, 1802)  
*Abra prismatica* (Montagu, 1808)  
*Abra renierii* (Bronn, 1831)  
*Pitar rudis* (Poli 1795)  
*Timoclea ovata* (Pennant, 1777)  
*Corbula gibba* (Olivi, 1792)  
*Antalis inaequicostata* (Dautzenberg, 1891)

## Crustacea

*Iphinoe rhodaniensis* Ledoyer, 1965  
*Iphinoe serrata* Norman, 1867  
*Apseudes acutifrons* G. O. Sars, 1882  
*Apseudes elisae* Bacescu, 1961  
*Apseudes latreilli* (Milne-Edwards, 1828)  
*Tuberapseudes echinatus* (G.O. Sars, 1882)  
*Leptocheilia savignyi* (Kroyer, 1842)  
*Arcturella dilatata* (G.O. Sars, 1883)  
*Gnathia* sp.  
*Anthura gracilis* (Montagu, 1808)  
*Cirolana borealis* Lilljeborg, 1852  
*Cirolana* sp.  
*Ampelisca diadema* (A Costa, 1853)  
*Ampelisca spinifer* Reid, 1951  
*Ampelisca spinipes* Boeck, 1861  
*Ampelisca typica* (Bate, 1856)  
*Haploops dellavallei* Chevreux, 1900  
*Haploops nirae* Kaim Malka, 1976  
*Leptocheirus guttatus* (Grube, 1864)  
*Leptocheirus mariae* G Karaman, 1973  
*Medicorophium rotundirostre* (Stephensen, 1915)  
*Photis longicaudata* (Bate & Westwood, 1862)  
*Leucothoe incisa* Robertson, 1892  
*Leucothoe lilljeborgi* Boeck, 1861  
*Leucothoe oboa* G. Karaman, 1971  
*Lilljeborgia dellavallei* Stebbing, 1906  
*Hippomedon massiliensis* Bellan-Santini, 1965  
*Maera grossimana* (Montagu, 1808)

**Table 2.** (Continued.)

*Othomaera schmidtii* (Stephensen, 1915)  
*Westwoodilla rectirostris* (Delia Valle, 1893)  
*Harpinia agna* G Karaman, 1987  
*Harpinia ala* G. Karaman, 1987  
*Harpinia antennaria* Meinert, 1890  
*Harpinia karamani* King, 2004  
*Harpinia* sp.  
*Metaphoxus fultoni* (Scott, 1890)  
*Phtisica marina* Slabber, 1769  
*Alpheus glaber* (Olivi, 1792)  
*Athanas nitescens* (Leach, 1814)  
*Processa canaliculata* Leach, 1815  
*Callianassa subterranea* (Montagu, 1808)  
*Goutretia denticulata* (Lutze, 1937)  
*Jaxea nocturna* Nardo, 1847  
*Paguristes eremita* (Linnaeus, 1767)  
*Anapagurus laevis* (Bell, 1845)  
*Anapagurus serripes* (Hope, 1851)  
*Pagurus cuanensis* Bell, 1845  
*Medorippe lanata* (Linnaeus, 1767)  
*Ebalia deshayesi* Lucas, 1845  
*Liocarcinus maculatus* (Risso, 1827)  
*Goneplax rhomboides* (Linnaeus, 1758)

## Polychaeta

*Capitella capitata* (Fabricius, 1870)  
*Heteromastus filiformis* (Claparede, 1864)  
*Leiocapitella glabra* Hartman, 1947  
*Notomastus aberans* Day, 1957  
*Notomastus latericeus* Sars, 1850  
*Notomastus lineatus* Claparede, 1870  
*Pseudoleiocapitella fauveli* Harmelin, 1964  
*Cossura soyeri* Laubier, 1964  
*Clymenura clypeata* (Saint-Joseph, 1894)  
*Praxillella affinis* (M. Sars, 1872)  
*Praxillella gracilis* (M. Sars, 1872)  
*Maldane glebifex* Grube, 1860  
*Maldane sarsi* Malmgren, 1865  
*Nematonereis unicornis* (Schmarda, 1861)  
*Palola siciliensis* (Grube, 1840)  
*Metasychis gotoi* (Izuka, 1902)  
*Nicomache lumbricalis* (Fabricius, 1780)  
Maldanidae gen.sp  
*Polyopthalmus pictus* (Dujardin, 1839)  
*Polyodontes maxillosus* (Ranzani, 1817)  
*Harmothoe longisetis* (Grube, 1863)  
*Lepidonotus clava* (Montagu, 1808)  
*Lepidonotus squamatus* (Linnaeus, 1767)  
*Malmgreniella lunulata* (Delle Chiaje, 1830)  
*Sthenelais boa* (Johnston, 1833)  
*Podarkeopsis arenicola* (La Greca, 1947)  
*Pilargis verrucosa* (Saint-Joseph, 1899)  
*Sigambra tentaculata* (Treadwell, 1941)  
*Glycera alba* (O.F. Muller, 1776)  
*Glycera tessellata* Grube, 1863  
*Glycera unicornis* Savigny, 1818  
*Glycinder nordmanni* (Malmgren, 1866)  
*Goniada maculata* Oersted, 1843  
*Nephtys hombergi* Savigny, 1818

Table 2. (Continued.)

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<i>Nephtys hystrix</i> McIntosh, 1900
<i>Paralacydonia paradoxa</i> Fauvel, 1913
<i>Phyllodoce lineata</i> (Claparede, 1870)
<i>Dorvillea (Schistomeringos) neglecta</i> (Fauvel, 1923)
<i>Dorvillea (Schistomeringos) rudolphii</i> (Delle Chiaje, 1828)
<i>Aglaophamus rubellus</i> (Michaelsen, 1897)
<i>Eunice pennata</i> (O.F. Muller, 1776)
<i>Eunice vittata</i> (Delle Chiaje, 1828)
<i>Lysibranhia paucibranchiata</i> Cantone, 1983
<i>Marphysa belli</i> (Audouin & Milne-Edwards, 1833)
<i>Marphysa kinbergi</i> McIntosh, 1910
<i>Lumbrineriopsis paradoxa</i> (Saint-Joseph, 1888)
<i>Lumbrineris gracilis</i> (Fillers, 1868)
<i>Lumbrineris latreilli</i> Audouin & Milne Edwards, 1834
<i>Scoletoma emandibulata-mabiti</i> (Ramos, 1976)
<i>Scoletoma fragilis</i> (O.F. Muller, 1776)
<i>Scotetoma tetrava</i> (Schmarda, 1861)
<i>Arabella tricolor</i> (Montagu, 1804)
<i>Drilonereis filum</i> (Claparede, 1870)
<i>Apomtphis bilineata</i> (Baird, 1870)
<i>Apomtphis brementi</i> (Fauvel, 1916)
<i>Apomtphis fauveli</i> (Rioja, 1918)
<i>Hyalinoecia tubicola</i> (O.F. Muller, 1776)
<i>Myriochele oculata</i> Zachs, 1923
<i>Owenia fusiformis</i> Delle Chiaje, 1841
<i>Aphelochaeta marioni</i> (Saint-Joseph, 1894)
<i>Caulleriella mitltibranchiis</i> (Grube, 1863)
<i>Chaetozone caputesocis</i> (Saint-Joseph, 1894)
<i>Chaetozone setosa</i> Malmgren, 1867
<i>Monticellina dorsobranchialis</i> (Kirkegaard, 1959)
<i>Brada villosa</i> (Rathke, 1843)
<i>Diplocirrus glaucus</i> (Malmgren, 1867)
<i>Flabelligera affinis</i> M. Sars, 1829
<i>Sternaspis scutata</i> (Ranzani, 1817)
<i>Amage adspersa</i> (Grube, 1863)
<i>Amage gallasii</i> Marion, 1875
<i>Ampharete acutifrons</i> (Grube, 1860)
<i>Amphicteis gunneri</i> (M. Sars, 1835)
<i>Anobothrus gracilis</i> (Malmgren, 1866)
<i>Eclysippe vanelli</i> (Fauvel, 1936)
<i>Lysippe labiata</i> Malmgren, 1866
<i>Sabellides octocirrata</i> (M. Sars, 1835)
<i>Melinna palmata</i> Grube, 1870
<i>Pectinaria auricoma</i> (O. F. Muller, 1776)
<i>Pectinaria koreni</i> (Malmgren, 1866)
<i>Pista brevibranchia</i> Caullery, 1915
<i>Pista cnstata</i> (O. F. Muller, 1776)
<i>Streblosoma bairdi</i> (Malmgren, 1866)
<i>Terebellides stroemi</i> M. Sars, 1835
<i>Magellona spl</i>
<i>Magelona sp2</i>
<i>Spiochaetopteus costarum</i> (Claparede, 1868)
<i>Aonides paucibranchiata</i> Southern, 1914
<i>Laonice cirrata</i> (M. Sars, 1851)
<i>Minuspio cirri/era</i> Wiren, 1883
<i>Paraprionospio pinnata</i> (Fillers, 1901)
<i>Prionospio caspersi</i> Laubier, 1962
<i>Prionospio ehlersi</i> Fauvel, 1928

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Table 2. (Continued.)

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<i>Prionospio fallax</i> Soderstrom, 1920
<i>Prionospio steenstrupi</i> Malmgren, 1867
<i>Scolelepis bonnieri</i> (Mesnil, 1896)
<i>Scolelepis foliosa</i> (Audouin & Milne-Edwards, 1833)
<i>Spio decoratus</i> Bobretzky, 1870
<i>Spio filicornis</i> (O. F. Muller, 1776)
<i>Spio multioculata</i> (Rioja, 1918)
<i>Spiophanes bombyx</i> (Claparede, 1870)
<i>Spiophanes kroyeri</i> Grube, 1860
<i>Spiophanes kroyeri reysii</i> Laubier, 1961
<i>Poecilochaetus serpens</i> Alien, 1904

## Echinodermata

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<i>Pseudotrachytyone</i> sp.
<i>Trachythyone elongata</i> (Duben Koren, 1844)
<i>Trachythyone tergestina</i> (M. Sars, 1857)
<i>Thyone fusus</i> (O.F. Muller, 1776)
<i>Phyllophorus urna</i> Grube, 1840
<i>Labidoplax digitata</i> (Montagu, 1815)
<i>Amphiura chiajei</i> Forbes, 1843
<i>Amphiura filiformis</i> (O.F. Muller, 1776)
<i>Ophiopsila aranea</i> Forbes, 1843
<i>Ophiura albida</i> Forbes, 1839
<i>Schizaster canaliferus</i> (Lamarck, 1816)

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characterised by high species richness and diversity. Concerning the grain size distribution of the sediments, some grain size variations were observed after the dredgings, both inside and outside the dredged areas. In particular, a significant increase in the sandy fraction (from 28% to 94.3%) was observed after the first dredging in station 5 (inside the dredged area) and another (from 47% to 88.7% of sand) was recorded after the third dredging in station 6 (inside the dredged area). No relevant grain size variations were reported in the other stations.

## Discussion

The results obtained from this study, as expected and in accordance with some authors (Blake *et al.* 1996; Newell *et al.* 1998; Sardà *et al.* 2000; Van Dalftsen *et al.* 2000; Boyd & Rees 2003; Simonini *et al.* 2005), highlighted that the direct effects of relict sand dredgings on macrobenthos assemblages were limited to the dredged areas. In particular, all the stations located inside the dredged areas during the first (station 5) and the third dredging (stations 3, 4 and 6) showed a strong decrease in ecological indices as a consequence of the complete removal of superficial sediments. Despite the lack of data, both before and during the second dredging, it is important to highlight the case of station 6, where both the low values of the ecological indices recorded a few months after the second extraction and its position (inside the second dredged area) allowed us to hypothesise that this station was dredged during the second extraction.

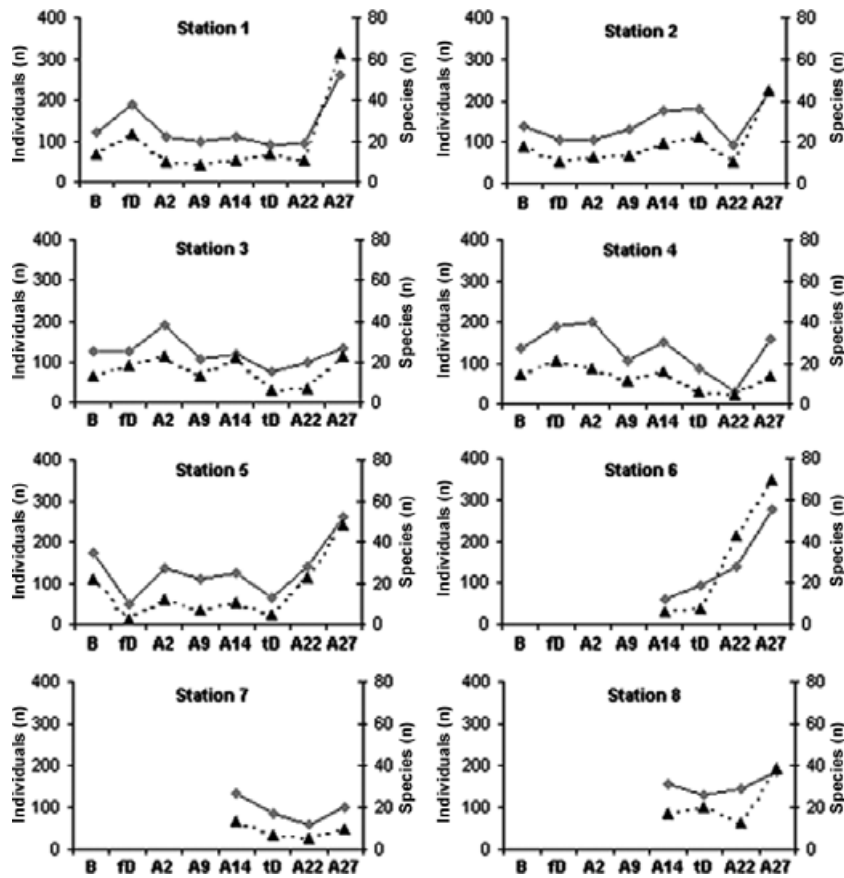


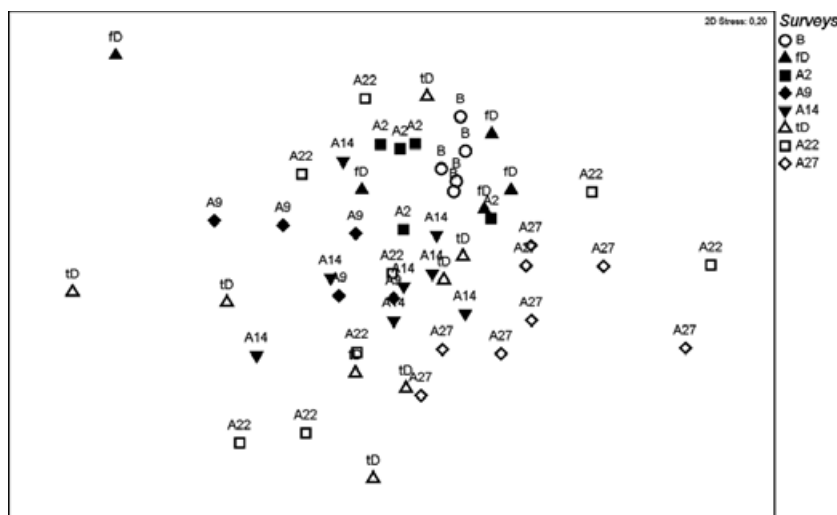
Fig. 2. Number of individuals (black line) and species (grey line) collected at each station over time.

Table 3. Species richness (d) and Shannon diversity (H') values calculated for each station over time.

Stations		B	fD	A2	A9	A14	tD	A22	A27
1	d	5.74	8.03	5.62	5.34	5.86	4.37	5.06	9.37
	H'	4.21	4.86	4.10	3.97	4.14	3.30	3.95	4.67
2	d	6.40	5.35	5.29	6.53	7.92	7.90	5.15	8.62
	H'	4.05	4.02	3.99	4.50	4.77	4.87	3.77	4.96
3	d	5.96	5.94	8.05	5.32	5.23	4.25	5.64	5.93
	H'	4.24	4.36	4.71	3.97	3.88	3.51	4.11	4.07
4	d	6.18	8.68	8.93	5.35	7.34	5.03	1.85	7.77
	H'	3.95	4.93	5.00	4.11	4.64	3.91	2.15	4.64
5	d	7.61	3.75	6.87	6.07	6.51	3.96	6.40	9.99
	H'	4.54	3.28	4.50	4.25	4.47	3.36	4.38	5.11
6	d	-	-	-	-	3.97	5.19	5.31	9.60
	H'	-	-	-	-	3.45	4.09	3.34	4.07
7	d	-	-	-	-	6.75	4.75	3.74	5.30
	H'	-	-	-	-	4.47	3.71	3.22	4.03
8	d	-	-	-	-	7.19	5.85	7.09	7.31
	H'	-	-	-	-	4.63	4.28	4.53	4.35

This study highlighted that the impacts of relict sand dredgings on macrobenthos assemblages were observed in the zones in proximity to the dredged areas. These indirect impacts were due to the re-suspension and subse-

quent deposition of fine sediments caused by sand-extraction operations and was mainly evident at stations 5 and 7, which were located in proximity to the third dredged area. The increase in the fine fraction of superficial



**Fig. 3.** 2D-nMDS ordination plot of abundance data of each station and each sampling period.

sediments observed in station 5 after dredging confirmed that fine sediment re-deposition had occurred.

These results also highlighted that a stronger sediment suspension was generated by the trailer dredge (used for the second and the third dredging), whose impact was greater than that of the anchor dredge (used for the first dredging).

Concerning the analysis of the recolonisation processes of macrobenthos assemblages, our results showed that a few months after the end of dredgings, the recolonisation processes could still be observed at all the impacted stations, in accordance with Green (2002), Boyd & Rees (2003), Simonini *et al.* (2005). In general, these processes are mainly due to the settlement of new recruits from the planktonic larvae and immigration of the adults from the neighbouring areas (Bonvicini Pagliai *et al.* 1985; Rees & Dare 1993; Newell *et al.* 1998; Van Dalen *et al.* 2000), but recolonisation processes are difficult to predict because they are strongly influenced by many different factors (*e.g.* biological cycles of different species, hydrodynamic regime, changes in sediment structure depth).

This study also revealed differences in the recolonisation processes of the impacted stations. The gradual recolonisation process was observed at stations 3, 4 and 7, whereas different processes (with an exponential trend) were observed at stations 5 and 6. These stations were initially characterised by the abundance of a few opportunistic species (*e.g.* *Corbula gibba* and *Terebellides stroemi*) and, subsequently (in the last monitoring), by an increase in abundance and in the number of sabulicolous species (*e.g.* *Streblosoma bairdi*, *Nephtys hombergi* and *Diplocirrus glaucus*) which had not been collected in the previous investigated periods. This phenomenon is normally observed in dredged substrata where the defaunation allows the opportunistic species to form dense popula-

tions in the first phase of the recolonisation process, followed by an increase in the number of species and individuals (Bonsdorff 1980; Kenny & Rees 1994, 1996; Newell *et al.* 1998; Sardà *et al.* 2000; Van Dalen *et al.* 2000; Nicoletti *et al.* 2004). The differences between two recolonisation processes at the impacted stations were probably related to the fact that the first group of stations (3, 4 and 7) was influenced exclusively by only one dredging (the third one), whereas the second group (5 and 6) was affected by two dredgings (respectively the first and the third one for station 5 and the second and the third one for station 6). Moreover, these differences could be related to the intensity of dredging operations in terms of dredging frequency, as also observed by Boyd & Rees (2003), Newell *et al.* (2004), Robinson *et al.* (2005) and Cooper *et al.* (2007).

This study has confirmed the observations of some authors (Robinson *et al.* 2005; Smith *et al.* 2006) concerning the difficulties in evaluating the effects over time of relict sand dredgings on benthic assemblages, due to the high number of factors involved. In our specific case, the analysis of the impact on the assemblages was further complicated by the use of two different types of dredge, and by the fact that dredging activities were repeated within a relatively short period of time, as well as in areas that are very close to one another. Further, medium-term monitoring surveys will provide a more detailed description of how the recolonisation process of macrobenthos assemblages affected by sand dredging will occur, as well as how long this will take.

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## ORIGINAL ARTICLE

# Effect of observation method on the perception of community structure and water quality in a brackish water ecosystem

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## Keywords

Baltic sea; macrophytes; benthic invertebrates; scale; water quality.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

The EU Water Framework Directive is a Community legislative instrument in the field of environmental protection that establishes a common framework for keeping water quality at a favourable level. To implement the directive, classification systems need to be established that allow detection of human impacts at early stages and, thus, more effective management of coastal communities. Due to the spatial variability of communities, however, the results of any assessment are highly dependent on the selection of data. In this study we identified local spatial scales in which variability of macrophyte communities was maximised, quantified links between observed patterns of sediment types and communities and estimated how selection criteria impacted the outcome of the assessment of indicator class value in four different communities of the Northern Baltic Sea. The main findings of the study were that: (i) there were no clear local spatial scales in which the variability of benthic communities was maximised; (ii) hard-bottom communities were better predicted by the spatial arrangement of sediment characteristics than soft-bottom communities; (iii) the selection of method had no effect on the estimates of macrophyte cover and indicator class; but (iv) method impacted independently of habitat type on error estimates of macrophyte cover and indicator class. To conclude, in such homogeneous and low diversity macrophyte communities it is preferable to use methods that result in lower error estimates of algal coverage and, thus, result in lower uncertainties of estimates in the water quality class.

## Problem

The EU Water Framework Directive is a Community legislative instrument in the field of environment protection that establishes a common framework for keeping water quality at a favourable level. To implement the directive, classification systems need to be established that allow us to assign each waterbody one of five ecological quality classes. This classification system has to be developed using mainly biological indicators (Water Quality Elements). Submerged Aquatic Vegetation is one of those Quality Elements and relevant indicators have to be

developed and intercalibrated to be useable in the classification system (Anonymous 2000). The choice of indicators and assessment method depends on the spatial patterns of the pressures and communities involved.

To identify the most important governing factors one needs to determine the scales at which communities have the largest variability and where the links between environmental and biotic patterns are the strongest (Platt & Denman 1975; Steele & Henderson 1994). Benthic communities have high structural variability at a multitude of scales and this variability is closely linked with physical setting. Therefore the mapping studies should incorporate

the relevant scales of variability to understand factors and processes generating patterns in biotic and abiotic components of ecosystems (Menge & Olson 1990; Levin 1992; Karlson & Cornell 1998). To quantify marine biodiversity, conserve marine ecosystems and assess impacts of human activities, understanding the spatial distributions of biota at local scales (10s or 100s of metres) is important for coastal management (Kotta *et al.* 2008c).

To date a number of quantitative methods have been developed to map benthic macrophyte communities at the local scale. Except for one study (Leujak & Ormond 2007), earlier studies have failed to provide any clear guidance as to which method is to be preferred. With the introduction of digital video cameras with greatly improved resolution, filming and assessing benthic communities along transects has become an increasingly practical and popular method (Murdoch & Aronson 1999; Riegl *et al.* 2001; Houk & Van Woessik 2006; Bucas *et al.* 2007). In general, the accuracy and precision of methods is dependent on the investment of resources but the resources are generally limited or fixed. Sample size is often the dominant factor in determining precision. If the sample size is too low, the study will lack the precision to provide reliable answers to the questions it is investigating. If the sample size is too large, time and resources will be wasted, often for minimal gain. Absolute accuracy and precision are not our ultimate goal; rather, an analysis of how data selection affects the outcome of water quality assessment is needed. Nevertheless, it is essential that the preferred method allows detection of already small changes in community structure (*i.e.* has low error estimate) and therefore can be used by the early warning systems.

In this study we: (i) attempted to identify local spatial scales at which the variability of benthic communities is maximised; (ii) quantified links between observed patterns of sediment types and communities; and (iii) estimated whether and how the criteria of data selection impacted the outcome of the assessment of indicator class value in four different communities of the Northern Baltic Sea. Our hypotheses were as follows: (i) the spatial patterns of macrophyte communities strongly reflect the patterns of seabed sediment, (ii) the communities that are more heterogeneous are more sensitive to the criteria of data selection and (iii) random sampling should produce better estimates of distribution of key species and communities as compared to uniform sampling.

## Material and Methods

The study was carried out in four shallow water communities of the Eastern Baltic Sea in summer 2007 (Fig. 1). The Baltic Sea is a geologically young semi-enclosed sea. There exists a strong salinity gradient

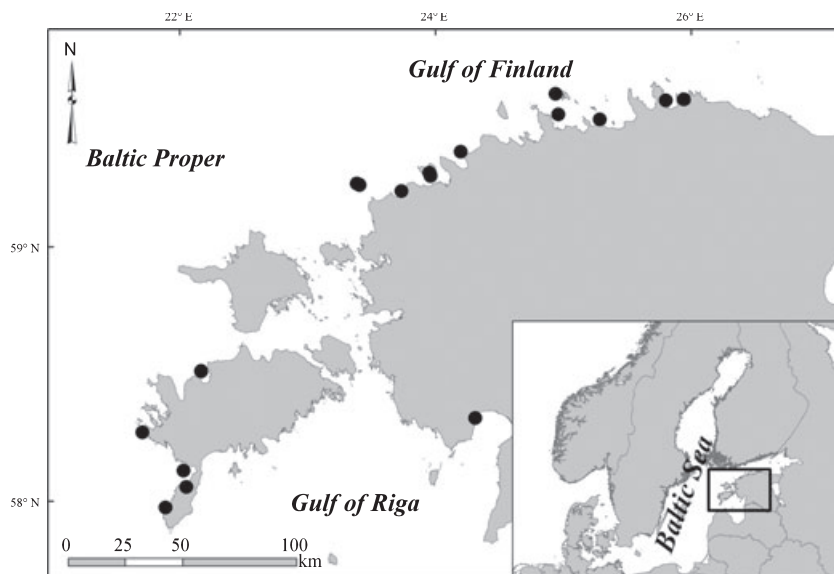
from west to east. Sand, silt and clay sediment prevail and hard substrata are usually found in shallow and exposed bays. Due to the prevalence of clay substrate and strong wave action, the water transparency is often very low, while the low salinity and strong seasonality in temperature and light conditions result in the number of aquatic plant species inhabiting the Northern Baltic Sea being small (Kotta *et al.* 2008a,b).

Using a remote underwater video device we estimated the spatial patterns of benthic vegetation and invertebrates in four different communities of the Northern Baltic Sea. The camera was set at an angle of 35° below horizontal to maximise the field of view and the range of the forward view was about 2 m in clear waters. The camera sled was towed from a 5-m-long boat 1 m above the sea floor at an average speed of 50 cm·s<sup>-1</sup>. Real-time video was captured with a digital video recorder. Depth and navigational data (from GPS) were recorded at 1-s intervals during camera deployments.

The four different communities investigated were: (i) bladder wrack *Fucus vesiculosus*, (ii) the red alga *Furcellaria lumbricalis*, (iii) the eelgrass *Zostera marina*, (iv) other higher plants (*Potamogeton* spp., *Myriophyllum spicatum*). The former two communities rely on hard substrate and the latter two on soft substrate.

For each community five transects of 87.5 m length were videotaped. In each transect the coverage of different sediment type (rock, boulders, pebbles, gravel, sand), benthic macrophyte and invertebrate species were estimated (i) in a continuous video mode and (ii) in a still picture mode based on 35 photographs taken with a step of 2.5 m. Additionally, the coverages were also estimated based on 5, 10 and 20 photographs selected using either uniform or random sampling of the respective pool of 35 photographs. The coverages were then averaged over transect and selection criteria, resulting in five replicate cover estimates of each community and selection criteria. These replicates were used later in statistical data analyses. A mean of sediment, algal and invertebrate coverages of all frames provided a baseline within the video technique when estimating the precision of different methods (Weinberg 1981; Leujak & Ormond 2007).

The ratios of annual to perennial macroalgae and of annual algae to *Z. marina* are potential indicators of eutrophication, as high nutrient concentrations are known to favour the growth of ephemeral flora (Sand-Jensen & Borum 1991; Kotta *et al.* 2000; Pedersen & Snoeijs 2001). The group of annual algae often includes ephemeral species such as *Chaetomorpha linum*, *Cladophora glomerata*, *Pilayella littoralis* and *Ectocarpus* sp. and sheet-formed species such as *Ulva lactuca*. Typically, they have thin tissues capable of fast rates of nutrient uptake and growth; they are relatively short-lived and represent



**Fig. 1.** Map of the studied transects in the NE Baltic Sea.

the so-called r-strategy of population growth. In contrast to the annual species, perennial species are slow-growing, often have thicker and more complex tissues and are represented by, for example, the genera *Fucus*, *Furcellaria* and *Laminaria*. Although the division into annual and perennial types is straightforward in many cases, there are some species that do not fit this classification scheme. Nevertheless, this indicator is used in several schemes of water quality assessment, especially in cases where more traditional indicators (e.g. depth penetration of vegetation) is not applicable due to substratum limitation or shallowness of coastal areas. In the Mediterranean area this indicator is used either independently or is incorporated into different index calculations (e.g. Orfanidis *et al.* 2001; Juanes *et al.* 2008). The indicator is often calculated as the share of perennial (or annual) species to total vegetation cover or biomass. In Estonia the indicator is calculated as the coverage of species and divided into five classes: high (share of perennial macroalgae >72%), good (72–45%), moderate (45–27%), poor (27–9%) and bad (<9%) (Anonymous 2008).

Factorial ANOVA (Statistica version 8.0) and ANOSIM (PRIMER version 6.1) analyses were used to test for significant differences in the coverage of key species and communities among observation methods. Dissimilarities between each pair of algal and invertebrate cover estimates along transects were calculated using a zero-adjusted Bray–Curtis coefficient and dissimilarities between each pair of sediment cover estimates along transects were calculated using Euclidean distance. The scatterplot between the obtained dissimilarity matrixes of coverages and the respective point distances of samples was used as a proxy of the scale-specific spatial variability in sediment characteristics and community cover.

## Results

Altogether, 16 taxa were identified. Most species were observed in *Zostera marina* (9) and *F. vesiculosus* (8) community. The mean coverage of community-forming species (i.e. *Z. marina*, other higher plants, *F. vesiculosus*, *F. lumbricalis*) exceeded 40% (Table 1).

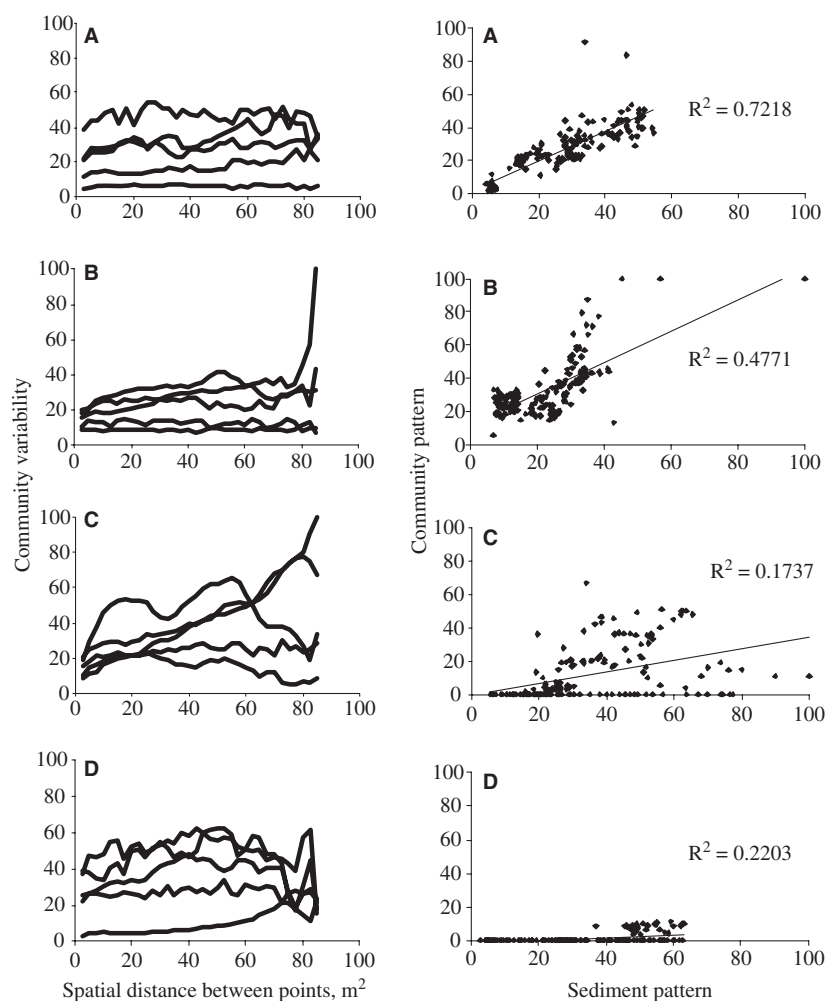
The scatterplots between the point distances of samples and dissimilarity matrixes of coverage (i.e. community variability) show that there were no clear local spatial scales in which the variability of benthic communities was maximised (Fig. 2). Hard bottom communities were less patchy compared to soft bottom communities. *Fucus vesiculosus* and *F. lumbricalis* communities tended to be relatively homogeneous through the range of the studied spatial scales. The spatial pattern of *Z. marina* and other higher plant communities largely varied along transects. These scatterplots also show that some transects had the largest variability at 20 or 50 m (indicating that the patch size of macrophyte communities was on average either 20 or 50 m). The variability of other communities either increased with spatial scale or, similarly to hard-bottom communities, did not vary with spatial scale (Fig. 2).

Changes in sediment characteristics largely explained the spatial arrangement of *F. vesiculosus* and *F. lumbricalis* communities on hard bottom. However, *Z. marina* and other higher plant communities on soft bottoms were weakly related to the spatial patterns of sediment characteristics (Fig. 2).

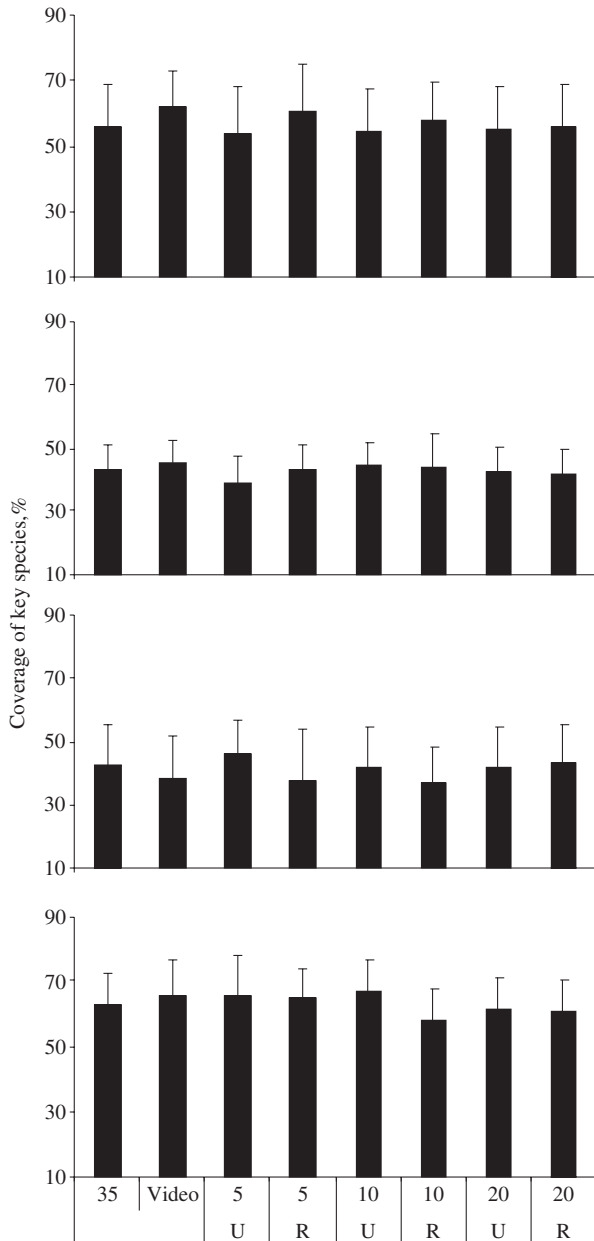
Different sampling frequencies and distributions had no effect on the mean estimates of the key species (Fig. 3), community coverage and the indicator class (ANOVA and ANOSIM  $P > 0.05$ ) (Table 2). However, different sampling frequencies and distributions produced

**Table 1.** Average coverage of benthic macrophyte and invertebrate species found in the investigated communities.

taxa	community			
	<i>Fucus vesiculosus</i>	<i>Furcellaria lumbricalis</i>	<i>Zostera marina</i>	higher plants
<i>Ceramium tenuicorne</i> (Kützinger) Waern	1.94	–	–	–
<i>Chara</i> sp.	–	–	–	3.94
<i>Chorda filum</i> (Linnaeus) Stackhouse	0.20	–	0.02	–
<i>Cladophora glomerata</i> (Linnaeus) Kützinger	11.31	–	0.03	–
<i>Cladosiphon zosterae</i> (J. Agardh) Kylin	–	–	2.75	–
<i>Fucus vesiculosus</i> Linnaeus	56.31	–	0.81	0.56
<i>Furcellaria lumbricalis</i> (Hudson) J.V. Lamouroux	–	43.51	–	–
<i>Myriophyllum spicatum</i> Linnaeus	–	–	–	11.07
<i>Pilayella littoralis</i> (Linnaeus) Kjellman	32.94	1.78	42.21	38.04
<i>Polysiphonia</i> sp.	–	38.81	–	–
<i>Potamogeton pectinatus</i> Linnaeus	–	–	4.48	41.19
<i>Potamogeton perfoliatus</i> Linnaeus	0.01	–	2.38	10.61
<i>Sphacelaria arctica</i> Harvey	–	0.37	–	–
<i>Zostera marina</i> Linnaeus	1.12	–	42.12	–
<i>Balanus improvisus</i> Darwin	1.04	3.76	–	–
<i>Mytilus trossulus</i> Gould	–	0.12	0.69	–

**Fig. 2.** Left column: variability of the macrophyte communities (Bray–Curtis dissimilarities) through the range of studied spatial scales. Right column: scatterplots of the dissimilarities of sediment characteristics and benthic macrophyte communities assessed separately for each pair of samples in a transect; R, linear correlation coefficient between the studied variables. Community code is as follows: A, *Fucus vesiculosus*; B, *Furcellaria lumbricalis*; C, *Zostera marina*; D, other higher plants.



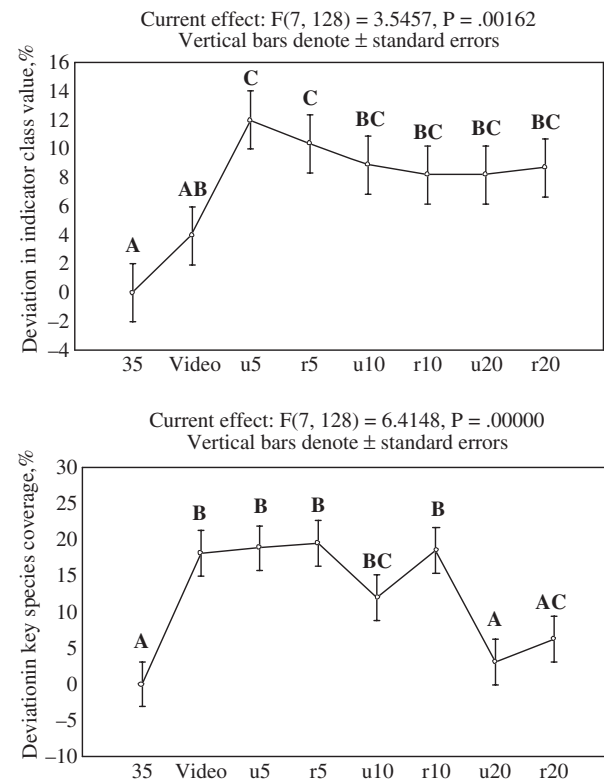


**Fig. 3.** Average coverage of key species (%) with SE according to different sampling frequencies and distributions applied. Treatments: video, estimates based on continuous video, 5, 10, 20 and 35 indicate the number of photographs included; U, uniform sampling; R, random sampling. Community code is as follows: A, *Fucus vesiculosus*; B, *Furcellaria lumbricalis*; C, *Zostera marina*; D, higher plants.

different error estimates. The cover estimates in a still picture mode based on 35 or 20 photographs had significantly lower error compared to other sampling rates. Similarly, the indicator class estimates in a continuous video mode and in a still picture mode based on 35 photographs had significantly lower error compared to other sampling rates (Fig. 4).

**Table 2.** Summary of the factorial anova analysis of the effect of sampling methods and community types on the evaluation of water indicator classes and key species coverage. Significant effects are indicated in bold.

effect	SS	DF	MS	F	P
key species coverage					
method	893	7	128	0.31	0.948
habitat	466	3	155	0.38	0.770
method × habitat	9305	21	443	1.08	0.381
key species coverage (absolute values)					
<b>method</b>	<b>8803</b>	<b>7</b>	<b>1258</b>	<b>6.41</b>	<b>0.000</b>
<b>habitat</b>	<b>2260</b>	<b>3</b>	<b>753</b>	<b>3.84</b>	<b>0.011</b>
method × habitat	4120	21	196	1.00	0.467
indicator classes					
method	287	7	41	0.27	0.964
habitat	951	3	317	2.09	0.104
method × habitat	1499	21	71	0.47	0.976
indicator classes (absolute values)					
<b>method</b>	<b>2024</b>	<b>7</b>	<b>289</b>	<b>3.54</b>	<b>0.002</b>
habitat	508	3	169	2.08	0.106
method × habitat	908	21	43	0.53	0.953



**Fig. 4.** Deviation in the estimates of indicator class and key species coverage for different sampling frequencies and distributions applied. A, B, C indicate different homogeneous groups (Fisher LSD *post-hoc* test).

## Discussion

The main findings of the study were that (i) there were no clear local spatial scales in which the variability of benthic communities was maximised; (ii) hard-bottom communities were better predicted by the spatial arrangement of sediment characteristics than soft bottom communities; (iii) the selection of method had no effect on the estimates of macrophyte cover and indicator class, but (iv) impacted independently of community the error estimates of macrophyte cover and indicator class.

In the Northern Baltic Sea bottom substrate is very heterogeneous and often characterised by mixed sediments. Different substrate types are found either within very small areas (e.g. patches of sand or stones that do not exceed 50–200 cm in diameter) or, alternatively, different sediment types are mixed at the same location (e.g. mixture of clay, sand and pebbles) (Kotta *et al.* 2008a,b). Our results suggest that the cover values of true hard-bottom species *i.e.* *F. vesiculosus* and *F. lumbricalis* clearly reflected such sediment heterogeneity. By far the strongest correlations between the cover of different substrate types and the patterns of macrophyte communities were observed in *F. vesiculosus* community. The availability of light and mineral nutrients is known to play a crucial role, with upwelling, turbulence, turbidity and grazing intensity as secondary factors in regulating the distribution of macroalgae (Field *et al.* 1998). The availability of hard substrate is a prerequisite for the establishment of the macroalgal species found in the study area and other limiting factors are known to have minor effects on the macroalgal communities in the study area (Kotta & Orav 2001; Eriksson & Bergström 2005; Kotta *et al.* 2006; Orav-Kotta *et al.* 2009).

On the other hand, there was only a weak link between sediment heterogeneity and soft-bottom macrophyte communities. It is likely that wave exposure rather than sediment characteristics determine the distribution of true soft-bottom macrophyte species. Large waves may cause considerable resuspension of sediments and result in prolonged periods of poor light conditions (Madsen *et al.* 2001) but, similarly, they may exert a mechanical disturbance of the benthic communities (Dernie *et al.* 2003; Kotta *et al.* 2007; Schiel & Lilley 2007).

It is plausible that the weak effects of the choice of methods can be attributed to the homogeneous distribution of benthic macrophyte communities in the study area and to the small range of scales investigated. Only a few transects on soft sediments were characterised by the patchy distribution of the key macrophyte species. Providing the low diversity of macrophyte communities in the study area (Kotta *et al.* 2008a,b) already, a few observations may capture the pattern of macrophyte

communities and provide accurate estimates of their parameters.

Earlier studies have shown that the variance of estimates decreases (and precision increases) with increasing sample size when sampling units are scaled up to standard units for comparison (Elliot 1979; Morin 1985). Similarly, in our study the precision of the estimate increased with sample size. Besides, the precision of different sampling frequencies and distributions varied depending on the variable considered (cover of key species *versus* indicator class value). Observation that was based on a continuous video mode resulted in large variability of estimates of the coverage of dominant macroalgal species (*F. vesiculosus*, *F. lumbricalis* and higher plants) compared to still picture mode. On the other hand, both video and still picture mode precisely captured the cover patterns of annual and perennial algae. This may be due to more clumped distribution (reflected in the greater variance of estimates) of the dominant species in relation to the broader categories such as perennial and annual species. In such macrophyte clumps an average speed of 50 cm·s<sup>-1</sup> was too high to capture the patterns of dominant macrophytes precisely.

To implement the EU WFD we need to build monitoring strategies that provide data with sufficient statistical confidence and develop efficient indicators (Anon 2003a,b). When measuring biological indicators a large amount of monitoring data is needed to reach this level of confidence. This goal is often difficult to achieve due to the limitation of resources. Currently evaluated techniques enable us to produce high amounts of data with acceptable quality, enabling the development of adequate assessment schemes.

To conclude, our estimates in a continuous video mode and in a still picture mode based on 35 and 20 photographs produced less variable results compared to other methods. In any long-term monitoring programme a high precision (ability to detect differences) is more important than high accuracy (ability to detect true value) (Andrew & Mapstone 1987). While reducing uncertainties of estimates, these methods should be preferred in the mapping of benthic macrophyte communities or in the assessment of water quality in the relatively homogeneous environments of the Northern Baltic Sea. When a method can only detect large changes, then it is often too late to undertake remedial actions.

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## ORIGINAL ARTICLE

**Macroalgal turfs in the Azores**Francisco M. Wallenstein<sup>1,2,3</sup>, Marlene R. Terra<sup>2</sup>, Joana Pombo<sup>2</sup> & Ana I. Neto<sup>2,3</sup>

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**Keywords**

Calcareous turf; intertidal; non-calcareous turf; rocky shores.

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E-mail: fwallenstein@uac.pt**Conflicts of interest**

The authors declare no conflicts of interest.

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**Abstract**

Studies on macroalgal communities of the Azores report algal turfs as one of the most conspicuous ecological entities occurring on the rocky shores of these islands. The present study investigates the influence of shore height and substratum on turf composition. Data were analysed using the software PRIMER and results confirmed the previous distinction between calcareous, dominated by articulated coralline algae, and non-calcareous turfs, characterized mainly by small red algae. However, no differences in species composition were found in either type of turf occurring on different substrata or at different shore levels.

**Problem**

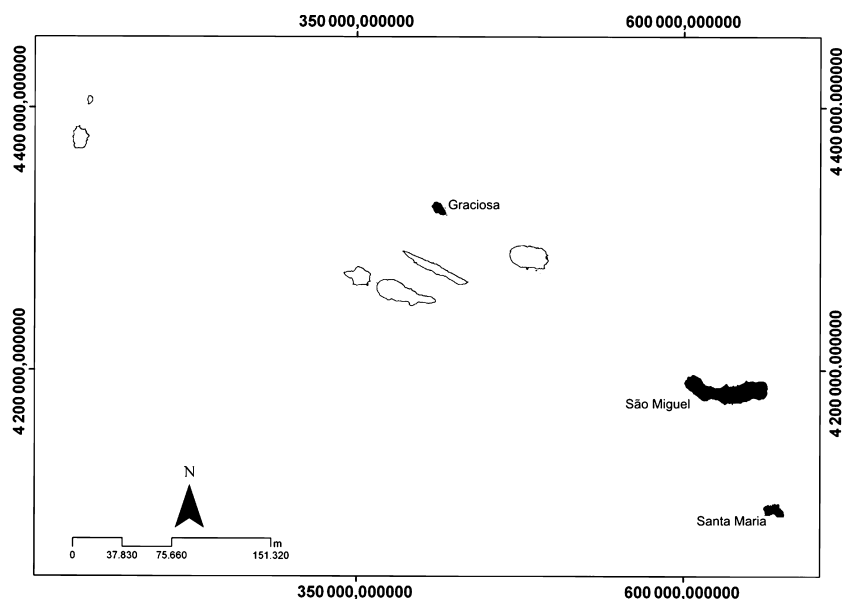
A suite of studies undertaken during the past 15 years has investigated the intertidal and subtidal algal communities of the Azores (Neto 1992, 2000, 2001; Neto & Tittley 1995; Tittley *et al.* 1998; Tittley & Neto 2000). Algal turfs are generally described as complex assemblages of macroalgae with compact growth, 5 cm in height and well developed entangled prostrate axes (Price & Scott 1992). Turfs are conspicuous features of warm temperate intertidal communities, and have been widely studied (Chapman 1955; Pryor 1967; Lawson & Norton 1971; Oliveira & Mayral 1976; Lawson & John 1977; Rogers & Salesky 1981; Stewart 1982; Neto & Tittley 1995; Morton *et al.* 1998). Some studies have classified communities systematically as biotopes based on broad ecological/taxonomic categories, such as green algae, calcareous and non-calcareous turfs and crusts (*e.g.* Neto & Tittley 1995; Tittley *et al.* 1998, Tittley & Neto 2000; Wallenstein & Neto 2006; Wallenstein *et al.* 2008). Turfs classified as calcareous or coralline occur with increasing abundance towards

the low-shore and are usually not very conspicuous on less stable substrata such as cobbles, whereas turfs classified as non-calcareous are more evenly distributed across the intertidal with higher abundances at mid-shore (Neto & Tittley 1995; Wallenstein & Neto 2006; Wallenstein *et al.* 2008). Classification of turfs into these two broad categories has been based on the abundance of erect coralline algae (recognizable by the naked eye) and can thus be subjective and artificial. To evaluate whether such a classification is artificial we have tested differences between species composition in samples of turf provisionally identified *in situ* as calcareous (*i.e.* dominated by erect coralline algae) or non-calcareous turf.

**Material and Methods**

Data for analysis were gathered in the course of intertidal biotope surveys undertaken on three islands of the archipelago – São Miguel, Santa Maria and Graciosa (Fig. 1) in the summer periods of 2004, 2005 and 2006, respectively. As macroalgae communities are not evenly distrib-





**Fig. 1.** Schematic representation of the Azores archipelago with the sampled islands in dark.

uted across the intertidal, both in terms of species composition and substratum coverage, they pose a difficulty in sampling designs that require the collection of an equal number of samples at differing ‘substratum × shore level’ combinations. Although the original aim was to acquire quantitative data for macroalgal communities at high-, mid- and low-shore, on three substrata (cobble, boulders and bedrock), high-shore samples had to be excluded from the pool of data because there were several sites where calcareous turf was absent. At mid- and low-shore, whenever possible two 10 × 10 cm areas (minimal intertidal scraping area defined by Neto 1997) of each type of turf (calcareous and non-calcareous) were cleared (scraped with a chisel into a nylon mesh bag) from the upper facing surfaces of three different substrata and brought to the laboratory for examination. Additionally, owing to an irregular distribution of substrata along the shores of the surveyed islands, an uneven sampling design had to be adopted (Table 1).

Turf constituents were identified with the help of a microscope and species abundances recorded using a semi-quantitative DAFOR scale (Dominant; Abundant; Frequent; Occasional; Rare). To test for differences between types of turfs (calcareous *versus* non-calcareous), substrata (cobble *versus* boulders *versus* bedrock) and shore levels (low-shore *versus* mid-shore), PERMANOVA analyses were run on species richness (as presence/absence data) and species relative abundance data with the software PRIMER (Anderson *et al.* 2008). PERMANOVA combines the best of traditional test-statistics (ANOVA designs) and flexible multivariate nonparametric methods, and can thus be based on symmetric dissimilarity or distance measures (or their ranks) providing

**Table 1.** Number of samples collected according to the surveyed islands, turf types, substrata and shore level.

	Graciosa	Santa Maria	São Miguel	Total
<b>Turf type</b>				
Calcareous	32	27	39	98
Non-calcareous	34	47	48	129
<b>Substrata</b>				
Cobble	22 (3 sites)	14 (4 sites)	28 (5 sites)	64 (12 sites)
Boulders	20 (3 sites)	19 (3 sites)	22 (4 sites)	61 (10 sites)
Bedrock	24 (3 sites)	41 (9 sites)	37 (6 sites)	102 (18 sites)
<b>Shore level</b>				
Low	32	40	40	112
Mid	34	34	47	115
<b>Total</b>	<b>66 (9 sites)</b>	<b>74 (16 sites)</b>	<b>87 (15 sites)</b>	<b>227 (40 sites)</b>

probability values (*P*; significant if <0.05; not significant if ≥0.05) using appropriate permutation methods. The greater the number of possible permutations, the stronger the result of the tests. The island where samples were collected was set as a random factor, as there was no hypothesis regarding differences between islands. Furthermore, SIMPER analysis on relative abundance data was used to identify the species responsible for the differences found between both types of turf.

## Results

### Species present

The turf samples contained 139 species of algae from 24 orders; the Ceramiales contained 60 species, the Gigartinales 11 species, and the Corallinales 9 species. These

**Table 2.** PERMANOVA analysis of differences in turfs according to substratum type (fixed factor with three levels: cobbles, boulders and bedrock) and to shore level (fixed factor with two levels: low-shore and mid-shore).

Source	df	PSpp. composition data/PA data	Permutations
<b>All turfs</b>			
Turf type	1	0.001/0.001 <sup>a</sup>	999
Residual	225		
Total	226		
<b>Calcareous turf</b>			
Substratum	2	0.321/0.23	998
Shore level	1	0.278/0.468	998
Island	2	0.001/0.001 <sup>a</sup>	998
Island × Substratum	4	0.001/0.001 <sup>a</sup>	998
Island × Shore level	2	0.114/0.069	999
Substratum × Shore level	2	0.846/0.625	997
Island × Substratum × Shore level	4	0.01/0.001 <sup>a</sup>	999
Residual	100		
Total	117		
<b>Non-calcareous turf</b>			
Substratum	2	0.393/0.289	998
Shore level	1	0.11/0.077	998
Island	2	0.001/0.001 <sup>a</sup>	998
Island × Substratum	4	0.001/0.001 <sup>a</sup>	998
Island × Shore level	2	0.006/0.037 <sup>a</sup>	998
Substratum × Shore level	2	0.724/0.821	998
Island × Substratum × Shore level	4	0.001/0.001 <sup>a</sup>	999
Residual	91		
Total	108		

<sup>a</sup>Significant test; df = degrees of freedom; P = probability value associated to the test; PA data = presence/absence data; Permutations = number of permutations on which the test was based.

three orders are all in the red algae (Rhodophyta). Other orders present had fewer species.

### Numerical analysis

PERMANOVA tests on species composition and relative abundance upheld the basic separation into calcareous and non-calcareous turfs (Table 2).

When analysing data separately for each turf type there were no significant differences in turfs associated with substratum or shore level (Table 2).

Dissimilarity between calcareous and non-calcareous turfs is greater when comparing these using the relative abundance of its constituents (87.62) rather than using species richness (78.32). Differences between the two types of turf are mainly due to the co-dominant, erect calcareous species within one type of turf and *Gelidium* (dominant) and *Ceramium* spp. within the other (Table 3).

### Discussion

The present study validates the empirical classification of two types of turf, calcareous, and non-calcareous, based on the presence of species of *Corallina*, *Jania* and *Halimnion* that occur in such abundance that they are visually recognizable (cf. Neto & Tittley 1995). Although differences between the two types of turf are mainly due to the dominance of calcareous species within calcareous turfs, *Corallina elongata* can also be an important constituent of non-calcareous turfs; likewise, *Laurencia* spp. are a noticeable component of both communities. The few other major constituents of these two types of turf differ slightly, but there is a long list of minor constituents that are common to both (Table 3) and these are mainly filamentous red algae. The definition of both turfs in the present study is in accordance with the provisional biotope definition study of Tittley & Neto (2000). However, regarding non-calcareous turf in the present study we add *Gelidium microdon* to the species list defined as 'soft algal turf' by Tittley & Neto (2000). This is the main structuring species in our non-calcareous turf samples, which is certainly due to the fact that it occurs mainly in the mid-to-low eulittoral, which coincides with the distribution reported for the association of *Gelidium microdon* and *Fucus spiralis* by Tittley & Neto (2000). Approximately 30% of the total flora of the Azores was identified within the turfs sampled for the present study.

Highly mobile substrata tend to inhibit the attachment and growth of macroalgae and favour thin, turf-like growths that are resistant to abrasion, namely fast-growing, opportunistic algae (mainly green algae and filamentous red and brown algae) that comprise non-calcareous turfs. Our finding of the absence of substratum specificities in non-calcareous turfs with increasing substratum stability (cobbles to boulders to bedrock) was unexpected (Table 2). Increased stability was expected to allow the development of more mature communities, namely turfs with a greater number of species. However, unusual stability conditions in cobble beaches as a result of reduced wave action in the summer might have allowed non-calcareous turf communities on less stable substrata to develop towards those common on more stable substrata. Accordingly, sampling at other times of year might have detected changes in communities on different types of substrata.

Species composition of both types of turf does not vary significantly in the narrow mid- to low-shore range, probably because of the limited shore extension in the Azores. Steep shores and small tidal ranges provide very little space for macroalgae to attach. A turf-like life form may present an advantage in the competition for space and help resist the strong wave action prevalent on most

**Table 3.** Species that contribute to 90% of the differences between calcareous and non-calcareous turfs (SIMPER analysis) and their respective occurrence rate in all turf samples and abundance scores (DAFOR; in bold the most common category for each species).

Spp.	Contribution%	Occurrence	DAFOR categories
Calcareous turf			
<i>Corallina elongata</i>	56.73	0.73	<b>DA</b>
<i>Halimnion virgatum</i>	9.63	0.32	<b>DA</b>
<i>Jania</i> spp. <sup>a</sup>	10.83	0.46	<b>DAFO</b>
<i>Laurencia</i> spp. <sup>b</sup>	7.37	0.51	<b>AFO</b>
<i>Chondria</i> spp. <sup>c</sup>	2.17	0.29	<b>AFO</b>
<i>Gelidium</i> spp. <sup>d</sup>		0.25	<b>AFO</b>
<i>Stypocaulon scoparium</i>		0.27	<b>FO</b>
<i>Chondracanthus acicularis</i>		0.25	<b>FO</b>
<i>Ceramium</i> spp. <sup>e</sup>	4.08	0.51	<b>FOR</b>
<i>Chaetomorpha</i> spp. <sup>f</sup>		0.26	<b>OR</b>
Other spp.(see below)		<0.25	<b>R</b>
Total	90.81		
Non-calcareous turf			
<i>Gelidium</i> spp. <sup>d</sup>	32.44	0.43	<b>DAF</b>
<i>Corallina elongata</i>	13.15	0.46	<b>AFO</b>
<i>Laurencia</i> spp. <sup>b</sup>	7.71	0.35	<b>AFO</b>
<i>Ceramium</i> spp. <sup>e</sup>	14.28	0.51	<b>AFOR</b>
<i>Jania</i> spp. <sup>a</sup>	2.66	0.26	<b>FO</b>
<i>Ulva</i> spp. <sup>g</sup>	2.48	0.28	<b>OR</b>
<i>Chaetomorpha</i> spp. <sup>f</sup>	3.22	0.35	<b>R</b>
<i>Herposiphonia</i> sp.	3.32		
<i>Polysiphonia</i> spp. <sup>h</sup>	2.68		
<i>Gymnogongrus</i> spp. <sup>i</sup>	2.52		
<i>Ahnfeltia plicata</i>	1.77		
<i>Osmundea</i> spp. <sup>j</sup>	1.57		
<i>Centroceras clavulatum</i>	1.53		
<i>Caulacanthus ustulatus</i>	1.4		
Other spp. (see below)		<0.25	<b>R</b>
Total	90.73		

<sup>a</sup>*Jania capillacea*, *Jania longifurca*, *Jania pumila*, *Jania rubens*.

<sup>b</sup>*Laurencia viridis*, *Laurencia* sp.

<sup>c</sup>*Chondria coerulescens*, *Chondria dasyphylla*, *Chondria capillaris*.

<sup>d</sup>*Gelidium microdon*, *Gelidium pusillum*, *Gelidium spinosum*.

<sup>e</sup>*Ceramium ciliatum*, *Ceramium circinatum*, *Ceramium diaphanum*, *Ceramium echionotum*, *Ceramium flaccidum*, *Ceramium virgatum*.

<sup>f</sup>*Chaetomorpha linum*, *Chaetomorpha pachynema*.

<sup>g</sup>*Ulva compressa*, *Ulva intestinalis*, *Ulva clathrata*, *Ulva rigida*.

<sup>h</sup>*Polysiphonia brodiei*, *Polysiphonia denudata*, *Polysiphonia elongata*, *Polysiphonia furcellata*.

<sup>i</sup>*Gymnogongrus griffithsiae*, *Gymnogongrus crenulatus*.

<sup>j</sup>*Osmundea hibrida*, *Osmundea pinnatifida*.

Other spp.: *Acrosorium venulosum*, *Aglaothamnion* sp., *Aglaozonia parvula*, *Ahnfeltia* sp., *Ahnfeltiopsis intermedia*, *Amphiroa* spp. (*Amphiroa beauvoisii*, *Amphiroa* sp.), *Anotrichium* spp. (*Anotrichium furcellatum*, *Anotrichium tenue*), *Antithamnion* sp., *Asparagopsis armata*, *Bachelotia antillarum*, *Boergeseniella* spp. (*Boergeseniella fruticulosa*, *Boergeseniella* sp.), *Bonnemaisonia asparagoides*, *Bryopsis* spp. (*Bryopsis cupressina*, *Bryopsis hypnoides*, *Bryopsis plumosa*), *Callithamnion* spp. (*Callithamnion corymbosum*, *Callithamnion tetragonum*, *Callithamnion* sp.), *Catenella caespitosa*, *Caulacanthus ustulatus*, *Centroceras clavulatum*, *Chondracanthus acicularis*, *Chondrophycus* sp., *Cladophora* spp. (*Cladophora albida*, *Cladophora coelothrix*, *Cladophora prolifera*, *Cladophora* sp.), *Cladophoropsis membranacea*, *Cladostephus spongiosus*, *Codium adhaerens*, *Compsothamnion decompositum*, *Cryptopleura ramosa*, *Cystoseira* spp. (*Cystoseira abies-marina*, *Cystoseira humilis*, *Cystoseira* sp.), *Dasya* spp. (*Dasya corymbifera*, *Dasya hutchinsiae*, *Dasya* sp.), *Dictyota* sp., *Diplothamnion* sp., *Dipterosiphonia* sp., *Drachiella minuta*, *Enderachne binghamiae*, *Erythrocytis montagnei*, *Falkenbergia rufolanosa*, *Fucus spiralis*, *Gastroclonium* spp. (*Gastroclonium ovatum*, *Gastroclonium reflexum*), *Gelidiella* sp., *Gelidiopsis* sp., *Gigartina pistillata*, *Grateloupia* spp. (*Grateloupia filicina*, *Grateloupia dichotoma*), *Griffithsia corallinoides*, *Halopteris filicina*, *Herposiphonia* sp., *Heterosiphonia crispella*, *Hypnea* spp. (*Hypnea arbuscula*, *Hypnea musciformis*), *Lomentaria articulata*, *Lophosiphonia* spp. (*Lophosiphonia obscura*, *Lophosiphonia reptabunda*), *Nemoderma tingitanum*, *Platysiphonia* sp., *Pleonosporium* sp., *Plocamium cartilagineum*, *Porphyra* sp., *Pterocliadiella capillacea*, *Pterosiphonia* spp. (*Pterosiphonia pennata*, *Pterosiphonia* sp.), *Rhodomenia holmesii*, *Sargassum* spp. (*Sargassum cymosum*, *Sargassum vulgare*), *Sphacelaria* spp. (*Sphacelaria cirrosa*, *Sphacelaria plumula*), *Sphondylothamnion multifidum*, *Stylonema alsidii*, *Stypocaulon scoparium*, *Symphyclocladia marchantioides*, *Valonia* spp. (*Valonia macrophysa*, *Valonia utricularis*).

shores of the Azores. Other advantages of the turf life form are the retention of water, the increased surface areas for attachment of admixed algae, increased resistance to herbivory, and increased ability in vegetative propagation (Price & Scott 1992).

No hypothesis was set regarding differences in turfs sampled on different islands. Sampling was done in different years on each island and differences in species composition might reflect variations in population dynamics of the turf constituents.

The turf life form may be an ecological response to severe environmental conditions, notably wave action. Although this life form is resistant to the prevailing harsh conditions, more delicate/fragile algae, such as the filamentous species of *Callithamnion* and *Dasya*, are able to survive as minor constituents within the turf community. Turfs are an important feature of the warm temperate shore communities that dominate intertidal communities in the Azores. They thus deserve special attention, not as major features of an ecosystem, but as a local ecosystem in their own right.

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## ORIGINAL ARTICLE

# Effect of exposure time on the bioaccumulation of Cd, Mg, Mn and Zn in *Cystoseira abies-marina* samples subject to shallow water hydrothermal activity in São Miguel (Azores)

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## Keywords

Hydrothermal activity; intertidal; macroalgae; metals.

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## Conflicts of interest

All authors declare no conflicts of interest.

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## Abstract

Shallow water hydrothermal vents can be compared to polluted places due to high concentrations of heavy metals, and are thus good models for bioaccumulation studies. The present study intended to estimate the time of exposure required for the accumulation of certain elements to stabilize in specimens of *Cystoseira abies-marina*, to be used as a reference in future work. *Cystoseira abies-marina* intertidal specimens were transplanted from Mosteiros (a non-hydrothermal and pristine site) to Ferraria (with hydrothermal activity) and left there. Transplanted samples were collected after 1, 2, 4 and 8 weeks and the concentrations of Cd, Mg, Mn and Zn were measured through flame atomic absorption spectrophotometry. Although further studies with increased periods of exposure are needed because the concentration of these elements never stabilized in the collected samples, there is strong evidence that increased time of exposure led to increased concentrations of Cd, Mg, Mn, but not Zn. These results are consistent with the assumption that *C. abies-marina* is bioaccumulating some of the heavy metals and can thus be a good indicator for polluted waters.

## Problem

Increased coastal pollution causes change in ecosystems, namely due to species adaptation to stressing environmental conditions (Chapman & Bulleri 2003). It is important to study the phenomenon of pollution, such as increased nutrient and heavy-metal load, and its impact on living organisms, as bioaccumulation and biomagnification of such elements along trophic chains increase their toxicity in the aquatic environment over time (Gochfeld 2003; Kamala-Kannan *et al.* 2007).

Hydrothermal environments are extraordinary scenarios with high concentrations of metals such as Cd, Cu, Fe, Mg, Mn, Rb and Zn that arise from discharges of sulphides (Von Damm 1990), which makes them appropriate

for the study of ecological impacts of those elements on communities that live there (Cosson & Vivier 1997; Ventox 2003).

Marine macroalgae are primary producers in coastal waters that accumulate such elements, which are further transferred along the trophic chain by herbivores and detritivores (Agadi *et al.* 1978). Many toxic pollutants are found only in trace amounts in the water, and often at elevated levels in sediments, thus risk assessments based only on data derived from water analyses may be misleading, and data from sediments may not be representative of pollutant concentrations in the overlying water column and cannot give information on patterns of contamination at higher levels of the food chain (Torres *et al.* 2008). As the concentration of metals in algae tissues is



proportional to their diluted concentration in the environment, they are important bioindicators of the environmental exposure to those elements, thus useful as test organisms for marine pollution studies (Fletcher 1991). Brown algae tend to have a higher affinity for metal accumulation than green and red algae (Markham *et al.* 1980), mainly due to the polyphenolic substances and polysaccharides that constitute their cellular walls (Forsberg *et al.* 1988).

The present work focused on the bioaccumulation of cadmium, magnesium, manganese and zinc in *Cystoseira abies-marina* in a site with shallow water hydrothermal activity as a natural pollution source. It constitutes a preliminary approach to the use of this species as a tool to monitor water quality in the Azores.

### Study Area

The present study was conducted at São Miguel island (Azores), consisting of transplanting *Cystoseira abies-marina* specimens from a site with no hydrothermal activity to a small enclosed bay with shallow water hydrothermal activity (Mosteiros and Ferraria, respectively; Fig. 1).

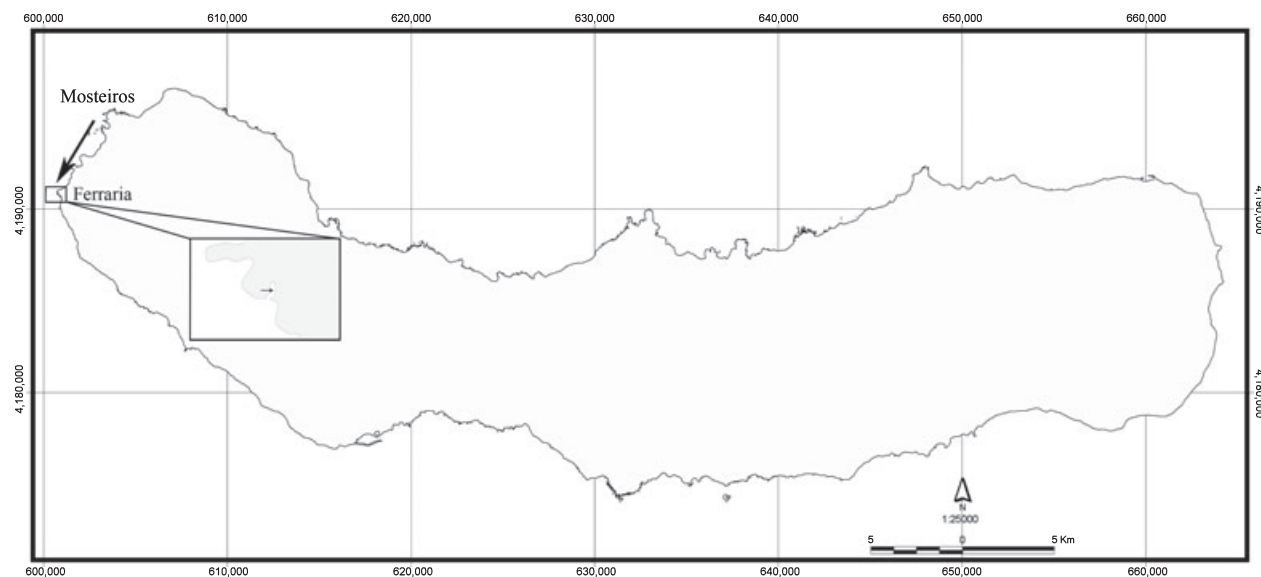
### Material and Methods

Specimens were collected with a chisel from Mosteiros, transported to Ferraria and placed within the hydrothermally active basin of Ferraria. *Cystoseira abies-marina* specimens were entangled in a 20 cm × 10 cm square of plastic-coated wire mesh (1 cm × 1 cm), and subsequently screwed to the rock (Fig. 2). Specimens

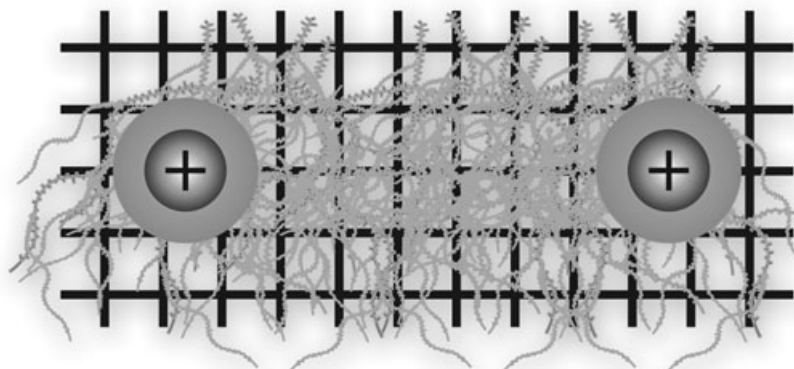
were collected for analysis at  $t = 0$  and transplanted specimens were collected after being exposed for 1 week, 2 weeks, 4 weeks and 8 weeks, and brought to the laboratory. They were then cleaned of epiphytes with a soft nylon brush, dried to constant weight in a drying oven at 70 °C, and ground with a mortar and pestle. Subsequently, samples were subject to acid digestion: (i) 3 ml of HNO<sub>3</sub> (65%) was added to approximately 0.5 g of ground sample and kept 43 h at room temperature; (ii) subsequently these were diluted 10 times and transferred to a heating plate at 100 °C for 4 h; (iii) then 1 ml of H<sub>2</sub>O<sub>2</sub> (30%) was added and the samples kept on the heating plate at 100 °C for one extra hour. Samples were then ultrasonicated (2 min at 50 Hz.) and spun at 3220 g for 10 min. The levels of Cd, Mg, Mn and Zn in the liquid fraction were quantified with flame atomic absorption spectrometry. EU-certified reference material (BCR-279 – *Ulva lactuca*; reference values for Cd and Zn) was used to validate the metal extraction process, and blank samples were used to validate sample treatment procedures. ANOVA procedures were used to test differences in metal levels of samples subject to different hydrothermal activity exposure times.

### Results

Blank samples did not indicate contamination for any of the analysed elements. Metal extraction efficiency from reference material was greater for Zn (96.8%) than for Cd (60.9%); there were no certified values for Mg and Mn (Table 1).



**Fig. 1.** São Miguel Island, indicating where samples were collected (Mosteiros) and transplanted to (Ferraria).



**Fig. 2.** Schematic representation of the transplantation method.

**Table 1.** Levels of Cd, Mg, Mn and Zn in *Cystoseira abies-marina* samples exposed to shallow water hydrothermal activity for 1 week, 2 weeks, 4 weeks and 8 weeks.

	Cd mg·kg <sup>-1</sup> dry weight	Mg mg·kg <sup>-1</sup> dry weight	Mn mg·kg <sup>-1</sup> dry weight	Zn mg·kg <sup>-1</sup> dry weight
0 weeks	1.014	666.639	4.054	25.655
	1.052	781.874	3.859	25.510
	0.552	712.627	3.567	31.172
1 week	0.000	1016.697	41.101	28.899
	0.000	1060.525	21.100	216.468
	0.000	870.383	25.285	29.113
2 weeks	0.195	1080.098	43.220	26.146
	0.087	962.674	50.868	26.997
	0.000	1016.551	29.684	35.140
4 weeks	0.412	936.294	257.647	20.529
	1.197	7826.590	179.356	21.057
	1.495	901.910	174.086	17.442
8 weeks	1.196	1094.616	1830.508	23.629
	1.811	8842.144	2164.510	26.691
	1.691	8649.610	2690.373	42.194
reference material	0.330	12,584.830	1606.083	59.692
(Cd = 0.274; Zn = 51.3)	0.058	15,195.579	1716.373	42.038
blank	0.000	0.000	0.000	0.004
	0.000	0.005	0.000	0.014
	0.000	0.003	0.000	0.009

Except for Zn, the concentration of all the elements showed an increase after the samples had been exposed to hydrothermal activity for 8 weeks relative to the time they were collected at the non-hydrothermal site (Table 1). However, for shorter periods these values varied for each element, and differences were significant only for Cd and Mn (Fig. 3). Compared to the initial Cd concentrations, there was first a reduction and then an increase to levels above the initial ones (Fig. 3a), whereas Mn concentrations started increasing significantly only after being

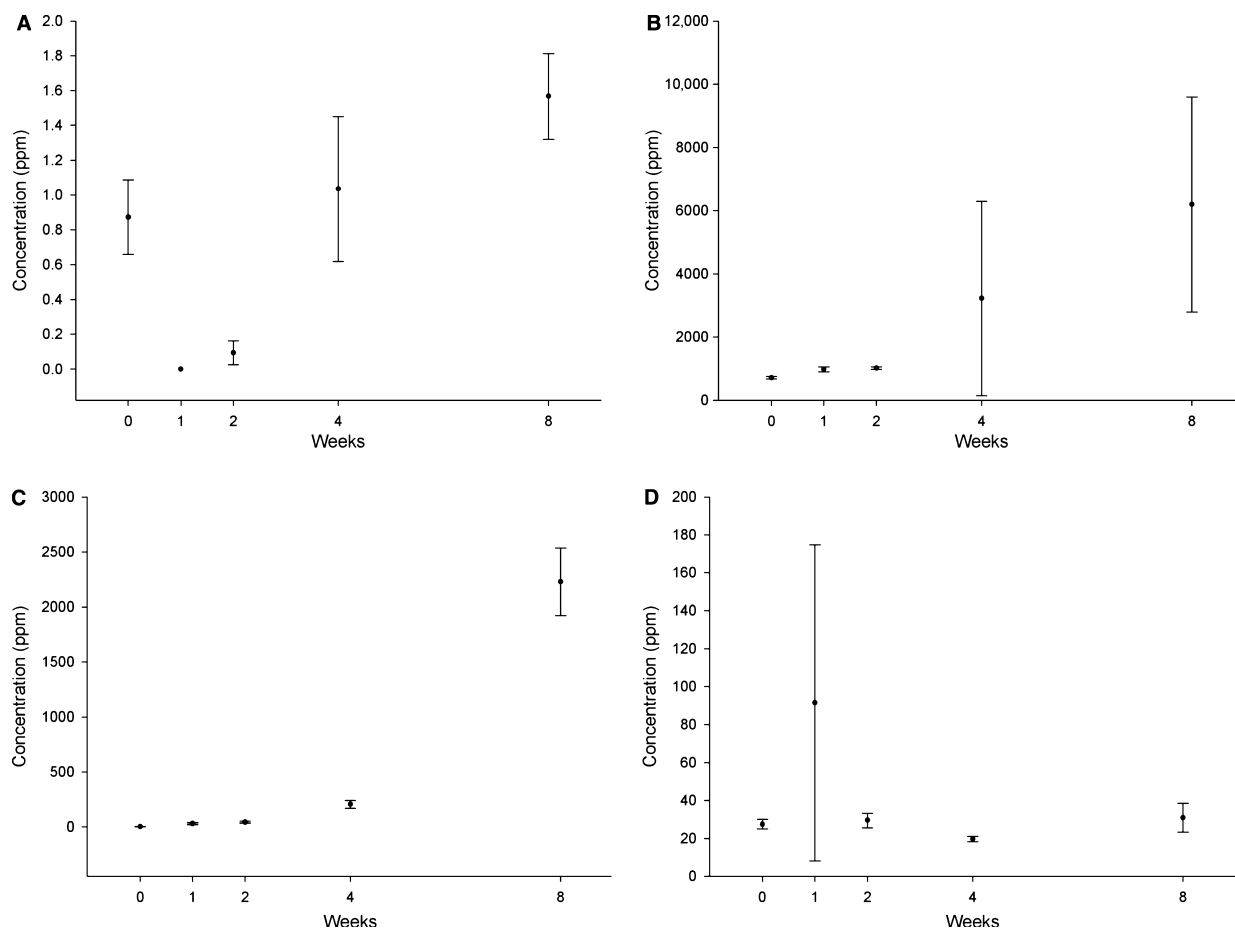
exposed for 4–8 weeks (Fig. 3c). Although not significant, Mg concentrations also started increasing in a greater proportion after being exposed for 4 weeks (Fig. 3b).

## Discussion

Cadmium levels are generally low and usually require accurate detection methods, ideally graphite furnace AAS. Flame AAS might not be accurate enough for detecting low Cd levels and thus reveal low extraction efficiency. Further assays using graphite furnace AAS are planned to confirm this theory, and if this has not been the issue, than alternative digestion protocols need to be tested to optimize results. Zn is generally present in much higher amounts and thus flame AAS is appropriate for its detection, as evidenced by the extraction efficiency obtained for the reference material.

The only work found on heavy metal accumulation in *Cystoseira abies-marina* (Lozano *et al.* 2003) reports Cd accumulation values similar to those of the present study associated with polluted sites in the Canary Islands. Other studies on heavy metal accumulation in different *Cystoseira* species focus on many elements, including Cd, Mg, Mn and Zn (Caliceti *et al.* 2002; Al-Masri *et al.* 2003). However, it is difficult to compare between species that might have different physiological response to heavy metal availability in the water. Given the objective of finding a tool to monitor water quality in the Azores, it seems more appropriate to study the usefulness of *C. abies-marina* in reflecting heavy metal concentration of surrounding waters rather than comparing its accumulation capacity throughout some geographical range, or with other related species.

Specimens collected in the non-hydrothermal site presented cadmium levels below what Lozano *et al.* (2003) report as polluted (<1 ppm). Polluted levels (1–2 ppm) were reached only after 4 weeks in the hydrothermal site. The initial decline in Cd levels is possibly related to the



**Fig. 3.** Average concentration of Cd (a; anova  $P = 0.006$ ), Mg (b; anova  $P = 0.1293$ ), Mn (c; anova  $P = 0.0000$ ) and Zn (d; anova  $P = 0.4160$ ) in samples of *Cystoseira abies-marina* exposed to hydrothermal activity for 1 week, 2 weeks, 4 weeks and 8 weeks.

stressing new environment (on average, temperatures 6–8 °C higher and pH 1–1.5 lower than those of the open ocean in the Azores). Such conditions can induce a physiological response like the release of polyphenolic molecules (phlorotannins) that are strong chelators to heavy metals in solution (Toth & Pavia 2000; Topcuoglu *et al.* 2003; Stengel 2006). The gradual increase of Cd levels in the second week and take off after the fourth week indicate a possible physiological adaptation to the new conditions and the gradual synthesis of new polyphenols. Eight weeks of exposure were not enough for the levels of cadmium to stabilize, which probably indicates high concentrations of this element in the surrounding water.

The initial period of unresponsiveness in the accumulation of Mg and Mn is likely to be also related to the release of polyphenols under stressing conditions. Synthesis of new polyphenolic molecules is likely to take time, and thus the reduced absorption and/or adsorption capacity in an initial period after being exposed to the hydrothermal activity. Unresponsiveness of Zn accumula-

tion suggest that there are no differences in Zn concentrations in hydrothermal and non-hydrothermal waters, which contradicts previous work conducted in the Azores in similar environments (Zaldibar *et al.* 2006; Amaral *et al.* 2007, 2008; Cunha *et al.* 2008). Zn is structurally present in many enzymes and is thus involved in physiological processes. Consequently, a delay in its accumulation might be related to the interference of adverse environmental conditions (increased temperature and acidity) with enzymatic activity, which might take longer, if ever, to respond.

## Conclusions

*Cystoseira abies-marina* proved to accumulate heavy metals differently in hydrothermal and non-hydrothermal sites. This means that: (i) *Cystoseira abies-marina* can be used to monitor heavy metal levels in the water; and (ii) shallow water hydrothermally active sites can be used as models for natural pollution studies. However, further

studies are needed to assess the maximum metal accumulation capacity of this species and the time required to reach it, and also relate it to heavy metal concentration in the surrounding water.

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## ORIGINAL ARTICLE

# Effects of experimental small-scale cockle (*Cerastoderma edule* L.) fishing on ecosystem function

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## Keywords

Biological traits analysis; ecological function; fishing disturbance; intertidal assemblages; macrofauna; redundancy hypothesis; UK.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

The cockle *Cerastoderma edule* L. occurs at commercially exploited densities within both species-poor and species-rich assemblages, dominating the faunal biomass and potentially acting as a key contributor to a number of ecological functions. The questions considered were: Is the delivery of ecological functions affected by the removal of *C. edule*? Does the biodiversity of the affected assemblage affect the extent to which it is altered? To explore these questions, adult cockles were removed from experimental plots at two shores in the North-West of England: a species-poor shore at Warton Sands, Morecambe Bay, and a more diverse shore at Thurstaston, Dee estuary. Impacts on the functioning of the systems were determined during a 4-month study in summer 2007 by direct measurements of benthic primary production, organic matter content and sediment granulometry, and by indirect analyses using Biological Traits Analysis. Removal of adult cockles led to significant changes in faunal assemblage composition and in the distribution of biological traits, with increased biodiversity and an increased prevalence of traits relating to opportunistic taxa observed following the removal of *C. edule*. These changes occurred at both study sites and hence were independent of the initial biodiversity of the assemblages. Sediment granulometry was significantly affected; however, surface chlorophyll and organic matter were not affected. This implies a minimal impact on these functions and a high degree of functional redundancy within the macrofauna. The implications of these findings and future areas of research are discussed.

## Problem

There is an increasing drive to consider ecosystem dynamics in terms of ecological functions, the maintaining of these functions being seen as a key factor in sustaining the goods and services provided by ecosystems (e.g. E.C. 2007). Ecosystem functions, as defined by Naeem *et al.* (2002), are the 'activities, processes or properties of ecosystems that are influenced by their biota'. In littoral systems, these functions include the cycling of organic carbon and nutrients, food provisioning, biological productivity and energy transfer through the system (Christensen *et al.* 1996).

There has been much debate as to the role that species diversity plays for the functioning of ecosystems (e.g. Kaiser 2000) and a number of hypotheses have been developed to explain the effects of species on ecosystem function (Huston 1997; Loreau & Hector 2001). Species do not evolve in order to provide ecological functions. Instead, the delivery of functions is a consequence of the methods that taxa have developed to survive and reproduce, with different taxa developing different approaches to, for example resource capture and reproduction. The 'rivet hypothesis' (Ehrlich & Ehrlich 1991) suggests that each species in an assemblage plays a unique and specialized role, providing a range of functions unique to that



species and that the extinction of a species will result in reduced ecosystem function. Therefore, within highly diverse assemblages, an increased number of species provide a greater range of ecological functions.

Species-rich assemblages may also be more resilient to species extinction than less diverse assemblages, providing a greater insurance against the loss of taxa. This concept is an integral part of the 'redundancy hypothesis' (Walker 1992; Naeem 1998) in that many species in the assemblage are able to deliver each ecological function and the localized extinction of one species is compensated for by other taxa carrying out the same functional roles. The identity of taxa removed, however, is likely to be of importance, with individual taxa making different contributions to each ecological function. Some taxa make a key contribution to one function, whereas other taxa have very little influence upon it. This combination of the rivet and redundancy hypotheses is the basis of the 'keystone hypothesis' (Lavorel & Garnier 2002; Hooper *et al.* 2005). For example, dense aggregations of the filter-feeding mussel *Mytilus edulis* L. have an influence on both the regeneration of nutrients and the primary productivity within coastal systems (Prins & Smaal 1994). *Mytilus edulis* is an efficient filter feeder, removing large amounts of phytoplankton. Dense aggregations also influence near-bed hydrodynamics, causing increased levels of biodeposition and mineralization of biodeposited particles, and resulting in increased levels of nutrient regeneration within mussel beds (Prins & Smaal 1994). *Mytilus* aggregates also provide refugia for other fauna, increasing biodiversity and thus influencing energy flow through the system (Ragnarsson & Raffaelli 1999).

The cockle *Cerastoderma edule* L. is common in intertidal assemblages on all British coasts and has been the target of a commercial fishery for over a century (Dare *et al.* 2004). *Cerastoderma edule* are a major prey item for birds (Drinnan 1957) and demersal fish (Pihl 1985) foraging on intertidal flats and are active filter-feeders, consuming planktonic flora and fauna and re-suspended microphytobenthos (Kamermans 1993, 1994; Sauriau & Kang 2000). At certain times of the year a large proportion of the diet of *C. edule* can be the planktonic juvenile stages of benthic organisms, including *C. edule* itself (Flach 1996). Bivalves eject undigested particulate material as mucous-coated pseudofaeces, which can alter the resuspension characteristics of the benthic boundary layer and add to the organic matter content of surface sediments, acting as a nutrient source for primary producers and a food source for surface deposit-feeding fauna (Hempel 1957; Swanberg 1991; Giles & Pilditch 2006). *Cerastoderma edule* therefore has the potential to impact upon the structuring of intertidal assemblages and be of critical importance in the delivery of ecological functions

in these systems, either directly (*e.g.* impacting on energy flow through the removal of settling juveniles) or indirectly (*e.g.* providing a food source to detritivores via the biodeposition of pseudofaeces). Dense beds of *C. edule* occur in situations where the faunal assemblage is species-diverse and productive, but also where very few taxa are present (*e.g.* Ivell 1981; Attrill 1998). This raises the possibility that these systems deliver quite different ranges and quantities of ecological functions, or that the role of *C. edule* varies in response to the nature of the assemblage. The removal of *C. edule* by a fishery would, presumably, alter the dynamics and ecological functioning of these systems, with the possibility that the response of the more diverse assemblages would differ from that of species-poor assemblages.

The large size of the organism and the availability of harvesting techniques provide an opportunity for the manipulation of cockle diversity within both species-rich and species-poor assemblages. This allows *in situ* field investigations to be carried out: investigating the impact of an experimentally reduced population of a potentially key species on ecological functioning. To date, manipulative experimental investigations of the impacts of altered assemblage composition on ecological functioning have principally been limited to laboratory-based microcosm studies (*e.g.* Michaud *et al.* 2006; Norling *et al.* 2007). Although such studies confer a high degree of control, allowing specific ecological questions to be approached, by their nature such designs remove a large number of unknown variables that would be present within natural systems. Microcosm experiments generally include only a limited number of taxa and hence only a limited representation of the complexity inherent within 'real' systems (Duffy *et al.* 2001; Bulling *et al.* 2008; Olsgard *et al.* 2008). The outcomes of such studies therefore, are generally less able to be applied to natural assemblages and ecosystems. Although *in situ* approaches introduce a greater number of unmeasured variables into experimental systems (and hence limit the degree to which the causality behind any responses can be assigned), they allow inference to a greater extent as to what the response of a 'natural' assemblage may be.

Traditional methods of assessing ecosystem functioning are based upon the direct or proxy measurements of a limited number of ecological functions, such as chlorophyll concentration, sediment organic matter content and sediment granulometry (Thrush *et al.* 2006; Sundbäck *et al.* 2007). However, this approach alone is somewhat limited in that it provides an incomplete view of the functioning of the system as a whole. That is, the responses of one function to a particular effect may be negligible and the observation of a limited number of functions may mean that more subtle changes to ecologi-

cal functioning, *i.e.* those difficult to predict *a priori*, are missed. Biological Traits Analysis (BTA) is a complimentary approach to these 'direct' methods. BTA was developed primarily for lotic systems (Charvet *et al.* 1998) but it has been increasingly applied to marine systems (Frid *et al.* 2000; Bremner *et al.* 2003; Tillin *et al.* 2006; Kenchington *et al.* 2007).

The delivery of ecological functions is, by definition, a consequence of the life history, ecological and physiological strategies that taxa within a system have evolved. Different strategies employed by species affect different ecological functions. For example, the living habit employed by a taxon (*e.g.* being a surface dweller, tube-dweller, active burrower, *etc.*) has an influence on the depth of sediment into which oxygen-rich water can penetrate, thus influencing the regeneration of nutrients and the circulation of toxins within the benthos (Aller 1983; Ziebis *et al.* 1996). Mapping the occurrence of these functionally important traits within a system may therefore be used as a proxy for the analysis of ecological functions. Species may have a number of traits in common; hence BTA allows a comparison between systems where the finite biogeographic ranges of species make the interpretation of taxa-based approaches difficult (Charvet *et al.* 2000). Changes to the prevalence of biological traits within a system can provide an insight as to how the ecological functioning of the assemblage has been affected by an external stressor.

The aim of the current study was to test the redundancy and rivet hypotheses *in situ* within two contrasting estuarine intertidal assemblages. The following questions were asked: Is the delivery of ecological functions affected by the removal of a potentially keystone species? Does the biodiversity of the affected assemblage affect the extent to which it is disturbed? To facilitate this, *C. edule* was experimentally removed from the benthos and ecological functioning assessed through measurements of surface chlorophyll as a proxy for microphytobenthic primary production, organic matter within the sediment as a proxy for benthic community metabolism, and sediment granulometry as a measure of changes to the physical habitat. BTA was carried out on faunal assemblages to assess changes to the functional structure of the faunal communities.

## Material and Methods

### Site description and experimental design

Experimental manipulations were carried out on two tidal flats in North-West England, UK. Thurstaston (53°20'N, 3°9'W) is located within the Dee estuary and Warton Sands (54°6'N, 2°49'W) within Morecambe Bay. Both are sandy shores, with mean silt contents (<63 µm) of

10.1 ± 1.3% and 15.9 ± 1.9% for Thurstaston and Warton Sands, respectively. The faunal biomass at both sites is dominated by *Cerastoderma edule*, the Baltic tellin *Macoma balthica* (L.), the mud snail *Hydrobia ulvae* (Pennant) and the spionid polychaete *Pygospio elegans* (Claparède). Biomass at Thurstaston is also dominated by the bivalves *Scrobicularia plana* (da Costa) and *Mya arenaria* (L.) and the polychaete *Hediste diversicolor* (OF Müller). A large proportion of the biomass at Warton Sands consists of the predatory polychaete *Nephtys hombergii* (Savigny). Thurstaston contains a more diverse macrofaunal assemblage (*c.* 30 species) than Warton Sands (*c.* 18 species; C. Cesar unpublished observations). Both sites have in the past been commercially fished cockle beds; however, at the time of the experiment, both had been closed to fishing for ~2 years.

At each shore, four replicate 2 × 2 m plots were established for each of three treatments:

- (1) Fished – surface sediment was hand-raked to a depth of ~10 cm and passed through a 6-mm-square mesh. All *C. edule* retained on the mesh were removed and all other organisms were returned to the plot.
- (2) Procedural control – surface sediment was raked as above and all organisms returned to the plot.
- (3) Undisturbed control.

The 12 plots on each shore were arranged in a randomized block design, with plot locations marked by a 30-cm plastic peg at each corner of the plot. To minimize interactions between plots, each was located at least 5 m from other plots.

The experiment was initiated in June 2007 and plots were sampled every 4 weeks until September 2007. To minimize the risk of creating artifacts from the repeated sampling of plots, each plot was split into four sub-plots (each representing an area of 1 m<sup>2</sup>). At each sampling event, samples were removed from random locations within the central 50 × 50 cm (to avoid edge effects) of only one randomly selected sub-plot; each sub-plot was sampled once only during the course of the experiment.

Every 4 weeks, four surface chlorophyll *a* samples were taken using plastic cores (internal diameter 20 mm) pushed into the sediment to a depth of 15 mm. Samples were wrapped in foil and stored in a cool box until return to the laboratory. Prior to analysis, chlorophyll *a* samples were stored at –80 °C. Sediment granulometry and organic matter content were ascertained from the removal of one sediment sample from each plot (PVC core, internal diameter 30 × 100 mm). On the first and final sampling occasions, four sediment core samples were taken to investigate the faunal assemblage composition at each plot (PVC core, internal diameter 110 × 200 mm depth). These samples were washed over a 500-µm sieve and the residue fixed in 4% formaldehyde.

### Infaunal assessment

Macrofauna were identified to the lowest taxonomic level practical and biomass ascertained by wet weight ( $\pm 0.0001$  g). Biomass was chosen as the faunal 'abundance metric' in this investigation as biomass is likely to better represent the distribution of resources within the system than species abundance measures (e.g. Chiarucci *et al.* 1999). A high abundance of very small organisms are not likely to partition as great a proportion of ecosystem resources (particularly organic carbon) as fewer, much larger taxa occupying a greater biovolume than their low abundance alone would suggest. This is particularly important when we wish to assay the ecological roles being played by taxa, rather than biodiversity *per se* and this approach is supported by other literature (e.g. Wilson 1991; Chiarucci *et al.* 1999; McGill *et al.* 2007). Comparisons of macrofaunal communities were carried out using permutational analysis of variance (PERMANOVA, Anderson 2001) based on Bray–Curtis dissimilarities and probability values calculated from 4999 permutations (see Manly 1997) using the FORTRAN program PERMANOVA (Anderson 2005). When assemblages significantly differed between factors (Shore, Treatment and Time) ( $\alpha < 0.05$ ), *a posteriori* pairwise comparisons were carried out using PERMANOVA and the significantly different factors investigated using the SIMPER procedure in the PRIMER v.6.1.6 software package (Clarke & Warwick 1994; Clarke & Gorley 2006), to identify the contribution of individual taxa to differences between factors.

### Biological traits analysis

To investigate the distribution of biological traits within the species assemblages, taxa were scored against 14 biological traits to reflect the life history, morphological and ecological strategies of taxa and to provide a proxy analysis of a number of ecological functions. Each trait was divided into a number of modalities (sub-categories) (Table 1) and a fuzzy coding approach used to score the affinity of each taxon against all of the modalities within each trait (Chevenet *et al.* 1994). Trait information was obtained from a range of sources; where possible, information came from published peer-reviewed literature. Alternatively, information was taken from grey literature or from expert opinion on the taxa. Where trait information for a taxon could not be obtained (in only 3% of cases), 0 scores were used for each modality and hence did not influence the analysis (Chevenet *et al.* 1994). Fuzzy scores were standardized prior to analysis so that within each trait, the modality scores for each taxa summed to one.

**Table 1.** Biological traits and modalities used in the fuzzy coding of taxa for biological traits analysis (BTA) to describe functional diversity (adapted from Bremner *et al.* 2006; Frid *et al.* 2008). Codes refer to Fig. 2.

trait	modality	code
adult mobility/dispersal potential (distance per year)	none	D0
	<10 m <sup>2</sup>	D1
	10–100 m <sup>2</sup>	D2
	>100 m <sup>2</sup>	D3
age at sexual maturity (years)	0.08–0.5	M1
	0.5–1	M2
	1–2	M3
	>2	M4
fecundity (eggs released per reproductive event)	10 s	F1
	100 s	F2
	1000 s+	F3
food type	Phytoplankton	Ph
	Benthic Invertebrates	Bi
	Carrion	Cr
	Benthic POM	Bp
	Plankton	Pl
	Pelagic POM	Pp
living habit	Tube	Tu
	Burrow	Bu
	Epizoic	Ep
	Free	Fr
living location	Pelagic	Pe
	Surface	Su
	Interface	In
	Shallow (0–20 cm)	Sh
	Deep (>20 cm)	Dp
longevity (years)	<0.5	L1
	0.5–1	L2
	1–2	L3
	2–5	L4
	>5	L5
maximum size (mm)	0–50	S1
	50–100	S2
	100+	S3
movement method	None	Nm
	Swim	Sm
	Crawl	Cw
	Burrow	Br
propagule dispersal	None	N
	Planktonic (0.04 years)	P1
	Plank (0.04–0.08 years)	P2
	Plank (0.08–0.5 years)	P3
	Benthic crawl	Bc
reproductive frequency	Raft	Rf
	Continuous	Co
	1 event per year	Y1
	2+ per year	Y2
reproductive method	Less than annual	Yx
	Asexual	Ax
	Sexual (spawn)	Ss
	Sexual (ovigerous)	So
	Sexual (direct)	Sd

**Table 1.** Continued

trait	modality	code
resource capture	Deposit	De
	Suspension: active	Sa
	Suspension: passive	Sp
	Opportunist/Scavenger	Sc
	Grazer	Gr
	Predator	Pr
tissue components	Calcareous	Ca
	Fleshy	Fl

To investigate differences in the distribution of biological traits between assemblages, the taxa-trait table was weighted by multiplying the trait scores for each taxon by the biomass of the taxon for each sample. The scores for each modality within each trait were summed. This resulted in a table containing the total biomass of organisms within an assemblage that displayed each trait. The resulting biomass-weighted trait-by-station table was ordinated using fuzzy correspondence analysis (FCA) (Chevenet *et al.* 1994). The ordinate scores for FCA axes one and two were analysed using Kruskal–Wallis analysis against the factors Shore, Treatment and Time. BTA was carried out using the *ade4* (version 1.4–5) package for R (version 2.6.2, CRAN 1999; Dray & Dufour 2007).

#### Sediment properties and chlorophyll *a* concentration

Changes to sediment granulometry were ascertained from the 30 × 100 mm sediment cores which were dried in a cool (60 °C) oven and weighed ( $\pm 0.01$  g). Sediments were soaked overnight in sodium hexametaphosphate solution and washed over a 63- $\mu$ m sieve. After drying at 80 °C, retained sand (>63  $\mu$ m) was reweighed and percentage silt contents (*i.e.* <63  $\mu$ m) were inferred by subtraction.

Changes to the organic matter content of the sediments were assayed by loss on ignition (LOI). Samples of *c.* 10 g of dried sediment were ground with a pestle and mortar to even consistency and weighed ( $\pm 0.0001$  g) before and after ignition at 500 °C for 5 h.

Surface chlorophyll *a* concentrations were taken as an index of microphytobenthic biomass (MacIntyre *et al.* 1996). Surface sediment samples were agitated for 15 min in 90% acetone solution and centrifuged for 15 min at 1200 g. Chlorophyll *a* concentration in the supernatant was determined by spectrophotometry, following Lorenzen (1967) and expressed as mass of chlorophyll *a* per unit mass of dry sediment ( $\mu$ g Chl *a* g<sup>-1</sup>).

Statistical analyses were carried out for each of the sediment properties using linear mixed models (LMM) with the first order auto-regressive (AR1) covariance structure fitted according to the criteria of Wang & Goonewardene

(2004), with Time as a repeated variable and experimental Treatment (*i.e.* 'Hand-raked' and 'Hand-raked & cockles removed') and Shore as fixed factors. LMM were selected as the assumption of sphericity, as required in general linear models (GLM), is unlikely to be satisfied (Rowell & Walters 1976). Additionally, unlike GLM, LMM is equally efficient for both balanced and unbalanced designs and hence allows for a degree of missing data and for the fact that due to tidal variations, sampling times were not exactly 4 weeks apart (Wang & Goonewardene 2004; Spilke *et al.* 2005). Percentile data (loss on ignition and silt content) were  $\sqrt{\cdot}$ -transformed prior to analysis; for clarity however, untransformed values were used in presented figures. LMM analyses were carried out in SPSS v15 (SPSS, Inc.).

## Results

### Infaunal assemblages

Analyses revealed significant differences in *Cerastoderma edule* biomass between the factors Treatment (LMM,  $F = 9.79$ ,  $P = 0.001$ ) and Time ( $F = 7.13$ ,  $P = 0.013$ ), but no significant differences within the interactions between factors. Plots subjected to the treatment Fishing had a mean *C. edule* biomass of  $89.15 \pm 52.41$  g·m<sup>-2</sup>; assemblages within Procedural Controls contained  $448.91 \pm 245.54$  g·m<sup>-2</sup> *C. edule* and Undisturbed Controls contained  $484.09 \pm 176.78$  g·m<sup>-2</sup>. Therefore the Fishing treatment did result in a significantly reduced biomass of *C. edule* within experimental plots. *Cerastoderma edule* biomass was also found to increase under the factor Time ( $T_1 = 268.08 \pm 124.13$  g·m<sup>-2</sup>,  $T_2 = 413.35 \pm 181.92$  g·m<sup>-2</sup>), presumably due to the immigration and/or growth of the organisms over the summer period (Flach 1996).

Total macrofaunal biomass was greater at Thurstaston than at Warton Sands (mean total macrofaunal biomass for Thurstaston =  $768.7 \pm 429.0$  g·m<sup>-2</sup>, for Warton Sands =  $353.1 \pm 283.6$  g·m<sup>-2</sup>, Mann–Whitney  $U = 779$ ,  $P < 0.001$ ). A total of 35 taxa were identified over both assemblages, with 33 at Thurstaston and 19 at Warton Sands (see supplementary Tables S1 and S2 in appendices). Thurstaston showed greater macrofaunal diversity than Warton Sands (Shannon–Weiner mean  $H'$ <sub>Thurstaston</sub> =  $1.30 \pm 0.37$ ;  $H'$ <sub>Warton Sands</sub> =  $0.97 \pm 0.23$ , Mann–Whitney  $U = 742$ ,  $P = 0.002$ ). Significant differences in faunal assemblage compositions were identified between the two shores (Table 2 and MDS plot, Fig. S1, in supplementary appendices). Faunal biomass within both assemblages was dominated by *C. edule*, contributing  $45.3 \pm 28.34\%$  of total faunal biomass at Thurstaston and  $52.9 \pm 28.6\%$  at Warton Sands. Other major contributors to faunal biomass at both assemblages were the bivalve *Macoma*

**Table 2.** PERMANOVA and pairwise *a posteriori* comparisons between faunal biomass compositions within two assemblages (TH = Thurstaston and WS = Warton Sands) under different experimental treatments (F = Fished, P = Procedural Control and C = Undisturbed Control) at two different times (1 = June 2007 and 2 = October 2007). Significant ( $P < 0.05$ ) differences in the PERMANOVA are shown in bold. For the pairwise *a posteriori* comparisons, only significant results ( $P < 0.05$ ) are shown. For example, TH, 1: F  $\neq$  P means that there was a significant difference in assemblages at Thurstaston at Time 1 between the Fished and Procedural control plots.

source of variation	df	MS	F	P(perm)	<i>a posteriori</i> comparisons
shore	1	16,940.02	18.60	<b>&lt;0.01</b>	TH $\neq$ WS
treatment(Shore)	4	2621.31	2.88	<b>&lt;0.01</b>	TH: F $\neq$ C, P $\neq$ C; WS: F $\neq$ C
time	1	549.68	0.60	0.644	
shore $\times$ time	1	618.02	0.68	0.586	
treatment(shore) $\times$ time	4	2861.33	3.14	<b>&lt;0.01</b>	TH, 1: F $\neq$ P, F $\neq$ C; TH, 2: F $\neq$ P, P $\neq$ C
error	36	910.58			

*balthica* (contributing  $20.7 \pm 13.1\%$  at Thurstaston and  $20.7 \pm 15.2\%$  at Warton Sands) and the gastropod *Hydrobia ulvae* (contributing  $7.3 \pm 9.2\%$  at Thurstaston and  $12.8 \pm 21.8\%$  at Warton Sands). Most of the difference between the two shores was derived from differences in the biomass of the bivalve species *C. edule*, *M. balthica* and *Scrobicularia plana*, the polychaete *Hediste diversicolor* and the gastropod *H. ulvae* (Table 3). With the exception of *H. ulvae*, which showed a greater biomass at Warton Sands, these taxa were either absent at Warton Sands or present at much reduced biomass.

Total macrofaunal biomass (including biomass of *C. edule*) present within assemblages was significantly reduced following the removal of *C. edule* (LMM,  $F = 16.27$ ,  $P < 0.001$ ). There was also a significant increase of biomass with sampling time, with samples taken in October housing a greater biomass than those in June (LMM,  $F = 7.25$ ,  $P = 0.010$ ). However, when the biomass data for *C. edule* were excluded from the analysis, although total biomass at Thurstaston was still greater (LMM,  $F = 84.97$ ,  $P < 0.001$ ), no significant changes under the experimental treatments were detected and hence non-*C. edule* assemblage biomass was not signifi-

cantly changed by the treatments. The removal of *C. edule* had a significant effect on biodiversity both with (Shannon–Wiener Index, LMM,  $F = 11.94$ ,  $P = 0.002$ ) and without (Shannon–Wiener Index, LMM,  $F = 4.56$ ,  $P < 0.05$ ) the *C. edule* data being included in the analyses. Fished plots showed increased biodiversity compared with both Procedural and Undisturbed Controls.

PERMANOVA revealed no significant changes over Time and no Shore  $\times$  Time interaction within faunal assemblage compositions within either assemblage (Table 2). However, significant differences were observed with regard to Treatment and within the Treatment  $\times$  Time interaction (Table 2). *A posteriori* pairwise analyses were carried out on the significant outcomes of the PERMANOVA. At Thurstaston, differences in faunal biomass compositions were observed between the Undisturbed Control plots and both the experimentally Fished and the Procedural Controls. SIMPER analyses (Table 3) revealed that much of the differences was due to a reduced biomass of the bivalves *C. edule*, *M. balthica*, and *S. plana* in Fished plots and increased biomass of *H. ulvae* in Undisturbed Control plots. Procedural Control plots showed an increased biomass of the *M. balthica* and

**Table 3.** Output of the SIMPER analyses displaying the taxa responsible for 90% of the difference between experimental treatments (F = Fished, P = Procedural Control, C = Undisturbed Control) within two assemblages (TH = Thurstaston and WS = Warton Sands) both in terms of overall differences and over two sampling occasions. Only differences revealed as significant by *a posteriori* pairwise comparisons are displayed. Values represent the percentage contribution made to the differences by each taxon. Superscript *a* indicates that the former of the pairwise comparisons has the greater biomass, *b* indicates that the latter comparison has greatest biomass, e.g. Thurstaston has a greater biomass of *Hediste diversicolor* than Warton Sands does.

species	TH versus WS	Thurstaston						Warton Sands
		overall		June 2007		October 2007		overall
		F versus C	P versus C	F versus P	F versus C	F versus P	P versus C	F versus C
<i>Hediste diversicolor</i>	4.86 <sup>a</sup>						2.95 <sup>b</sup>	
<i>Hydrobia ulvae</i>	4.02 <sup>b</sup>	4.14 <sup>a</sup>	3.76 <sup>b</sup>	4.83 <sup>b</sup>	6.27 <sup>a</sup>		3.27 <sup>b</sup>	
<i>Cerastoderma edule</i>	54.40 <sup>a</sup>	64.74 <sup>b</sup>	65.77 <sup>b</sup>	68.15 <sup>b</sup>	57.98 <sup>b</sup>	73.95 <sup>b</sup>	61.68 <sup>a</sup>	83.60 <sup>b</sup>
<i>Macoma balthica</i>	15.03 <sup>a</sup>	11.21 <sup>b</sup>	10.81 <sup>a</sup>	17.26 <sup>a</sup>	18.59 <sup>a</sup>	7.60 <sup>b</sup>	7.47 <sup>a</sup>	8.79 <sup>b</sup>
<i>Scrobicularia plana</i>	14.36 <sup>a</sup>	10.17 <sup>b</sup>	10.78 <sup>b</sup>		9.70 <sup>b</sup>	10.21 <sup>b</sup>	15.64 <sup>a</sup>	



reduced biomass of *C. edule*, *S. plana* and *H. ulvae* compared with Undisturbed Control plots.

At Thurstaston, Treatments were also significantly different between the two sampling times. In June 2007, Fished plots were different from both Procedural and Undisturbed Control plots (Table 2). Fished plots had a reduced biomass of *C. edule* and increased biomass of *M. balthica* compared to both controls. Plots subjected to Fishing had a reduced biomass of *H. ulvae* compared with Procedural Controls, but a greater biomass than Undisturbed Control plots. In October 2007, Procedural Controls were significantly different from both Fished and Undisturbed Control plots, with most of the difference attributable to increased biomass of *C. edule*, *S. plana* and *M. balthica* within Procedural Controls compared to the other two treatments (Table 3). Procedural Control plots were found to have a decreased biomass of *H. ulvae* and *H. diversicolor* in relation to the Undisturbed Control plots.

At Warton Sands, the only significant difference in taxon biomass was between Fished and Undisturbed Control plots; most of the difference between the Treatments was due to decreased *C. edule* and *M. balthica* biomass within Fished plots (Table 3). This effect was irrespective of the factor Time.

#### Biological traits analysis

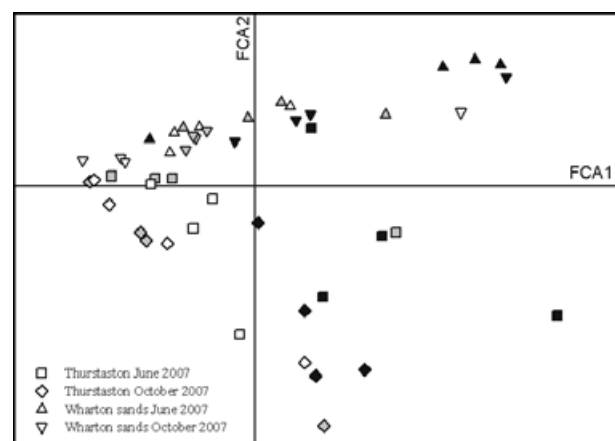
The first two axes of the FCA explained over 78% of variance, with 45% across fuzzy correspondence axis 1 (FCA1) and 34% of variance across axis 2 (FCA2) (Table 4). Much of the variance on FCA1 was represented within traits relating to faunal dispersal (propagule dispersal and adult mobility/dispersal potential), longevity and feeding-related traits (Resource capture and Food type) (Table 4). Ordination scores were compared for FCA1 and revealed a significant separation of samples under the factor Treatment (Kruskal–Wallis,  $H = 16.82$ ,

**Table 4.** Relative inertia and correlation ratios of biological traits on the first two axes of the fuzzy correspondence analysis. Correlation ratios represent the proportion of variance explained by the different traits across each axis.

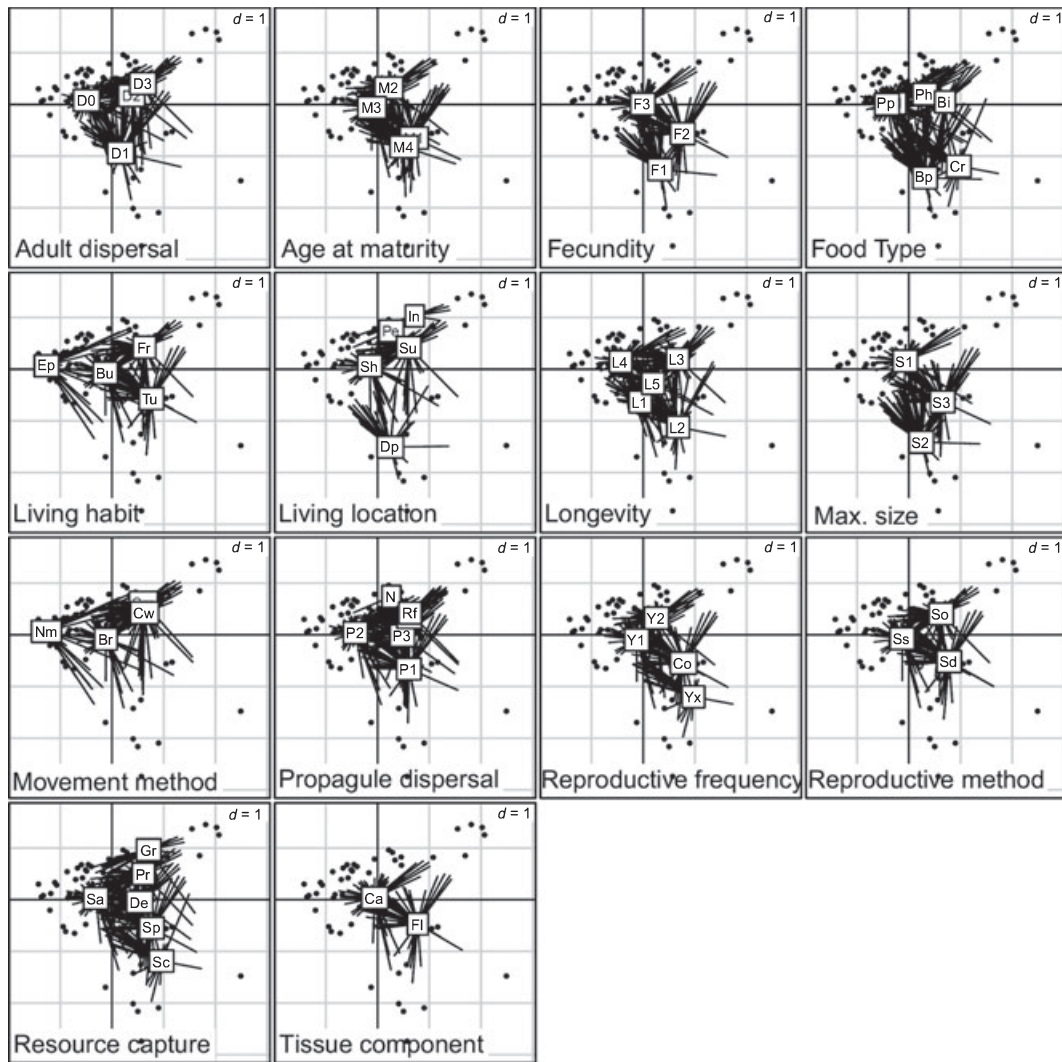
	axis 1	axis 2
relative inertia (%)	44.92	33.57
correlation ratio (%)		
propagule dispersal	17.07	6.05
adult dispersal	13.55	13.97
longevity	13.15	4.45
resource capture	12.75	2.18
food type	8.14	12.35
location	5.82	20.42
Max. size	2.56	18.04

$P < 0.001$ ), with Fished plots tending towards a positive value and the Procedural and Undisturbed Control treatments tending towards negative values on FCA1 (Fig. 1). No significant differences were detected on FCA1 under the factors Shore or Time. Fished samples tended towards a reduction in non-motile fauna, with an increased proportion of highly motile fauna (adult mobility  $>100 \text{ m}^2$ ). Fished plots had an increased prevalence of relatively short-lived fauna (0.5–1 and 1–2 year modalities) and a reduced biomass of long-lived organisms (2–5 years). Scavengers were more prominent within Fished plots and these plots had reduced prevalence of active suspension feeders relative to the controls (Fig. 2). The removal of *C. edule* therefore explained much of the variability within the data (factor = Treatment within FCA1) and much of this variability was explained by traits relating to the dispersive abilities, longevities and feeding methods of fauna.

Much of the variance within FCA2 was explained by the living location and maximum size of taxa, the dispersive potential of adult organisms and the food resources exploited (Table 4). No separation of samples on FCA2 was found relating to the factors of Treatment or Time. Significant separation of samples was observed along FCA2 under the factor Shore (Kruskal–Wallis  $H = 32.62$   $P < 0.001$ ), with samples taken at Warton Sands tending towards positive values and at Thurstaston tending towards negative values on FCA2 (Fig. 1). Assemblages at Warton Sands tended towards a prevalence of smaller (0–50 mm), more motile ( $>100 \text{ m}^2\text{-year}^{-1}$ ) taxa, dwelling at the sediment-water interface and assemblages at Thurstaston tended towards larger (50–100 mm), deeper-dwelling ( $>20 \text{ cm}$ ) and less-motile ( $<10 \text{ m}^2\text{-year}^{-1}$ ) fauna (Fig. 2).



**Fig. 1.** Ordination of the first two axes of the FCA for the two experimental assemblages. FCA1 explained ~45% of variance in the data and FCA2 explained ~34%. Sample treatment is indicated by symbol shading: solid black = Fished, grey = Procedural Control and clear = Undisturbed Control.



**Fig. 2.** Ordination of biomass-weighted biological trait categories from the first two axes of the FCA, allowing interpretation of the traits responsible for the differences between assemblages. Points represent the FCA coordinates for each experimental plot (as in Fig. 1) and label locations represent the centroid for each trait modality and lines link the plots to the modalities. Modality labels refer to Table 1.

When *C. edule* were excluded from the BTA, no significant changes to the distribution of biological traits was observed on either FCA1 or FCA2 (Kruskal–Wallis  $H = 0.53$ ,  $P = 0.766$  and  $H = 1.21$ ,  $P = 0.547$ , respectively). This suggests that the differences in the prevalence of traits following the removal of large *C. edule* are directly caused by *C. edule*, rather than by a shift in the underlying community structure. Differences were observed on FCA1 between the two Shores ( $H = 33.57$ ,  $P < 0.001$ ) and there were significant differences between the two sampling Times on both Shores (Thurstaston:  $H = 10.08$ ,  $P = 0.001$ ; Warton Sands:  $H = 5.60$ ,  $P = 0.018$ ), with increased prevalence of deep-dwelling, relatively non-motile and larger trait characteristics between the June and October sampling occasions. No

significant differences were observed on FCA2 for any of the factors.

#### Sediment properties and chlorophyll *a* concentration

Sediment properties differed between the two study sites, with significantly higher concentrations of chlorophyll *a* and organic matter content at Thurstaston (Table 5, Fig. 3). Sediment granulometry (indicated by silt content ( $63 \mu\text{m}$ )) at Warton Sands was less coarse than that at Thurstaston and changed over time at both shores (Table 5, Fig. 3). No significant differences in chlorophyll *a* concentration or loss on ignition were observed with regards to experimental treatment. However, sediment granulometry did show significant change; sediments in

**Table 5.** Summary of the linear mixed model analyses for the sediment parameters quantified to investigate ecological functions. Significant values ( $\alpha = 0.05$ ) indicated in bold.

source	df	chlorophyll <i>a</i>		LOI		silt content	
		F	P	F	P	F	P
shore	1	<b>127.42</b>	<b>&lt; 0.001</b>	<b>15.47</b>	<b>&lt; 0.001</b>	<b>52.21</b>	<b>&lt; 0.001</b>
raked	1	0.18	0.673	0.04	0.841	0.16	0.690
cockles removed	1	0.30	0.589	1.75	0.198	<b>5.39</b>	<b>0.026</b>
time	4	1.01	0.379	1.21	0.315	1.94	0.111
shore × raked	1	0.24	0.630	0.05	0.834	0.72	0.403
shore × cockles removed	1	0.03	0.858	0.66	0.425	0.19	0.666
shore × time	4	0.42	0.797	1.78	0.145	<b>2.86</b>	<b>0.028</b>
raked × time	4	0.27	0.894	0.39	0.818	1.00	0.414
cockles removed × time	4	0.16	0.960	2.11	0.091	0.86	0.491
shore × raked × time	4	0.19	0.944	0.15	0.963	0.92	0.459
shore × cockles removed × time	4	0.05	0.994	1.15	0.342	2.43	0.054

Fished plots contained higher silt contents ( $3.99 \pm 0.06\%$ ) than non-fished plots did ( $3.70 \pm 0.10\%$ ).

To investigate the power of the experiment to detect significant changes, iterative analyses were carried out on the chlorophyll and organic matter data. Under the current design, a change in surface chlorophyll values of  $\sim 16\%$  and a change in the organic matter content data of  $\sim 2.5\%$  would be necessary to give statistically significant changes.

## Discussion

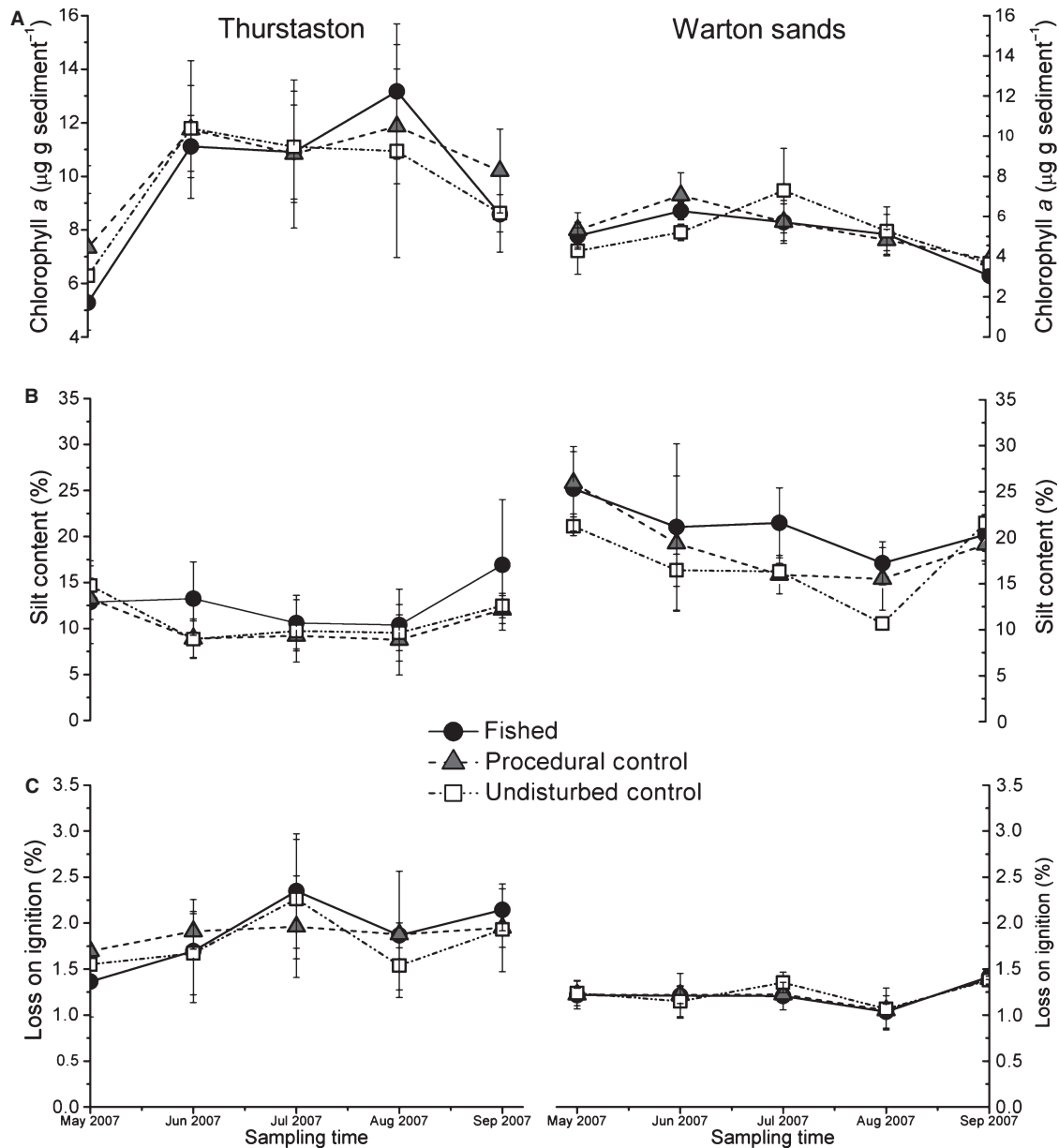
This study has shown that the removal of *Cerastoderma edule* led to a marked increase in faunal diversity within manipulated plots, irrespective of the pre-impact diversity at the site. This suggests that other taxa are prevented from establishing themselves by the presence of *C. edule* even within species-poor assemblages. Following the removal of large *C. edule*, other taxa established within the system, either exploiting the resources liberated by the loss of *C. edule* or in response to the physical disturbance of the habitat. Such changes have been reported in a range of systems (e.g. Tuck *et al.* 1998; Sparks-McConkey & Watling 2001; de Juan *et al.* 2007).

The observed changes to macrofaunal communities were reflected in changes to the distribution of biological traits within the assemblages; however, this change was due to the removal of large *C. edule* themselves, rather than to changes in the distribution of underlying traits following removal. Removal of *C. edule* resulted in an increased prevalence of motile, short-lived taxa, with increased occurrences of scavengers, coupled with a relative reduction in non-mobile, longer-lived suspension-feeding fauna. This adds support to the potential of *C. edule* as key contributors to ecological functioning, in that their removal significantly altered the distribution

and prevalence of functionally important biological traits. This occurred within both study assemblages and is consistent with other studies investigating the impacts of disturbance on benthic communities. At a smaller scale, Norkko & Bonsdorff (1996) for example found increased abundances of opportunistic taxa beneath experimentally placed algal mats. Larger-scale analyses of North Sea benthos also found a trend towards increased numbers of motile predators and scavengers with high levels of fishing disturbance (Bremner *et al.* 2003; Tillin *et al.* 2006).

Following the observed changes to the macrofaunal communities and distribution of biological traits within the two assemblages, it could be expected that changes to the delivery of the ecological functions would be evident. However, this was not the case with respect to the functions quantified here. *Cerastoderma edule* is an active suspension feeder and a significant portion of its diet can be re-suspended microphytobenthos (Sauriau & Kang 2000; Page & Lastra 2003). Removal of *C. edule*, a principal contributor to macrofaunal biomass, would therefore reduce grazing pressure and result in an increased biomass of primary producers. This was not observed in the current study, with chlorophyll *a* concentration (as a proxy for primary producer biomass) showing no significant relationship to experimental treatment on either study shore.

Organic matter content within the sediment was also studied as a proxy for the overall community metabolism within the assemblages (van Duyl *et al.* 1992). A shift towards a more motile fauna, for example, can influence the rate at which organic matter is incorporated into the sediment, influencing the resources available to the microbial community; this has subsequent impacts onto the provision of oxygen and the mineralization of organic materials within the sediment (Gilbert *et al.* 1995; Fenchel 1996). Assemblages with shorter life histories and more



**Fig. 3.** Mean values ( $\pm$ SD) of the sediment parameters over 4 months following the experimental removal of *Cerastoderma edule* at two locations: (a) chlorophyll *a* concentration ( $\mu\text{g}\cdot\text{g}$  per dry sediment), (b) silt content (% <63  $\mu\text{m}$ ) and (c) organic matter (as % loss on ignition).

opportunistic resource-gathering methods would also influence the trophic relationships throughout the assemblages and hence also the overall metabolism within the system. Therefore, the removal of *C. edule* was expected to result in a change in the availability of organic matter within the benthos. Again, no significant relationship with experimental treatment was observed at either study site.

The third ecological indicator considered was the granulometry of sediments within the assemblages. This is important to the functioning of systems, impacting upon sediment porosity and hence the depth of the redox layer

(Huettel & Rusch 2000) with implications for the settlement of larvae into the benthos (Pinedo *et al.* 2000; Duchêne 2004). *Cerastoderma edule* have been shown to loosen and destabilize sediments (Flach 1996), leaving finer grained particles more prone to resuspension, resulting in a coarsening of sediment granulometry (Ciutat *et al.* 2006, 2007). The findings of the current study concur with this: the removal of large *C. edule* resulted in a higher silt content within plots.

Within the current study, the removal of large *C. edule* had no significant impact on the measured ecological

functions relating to the biological productivity (as indexed by organic matter content and primary producer biomass) within the benthos. These responses, or lack of them, were common to both study shores and hence were independent of the initial biodiversity of the assemblages. Following the removal of large *C. edule*, both the species-rich assemblage at Thurstaston and the species-poor assemblage at Warton Sands showed a shift towards assemblages dominated by traits common to opportunist taxa. It appears, therefore, that ecological functions were conserved following the removal of large *C. edule*, regardless of the underlying macrofaunal diversity present at the sites. A number of possible explanations may account for this. First, the statistical power of the current study may be insufficient to detect subtle changes to the measured functions. Iterative power analyses revealed that, with the natural variability observed in this study, chlorophyll and organic matter contents would have to alter by ~16% and 2.5%, respectively, to detect a significant change. Such changes are not unfeasible. Swanberg (1991), for example, observed that the presence of *C. edule* resulted in a change in microphytobenthic biomass in the region of 90%. Therefore, it appears that surface chlorophyll and organic matter content did not change significantly under the experimental treatments. Two alternative explanations may also account for this observation: (i) the removal of large *C. edule* and the subsequent changes to the macrofaunal communities (and therefore the distribution of biological traits) within the study systems have no effect on the investigated functions, or (ii) other processes are buffering or masking the response of the systems to the removal of *C. edule* and the subsequent shift in assemblage and trait structure.

Previous, largely laboratory-based, experimental investigations have shown that *C. edule* can influence ecological functions, for example enhancing primary productivity (Swanberg 1991); *C. edule* has also been shown to significantly alter near-bed hydrodynamics and sediment stability (Ciutat *et al.* 2007). Additionally, shifts in the distribution of biological traits within an assemblage have been shown to affect the delivery of ecological functions, for example changes to the prevalence of different methods of bioturbation affect the regeneration of nutrients and penetration of oxygen within sediments (Mermillod-Blondin *et al.* 2005; Waldbusser & Marinelli 2006; Gilbert *et al.* 2007). It is therefore likely that the removal of *C. edule* and the subsequent shift in biological traits would have some effect on the functions under investigation unless other factors were simultaneously changing and compensating for it.

The findings of the current study imply that the ecological functions measured are largely unaffected by significant reductions in the biomass of a potentially key

contributor to ecological function. This is contrary to a number of other studies that have identified substantial changes to ecological functions following the removal of large taxa. Kanaya *et al.* (2005), for example, found that deposit-feeding bivalves had significant impacts on primary producer biomass, and Volkenborn (2005) found that the exclusion of the lugworm *Arenicola marina* from intertidal plots had significant effects on a number of ecosystem functions. Conversely, however, Bolam *et al.* (2002) found no effects of macrofaunal biomass or species richness on ecological functioning within a Scottish intertidal mudflat.

In the present study, BTA revealed significant changes to the prevalence of a number of traits within the macrofauna following the removal of large *C. edule*. However, there were no significant changes in the distribution of traits likely to impact upon the composition of the microbial community which primarily drives benthic primary production and microbial metabolism (Azam *et al.* 1993; Paerl 1997; Azam 1998). Additionally, Franklin & Mills (2006) showed that microbial communities display a high degree of functional redundancy, and even considerable changes to the microbial community composition are not reflected by changes to ecological functioning. Therefore, even if changes to the macrofaunal community did alter the microbial community, the substantial functional redundancy within these communities means that the measured functions were unlikely to be affected.

The conservation of functions observed in this investigation is likely, to some degree, to be related to the scale of the study. Subtle and/or small-scale changes in ecological processes are likely to be masked by the substantial natural variability over small scales and larger-scale processes occurring within the habitat (Kendrick *et al.* 1996). A larger-scale study would be more likely to reveal observable impacts (*e.g.* Watling *et al.* 2001; Falcão *et al.* 2003). However, as the aim of the current study was to assay the impacts of small-scale cockle hand-raking on ecological functioning, the spatial and temporal scales used reflected those used within the commercial fishery.

A limitation of the fuzzy-coding approach in BTA requires further consideration. This technique can only provide an indication, based on published and expert information, as to how a taxon may behave. It cannot provide any indicator of how an organism actually does act in a given situation. *Hediste diversicolor*, for example, is able to utilize a number of feeding strategies, depending upon resource availability (Fauchald & Jumars 1979) and *Pygospio elegans* can adopt a wide range of reproductive strategies (Gudmundsson 1985; Anger *et al.* 1986). Additionally, BTA does not account for ontogenetic differences within taxa. In this investigation, large *C. edule* were removed from assemblages and



the remaining *C. edule* were scored identically to large *C. edule* for the purposes of BTA. However, it is likely that younger (*i.e.* smaller) individuals behave differently and display a different range of functional traits than adult individuals, for example displaying different feeding preferences (Sauriau & Kang 2000). It is impossible to ascertain which strategy or strategies an organism is utilizing at any time and whether a change in conditions causes an organism to change its strategy, as the fuzzy coding technique includes all the strategies that can potentially be used by a species. It is necessary to address whether conspecifics under different disturbance regimes adopt the same behavioural/life-history strategies as this would potentially affect how the assemblage is delivering ecological functions. Studies involving BTA should therefore aim to incorporate some inference of the behavioural responses of taxa within affected assemblages.

The conservation of the measured functions does not necessarily mean that fished systems will continue to deliver other ecological functions. The availability of food resources within the system for example, is a key aspect of ecological functioning (Frid *et al.* 2008). As well as being the target of a fishery, *C. edule* are an important food source to demersal fish and shorebird species and are a major contributor to macrofaunal biomass. Large-scale removal of *C. edule* will therefore affect the food availability within and, by definition, the functioning of affected systems. Over longer timescales, this will potentially impact upon the regeneration and sustainability of *C. edule* stocks (Piersma *et al.* 2001; Kraan *et al.* 2007) and directly affect taxa relying on *C. edule* for food (Beukema & Dekker 2006). Therefore, the implications of small-scale, non-ubiquitous cockle harvesting depend upon the aspect of the system under concern. In terms of microbial and small-scale processes, there appears to be little impact; however, the removal of large *C. edule* from the system does impact upon the total macrofaunal biomass within the system and the potential availability of food resources available within the system.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Three-dimensional multidimensional scaling ordination of biomass-weighted community assemblages at two experimental assemblages. Sample treatment is indicated by symbol shading: solid black = Fished, grey = Procedural Control and white = Undisturbed Control. Stress = 0.06.

**Table S1.** Mean abundance (abund, number  $m^{-2}$ ) and biomass (biom,  $g\cdot m^{-1}$ ) of taxa observed at Thurstaston in experimentally Fished and Procedural Control and Control plots on two dates.

**Table S2.** Mean abundance (abund, number  $m^{-2}$ ) and biomass (biom,  $g\cdot m^{-1}$ ) of taxa observed at Warton Sands in experimentally Fished and Procedural Control and Control plots on two dates.

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## ORIGINAL ARTICLE

# The zebra mussel (*Dreissena polymorpha*) and the benthic community in a coastal Baltic lagoon: another example of enhancement?

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## Keywords

Alien species; Baltic Sea; *Dreissena polymorpha*; macrobenthos; meiobenthos; organic enrichment; Szczecin Lagoon.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

The zebra mussel (*Dreissena polymorpha*), a sessile suspension feeder, has been known to enhance the benthos in the sediment around mussel beds and druses. The enhancement is mainly due to biodepositing activity of the mussel and the resultant organic enrichment of the sediment. In addition, mussel beds increase habitat complexity. In June 2007, we studied the distribution of *D. polymorpha* beds and their effects on the benthos in Kleines Haff, the western part of the Szczecin Lagoon, a Southern Baltic coastal water body. We expected that: (i) the sediment near a *Dreissena* bed will support meio- and macrobenthic assemblages more abundant than those living away from the bed, and (ii) the meio- and macrobenthic assemblages close to the *Dreissena* bed will show a stronger dominance of the taxa relying on the organic enrichment (nematodes among the meiofauna; oligochaetes and chironomids among the macrobenthos). Meiobenthos was sampled at two localities, one (MB4) featuring *D. polymorpha* druses and the other (MB5) supporting a distinct zebra mussel bed. The two localities differed in their meiobenthic communities: whereas no difference in abundance and composition was observed between the sets of samples collected at MB4, MB5 showed a distinctly more abundant meiobenthos in the uppermost sediment layer near the zebra mussel bed, but the overall abundance was lower than that away from the bed. We conclude that the meiobenthic response to the presence of *D. polymorpha* was, at least in part, mediated by site-specificity of sediment characteristics. Macrozoobenthos was sampled at four stations in the vicinity of MB5 and around another *Dreissena* locality (MB10), and was also identified in the sediment samples collected for the meiobenthos. The macrobenthic abundance was higher by a factor of 2.4 (sandy sediment) to 4.9 (silty bottom) near the *Dreissena* beds than away from them. Irrespective of their situation relative to *Dreissena* aggregations, both meio- and macrobenthos were dominated by the taxa associated with organic enrichment. The macrobenthos associated with *Dreissena* beds included *Gammarus tigrinus*, a non-indigenous amphipod, whereas another non-native species, the polychaete *Marenzelleria neglecta*, was present in the macrobenthos of sandy sediments of the area.



## Problem

Sessile suspension feeders, particularly bivalves, are commonly regarded as keystone species in aquatic ecosystems (Covich *et al.* 1999) due to the variety of effects they produce. Their feeding mode renders them important in benthic-pelagic couplings (Newell 2004) via the effects they produce both in the water column and on the bottom. The effects observed in the water column include changes in seston flux (Asmus & Asmus 1991; Muschenheim & Newell 1992; Baker *et al.* 1998), improvement of water quality and nutrient removal (Nakamura & Kerciku 2000; Yamamuro *et al.* 2000; Newell *et al.* 2002) as well as removal of bacteria (Silverman *et al.* 1997). In the benthic system, they modify the bottom architecture (Buschbaum *et al.* 2009; Crooks & Khim 1999) and provide additional habitats for various other taxa (*e.g.* Morgado & Tanaka 2001), including alien species (Çinar *et al.* 2008), but their major role there is through the formation of biodeposits, silt production, and sediment organic enrichment, whereby the energy transfer to the benthos is facilitated (Kautsky & Evans 1987; Jaramillo *et al.* 1992; Haven & Morales-Alamo 1996; Kröncke 1996; Ragueneau *et al.* 2005; Kooijman 2006). The ensuing effects involve epifaunal attraction to, as well as their facilitation and enhancement in, the suspension feeder's settlement sites (*e.g.* Krantz 1973; Radziejewska 1986; Jacobi 1987a,b; Commito & Boncavage 1989; Svane & Setyobudiandi 1996; Crooks 1998; Thiel & Ulrich 2002; Tsuchiya 2002; Duarte *et al.* 2006; Vaughn & Spooner 2006). However, biotic effects of sessile suspension feeders are not always clearly identifiable, and multiple and divergent results in complex systems have been observed (Cummins *et al.* 2001; Spooner & Vaughn 2006).

The zebra mussel (*Dreissena polymorpha*) is a sessile suspension feeding bivalve that has attracted immense attention as an invader in various aquatic ecosystems of the Northern hemisphere (Minchin *et al.* 2002), but also as a keystone species involved in benthic-pelagic couplings (Fréchette & Bourget 1985; Ackerman *et al.* 2001; Daunys *et al.* 2006) that exerts multiple effects in the reservoirs it inhabits. Zebra mussels have been reported to affect aquatic ecosystem energy budgets and food-web structure (*e.g.* Hamburger *et al.* 1990; MacIsaac *et al.* 1991, 1995; Richardson & Bartsch 1997; Thayer *et al.* 1997) by, among others, interaction with microorganisms (Frischer *et al.* 2000) as well as with bacterio-, phyto-, and zooplankton (*e.g.* Cotner *et al.* 1995; Lavrentyev *et al.* 1995; MacIsaac *et al.* 1995; Bastviken *et al.* 1998), by affecting fish predation and spawning (Prejs *et al.* 1990; Gonzalez & Downing 1999; Marsden & Chotkowski 2001; McCabe *et al.* 2006), and by augment-

ing food resources available to waterfowl, *e.g.* the coot (*Fulica atra*) (Piesik 1983). The major effects, however, involve – as in other sessile suspension feeder bivalves – an increase in architectural complexity of the bottom as well as energy transfer to the bottom via biofiltration of water, biodeposition, and organic enrichment of the sediment, and the resultant facilitation and enhancement of invertebrate communities (recently summarised by Ward & Ricciardi 2007).

The biofiltering capacity of the zebra mussel (Horgan & Mills 1997; Orlova *et al.* 2004) has rendered the species attractive as a potential tool for water quality management (Piesik 1983; Smit *et al.* 1993; Mackie & Wright 1994; Fenske 2002, 2005; Gulati *et al.* 2008). For this potential to be taken advantage of in a given water body, it is necessary to have information on the *Dreissena* distribution and the role it plays in the reservoir to be managed. With this general objective in mind, a research programme aimed at mapping *Dreissena* distribution and the bivalve's potential for affecting the local benthic communities was launched in June 2007 in the Kleines Haff, the western part of the Szczecin Lagoon (a Southern Baltic coastal water body; Radziejewska & Schernewski 2008). We asked whether, and to what extent, the presence of *Dreissena* aggregations on the bottom could be important in structuring assemblages of meio- and macrobenthos in the Kleines Haff, and whether the effects observed would conform to the universality of interactions between sessile suspension feeders and other invertebrates, proposed by Ward & Ricciardi (2007) (but see Buschbaum *et al.* 2009, who found exceptions to the seemingly universal pattern).

To summarise, the structural complexity of a *Dreissena* bed or a druse offers a variety of refuges and microhabitats to small invertebrates (Botts *et al.* 1996). In addition, as a result of the zebra mussel feeding mode involving ingestion of suspended particulates and release of faeces and pseudofaeces, the presence of the bivalve on the bottom results in organic enrichment of the sediment surrounding the aggregation (Griffiths 1993). In this way, *D. polymorpha* acts as a habitat modifier that facilitates and enhances the development of benthic invertebrate communities (Ward & Ricciardi 2007). This line of reasoning led us to expect that:

- 1 The sediment near a *Dreissena* bed will support meio- and macrobenthic assemblages more abundant than those living away from the bed, as a result of facilitation and enhancement rendered by organic enrichment produced by *Dreissena*;
- 2 The meio- and macrobenthic assemblages in the sediment adjacent to the *Dreissena* bed will show a stronger dominance of those taxa relying on the organic enrich-

ment, primarily nematodes among the permanent meiofauna, and oligochaetes and chironomids among the macrobenthos.

In addition, our study was meant to provide qualitative and quantitative data on the Kleines Haff benthos; such data, particularly with respect to the meiobenthos, are scarce, while being urgently needed for a comprehensive assessment of the Haff's biota.

## Material and Methods

### Area of study

The Kleines Haff (Fig. 1) is the western part of the Szczecin Lagoon (German: Oderhaff), a coastal Southern Baltic water body with a long history of pollution and eutrophication (Radziejewska & Schernewski 2008). The Kleines Haff is shallow (average depth of 4.4 m), its average salinity being 1 ppt. The bottom in the nearshore zone is sandy and becomes more and more fine-grained towards the central part where muddy sediment (<0.063 mm grain size fraction accounting for >80%) prevails (Osadzuk 2004). Data on hydrographical parameters measured during the sampling campaign are summarised in Table 1.

Several areas supporting zebra mussel aggregations were revealed during the June 2007 campaign (*Dreissena* areas MB1–MB12; Fig. 2). The zebra mussels were estimated to cover a total of 6.56 km<sup>2</sup> of the Kleines Haff bottom (*i.e.* 2.4% of the bottom area). The *Dreissena* biomass was estimated at 8812.65 × 10<sup>3</sup> kg, with abundances ranging from 864 to 10,444 ind·m<sup>-2</sup>.

### Field work

Samples for the study of the zebra mussel-associated meiobenthos were collected from two localities (*Dreissena* areas MB4 and MB5, *cf.* Fig. 2), two sites being sampled in each. The idea was to sample the sediment as close as possible to a *Dreissena* bed and away from it, and to compare the composition and abundance of meiobenthic assemblages from the two sites.

At MB4 (Fig. 2), located at the depth of about 3 m and about 150 m away from the shore, *Dreissena* occurred in scattered clumps (druses) anchored in the bottom rather than forming a distinct bed. Consequently, the two sites sampled in the locality (denoted A and B) could not be sharply contrasted for the *Dreissena*-close versus *Dreissena*-away effect, although an effort was made to collect the sediment close to the druses (site A) and, as much as possible, away from any druse (site B). Visual observations of the sediment in samples from the two sites showed hardly any difference between them; the sediment consisted of sand, abundant shell debris, and coarse and fine detritus.

*Dreissena* area MB5 supported a distinct zebra mussel bed, and was sampled at sites denoted R (sediment cores collected close to the mussel bed) and O (about 10 m away from the bed). Visual observations of the sediment in samples showed fairly distinct differences between the two sites: whereas the site O sediment consisted of sand, shell debris, and rather fine detritus, the site R samples contained sediment with a clearly higher amount of shell debris and detritus. In addition, the site R cores showed a characteristic sediment composition whereby about 1 cm of heavily organically enriched sand mixed with shell debris

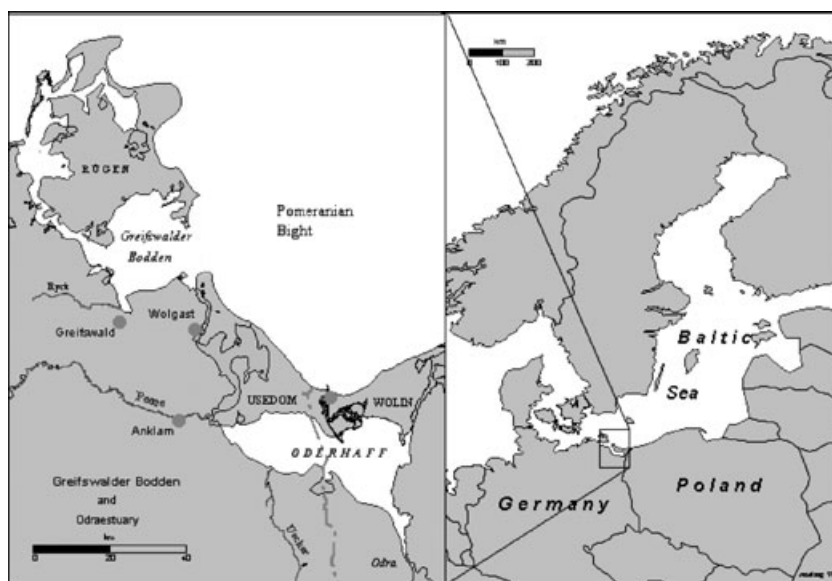


Fig. 1. The Szczecin Lagoon (Oderhaff): general location; the Kleines Haff occupies the western part of the Szczecin Lagoon.

**Table 1.** Hydrographic parameters measured at the sites sampled during the June 2007 sampling campaign in the Kleines Haff.

parameter	average or mean (± SD) value (range)
Secchi depth (m)	0.57 (0.4–1.7)
dissolved oxygen content (mg·dm <sup>-3</sup> )	10.12 ± 1.72 (1 m below surface), 8.71 ± 2.19 (0.4 m above bottom)
PO <sub>4</sub> -P content (mg·dm <sup>-3</sup> )	0.1 (0.03–0.2)
total P (mg·dm <sup>-3</sup> )	0.2 (0.16–0.32)
total N (mg·dm <sup>-3</sup> )	1.21 (0.9–1.9)
BOD <sub>5</sub> (mg·dm <sup>-3</sup> )	3.72 (2.6–5.39)
suspended particulates concentration (mg·dm <sup>-3</sup> )	13.7 (9.5–18.1)
chlorophyll a content (µg·dm <sup>-3</sup> )	61.3 ± 9.5

ris overlay a peat-like deposit consisting of tightly packed large plant remains interspersed by shell fragments and sand grains.

#### Meiobenthos sampling

Sediment samples for the study of meiobenthos were collected by divers using hand-held corers (22 mm inner diameter) consisting of transparent polycarbonate tubes. At each site, five sediment cores were retrieved. The tubes were inserted into the sediment to the depth of about 10 cm. When in the sediment, the tube was closed from above with a rubber stopper and was carefully pulled out; once out of the sediment, the lower end of the tube was closed with a rubber stopper to prevent sample loss. Following retrieval, the near-bottom water overlying the sediment was siphoned off into a container, and the entire core was pushed up, from below, by a piston. The

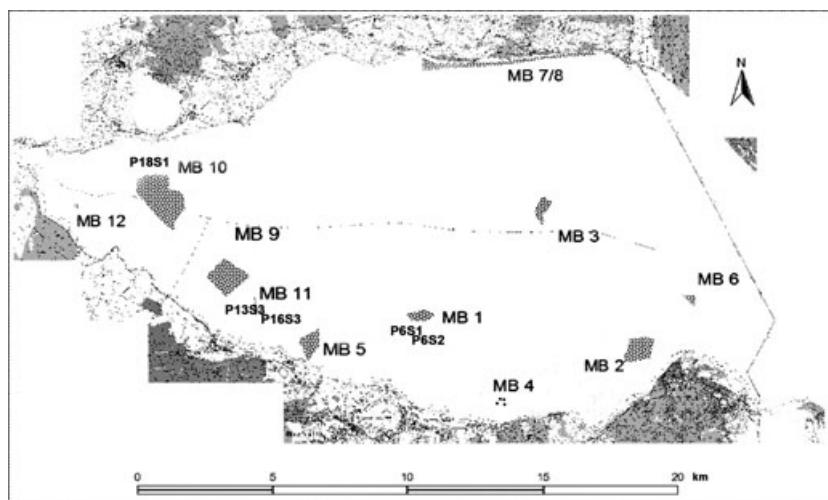
uppermost 1-cm-thick layer was cut off to form the ‘upper sediment’ sample and transferred to a container, and the remainder of the core (the ‘lower sediment’ sample) was placed in another container. The water and sediment samples were preserved with 10% buffered formalin and stained with Rose Bengal. At each station, five sediment cores were collected.

#### Macrobenthos sampling

Macrobenthos was sampled at five stations representative of various sedimentary habitats in the Lagoon. Four of the sites were close to the meiobenthos sampling stations (near MB5; Fig. 2), one being located in the western part of the Kleines Haff (near MB10; Fig. 2). At three sampling stations (P16S3, P6S1, P6S2; Fig. 2), macrobenthos was collected away from *Dreissena* aggregations; the sediment at those stations was silt, silty sand and sand, respectively. The two remaining stations (P13S3, P18S1; Fig. 2) supported *Dreissena* druses or a *Dreissena* bed, the underlying sediment containing a high proportion of shell debris and detritus. The samples were collected from RV *Bornhöft* with Günther box corers (10 cm × 10 cm or 10 × 6 cm surface area, both penetrating about 20 cm into the sediment). At each site, three sediment samples were collected. The samples were sieved through an 0.5-mm sieve and the sieving residue was fixed in 10% buffered formalin.

#### Laboratory procedures

In the laboratory, the content of each meiobenthos sample container was wet-sieved on a series of sieves (mesh sizes used: 1.00 and 0.500 mm to separate the macrobenthic animals; 0.180; 0.090 and 0.032 mm). The residues from the two coarsest sieves were retained for



**Fig. 2.** Zebra mussel beds in the Kleines Haff and location of macrobenthos sampling stations.

identification of any macrobenthic animals present (see below). The residues from the 0.032–0.500-mm sieves were transferred, in portions, onto ruled Petri dishes and examined under a stereomicroscope. Meiobenthic organisms were identified to the lowest taxon possible and enumerated.

Macrobenthic animals were removed from the sieving residue, rinsed with tap water (on a 0.5-mm sieve), examined under a stereomicroscope, identified to the lowest taxon possible, and enumerated.

### Statistical treatment

Significance of differences between mean abundances and mean relative abundances of the benthic assemblages was tested by means of non-parametric Kruskal–Wallis test (Sokal & Rohlf 1995).

## Results

### Meiobenthos

The number of meiobenthic taxa present in the sediment (Table 2) was found to depend on both the site and the core section, the uppermost sediment layer usually supporting more diverse meiofauna. In terms of the total number of taxa found in the core, meiobenthic assemblages at sites A and B proved more diverse than those at the other pair of sites, Site R showing the widest within-site variability in the number of taxa.

The meiobenthic assemblages were found to be dominated by nematodes (Fig. 3A,B). In terms of the entire core (Fig. 3B), the mean percent contribution of nematodes to the entire assemblage ranged from about 35% at site B to about 85% at site O, the nematode domination at the MB5 sites, particularly at site O, being much stronger than at the MB4 sites. Other taxa of importance were ostracods, oligochaetes, chironomid larvae, and harpacticoid copepods (*cf.* Fig. 3).

The total meiobenthos abundance was found to range from a minimum of  $255.11 \text{ ind}\cdot(10 \text{ cm}^2)^{-1}$  to a maximum of  $3084.99 \text{ ind}\cdot(10 \text{ cm}^2)^{-1}$ . Generally, the MB4 sites supported meiobenthic communities that were significantly

( $P < 0.05$ ) less abundant than those at MB5 (Fig. 3A). The whole-core meiobenthos abundances at the MB4 sites were very similar; on the other hand, at MB5, the meiobenthos at site O (away from the *Dreissena* bed) was much more abundant than that at site R, although the difference was not significant ( $P > 0.05$ ) due to the high within-site variability.

At all sites, the top centimetre of the sediment was inhabited by most of the meiobenthic animals found in the entire core (Table 3). This trend was at its weakest at site O where more than half of the meiobenthic animals (about 56%) dwelt below the uppermost sediment layer. At MB4, there were no significant between-sites differences ( $P > 0.05$ ) in the partitioning of the meiobenthic assemblages between sediment layers; conversely, the two MB5 sites differed significantly in this respect ( $P < 0.05$ ), the site located next to the *Dreissena* bed (R) showing a marked aggregation of the meiobenthos (about 86% of the total abundance) in the uppermost sediment layer.

Meiobenthic abundances in the uppermost sediment layer (Fig. 3C) repeated some of the pattern evidenced by the total abundances, namely significantly lower ( $P < 0.05$ ) abundances at MB4 than at MB5 and no significant between-site differences ( $P > 0.05$ ) at either of the site pairs. At MB5, however, the meiobenthos was much more abundant in the uppermost sediment layer of site R than of site O, although the difference was not significant ( $P > 0.05$ ) due to the high within-site variability.

### Macrobenthos

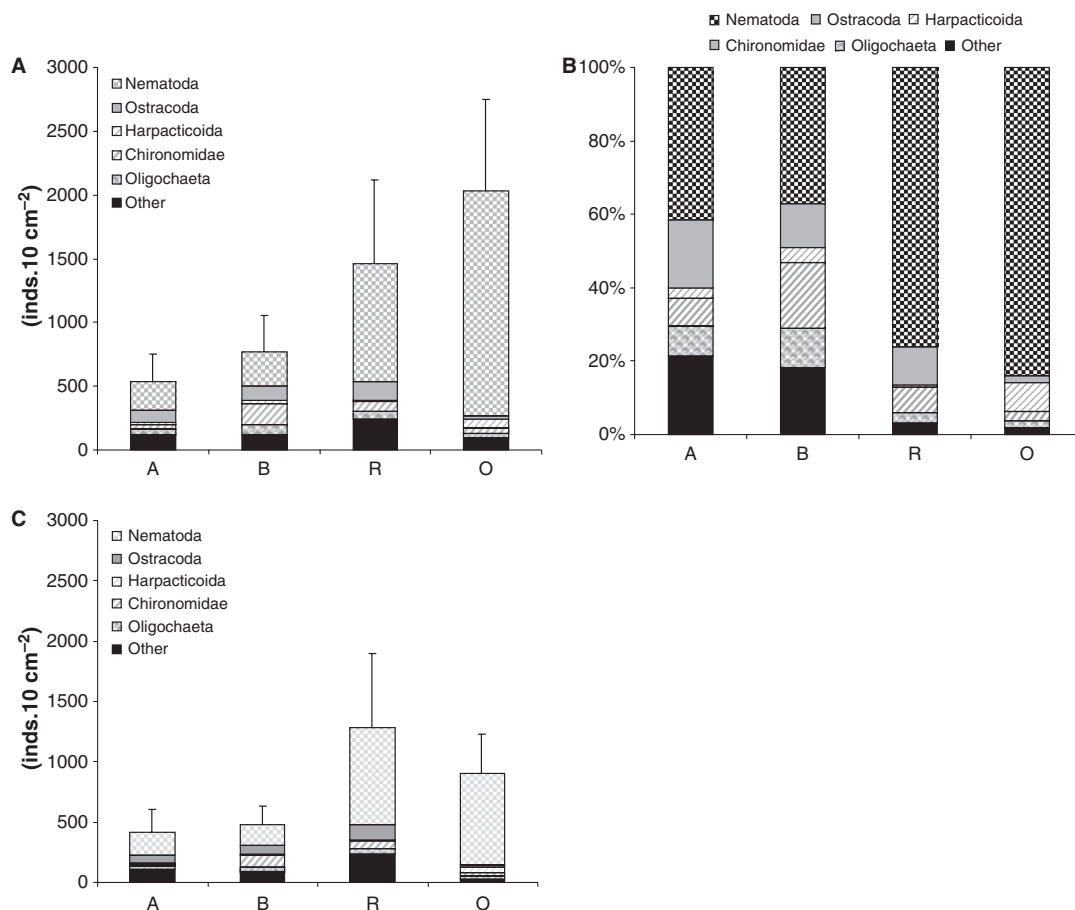
The number of macrobenthic taxa per sample varied from 2 to 8. Although we found a total of 27 macrobenthic taxa, the maximum taxon richness per sample was rather low, even at the zebra mussel bed stations. Oligochaetes and chironomid larvae were the dominant taxa at most stations (Fig. 4A), except for one mussel bed station dominated by *D. polymorpha*, abundant also at the other *Dreissena* bed sampling site (Fig. 4A).

The macrobenthic abundance varied greatly over the area sampled: the mean total abundances ranged from 3367 to  $18,333 \text{ ind}\cdot\text{m}^{-2}$  (Table 4; Fig. 4B). Higher abundances were found at the station in the *Dreissena* area MB10 (P13S3), with live *Dreissena* and empty shells (Fig. 4B). The mean total abundances of the macrobenthos sampled in the vicinity of the *Dreissena* beds were much higher than away from the beds; however, due to the high within-site variability (*cf.* high standard deviations in Table 4 and Fig. 4B), the difference between the two groups of stations was not significant ( $P > 0.05$ ).

Macrobenthic animals retrieved from the meiobenthic samples (Table 5) represented six major taxa (Oligochaeta, Hirudinea, Gastropoda, Bivalvia, Crustacea, and Dip-

**Table 2.** Taxon richness of meiobenthic assemblages at sites sampled: ranges of the number of taxa.

locality	MB4		MB5	
	site A	site B	site O	site R
core part				
uppermost	11–13	9–12	7–9	6–12
lower sediment	6–9	7–10	5–8	3–9
total	11–14	9–13	8–10	6–13



**Fig. 3.** Meiobenthos in the Kleines Haff. (A) Total meiobenthos abundance. (B) Composition of the Kleines Haff meiobenthos (total core). (C) Meiobenthos abundance in top sediment layer.

**Table 3.** Partitioning of meiobenthic abundance between the core parts examined: mean ( $\pm$  SD) percentages of total abundance at the core parts.

locality	site	U upper core part	Lower sediment part	significance of within-locality difference
MB4	A	74.79 $\pm$ 5.56	25.21 $\pm$ 5.57	not significant ( $P > 0.05$ )
	B	62.67 $\pm$ 4.85	37.33 $\pm$ 4.85	
MB5	R	88.76 $\pm$ 12.98	11.24 $\pm$ 12.98	significant ( $P < 0.05$ )
	O	44.16 $\pm$ 6.36	55.83 $\pm$ 6.36	

tera). Generally, a higher taxon richness was evident at sites A and R (closest to *Dreissena* druses or beds). The macrobenthos was dominated by chironomid larvae and oligochaetes, except for site A showing a high proportion of *Gammarus tigrinus* (prominent also at site R) and small specimens of *Dreissena polymorpha* (Fig. 5A). The mean total abundances varied from 64,924 to 87,273 ind·m<sup>-2</sup> (Table 5; Fig. 5B). Although the total abundances at sites A and R (closest to *Dreissena* aggrega-

tions) tended to be higher than elsewhere, the high within-site variability precluded statistical significance of the differences.

The sites sampled for macrobenthos revealed the presence of some non-indigenous taxa. Among those, the amphipod *G. tigrinus* was found at two of the five stations sampled, with the maximum abundance of 167 ind·m<sup>-2</sup> (cf. Table 4). Other invaders included *Obesogammarus crassus* and *Pontogammarus robustoides*, both found in an ancillary sample collected for the concurrent study of macrophytes at MB4; and the polychaete *Marenzelleria neglecta*. The latter occurred only at the sandy stations (P6S2) and was not very abundant (67 ind·m<sup>-2</sup>).

## Discussion

### Meiobenthos

Examination of cores collected within areas supporting *Dreissena* beds and druses in the Kleines Haff provided information on the composition and abundance of meio-



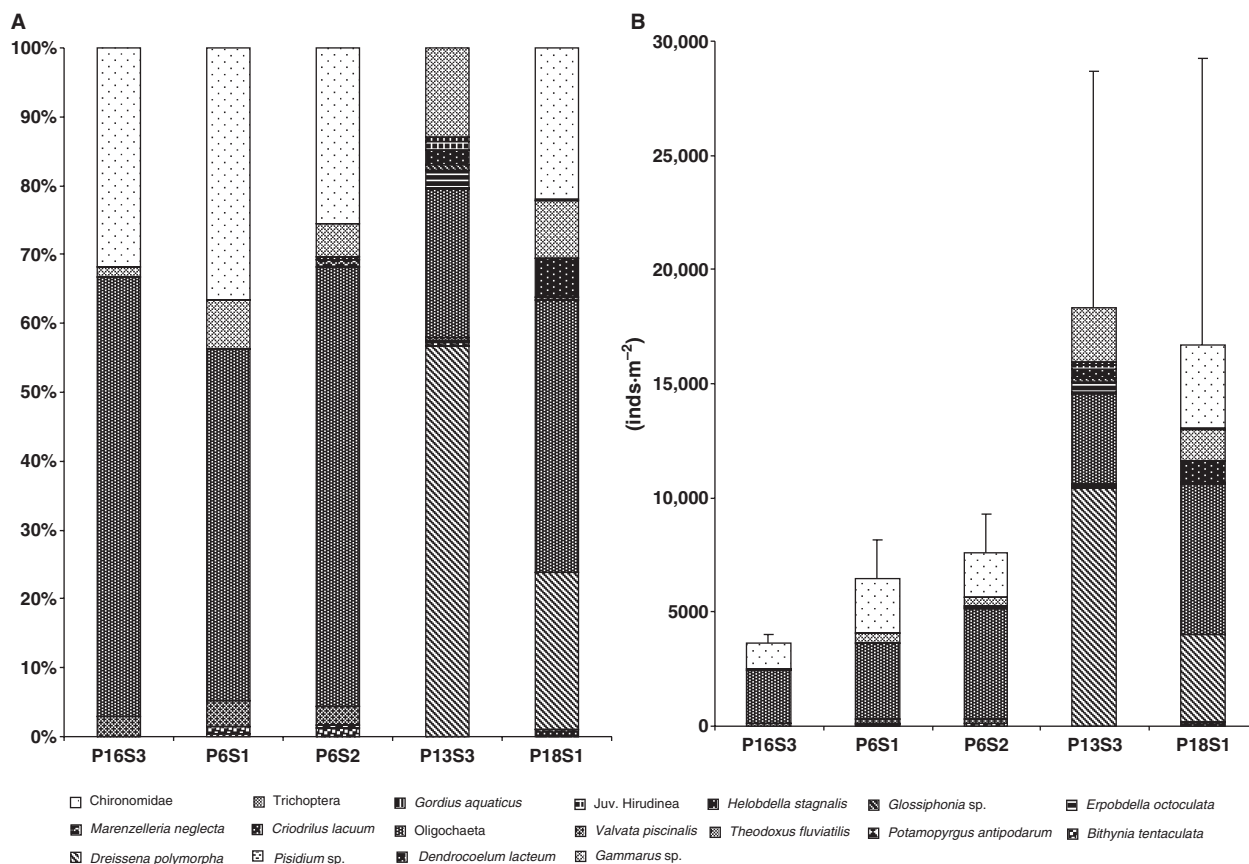


Fig. 4. Macrobenthos in the Kleines Haff. (A) Relative abundance of macrobenthic taxa. (B) Mean abundances ( $\text{ind}\cdot\text{m}^{-2} \pm \text{SD}$ ) of macrobenthos.

benthic assemblages inhabiting those areas. The meiobenthic aspect of *Dreissena*–benthos interactions has been largely ignored so far. It was only Dermott & Kerec (1997) who reported increased abundances of meiofauna, notably ostracods, nematodes, and harpacticoids following *Dreissena* settlement in the Eastern Lake Erie. They attributed the very highly significant increase in mean densities of those meiobenthic taxa to an increase in the amount of organic matter and associated bacteria in the faeces and pseudofaeces being deposited onto the sediment surface by *Dreissena*. In the present study, the abundance of meiobenthic assemblages, estimated from the total core data (Fig. 3A), only partially conformed to the expected pattern, in that the mean abundances at the *Dreissena* bed-supporting locality (MB5) were significantly higher than those at the locality with scattered zebra mussel druses (MB4). However, no significant difference in the mean total abundance was found between the two sites sampled within MB5, although a strong tendency towards higher abundances at the site away from the *Dreissena* bed was observed (cf. Fig. 3A). The effect could have been partially a result of sediment differences between the two sites:

whereas at site R the underlying sediment was very consolidated, peaty, and thus hardly penetrable by the fauna, site O supported sediment that was mostly sandy and thus amenable to colonisation by interstitial fauna throughout the entire depth sampled. On the other hand, had we considered the uppermost sediment layer only, the enhancement hypothesis (higher abundance near the *Dreissena* bed than away from it, i.e. at site R versus site O; cf. Fig. 3C) would have been borne out by the data from MB5. The results for the whole core, however, weaken the actual support for the hypothesis. It seems that the result observed could have been caused by a combination of factors: *Dreissena* facilitation (increased organic matter content in the sediment) on the one hand and purely physical effect (more available space in the penetrable uppermost sediment layer) on the other.

The lack of a clear-cut pattern in the meiobenthic assemblage response could also have been associated with seasonal effects. Aggregations of suspension feeders, including *Dreissena polymorpha*, were reported to exhibit seasonal variability (e.g. Cummings *et al.* 2001; Wolnomiejski & Woźniczka 2008). Coupled with high

**Table 4.** Mean ( $\pm$  SD) macrobenthic abundances (ind·m<sup>-2</sup>) at the Kleines Haff sites sampled for the macrobenthos during the June 2007 campaign.

	sampling site				
	P16S3	P6S1	P6S2	P13S3	P18S1
sediment type	silt	silty sand	sand	shells, <i>Dreissena</i> clumps	silt, <i>Dreissena</i> bed
taxon					
<i>Dendrocoelum lacteum</i>	0	0	0	0	166.67 $\pm$ 288.68
<i>Pisidium</i> spp.	0	33.33 $\pm$ 57.74	0	0	0
<i>Dreissena polymorpha</i>	0	0	0	10,388.9 $\pm$ 7874.8	3833.33 $\pm$ 2020.77
<i>Bithynia tentaculata</i>	0	66.67 $\pm$ 57.74	100.0 $\pm$ 100.0	111.11 $\pm$ 96.23	0
<i>Potamopyrgus antipodarum</i>	0	0	33.33 $\pm$ 57.74	0	0
<i>Theodoxus fluviatilis</i>	0	0	0	55.56 $\pm$ 96.23	0
<i>Valvata piscinalis</i>	111.11 $\pm$ 96.23	233.33 $\pm$ 251.66	200.0 $\pm$ 100.0	55.56 $\pm$ 96.23	0
Oligochaeta	2333.33 $\pm$ 0	3300.0 $\pm$ 1664.3	4833.33 $\pm$ 1026.3	3944.44 $\pm$ 1512.3	6611.11 $\pm$ 4715.12
<i>Criodrilus lacuum</i>	0	0	0	55.56 $\pm$ 96.23	0
<i>Marenzelleria neglecta</i>	0	0	66.67 $\pm$ 57.74	0	0
<i>Erpobdella octoculata</i>	0	0	0	444.44 $\pm$ 384.91	0
<i>Glossiphonia</i> sp.	0	0	0	166.67 $\pm$ 166.67	55.56 $\pm$ 96.3
<i>Helobdella stagnalis</i>	0	0	0	388.89 $\pm$ 254.59	944.44 $\pm$ 693.90
Hirudinea juv.	0	0	0	222.22 $\pm$ 192.45	0
<i>Gordius aquaticus</i>	0	0	33.33 $\pm$ 57.74	111.11 $\pm$ 192.45	0
<i>Gammarus</i> spp.	0	466.67 $\pm$ 230.94	199.99 $\pm$ 346.41	0	1222.22 $\pm$ 2116.99
<i>G. zaddachi</i>	0	0	166.67 $\pm$ 288.68	2388.89 $\pm$ 1170.7	0
<i>G. tigrinus</i>	55.56 $\pm$ 96.23	0	0	0	166.67 $\pm$ 166.67
Trichoptera	0	0	0	0	55.56 $\pm$ 96.23
Chironomidae	1166.67 $\pm$ 166.67	2366.67 $\pm$ 603.68	1933.33 $\pm$ 608.49	0	3666.67 $\pm$ 3355.18
total	3666.67 $\pm$ 333.34	6466.67 $\pm$ 1680.3	7566.67 $\pm$ 1721.4	18,333.3 $\pm$ 10,332.2	16,722.22 $\pm$ 12,549.41
no. of taxa per sample	2–4	4–6	4–7	6–8	5–7

susceptibility of meiobenthic assemblages to temporal variability (Radziejewska & Chabior 2004), the seasonal effect could have masked or obliterated the responses expected, had they occurred.

The meiobenthos at all sites was strongly dominated by the taxa relying on the organic enrichment, *i.e.* nematodes, chironomids, and ostracods. The Kleines Haff, like the entire Szczecin Lagoon, has been heavily eutrophic for decades (Radziejewska & Schernewski 2008), hence the sediment is highly organically enriched as a result of background sedimentation. Nematodes, chironomids, and ostracods have been reported as absolute dominants of the Lagoon meiobenthos (Radziejewska & Drzycimski 1988, 1990; T. Radziejewska, unpublished observations). It seems then that the enhancement hypothesis is only partially borne out by the meiobenthos data, and that site-specific effects could play an important role in shaping the meiobenthic assemblages associated with *Dreissena* aggregations in the Kleines Haff. In a different setting, Norkko *et al.* (2001) pointed out the importance of site-specific effects, related to a sedimentation gradient, leading to changes in interactions between the suspension-feeding bivalve *Atrina zelandica* and the benthos in a coastal ecosystem.

It is noteworthy that the maximum meiobenthic mean total abundance recorded in this study, 3084.99 ind·(10 cm<sup>2</sup>)<sup>-1</sup>, proved higher than any meiobenthic abundance reported so far from the entire Szczecin Lagoon (Radziejewska & Drzycimski 1988, 1990; Radziejewska & Schernewski 2008). The only other set of Kleines Haff meiobenthos abundance data on hand (T. Radziejewska, unpublished observations) originated from research carried out in the early 1990s, based on samples collected in the central part of the area (muddy bottom). The maximum abundance recorded then was about 380 ind·(10 cm<sup>2</sup>)<sup>-1</sup> and the meiobenthic assemblages were strongly dominated by ostracods.

#### Macrobenthos

Compared to the meiobenthos, the macrobenthic assemblages sampled in this study showed a more pronounced abundance pattern supporting the facilitation and enhancement hypothesis, and conforming to situations reported in other studies (*e.g.* Beekey *et al.* 2004). The macrobenthic abundances near the zebra mussel bed were substantially higher than the abundances recorded at the bottom away from the *Dreissena* bed (*cf.* Fig. 5). It is worth mentioning

**Table 5.** Mean ( $\pm$  SD) total macrobenthic abundances (ind·m<sup>-2</sup>) at the Kleines Haff sites sampled for the meiobenthos during the June 2007 campaign.

taxon (% frequency)	sampling locality/site			
	MB4/A	MB4/B	MB5/R	MB5/O
Oligochaeta (12.1)	22,727.3 $\pm$ 28,747.98	19,090.9 $\pm$ 8743.36	38,181.8 $\pm$ 25,430.18	6818.2 $\pm$ 5868.16
Hirudinea (9.1)	3636.4 $\pm$ 5926.55	0.0	2272.7 $\pm$ 0.00	3030.3 $\pm$ 2624.32
<i>Valvata piscinalis</i> (6.1)	909.1 $\pm$ 2032.79	0.0	6818.2 $\pm$ 3214.12	0.0
<i>Valvata pulchella</i> (3.0)	0.0	0.0	4545.5 $\pm$ 0.00	0.0
<i>Pisidium nitidum</i> (6.1)	909.1 $\pm$ 2032.79	3409.1 $\pm$ 4351.94	0.0	0.0
<i>Dreissena polymorpha</i> (6.1)	17,272.7 $\pm$ 22,590.50	0.0	4545.5 $\pm$ 0.00	0.0
<i>Gammarus tigrinus</i> (6.1)	24,545.5 $\pm$ 47412.38	0.0	4545.5 $\pm$ 0.00	0.0
<i>Procladius</i> sp. (3.0)	0.0	2272.7 $\pm$ 0.00	0.0	0.0
<i>Chironomus</i> sp. (12.1)	11,818.2 $\pm$ 14766.48	25,000.0 $\pm$ 15414.39	4545.5 $\pm$ 0.00	3030.3 $\pm$ 2624.32
<i>Glyptotendipes</i> sp. (12.1)	2727.3 $\pm$ 4065.58	1515.2 $\pm$ 0.00	12,727.3 $\pm$ 7468.94	4545.5 $\pm$ 0.00
<i>Tanytarsus</i> sp.(9.1)	0.0	5681.8 $\pm$ 0.00	6818.2 $\pm$ 0.00	30,303.0 $\pm$ 31,926.22
Chironomidae indet. (9.1)	0.0	6818.2 $\pm$ 0.00	2272.7 $\pm$ 0.00	34,090.9 $\pm$ 0.00
Chironomidae pupae (6.1)	0.0	1136.4 $\pm$ 0.00	0.0	2272.7 $\pm$ 0.00
total	84,545.5 $\pm$ 102,982.4	64,924.2 $\pm$ 20,680.6	87,272.7 $\pm$ 28,640.0	84,090.91 $\pm$ 40,092.9
total number of taxa (range)	8.0 (4–6)	8.0 (2–7)	10.0 (2–6)	7.0 (0–5)

that under conditions of the highly polluted Rhine-Meuse estuary, the only site among those sampled by Smit *et al.* (1995) that showed elevated abundances of the macrobenthos was the locality supporting a *Dreissena* bed.

On the other hand, the macrobenthos data provided by the small meiobenthos sediment cores failed to reveal any pattern, perhaps due to too small sediment volume being available for projecting the macrobenthic abundance.

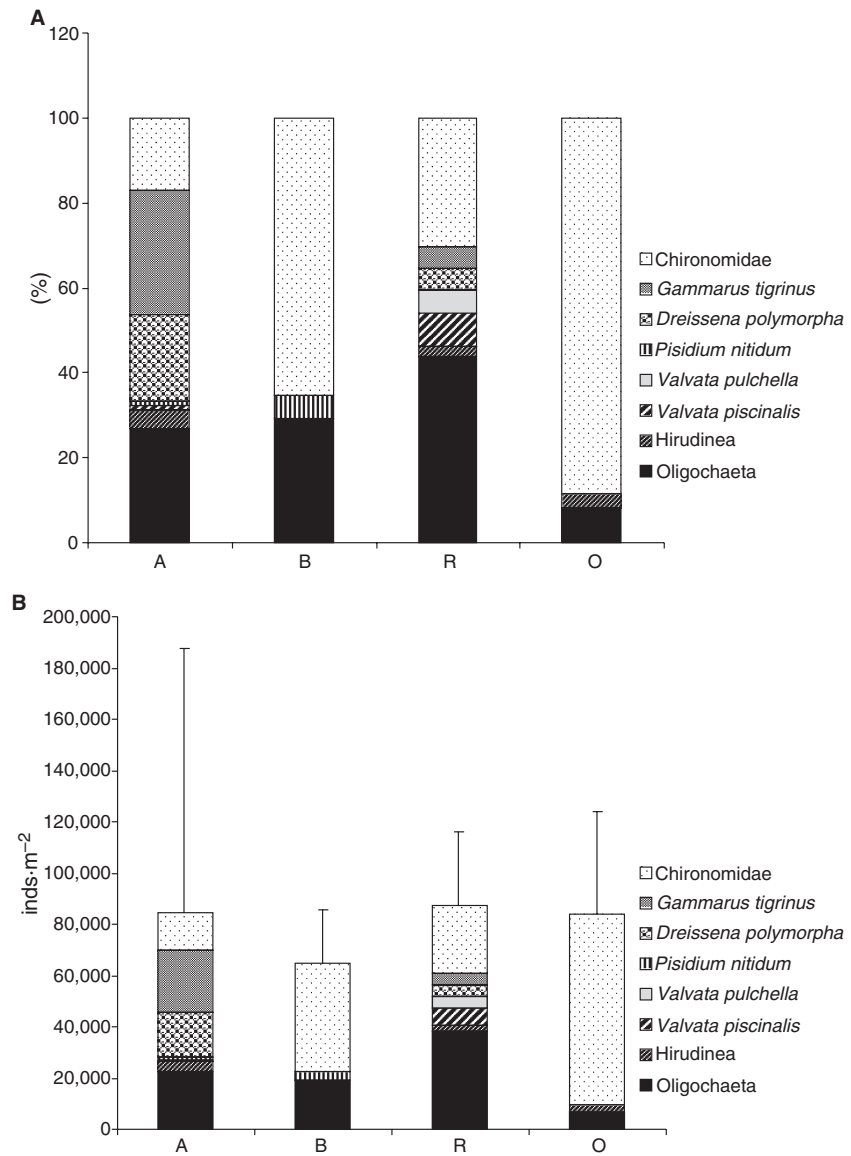
The most abundant macrobenthic assemblages differed in their composition and domination structure (*cf.* Fig. 4), but did show domination of the taxa known to be associated with organically enriched sediment (oligochaetes and chironomid species typical of muddy habitats). However, those taxa were prominent at other sites as well, for reasons already discussed above.

On the other hand, the sites supporting the highest macrobenthic abundance were not particularly taxa-rich. The station with the highest abundance (P13S3) supported 18,333 ind·m<sup>-2</sup>, but only 10 taxa. This finding lends support to the conclusion of Buschbaum *et al.* (2009) who, having analysed diversity of mussel bed-associated fauna in different parts of the world, contended that mussel beds themselves were hardly universal centres of biodiversity, and that their effects on associated species were site-specific. The macrobenthic community at the highest-abundance station showed the presence of chironomid [*Glyptotendipes* sp., *Polypedilum (nubeculosum* group), *Tanytarsus* sp.] and mollusc (*Valvata piscinalis*) species known for their association with sandy rather than muddy sediment.

It has to be remembered that facilitation and enhancement of macrobenthos by an aggregation of a suspension-

feeding bivalve are not absolute. Species that produce planktonic larvae, *e.g.* polychaetes, will not be enhanced, and may even be inhibited by a suspension feeder, as demonstrated by Duarte *et al.* (2006) for *Mytilus chilensis*, Ragnarsson & Raffaelli (1999) for *Mytilus edulis*, Crooks & Khim (1999) for *Musculista senhousia*, and Dermott & Kerec (1997) for *Dreissena polymorpha*. In the latter case, the detrimental effects were shown by the burrowing amphipod *Diporeia hoyi* and by fingernail clams (Sphaeriidae) (see also Lauer & McComish 2001). In contrast, non-planktonic developers (*e.g.* chironomid larvae, oligochaetes, amphipods) showed positive responses to the mussels, and might have even be favoured in the presence of suspension feeders such as *Dreissena* or *M. edulis* (see *e.g.* Commito & Boncavage 1989 for discussion of facilitative effects in the oligochaete *Tubificoides benedeni*).

The polychaete *Marenzelleria neglecta*, a non-indigenous species introduced in the 1980s, was very abundant on sandy sediments in the mid-1990s (averaging 8278 ind·m<sup>-2</sup> in 1996; Fenske 2003). In 2007, *M. neglecta* did occur, but in much lower abundances (67 ind·m<sup>-2</sup>). This is a phenomenon known from many biological invasions (Strayer *et al.* 2006): at first, the invader occurs at a very high abundance ('explosion'), but after a few years it becomes integrated in the existing community and its abundance becomes stabilised at a lower level on account of niche opportunity constraints (*sensu* Shea & Chesson 2002). Our study showed the *Dreissena* bed-associated macrobenthos to include another frequent and abundant non-indigenous species, the amphipod *Gammarus tigrinus*. It is also known to have been present in the Szczecin Lagoon since the late 1980s (Wawrzyniak-Wydrowska &



**Fig. 5.** Macrobenthos retrieved from meiobenthic samples. (A) Relative abundances of macrobenthic taxa. (B) Mean abundances ( $\text{inds}\cdot\text{m}^{-2} \pm \text{SD}$ ) of macrobenthos.

Gruszka 2005). *Gammarus tigrinus*, the commonest invader in the eastern part of the Lagoon (Wawrzyniak-Wydrowska & Gruszka 2005), was found at several stations sampled, including the sediment sampled for meiobenthos. The presence of this amphipod in the Kleines Haff is in line with two other interesting, interconnected findings reported in other accounts on research focusing on *Dreissena* bed-associated macrobenthos: a preference of amphipods for inhabiting *Dreissena* beds (e.g. Griffiths 1993; Dermott & Kerec 1997; Thayer *et al.* 1997; Gonzalez & Downing 1999) and facilitation of non-indigenous species' expansion in aquatic environments by *Dreissena* (e.g. Limén *et al.* 2005; Çinar *et al.* 2008). Positive amphipod response to *Dreissena* is thought to be a result of the amphipod's reliance on increased habitat complexity

(a physical effect; Botts *et al.* 1996) as a means to aiding the release from predation pressure exerted by, for example, fish (a biotic effect; Gonzalez & Downing 1999). Limén *et al.* (2005) used a stable isotope technique in an attempt to elucidate mechanisms of success of an invader amphipod, *Echinogammarus ischnus*, in replacing the native amphipod *Gammarus fasciatus*. Both species show positive responses to the presence of *Dreissena* beds (Palmer & Ricciardi 2004); exploitative competition for food (*Dreissena* faeces/pseudofaeces) was put forward by Limén *et al.* (2005) as an underlying cause for the *E. ischnus* success. However, evidence obtained showed that not to be the case. Palmer & Ricciardi (2004) postulated segregation of the two amphipod species along physicochemical gradients in the habitat, *E. ischnus* showing a stronger

preference towards coarse-grained sediment which *Dreissena* beds might resemble. By revealing the presence and abundance of the alien amphipods associated with the Kleines Haff *Dreissena* aggregations, our study contributes to the ongoing discussion on and concern about non-native species colonising the Southern Baltic coastal waters (Wawrzyniak-Wydrowska & Gruszka 2005).

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## ORIGINAL ARTICLE

# Distribution and spatial structure of pelagic fish schools in relation to the nature of the seabed in the Sicily Straits (Central Mediterranean)

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## Keywords

acoustic surveys; bottom and fish backscattering; echo-sounder; fish school; seabed; Sicily Channel.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

Hydroacoustic data collected during two echosurveys carried out in the Sicily Channel in 1998 and 2002 were analysed to investigate the distribution and spatial structure of small pelagic fish species in relation to the sedimentological nature of the sea bottom. The study was carried out on two contiguous areas (labelled ZONE 1 and ZONE 2) of the continental shelf off the southern coast of Sicily, characterised by different dominant texture, 'sand' for ZONE 1 and 'clayey-silt' for ZONE 2. Simultaneous information on small pelagic fish schools and the seabed was obtained using a quantitative echo-sounder (SIMRAD EK500) that measures echoes due to the scattering from both fish schools and the bottom surface. Acoustically determined fish school and seabed data were integrated, respectively, with information on species composition obtained by experimental fishing hauls, and with granulometric information obtained from the analysis of *in situ* sediment samples. The results indicate a general preference of small pelagic fish schools for seabeds of finer granulometry. First, the occurrence of fish schools was higher over the acoustically classified 'soft' seabeds of ZONE 2. Secondly, although ZONE 2 represents <30% of the total length of daytime acoustic tracks analysed in this study, in both surveys the bulk of fish biomass (>60%) was concentrated over 'soft' seabed substrates of ZONE 2. Different species composition and/or behaviour of fish schools in the two areas investigated were postulated in relation to seabed conditions. Specifically, over the hard and soft bottoms of ZONE 2, fish schools were found at lower depths and at shallower bottom depths compared to ZONE 1. Furthermore, over the softer bottoms of ZONE 2, fish schools exhibiting a more 'pelagic' behaviour (*i.e.* detected at a greater distance from the bottom) showed a preference for softer (and finer) seabed substrate conditions. Conversely, fish schools exhibiting a more 'demersal' behaviour (*i.e.* at a smaller distance from the bottom) were mostly found on relatively harder substrates.

## Problem

Small pelagic fishes are a significant component of the Mediterranean fisheries and they are also important to the ecosystem as they constitute the main prey of a large num-

ber of fish such as tuna, cod and mackerel. In the Mediterranean Sea there are eight main small pelagic species. Only four of them – anchovy (*Engraulis encrasicolus*), sardine (*Sardina pilchardus*), round sardinella (*Sardinella aurita*) and sprat (*Sprattus sprattus*) – are considered to be fully

pelagic, whereas Atlantic mackerel (*Scomber scomber*), chub mackerel (*Scomber japonicus colias*), bogue (*Boops boops*) and Atlantic horse mackerel (*Trachurus trachurus*) have more or less prolonged biological stages with pelagic behaviour (Alvares *et al.* 2003). Two of these species, the European anchovy and the European sardine, are important fishery resources in the Sicilian Channel, and historically have been exploited by fishermen from the north-western and southern coasts of Sicily.

The pelagic species are social animals that live in mono- or plurispecific schools with a highly patchy distribution (Fréon & Misund 1999). The distribution and the spatial structure of pelagic schools is a complex phenomenon that is controlled by a number of interactive mechanisms and factors (Maravelias 1999; Bahri & Fréon 2000; Soria *et al.* 2003).

Some authors also considered the nature of the seabed to be a possible factor affecting fish spatial distribution (Genin & Boehlert 1985; Dower *et al.* 1992; Maravelias 1999; Maravelias *et al.* 2000; Manik *et al.* 2006a). Specifically, the nature of the seabed was found to modulate the presence and relative abundance of Atlantic herring (*Clupea harengus*) within the northern North Sea, with species clustering in habitats with a depth between approximately 100 and 150 m and a gravel/sand type of seabed, which also carried the highest abundance of zooplankton (Maravelias 1999; Maravelias *et al.* 2000). Manik *et al.* (2006a), on the other hand, using hydroacoustic data collected during an echosurvey carried out during December 2003 in the Indian Ocean, on the southern coast of Jawa Island (Indonesia), generally observed a higher occurrence of fish schools (64 schools of the total of 66 detected) over sandy bottoms. Furthermore, the presence of seamounts may affect the aggregation and movement of small pelagic fishes (Allain *et al.* 2008). The presence of these topographic features on the ocean floor determines the uplifting of isotherms, isopycnals (Genin & Boehlert 1985) and nutrient isolines (Coméau *et al.* 1995). This upward deflection of deeper, more nutrient-rich waters can lead to an increase in primary production if its effects reach up into the euphotic zone, where there is enough light for photosynthesis (Furuya *et al.* 1995; Odate & Furuya 1998). The same effect could be produced by different geological structures such as benches, platforms, relieves or marine terraces (Allain *et al.* 2008).

Some topographic features, such as the degree of land enclosure and size of habitat, were also found to affect the spatial structure of anchovy and sardine schools in the Eastern Mediterranean, suggesting that environmental spatial heterogeneity, attributable to coastal topography, is able to affect the way fish schools become organised into aggregations (Giannoulaki *et al.* 2006).

Another important feature of habitat selection related to the seabed is the frequent association of pelagic species

with sudden breaks in the bottom depth due to submarine canyons, escarpment areas or deep water basins located close to the shore, as reported by Mais (1977) for the northern anchovies (*Engraulis mordax*) in the California current system.

Habitat selection related to the seabed could also be linked to the daily vertical migration of these species along the water column. For instance, Dias *et al.* (1989) and Zwolinski *et al.* (2007) reported that sardine and pelagic fish schools off Portugal are often observed on the bottom during the night, with schools descending towards the seabed during their expansion at dusk, and remain in close contact with the seabed throughout the night. Although the descent may be linked to the spawning behaviour of the species, with reproduction occurring at dusk close to the seabed (Zwolinski *et al.* 2007), similar patterns have been observed off Portugal outside the spawning season (Dias *et al.* 1989). This pattern of diel vertical migration contrasts with that reported for sardine in the Eastern Mediterranean (Giannoulaki *et al.* 1999) and for the round sardine (*S. aurita*) in Senegal. Nieland (1982) found a large proportion of mud and sediments that had a non-negligible energetic value in the stomach of these sardines (*S. aurita* off Senegal).

For pelagic fish, light above a certain threshold is needed for visual predation (Blaxter & Hunter 1982). For such species, upward migration at dusk has been identified as a strategy for increasing feeding opportunities at twilight (Blaxter & Holliday 1963; Cardinale *et al.* 2003). In contrast, filter-feeding clupeids can feed on abundant or small prey even in darkness (Batty *et al.* 1986). The presence of schools close to the bottom may be due to the presence of sufficient food or to processes unrelated to feeding, such as the use of the shelf for orientation during migration (Kim *et al.* 1993).

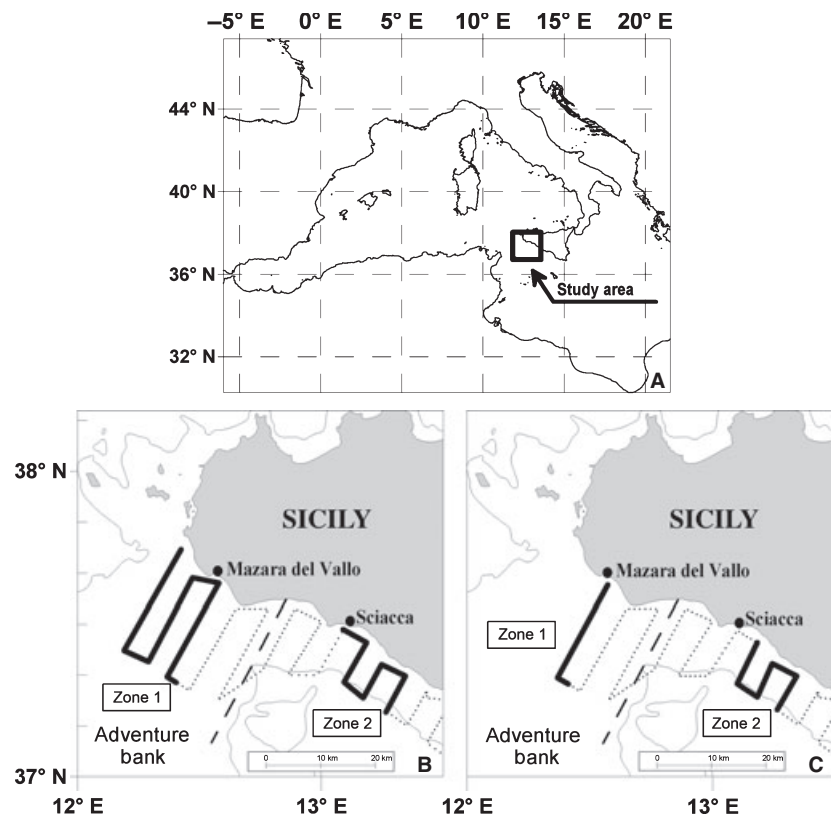
In the present study the geographical distribution and the spatial structure of pelagic fish schools off the southern coast of Sicily (Central Mediterranean) detected by image analysis algorithms applied to hydroacoustic data were examined to investigate their association with the nature of the seabed. This study is of primary interest for stock assessment and management aims because it could allow identification of favourable environmental conditions or micro-habitats at small time scales for the pelagic species in the Sicilian Channel.

## Material and Methods

### Study site description

This study is mainly based on acoustic information gathered in the Sicilian Channel over the two sectors labelled in Fig. 1 as 'ZONE 1' (north-western sector, over the





**Fig. 1.** Location of the study area (A), showing the echosurvey tracks (dotted line) and day-time transects (superimposed thick line) in 1998 (B) and in 2002 (C: only the survey tracks performed in the day time also in 1998, selected for comparative purposes, are displayed). The dashed line separates ZONE 1 from ZONE 2.

Adventure Bank; total length of acoustic transects: 83 nautical miles) and 'ZONE 2' (south-eastern sector, continental shelf off the central part of the Southern Sicilian coast; total length of acoustic transects: 34 nautical miles). These two sectors are characterised by different dominant seabed conditions, harder (and coarser) in ZONE 1 and softer (and finer) in ZONE 2.

### Fish schools

Data on the distribution and the spatial structure of pelagic schools were collected during two hydroacoustic surveys carried out on the continental shelf off the southern coast of Sicily aboard the N/O *Salvatore lo Bianco* in June 1998 and the N/O *G. Dallaporta* in July 2002.

For the aims of the present study, only day-time acoustic transects were used, as during night time fish schools tend to disperse in the water column. Acoustic data were acquired by means of the scientific echo-sounder Simrad EK-500. For each transect, the volume backscattering coefficient ( $S_v$ , dB per cubic metre ref. to  $1 \mu\text{Pa}$  at 1 m) along the water column at 38 kHz was measured, with a vertical resolution of 1 m and a horizontal resolution in the range 1–10 m (depending on the bottom depth).

Fish schools were identified from echograms using an adapted version of the algorithm introduced by Swartzman

*et al.* (1994, 1999). First, raw data were imported in ECHOVIEW v.3 (Sonar Data) post-processing software for the preliminary display and filtering of echograms and converted, using the same software, into ASCII format for the purposes of subsequent analysis. Only pixels of the echograms above the seabed and below the depth of 8.5 m (to remove portions of the echograms affected by air bubbles) were considered. Secondly, image analysis tools (morphological filters) were applied to echograms (Haralick & Shapiro 1992) with the aim of detecting and delimiting objects, in an intensity range for backscattering reflecting the target species, made by contiguous pixels (*i.e.* fish schools) of sufficient backscatter to distinguish them from background noise without smoothing their shape. MATLAB v.6 (The Mathworks) routines were used for this purpose.

Specifically, echograms at 38 kHz were first filtered to select pixels in the backscatter range ( $-58$ ,  $-36$  dB), setting all pixels outside this range to the background level. Then a morphological filter, created by the application of a closing operator followed by an opening operator with a  $2 \times 2$  pixel binary structuring element (*i.e.* two pixels on the horizontal axis and two pixels on the vertical axis), was applied to the image. Subsequently, the binary version of the resulting echogram (having '0' values for the background and '1' for pixels included in the analysis) was multiplied by the original echogram at 38 kHz. The



final echogram was therefore characterised by the backscattering levels of the original echogram for all identified fish schools and the background level ( $-100$  dB) for all the other pixels. Fish schools were then detected by means of a connected component algorithm, and for each detected school, parameters relative to position (*Depth of the school*, *Distance from bottom*), size (*Height*, *Length*, *Elongation*, *Area*), energy (*NASC*, nautical acoustic scattering coefficient) and density (*NASC/Area*) were estimated (Fig. 2). A minimum *Area* ( $4 \text{ m}^2$ ) threshold was adopted to determine whether a potential acoustical patch was considered a fish school.

Specifically, the following energetic, morphological and positioning descriptors were estimated for each detected fish school:

- 1 *NASC*: this value is related to the total biomass of the school,  $\text{m}^2 \cdot \text{nmi}^{-2}$  ( $\text{nmi} = \text{nautical mile}$ ;  $1 \text{ nmi} = 1852 \text{ m}$ );
- 2 *NASC/Area*: this value gives an indication on the average density of the school (Muiño *et al.* 2003); units:  $\text{nmi}^{-2}$ ;
- 3 *Length*: maximum length of the school, m;
- 4 *Height*: maximum height of the school, m (measured as the distance between the uppermost limit and the lowest limit of the school);
- 5 *Elongation*: ratio length and height of the school, m;
- 6 *Area*: surface of the projected area of the school over the vertical plane,  $\text{m}^2$ ;
- 7 *Depth of the school*: school position in the water column with reference to sea surface, m (measured in the centre of gravity of the school);
- 8 *Distance from the bottom*: minimum height of the school, m (measured as the distance between the bottom and the lower limit of the school).

Finally, fish school data were integrated with information on species collected with experimental biological hauls carried out during the echosurveys using a pelagic trawl net.

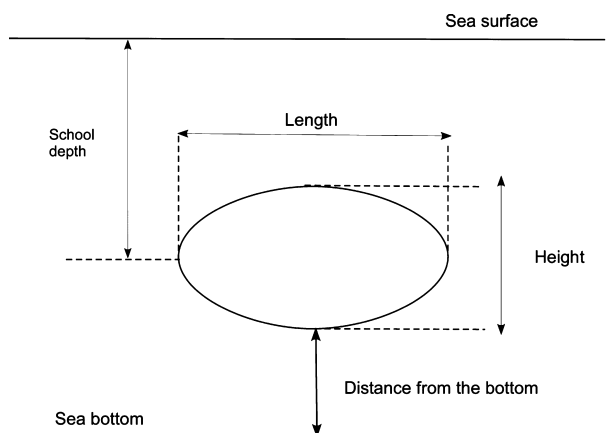


Fig. 2. Example of measured descriptors, giving the position and the geometry of the fish schools.

### Seabed data

Information about the nature of the seabed along the acoustic transects was provided using a SIMRAD EK 500 split-beam echo-sounder, originally developed for fisheries surveys. A direct relationship is expected to exist between the mean diameter of the seabed material and the bottom surface (and volume; see Manik *et al.* 2006b) backscattering coefficient: the larger the grain size, the stronger the bottom backscattering strength.

The reason for this is related to the bulk density of the sediments, which in turn is determined principally by porosity. The higher porosity of silt and clay compared to sand accounts for the lower bulk density of 'soft' seabed substrate types compared to 'hard' substrates, so determining the acoustic impedance of the sediment (Nolle *et al.* 1963; Hamilton & Richard 1982; Richardson 1997); the higher the sediment density, the higher the impedance, and the greater the scattering strength.

For each transect the  $S_V$  volume backscattering values of the seabed line (referred to as  $S_{VB}$ , or bottom  $S_V$ , measured in dB per cubic metre ref. to  $1 \mu\text{Pa}$  at 1 m) were extracted by means of ECHOVIEW v.3 software. Then a moving average to 50 terms (= acoustic pings), corresponding to about 200–250 m, was calculated to reduce variability in the  $S_{VB}$  values. The median value of these averaged  $S_{VB}$  data ( $-33.4$  dB) was used to classify the bottom type (0 for  $S_{VB}$  values < median value, identifying 'soft' seabed areas, and 1 for  $S_{VB}$  > median value, identifying 'hard' seabed areas).

Sediment samples, collected by a grab and box-corer (sample taken at the surface of the seabed) in proximity with the acoustically prospected transects, were also analysed to obtain information on the nature of the bottom type of the two investigated zones in terms of average particle size.

The analysis of the particle size of sediment samples required a phase of sample pre-treatment (digestion with hydrogen peroxide, washing, separation of particles with a diameter lower than  $500 \mu\text{m}$  and drying) and subsequent analysis by a laser diffraction instrument (Fritsch model Analysette 22). Analysis of particle size distribution was conducted using the Wentworth scale, and classification into materials was based on Shepard's method (Shepard 1954).

## Results

### Fish school data

The analysis of data from 1998 showed that fish schools had a preference for ZONE 2 compared to ZONE 1. Fish schools were more frequent in ZONE 2 (Table 1; the occurrence of fish schools in ZONE 2,  $131/294 = 0.45$

**Table 1.** ZONE 1 and 2 features ('N' indicates the number of detected fish schools) and mean values of fish school descriptors by ZONE. Only schools with a bottom depth <200 m are considered. For each descriptor, standard errors are also given within brackets below the average values.

ZONE	zone features		fish school descriptors								
	track length (nmi)	schools:nmi <sup>-1</sup>	bottom depth (m)	distance from bottom (m)	area (m <sup>2</sup> )	length (m)	height (m)	elongation (length/height)	school depth (m)	NASC (m <sup>2</sup> :nmi <sup>-2</sup> )	NASC/area (nmi <sup>-2</sup> )
1 (N = 163)	83	1.96	80.6 (3.2)	13.7 (1.5)	71.3 (9.5)	17.1 (1.1)	3.97 (0.3)	4.80 (0.23)	64.9 (3.2)	3222 (589)	88.2 (20.9)
2 (N = 131)	34	3.85	66.3 (3.1)	23.9 (3.6)	67.7 (9.5)	17.6 (1.3)	3.91 (0.3)	5.07 (0.27)	40.4 (1.8)	5366 (687)	122.2 (15.3)

was significantly higher than the proportion of survey tracks in ZONE 2, 34/117 = 0.29; normal test,  $Z = 5.9$ ,  $P < 0.001$ ) compared to ZONE 1, and despite the shorter transect length (34 *versus* 83 nmi), their biomass represented up to 57.2% of the total. On average, morphological descriptors of the analysed fish schools (with bottom depth <200 m) did not show significant differences between the two zones (ANOVA;  $P > 0.37$ ). Conversely, significant differences were found in positioning (ANOVA;  $P < 0.01$ ) and energetic (NASC, ANOVA,  $F_{1,292} = 5.7$ ;  $P = 0.02$ ) descriptors. Specifically, fish schools detected in ZONE 2 had a greater energy and were found at a greater average distance from the sea bottom than schools in ZONE 1, whereas the greater average school depth in ZONE 1 appears to reflect its higher average bottom depth (Table 1). In fact, all school descriptors, with the exceptions of Area and Height, were significantly (and positively) correlated with bottom depth (ANCOVA;  $P < 0.001$ ).

A similar pattern was found when analysing schools with a centre of mass in the lower half of the water column (schools with ratio between fish school depth and bottom depth >50%). This selection accounted for most of the total fish biomass, including 247 of the total 294 detected fish schools, but removing shallower and less dense aggregations, mostly related to schools in formation

or dispersion, which greatly affected the average distance from the bottom of detected schools in the two zones (see Table 2). Hydroacoustic data collected during the 2002 survey were analysed and compared to the 1998 data only in relation to bottom substrate conditions (see below).

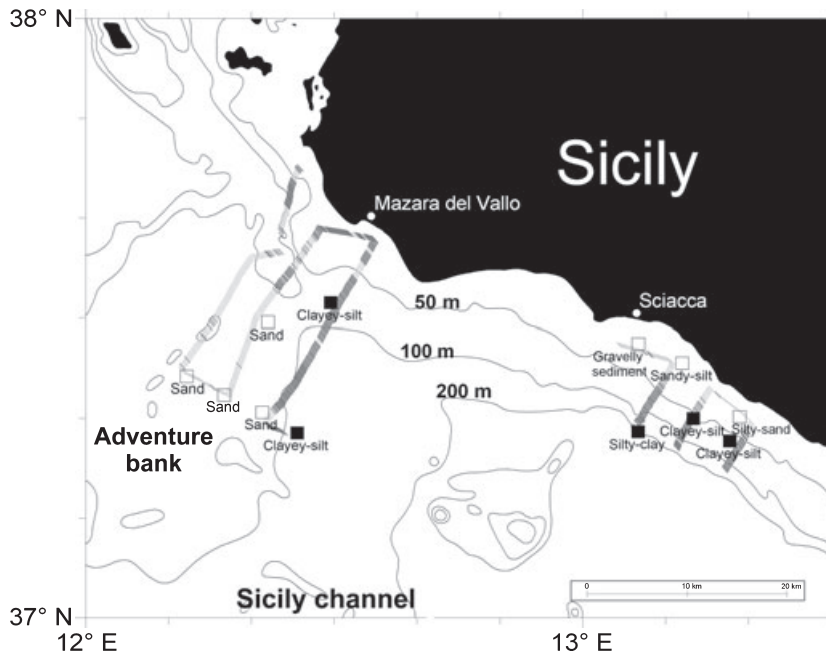
#### Seabed data

The results of the acoustic-based bottom type classification are displayed in Fig. 3, together with information obtained from the analysis of sediment samples collected over the study area. The acoustic classification demonstrates the differences in terms of bottom types between the two zones along the investigated transects. Although the average bottom depth of the prospected transects is higher in ZONE 1, this area (in particular the western sector over the Adventure Bank) is dominated by substrates with greater scattering strength, indicating relatively 'harder' (and coarser) bottom types. In contrast, in ZONE 2 the 'hard' substrate is confined to the shallower inter-transect regions, with the bulk of the seabed deeper than 50 m classified as 'soft' bottom.

The granulometric analysis of sediment samples is consistent with the results of the acoustic classification of the seabed. Although the sediment sample sites were available

**Table 2.** ZONE 1 and 2 features ('N' indicates the number of detected fish schools) and mean values of fish school descriptors by ZONE. Only schools with a bottom depth <200 m and a ratio between fish school depth and bottom depth >50% are considered. For each descriptor, standard errors are also given within brackets below the average values.

ZONE	zone features		fish school descriptors								
	track length (nmi)	schools:nmi <sup>-1</sup>	bottom depth (m)	distance from bottom (m)	area (m <sup>2</sup> )	length (m)	height (m)	elongation (length/height)	school depth (m)	NASC (m <sup>2</sup> :nmi <sup>-2</sup> )	NASC/area (nmi <sup>-2</sup> )
1 (N = 134)	83	1.61	78.3 (3.7)	5.51 (0.6)	74.7 (11)	16.1 (1.2)	4.24 (0.3)	4.2 (0.2)	70.7 (3.7)	3843 (705)	104.7 (25.3)
2 (N = 113)	34	3.32	55 (1.9)	8.32 (0.7)	68.3 (11)	16.5 (1.2)	4.08 (0.4)	4.6 (0.2)	44.7 (1.7)	6165 (770)	139.4 (17.2)



**Fig. 3.** Acoustic classification of the seabed (1998 day time data), based on bottom  $S_{VB}$  values recorded during the day time transects in 1998, binned and averaged every 0.1 nmi. 'Hard' seabeds are displayed in light grey ( $S_{VB} \geq$  median value), and 'Soft' seabeds are displayed in dark grey ( $S_{VB} <$  median value). The sediment sample sites are also shown, with white squares representing samples with a median particle size  $\geq 8 \mu\text{m}$  and black squares those with a median particle size  $< 8 \mu\text{m}$ . Shepard's sediment classification labels are also given under the symbols.

only in proximity to the acoustically prospected transects (within a distance of mostly  $< 1$  nmi), it is worth noting that the use of a threshold value of  $8 \mu\text{m}$  for the median particle size of the analysed sediment samples permitted us to separate finer (and softer) substrate locations (classified as clayey-silt and silt-clay), corresponding at the nearest position along the acoustic transect to  $S_{VB}$  values lower than the global median value, from coarser (and harder) sediment sites (facies: silty-sand, sandy-silt, sand and gravelly sediment) matching with higher  $S_{VB}$  values.

#### Combination of fish school and seabed data

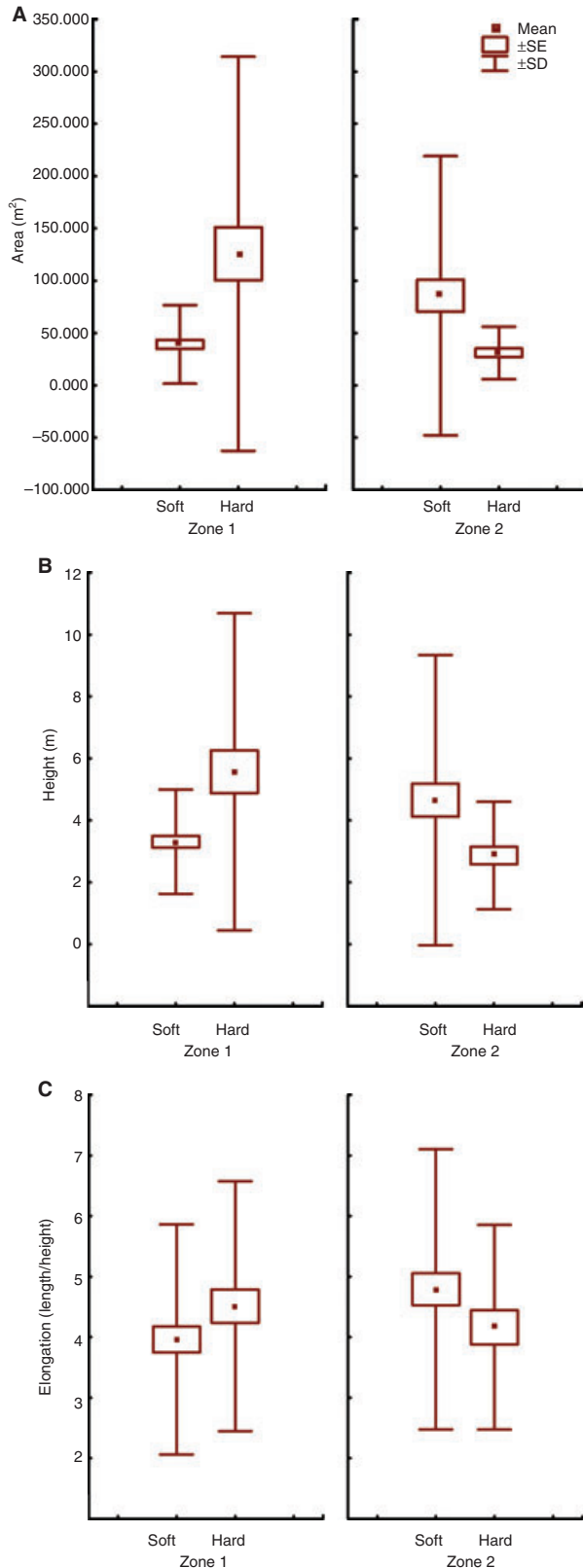
The spatial structure and distribution of fish schools detected over the continental shelf (bottom depth  $< 200$  m) and the centre of mass in the lower half of the water column were linked to the type of seabed.

In terms of morphological school descriptors, in ZONE 1 the average elongation, height and area of fish schools observed during the 1998 survey tended to be positively associated with higher sea bottom  $S_{VB}$  values, which were directly proportional to the mean granulometric dimension of the seabed sediment (*i.e.* harder bottom), but this association reverted in ZONE 2 (Fig. 4). However, no significant zone or substrate type effect was found (ANCOVA;  $P > 0.05$ ). School area, length, height ( $P < 0.05$ ), and depth ( $F_{1,239} = 321.3$ ;  $P < 0.001$ ) were positively correlated with bottom depth. Similar patterns, though less pronounced, were also observed during the 2002 survey.

The occurrence of fish schools over the whole study area during the 1998 survey was significantly higher on

relatively 'softer' grounds ( $167/247 = 0.68$ ; normal test,  $Z = 5.5$ ;  $P < 0.001$ ). The preference of fish schools for seabeds with finer granulometry was also observed when considering the two investigated areas separately, as the proportion of schools detected over acoustically 'softer' grounds in both ZONE 1 and ZONE 2 (respectively,  $89/134 = 0.66$  and  $78/113 = 0.69$ ) was significantly higher (normal tests,  $Z = 4.0$  and  $Z = 3.4$ ;  $P < 0.001$ ) than the fraction detected over acoustically classified 'soft' seabed in the corresponding zones (respectively, 49% and 53%).

Comparison of the distribution patterns of detected fish schools on 'hard' and 'soft' seabed substrates between the two echosurveys considered in the present study, carried out using only data from day-time transects (Fig. 1B), demonstrated a certain stability over time. In fact, even though fish abundance was considerably lower in the 2002 survey compared to 1998, in both years the occurrence of fish schools over 'soft' and 'hard' seabeds of ZONE 1 was not significantly different. Conversely, in ZONE 2, representing just 29% of the total length of the analysed day-time acoustic tracks (about 53 nmi) but accounting for 57% of seabeds classified as 'soft', fish schools were significantly more frequent (76% in 1998 and 88% in 2002) over bottoms with finer granulometry (year 1998: normal test,  $Z = 3.8$ ,  $P < 0.001$ ; year 2002: normal test,  $Z = 4.4$ ;  $P < 0.001$ ) and accounted for the bulk ( $> 60\%$ ) of total estimated fish biomass. The analysis of school descriptors for the 1998 survey did not show any significant ZONE or substrate effect (ANCOVA;  $P > 0.05$ ). Again, bottom depth was found to affect

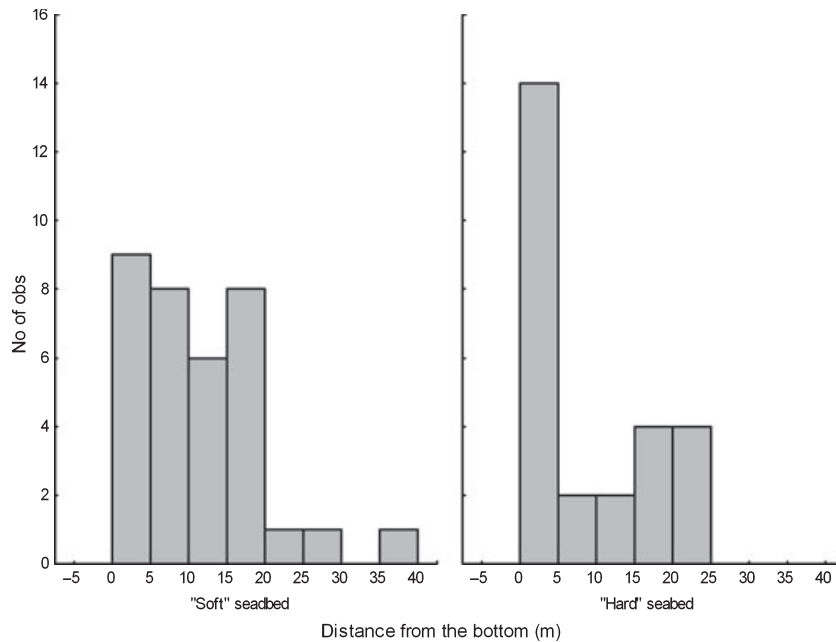


**Fig. 4.** Box plots of school area (A), height (B) and elongation (C), by type of seabed and by zone, 1998 survey. 'Soft' substrate:  $S_{VB} < \text{median value}$ ; 'Hard' substrate:  $S_{VB} \geq \text{median value}$ .

school depth (ANCOVA;  $F_{1,142} = 140.8$ ;  $P < 0.001$ ). The same statistical analysis approach (ANCOVA) was used with 2002 data, evidencing the same positive relationship with school area ( $F_{1,114} = 4.2$ ;  $P = 0.04$ ) and school length ( $F_{1,114} = 6.8$ ;  $P = 0.01$ ), whereas with school density the relationship was the inverse ( $F_{1,114} = 7.5$ ;  $P < 0.01$ ). In addition, there was a significant substrate effect on school length ( $F_{1,114} = 6.5$ ;  $P = 0.01$ ) and elongation ( $F_{1,114} = 4.9$ ;  $P = 0.03$ ), with a tendency for longer schools on 'hard' seabeds, and a significant ZONE\*Substrate interaction effect on school density ( $F_{1,114} = 7.1$ ;  $P < 0.01$ ).

However, the distribution pattern of fish schools between 'soft' and 'hard' bottoms that was observed during the 1998 survey in ZONE 2 was found to be somewhat linked to the different bathymetry characterising the two types of substrates. Actually, in ZONE 2 the 'hard' sea bottom substrate was restricted to bottom depths shallower than 40–50 m, where low numbers of fish schools were observed.

Using  $S_{VB}$  data collected in ZONE 2 only and at depths  $>50$  m, so excluding most of the seabeds previously classified as 'hard', and reclassifying the remaining seabed in relation to the resulting new median of  $S_{VB}$  values ( $-35.71$  dB), no significant preference of fish schools for the resulting new 'softer' seabed was found (only  $34/60 = 57\%$  of fish schools were detected over seabeds with  $S_{VB}$  values lower than the above median threshold). However, even on these deeper seabeds, which were mostly classified as 'soft' when analysed together with ZONE 1  $S_{VB}$  data, it is worth noting that fish schools detected over seabeds that were newly classified as relatively softer tended to be at a greater distance from the bottom, similarly to that previously observed when comparing fish school distribution between ZONE 1 and ZONE 2 during the 1998 survey. In fact, the proportion of fish schools found at a distance from the bottom  $>5$  m was significantly higher on the 'soft' substrate compared to the 'hard' substrate ( $14/26 = 54\%$  versus  $9/34 = 23\%$ , one-sided normal test, Yates' correction,  $Z = 1.89$ ;  $P = 0.03$ ) (Fig. 5). These results suggest selection for a relatively coarser substrate by fish schools having a more demersal behaviour, whereas more 'pelagic' fish schools prefer finer substrates. The same pattern for fish schools was observed in the 2002 survey, not only in ZONE 2 but also in ZONE 1.



**Fig. 5.** Fish school distribution in ZONE 2 by seabed type as a function of the distance from the bottom, 1998 survey. The 'Soft' and 'Hard' seabed type refers to ZONE 2 for which grounds with bottom depths >50 m were acoustically reclassified (see text for details).

## Discussion

In this study, image analysis algorithms were applied to hydroacoustic data with the aim of characterising the geographical distribution and the spatial structure of fish schools detected during two acoustic surveys carried out in 1998 and 2002 in two separate shelf areas (ZONE 1 and ZONE 2) off the Southern Sicilian coast, and verifying the influence of the seabed substrate, as classified by volume backscattering strength measurements and corroborated by granulometric compositional data, on fish distribution.

The results indicate that fish schools prefer seabeds with a finer granulometry, as in ZONE 2. To our knowledge, this is the first time that such a relationship between fish schools and the nature of the seabed has been established in the Mediterranean Sea, and the first as far as concerns anchovy and sardine, as previous studies were on the Atlantic herring (*Clupea harengus*) in the Northern North Sea (Maravelias 1999; Maravelias *et al.* 2000) or even did not explicitly refer to any particular fish species (Genin & Boehlert 1985; Dower *et al.* 1992; Manik *et al.* 2006a). In addition, the only papers considering the sedimentological nature of the sea bottom (Maravelias 1999; Maravelias *et al.* 2000; Manik *et al.* 2006a) obtained different results compared to those of the present study, as the identified preferred habitats were gravel/sand bottoms

Different species composition and/or fish school behaviour between the two areas investigated is postulated to explain the patterns observed during the 1998 survey. In fact, sardines and anchovies were only found in experimental trawls from ZONE 2, whereas in ZONE 1 horse

mackerel was dominant, this being a semi-pelagic fish species whose schools have been reported to occur at small distances from the bottom (Massé *et al.* 1996). However, the patterns observed in the 1998 fish schools in terms of the distance from the bottom were not confirmed by the 2002 data, so inter-survey variability in the distribution pattern of the main pelagic fish species is suggested.

The analysis carried out within ZONE 2 on the relatively 'softer' substrates characterising the seabed at depths >50 m give further insights on the relation between fish school spatial structure and bottom conditions, as there is evidence that the relatively more 'pelagic' (*i.e.* detected at a greater distance from the bottom) fish schools have a preference for softer (*i.e.* finer) seabed substrates. Conversely, fish schools exhibiting a more 'demersal' behaviour (*i.e.* detected at a smaller distance from the bottom) were mostly found on relatively harder (*i.e.* coarser) substrates. So, even relatively small differences in substrate type might provide different optimal day-time habitat conditions for pelagic (or semi-pelagic) fish species.

We consider these results of primary interest not only from the ecological point of view but also to the aims of fisheries management, as the understanding of the effects of environmental factors on fish distribution patterns may represent key information able to improve significantly the quality of scientific advice on exploited fish populations.

Finally, in agreement with previous studies (Misund 1993; Scalabrin & Massé 1993; Ohshima 1996; Fréon & Misund 1999), data analysis showed that the average fish school length was always greater than average height, suggesting an elongated shape.



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## ORIGINAL ARTICLE

# Distribution and population structure of the fish *Helicolenus dactylopterus dactylopterus* (Delaroche, 1809) in the Central Mediterranean (Southern Tyrrhenian Sea)

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## Keywords

*Helicolenus dactylopterus dactylopterus*; recruitment pattern; Southern Tyrrhenian Sea; spatial distribution; trawl bottom.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

The blue-mouth, *Helicolenus dactylopterus dactylopterus* (Delaroche, 1809) (Pisces: Scorpaenidae), is a scorpionfish widespread in the whole Mediterranean basin, where it plays an important ecological role in deep-sea fish communities. The depauperation of this large-size sedentary and slow-growing fish can be an index of overexploitation. This species is found throughout all Italian seas; the juveniles are mainly located around 150–300 m depth, whereas the adult specimens are spread over a wider depth range from 200 m to as deep as 1000 m. The abundance in number and weight, the spatial distribution, the population structure, the growth and the recruitment pattern of *H. dactylopterus* were analysed in this study. The present paper integrates data from 11 bottom trawl surveys carried out in the Southern Tyrrhenian Sea (Central Mediterranean) along Calabrian and Sicilian coasts, within the framework of the MEDITS Project. A total of 1412 specimens of *H. dactylopterus* weighting 20.051 kg were caught. The species appeared in 40% of the 296 hauls analysed, throughout the whole depth range surveyed. The highest values of frequency of occurrence (>67%) were obtained in the slope, whereas in the shelf they fell to around 16%. On the continental shelf the species was found between 106 and 196 m depth, and in the slope between 400 and 645 m. The highest mean density index ( $N \cdot km^{-2}$ ) value was obtained in the 100–200 m depth stratum, whereas the highest mean biomass index ( $kg \cdot km^{-2}$ ) value was recorded in the deeper 200–500 m stratum. The length distribution of the specimens ranged between 2.5 and 24.5 cm. From the Von Bertalanffy growth model the following parameters for the whole population were obtained:  $L_{\infty}$ : 30 cm;  $K$ :  $0.16 \text{ year}^{-1}$ ;  $t_0$ :  $-0.02$ . The sampled population is composed mainly of the young-of-the-year fraction, which can reach up to 98% of the whole catch. Recruits (age 0+) and juveniles (age up to 4 years) are limited to waters shallower than 500 m, whereas adults are present below this depth.

## Problem

The blue-mouth, *Helicolenus dactylopterus dactylopterus* (Delaroche, 1809) (Pisces: Scorpaenidae), is a medium-sized scorpionfish widespread in the whole Mediterranean basin on the coarse and mud-sandy bottoms of the conti-

mental shelf and mostly on the slope as deep as 1000 m (Fischer *et al.* 1987). The blue-mouth is widely distributed in the Eastern Atlantic, from the Norwegian coasts to the south-west coast of Africa (Hureau & Litvinenko 1986). *Helicolenus dactylopterus*, common throughout all Italian seas (Relini *et al.* 1999), represents, among bathyal

teleosts, more than a negligible fraction of the commercial catch in several Italian fisheries (IREPA, 2008). In particular, this species is exploited in deep-sea fisheries targeted at deep-water crustaceans. The study of *H. dactylopterus* population dynamics is important because the depauperation of this large-size sedentary and slow-growing fish can be an index of the overexploitation of fishing grounds. The life history of *H. dactylopterus* is well known in the Mediterranean Sea, both in the western and the central basin: (i) bathymetric distribution and population structure by D'Onghia *et al.* (1992) in the Ionian Sea and by Massuti *et al.* (2001) and Ribas *et al.* (2006) off the Iberian Peninsula; (ii) age and growth in the Ligurian Sea (Peirano & Tunesi 1986; Ragonese 1989), Strait of Sicily (Ragonese & Reale 1992, 1995), South-western Adriatic (Ungaro & Marano 1995), North-eastern Ionian Sea (D'Onghia *et al.* 1996), South-western Adriatic (Romanelli *et al.* 1997) and off the Iberian Peninsula (Massuti *et al.* 2000a,b); (iii) reproductive biology in the North-western Mediterranean by Muñoz *et al.* (1999, 2000, 2002a,b); (iv) diet by Froglià (1976) and Macpherson (1979); (v) exploitation rate by Ragonese & Reale (1992) and Ungaro & Marano (1995).

In the present paper, the abundance in number and weight, the spatial distribution, the population structure, the growth and the recruitment pattern of *H. dactylopterus* are discussed.

## Material and Methods

### Collection methods

*Helicolenus dactylopterus dactylopterus* specimens were collected during 11 bottom trawl surveys, carried out within the framework of international European MEDITS Project, from 1995 to 2005, in the southernmost part of the Tyrrhenian Sea (Central Mediterranean) along the Calabrian and Sicilian coasts (Bertrand *et al.* 2002). The surveyed area extended from Cape Suvero to Cape S. Vito, within the isobath of 800 m, for a total area of 7256 km<sup>2</sup> (Fig. 1).

Sampling procedures were the same in all surveys, according to the MEDITS project protocol (Bertrand *et al.* 2002, Anonymous 2007). Sampling was carried out randomly and the hauls were proportionately distributed in five bathymetric strata: stratum A: 10–50 m (622 km<sup>2</sup>); stratum B: 50–100 m (1003 km<sup>2</sup>); stratum C: 100–200 m (1224 km<sup>2</sup>); stratum D: 200–500 m (1966 km<sup>2</sup>); stratum E: 500–800 m (2441 km<sup>2</sup>). An experimental sampling gear with a cod-end mesh size of 20 mm was used. The haul duration was 30 min in the shelf (10–200 m), and 60 min in the slope (>200 m).

In each haul, the number and weight of each were recorded, and the total length (TL, cm) of the specimens

was measured to the nearest 0.5 cm. The sex was determined by macroscopic examination of the gonads.

### Data analyses

With regard to distribution and abundance of the species, three indicators were estimated:

- 1 frequency of occurrence (FOC), as % of positive (*i.e.* with at least one specimen) hauls;
- 2 abundance, as density index (DI) expressed as N·km<sup>-2</sup>;
- 3 abundance, as biomass index (BI) expressed as kg·km<sup>-2</sup>.

The mean abundance indices and their corresponding coefficient of variation (CV% = SD/mean) were estimated according to the classic swept-area approach (Gunderson 1993), but considering the area effectively trawled in the given stratum (Anonymous 2007).

The Spearman coefficient ( $r_0$ ) was used to test the significance of the correlation between abundance values, both in number and weight, of the whole area, and years.

Density and biomass indices by year were interpolated using GIS software (ARCMAP 9.2 by ESRI): data were mediated and normalized and then spatially elaborated by the deterministic interpolation method (IDW: Inverse Distance Weighting).

Moreover, given the extension of the studied area, the abundance in number and weight was analysed according to three geographic sectors distinguished by the homogeneity of their physiographic characteristics: (i) from Cape S. Vito to Cape Cefalù (Sicily); (ii) from Cape Cefalù to Cape Rasocolmo (Sicily); and (iii) from Cape Rasocolmo (Sicily) to Cape Suvero (Calabria) (Fig. 1).

ANOVA was used to check the null hypotheses that abundance and biomass do not change among years, sectors and depth strata. Before ANOVA, the assumptions of normality of data and homogeneity of variance were tested using one-way Kolmogorov–Smirnov (K–S) and Levene's tests, respectively. As a consequence of the non-normality of the DI dataset, the values were log-transformed [ $\ln(x + 1)$ ] in all analyses. The Student–Newman–Keuls test was applied to verify which of the mean values of biomass and abundance indices were significantly different from the others.

The Spearman non-parametric test was applied to test differences in time of the median length. Length frequency distributions (LFDs) for each year were computed. The differences between years were tested with the Kruskal–Wallis test. LFDs were resolved into Gaussian components with Bhattacharya's method implemented in the software package FISAT II (Gayaniolo *et al.* 2005). The separation of cohorts was used for the description of growth and to obtain numbers of recruits or the juvenile fraction of the population.

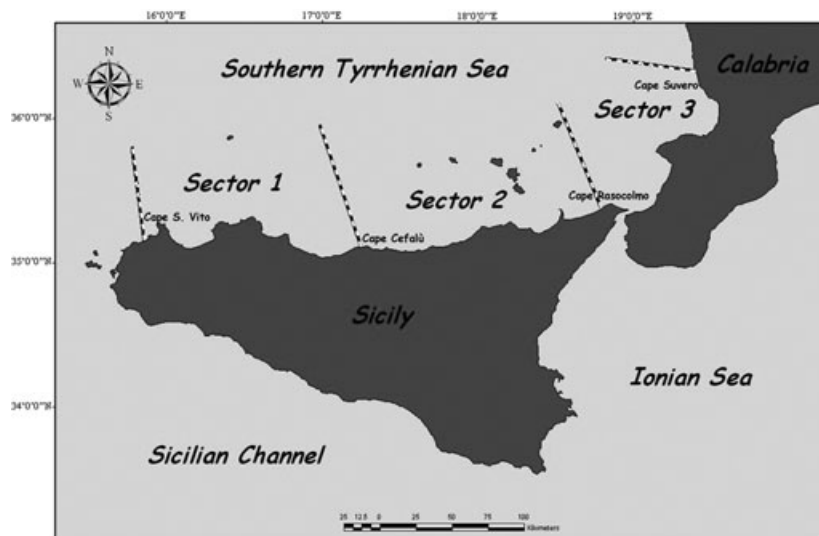


Fig. 1. Studied area with the relative sectors.

The Von Bertalanffy growth model was fitted after having assembled a series of length-at-age data using the length-at-age key provided by Massutì *et al.* (2001). Eight age classes were obtained. The growth performance index  $\Phi'$  was employed to compare growth rate (Munro & Pauly 1983).

The density of young-of-the-year (yoy per km<sup>2</sup>), defined as the first length class completely identified in each LFD, was obtained for each survey and stratum. To separate the yoy (in that case assimilable to recruits) from the older groups, cut-off values calculated for each survey were used (Table 1). These were derived by adding the mean length of the first group, obtained by Bhattacharya's separation, to the standard deviation. The yoy indices, calculated as the mean values of pooled years normalized to the highest value obtained in each survey, were represented graphically as 'bubble maps'.

Cluster analysis based on the resemblance between age composition by year, sector and stratum was applied to analyse differences in population structure. Only samples with more than 15 specimens were considered. The Bray-Curtis similarity index was used (Smith 2003). Analysis of similarity (ANOSIM) was carried out to test the null hypothesis that there were no differences in the age composition at different depth strata, sectors or years (Clarke & Green 1988). The statistical package PRIMER 5 was used to perform this analysis (Clarke & Warwick 2001).

## Results

### Abundance and spatial distribution

A total of 1412 specimens of *Helicolenus dactylopterus dactylopterus* weighing 20.051 kg were caught.

The species appeared in 40% of the 296 hauls analysed, throughout the whole depth range surveyed. The highest

values of frequency of occurrence were obtained in the 100–200 and 200–500 m depth strata (Table 2). In the first two strata (A = 10–50 m and B = 50–100 m) the species was not collected at all. A positive, although not significant (Spearman test), temporal trend of FOC values was observed.

On the continental shelf *H. dactylopterus* was found between 106 and 196 m depth, whereas in the slope the species was caught from 400 to 645 m. The highest mean density index value (Table 2) was obtained in the depth stratum 100–200 m, whereas the highest mean biomass index value (Table 3) was recorded in the deeper stratum (200–500 m). At this depth the mean biomass values showed a clear increase in 2005 compared with the previous years. A direct correlation between abundance indices and years was observed, for both number and weight, only in the 100–200 m stratum ( $r_0 = 0.752$ ;

**Table 1.** Mean total length, number of specimens (Nb), standard deviation (sd) of the component separated by Bhattacharya's method and cut-off values by year of *H. dactylopterus*.

year	mean length (TL, cm) of first group	SD	Nb	cut-off
1995	4.86	0.55	25.22	5.41
1996	4.77	0.58	29.43	5.3
1997*	–	–	–	–
1998	5.50	0.79	12.14	6.29
1999	4.31	0.50	78.71	4.81
2000	4.12	0.59	18.97	4.71
2001*	–	–	–	–
2002	5.58	0.99	35.52	6.57
2003	4.96	0.73	36.24	5.69
2004	3.67	0.56	149.02	4.23
2005	4.50	0.57	143.8	5.07

\*Data not sufficient for analysis.



**Table 2.** Density Index (N/km<sup>2</sup>), standard deviation (sd), coefficient of variation (CV) and percentage of positive hauls of *H. dactylopterus* by stratum and in the overall bathymetric range for each survey.

density (N·km <sup>-2</sup> )	year										
	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
<b>C 100–200 m</b>											
mean	14	0	0	10	327	17	3	127	139	776	455
SD	7	0	0	6	177	7	2	50	30	130	109
CV%	50	0	0	53	54	43	53	40	22	17	24
% positive hauls	17	0	0	17	50	33	17	60	60	100	100
<b>D 200–500 m</b>											
mean	99	127	62	106	97	169	54	51	62	69	431
SD	39	82	26	35	35	57	15	17	25	27	143
CV%	40	65	41	33	36	34	28	34	41	39	33
% positive hauls	75	86	71	86	86	100	88	67	50	67	100
<b>E 500–800 m</b>											
mean	33	7	10	2	2	2	0	11	6	2	2
SD	20	5	4	1	1	1	0	7	4	1	1
CV%	60	72	36	89	71	78	0	64	58	80	75
% positive hauls	50	29	57	14	17	14	0	33	33	17	17
<b>overall 100–800 m</b>											
mean	40	37	20	31	82	49	15	39	43	150	194
SD	13	22	7	10	31	16	4	10	9	23	43
CV%	31	61	35	31	38	32	27	26	20	15	22
% positive hauls	36	29	32	29	37	36	29	39	35	43	52

**Table 3.** Biomass Index (kg/km<sup>2</sup>), standard deviation (sd), coefficient of variation (CV) of *H. dactylopterus* by stratum and in the overall bathymetric range for each survey.

biomass (kg·km <sup>-2</sup> )	year										
	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
<b>C 100–200 m</b>											
mean	0.03	0.00	0.00	0.03	0.38	0.04	0.03	0.50	0.49	0.92	0.66
SD	0.02	0.00	0.00	0.02	0.18	0.02	0.01	0.19	0.11	0.14	0.08
CV%	50.44	0.00	0.00	53.32	47.75	41.45	53.30	38.77	22.24	14.84	11.70
<b>D 200–500 m</b>											
mean	1.69	1.60	2.09	3.40	2.06	2.63	2.01	0.82	1.06	1.01	6.13
SD	0.66	0.51	0.98	0.90	0.70	0.88	0.58	0.35	0.50	0.53	2.62
CV%	39.07	31.50	46.90	26.34	33.92	33.28	28.95	42.54	47.66	52.88	42.79
<b>E 500–800 m</b>											
mean	1.19	0.51	1.05	0.12	0.00	0.08	0.00	1.08	0.39	0.00	0.10
SD	0.57	0.39	0.35	0.11	0.00	0.06	0.00	0.60	0.24	0.00	0.08
CV%	48.25	76.60	33.37	88.57	71.35	77.62	0.00	55.39	60.98	79.58	75.38
<b>overall 100–800 m</b>											
mean	0.86	0.60	0.92	0.97	0.62	0.75	0.55	0.67	0.50	0.43	1.80
SD	0.26	0.19	0.29	0.25	0.19	0.24	0.16	0.22	0.16	0.15	0.71
CV%	30.47	31.25	31.61	25.34	30.76	31.92	28.70	33.56	31.85	33.98	39.39

$\alpha = 0.05$  and  $r_0 = 0.869$ ;  $\alpha = 0.05$ ,  $\alpha = 0.01$ ; for DI and BI indices, respectively).

Significant differences were found between sectors and strata for both density and biomass indices, but no significant variation between years was observed (Table 4). The density index showed highest values in sectors 1 and 3 in the 100–200 m depth stratum, whereas the highest bio-

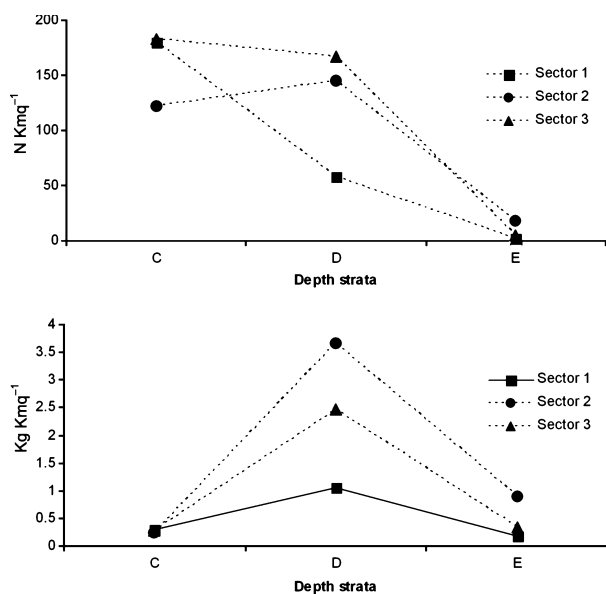
mass index value was obtained in sector 2 in the 200–500 m depth stratum (Fig. 2). The smaller individuals (recruits/juveniles) were observed in sector 3 in the 100–200 and 200–500 m depth strata, as determined by comparing DI and BI values (Fig. 2).

The spatial distribution of *H. dactylopterus* in the studied area was represented on GIS maps (Figs 3 and 4). The

**Table 4.** Summary of the statistic analysis to test the difference in fish biomass and density [ln (x + 1) transformed] between years (from 1995 to 2005), sectors (S1, S2 and S3) and strata (C, 100–200m; D, 200–500m and E, 500–800m). A) Test for homogeneity of variance Levene's test. B) Multifactor analysis of variance (ANOVA). C) Multiple range Student-Newman-Keuls test for density. D) Multiple range Student-Newman-Keuls test for biomass.

(A)										
		density		biomass		F = 0.17 (P = 0.05)		F = 0.81 (P = 0.05)		
		SS		MS		F-ratio				
		N·km <sup>-2</sup>	kg·km <sup>-2</sup>	df	N·km <sup>-2</sup>	kg·km <sup>-2</sup>	N·km <sup>-2</sup>	kg·km <sup>-2</sup>		
(B)										
main effects										
years (Y)		88.09	44.55	10	1.47	0.74	0.20	0.1		
sectors (S)		14.38	43.42	2	7.19	21.71	1.29	4.3*		
depth-strata (D-S)		262.93	179.00	2	131.46	89.50	30.34*	20.6*		
interactions										
Y × D-S		20.76	7.16	12	2.07	0.72	0.45	0.33		
Y × S		18.50	8.59	12	1.85	0.86	1.70	1.09		
S × D-S		17.67	8.48	4	8.84	4.24	21.3*	6.4*		
effect of density for each sector										
		mean density at S1			mean density at S2			mean density at S3		
		C	D	E	C	D	E	C	D	E
(C)	depth strata	2.16	2.36	0.48	2.15	3.70	1.29	1.25	4.54	0.72
	mean	<sup>e-c</sup> 1.69*	<sup>d-e</sup> 1.89*		<sup>e-c</sup> 0.86	<sup>d-e</sup> 2.41*		<sup>e-c</sup> 0.52	<sup>d-e</sup> 3.82*	
	difference	<sup>d-c</sup> 0.20			<sup>d-c</sup> 1.55*			<sup>d-c</sup> 3.29*		
effect of density for each depth stratum										
		mean density at stratum C			mean density at stratum D			mean density at stratum E		
		1	2	3	1	2	3	1	2	3
	sector	2.16	2.15	1.25	2.37	3.70	4.54	0.48	1.29	0.72
	mean	<sup>3-1</sup> 0.92	<sup>3-2</sup> 0.90		<sup>3-1</sup> 2.17*	<sup>3-2</sup> 0.84		<sup>3-1</sup> 0.25	<sup>3-2</sup> 0.56	
	difference	<sup>2-1</sup> 0.02			<sup>2-1</sup> 1.33*			<sup>2-1</sup> 0.81		
effect of biomass for each sector										
		mean biomass at S1			mean biomass at S2			mean biomass at S3		
		C	D	E	C	D	E	C	D	E
(D)	depth strata	0.29	1.05	0.18	0.24	3.66	0.89	0.21	2.46	0.35
	mean	<sup>e-c</sup> 0.11	<sup>d-e</sup> 0.86		<sup>e-c</sup> 0.65	<sup>d-e</sup> 2.77*		<sup>e-c</sup> 0.14	<sup>d-e</sup> 2.11*	
	difference	<sup>d-c</sup> 0.75			<sup>d-c</sup> 3.42*			<sup>d-c</sup> 2.25*		
effect of biomass for each depth stratum										
		mean density at stratum C			mean density at stratum D			mean density at stratum E		
		1	2	3	1	2	3	1	2	3
	sector	0.29	0.24	0.21	1.05	3.66	2.46	0.18	0.89	0.35
	mean	<sup>3-1</sup> 0.08	<sup>3-2</sup> 0.03		<sup>3-1</sup> 1.41*	<sup>3-2</sup> 1.19		<sup>3-1</sup> 0.16	<sup>3-2</sup> 0.54	
	difference	<sup>2-1</sup> 0.06			<sup>2-1</sup> 2.61*			<sup>2-1</sup> 0.70		

\*Denotes a statistically significant difference at the 95% confidence level.



**Fig. 2.** Density and Biomass overall total mean, calculated from all the years mean, of *H. dactylopterus* by sectors and depth-strata.

hot spots of both DI and BI indices were mainly observed in the Gulf of St Eufemia (sector 3, Calabria) and in the Gulf of Patti (sector 2, Sicily).

### Population structure and growth

The size of the specimens ranged from 2.5 to 24.5 cm of TL (Fig. 5). The median length showed a great variability between years (Fig. 6), although no significant temporal trend was observed (Spearman test).

Only specimens bigger than 9.5 cm TL could be sexed, although indeterminate individuals of up to 16.5 cm TL were still present. In particular, the length classes ranged from 10 to 24.5 cm TL for males and from 11 to 21.5 cm TL for females. Males predominated in length-classes between 13 and 24 cm TL ( $\chi^2 = 23.91$ ;  $P < 0.05$ ) (Fig. 7).

The length-frequency distributions of *H. dactylopterus* did not show significant differences among years (Kruskal–Wallis test). A first group characterized by small individuals (<7 cm) is well defined in almost all surveys (Fig. 5).

The dendrogram of similarities obtained from age composition allowed the identification of two main groups (Fig. 8). The first cluster includes all the hauls of the 100–200 m depth stratum and two samples of the 200–500 m stratum of the surveys carried out in 2004 and 2005. These samples were composed of fish 0–4 years of age, although more than 97% of specimens correspond to age class 0 and about 2% to age class 1. The second cluster includes samples of the 200–500 m depth stratum and one haul of the 500–800 m depth stratum in 1995. These samples were composed of fish 0–7 years of age; almost 50% of speci-

mens correspond to age class 0, about 20% to age class 1, 12% to age class 2 and about 8% to age class 3 and 4.

The age structure of samples was significantly different between depth strata (ANOSIM, global test,  $R = 0.558$ , significance level 0.01). There were no significant differences between samples by year (ANOSIM, global test,  $R = 0.152$ , significance level >0.1) or sector (ANOSIM, global test,  $R = 0.177$ , significance level >0.1).

The growth parameters, deriving from the Von Bertalanffy model, for the whole population were:  $L_{\infty}$ : 30 cm;  $K$ : 0.16 year<sup>-1</sup>;  $t_0$ : -0.02 (Table 5).

### Recruitment pattern

Recruits were collected in all surveys and showed a wide bathymetric distribution (100–610 m), although higher values are often recorded (Table 6) in the 100–200 m depth stratum. In particular, in this stratum very high values were observed in 2004 (552 N·km<sup>-2</sup>) and in 2005 (444 N·km<sup>-2</sup>). A significant positive direct correlation (Spearman's test) between youngest-of-the-year mean values and years was observed only for the 100–200 m depth stratum ( $r_0 = 0.731$ ;  $\alpha = 0.05$ ).

The distribution pattern of the recruits did not show significant variation over the 11 years and between sectors (Table 7). On the other hand, the number of recruits showed significant differences between the bathymetric strata analysed ( $F = 7.49$   $P = 0.05$ ) and between depth strata and sectors ( $F = 7.04$ ,  $P = 0.05$ ). In particular, in sector 1 the recruits occur at shallower depths (100–200 m) than in sector 3 (200–500 m) (Table 7).

Although a wide spatial distribution of the recruits was observed in the surveyed area, three zones characterized by the highest concentration of recruits (normalized number of recruits per km<sup>2</sup>) were observed (Fig. 9).

### Discussion

In the studied area there are two areas that have been banned to trawling since 1990: the Gulf of Castellammare and the Gulf of Patti (Sicily). Therefore only 65% (4716.4 km<sup>2</sup>) of the total studied area can be effectively trawled by commercial vessels.

The bottom of this area is characterized by a narrow continental shelf, sometimes entirely missing, and by a steep slope, with consequent spatial contiguity of the bathymetrics (Greco *et al.* 1998). The results reported in this paper show that the spatial distribution pattern of *Helicolenus dactylopterus dactylopterus* in this area is very similar over the time. No latitudinal gradient was observed. The density and biomass values showed differences between strata and sectors of the studied area, but not between years.

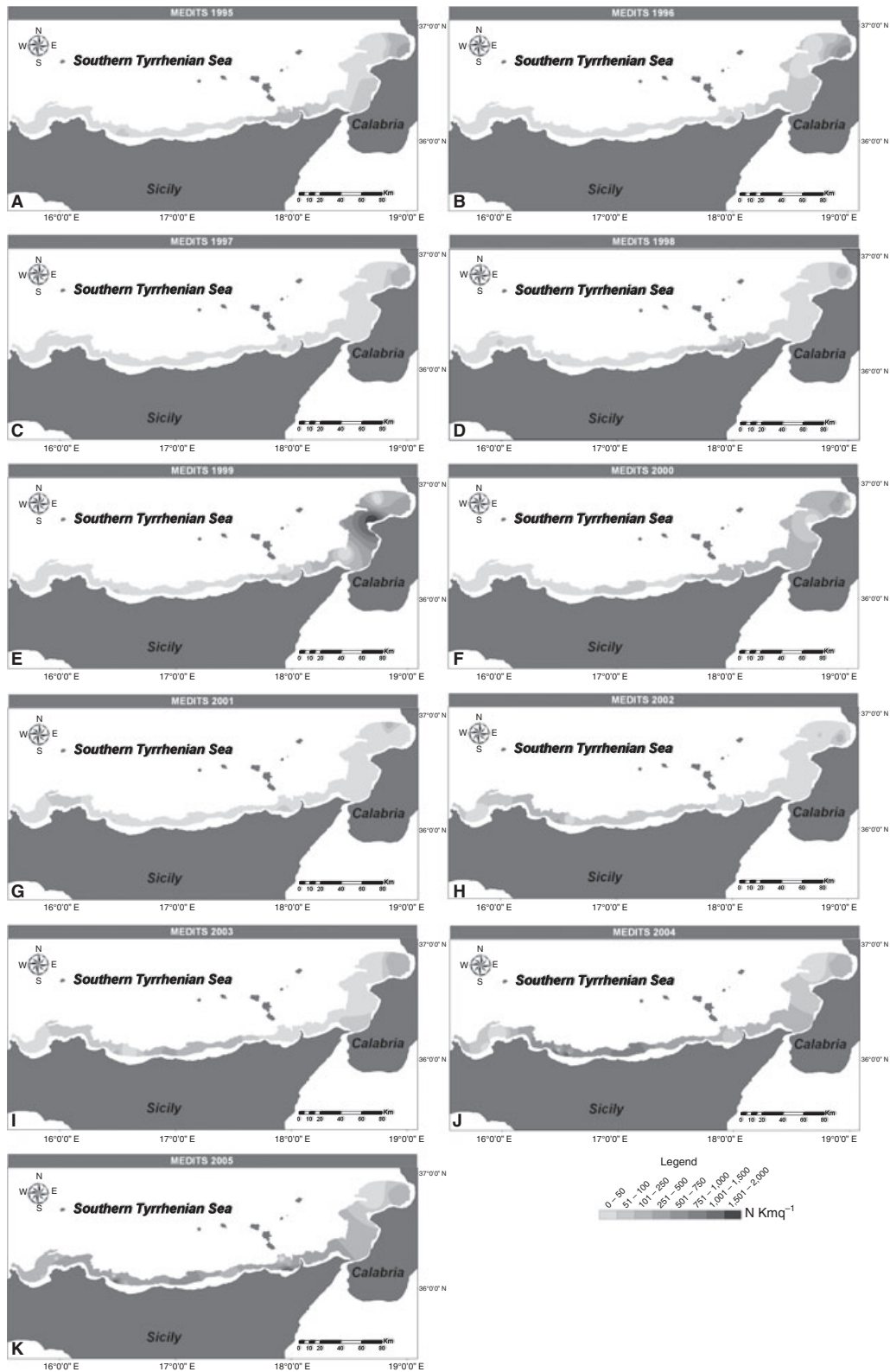


Fig. 3. Spatial distribution of *H. dactylopterus* in terms of density ( $N/km^2$ ), from 1995 to 2005 (a-k).

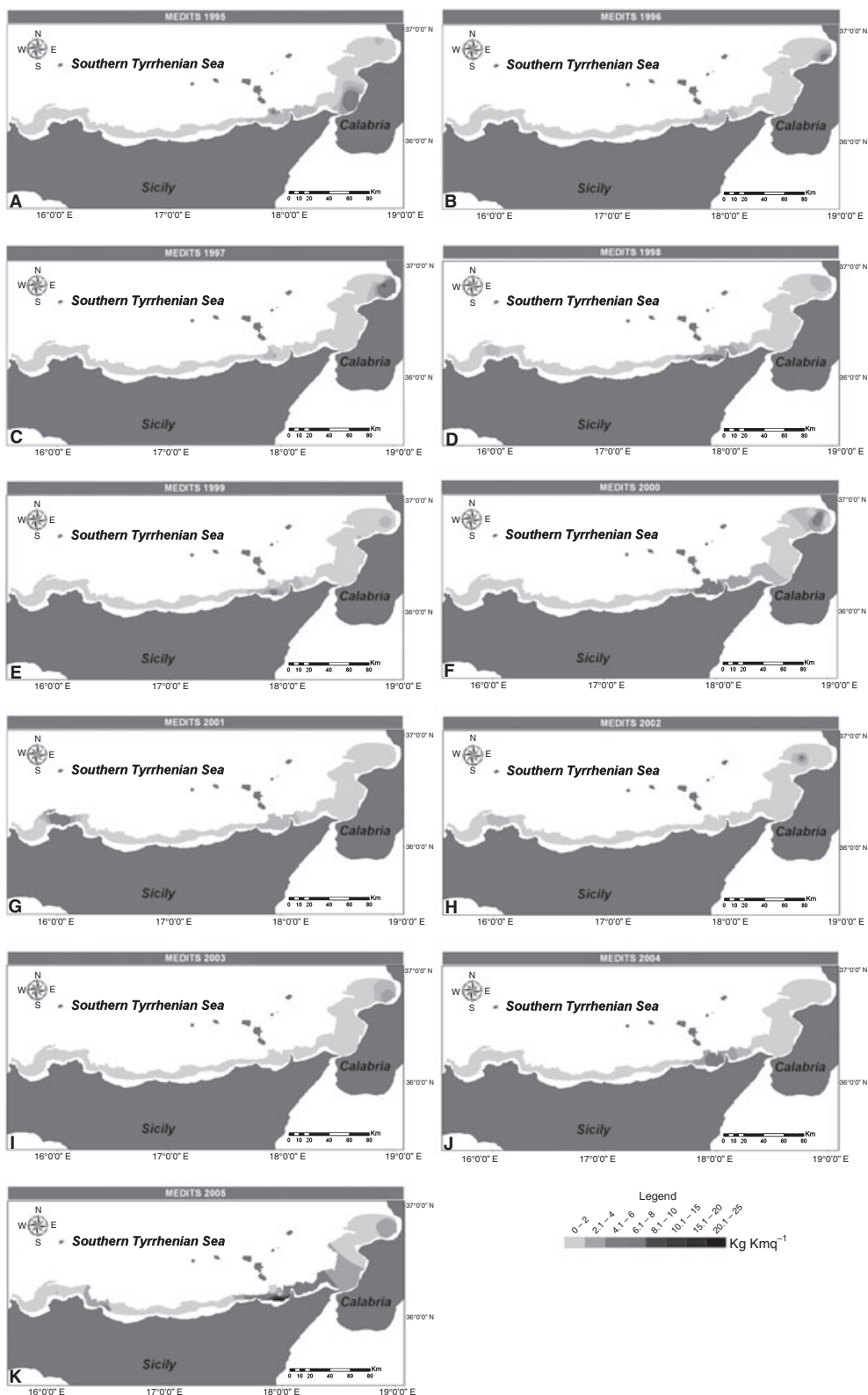


Fig. 4. Spatial distribution of *H. dactylopterus* in terms of biomass ( $\text{kg}/\text{km}^2$ ), from 1995 to 2005 (a-k).



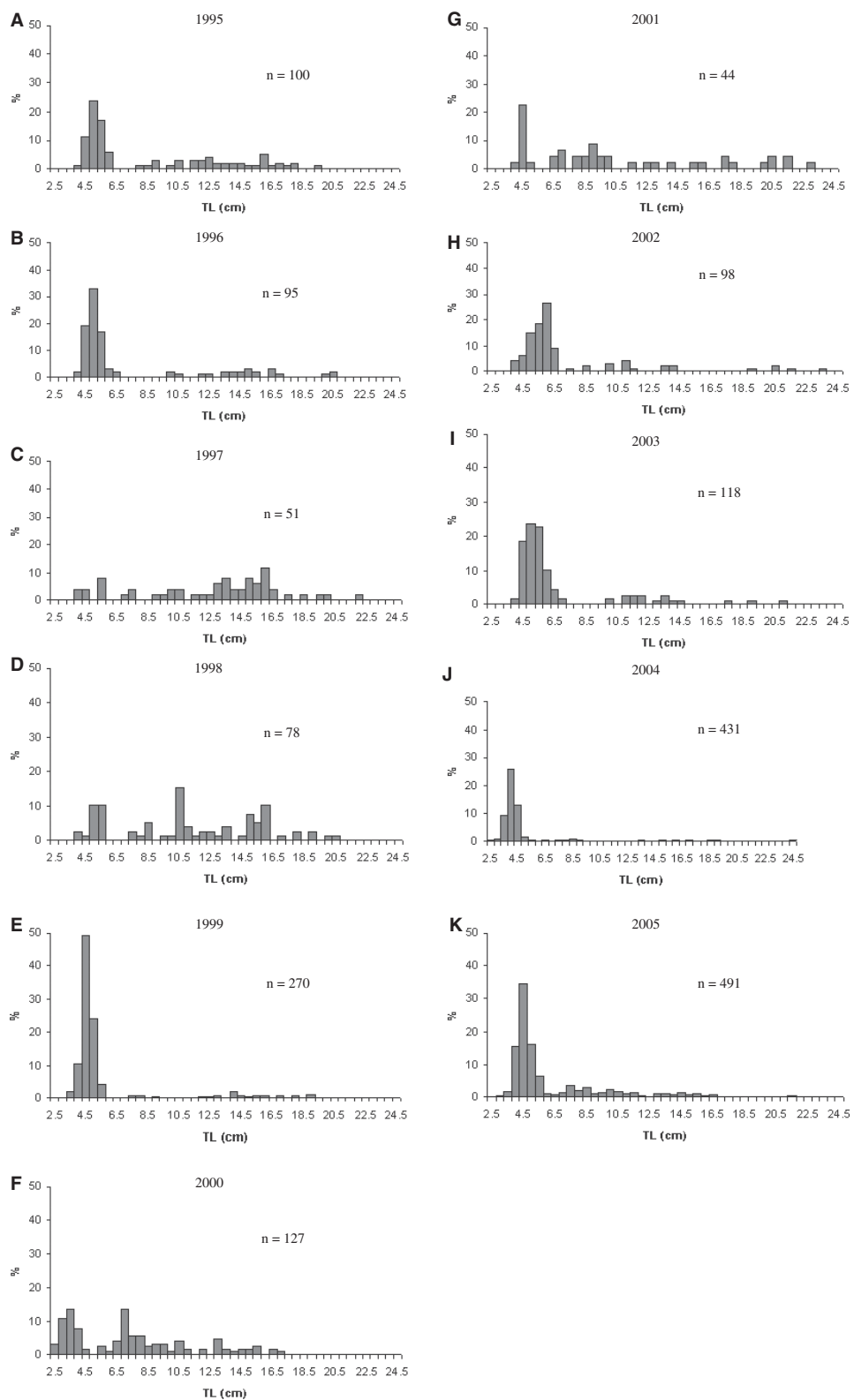


Fig. 5. Length frequency distributions of *H. dactylopterus* by survey.



**Table 6.** Recruits (N/km<sup>2</sup>), standard deviation (sd) and coefficient of variation (CV) of *H. dactylopterus* by stratum and by overall bathymetric range of each survey.

recruits (N·km <sup>-2</sup> )	year										
	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
<b>C 100–200 m</b>											
mean	10	0	0	10	213	10	3	127	121	552	444
SD	5	0	0	5	108	3	2	47	17	73	110
CV%	45	0	0	49	51	32	49	37	14	13	25
<b>D 200–500 m</b>											
mean	38	72	27	15	48	42	35	29	23	28	175
SD	23	53	6	10	23	18	10	9	10	11	66
CV%	60	74	21	65	49	44	29	32	43	38	38
<b>E 500–800 m</b>											
mean	8	0	0	0	2	0	0	0	0	2	0
SD	4	0	0	0	1	0	0	0	0	1	0
CV%	54	0	0	0	65	0	0	0	0	73	0
<b>overall 100–800 m</b>											
mean	15	20	7	6	49	13	10	29	27	101	122
SD	6	14	2	3	19	5	3	8	4	13	26
CV%	43	74	21	47	39	38	27	28	15	13	21

quently been observed for many demersal fish species (Macpherson & Duarte 1991) and for *H. dactylopterus* in the Mediterranean Sea (Ribas *et al.* 2006; Massuti *et al.* 2001; Ragonese & Reale 1995; D'Onghia *et al.* 1992; Peirano & Tunesi 1986). The different population structure observed along the bathymetric gradient persists over the years analysed.

The length structure of the whole population as well as the distribution pattern of recruits were quite homogeneous in the studied area. The recruits seem to be concentrated in the proximity of promontories and in particular in their eastern part. This phenomenon could be correlated to the western–eastern water flows in the Southern Tyrrhenian Sea and to the steep slope of the sea bottom (Della Croce 2000). In particular, the northern coasts of Sicily (sector 1 and 2) are influenced over the continental shelf by Modified Atlantic Water (MAW), characterized by temperatures of 14–15 °C, and in the slope by the Levantine Intermediate Water (LIW), marked by lower temperatures and higher salinity (Millot 1999). The Calabrian coasts (sector 3), where the recruits have a wider bathymetrical distribution, are probably influenced by local down-welling events, caused by wind forcing (Azzaro *et al.* 2006), which causes a major availability of trophic resources in deeper strata.

The growth parameters obtained in this study showed some differences compared with those reported from other Mediterranean areas; however, the  $\Phi'$  values obtained are similar (Peirano & Tunesi 1986; Ragonese & Reale 1995; D'Onghia *et al.* 1996; Ungaro & Marano 1995 and Massuti *et al.* 2000a). This could be due to differences in the range of sizes sampled, the methodology

applied and the different characteristics of the study areas. In fact, the estimation of growth parameters is strongly affected by sampling gear as well as by bias of age estimation (Massuti *et al.* 2000b).

*Helicolenus dactylopterus dactylopterus* is a long-lived species that can attain more than 30 years of age. Data related to the life span of this rockfish are influenced by estimation methods such as sliced or whole otolith readings (Abecais *et al.* 2006) and length-frequency distribution analysis. The differences of maximum age estimates are very important for management because the exploitation of the species is correlated to its life history (Jennings *et al.* 1998; Russ & Alcalá 1998; Denney *et al.* 2002). Deep-water species like the blue-mouth rockfish are particularly vulnerable to overfishing because of their biological characteristics (long life, large size, late maturity, slow growth and low mortality rate) and are strongly exploited by trawling fishing (e.g. red shrimps fishery).

The length-frequency distribution analysed in this research suggests that the population is made up almost exclusively of recruits/juveniles individuals (0–4 years). The larger animals (up to 20 cm TL) can reach up to 8 years of age.

Finally, the results obtained by this study confirm, in agreement with the available Mediterranean and Atlantic literature, that the population sampled by trawl nets is mainly composed of recruits and juveniles. Probably, the older population prefers rocky bottoms not accessible to trawl fishery. Therefore, to correctly evaluate the true exploitation of the species, data coming from trawl surveys should be integrated with those coming from studies using different sampling gear.

**Table 7.** Summary of the statistic analysis to test the difference in recruits density between years (from 1995 to 2005), sectors (S1, S2 and S3) and strata (C, 100–200 m; D, 200–500 m and E, 500–800 m). A) Test for homogeneity of variance Levene’s test. B) Multifactor analysis of variance (ANOVA). C) Multiple range Student-Newman-Keuls test for sectors and depth-strata.

(A)

		density		F = 0.91 (P = 0.05)	
	SS	df	MS	F-ratio	
	Y·km <sup>-2</sup>		Y·km <sup>-2</sup>		Y·km <sup>-2</sup>

(B)

effect of density for each sector								
main effects		mean density at S1		mean density at S2		mean density at S3		
	SS	df	MS	F-ratio				
	Y·km <sup>-2</sup>		Y·km <sup>-2</sup>		Y·km <sup>-2</sup>		Y·km <sup>-2</sup>	Y·km <sup>-2</sup>
years (Y)	2790.5	10	46.5	0.4				
sectors (S)	54.57	2	27.3	0.3				
depth strata (D-S)	1363.8	2	681.9	7.49*				
interactions								
Y × D-S	543.2	12	54.3	1.3				
Y × S	414.4	12	41.4	2.1				
S × D-S	56.8	4	28.4	7.04*				

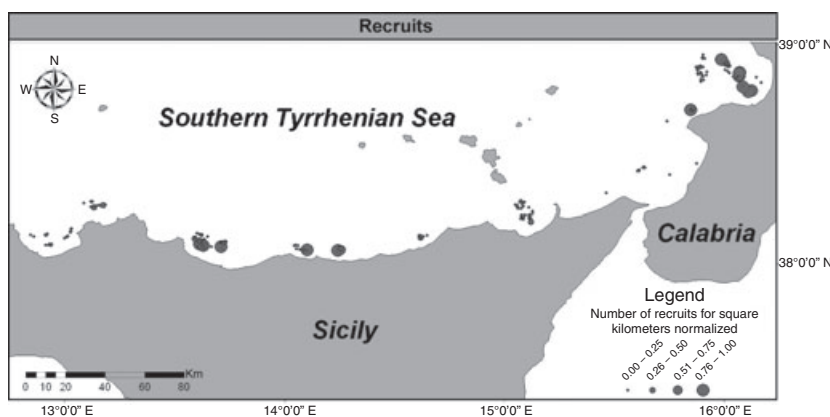
(C)

effect of density for each depth-strata									
mean density at stratum C			mean density at stratum D			mean density at stratum E			
	1	2	3	1	2	3	1	2	3
depth strata	7.41	3	0	5.14	1.91	0.31	5.82	8.04	0.06
mean	<sup>e-c</sup> 7.41*	<sup>d-e</sup> 3	0	<sup>e-c</sup> 4.82	<sup>d-e</sup> 1.60		<sup>e-c</sup> 5.76	<sup>d-e</sup> 7.98*	
difference	<sup>d-c</sup> 4.41			<sup>d-c</sup> 3.23			<sup>d-c</sup> 2.22		

(D)

effect of density for each sector									
mean density at stratum C			mean density at stratum D			mean density at stratum E			
	1	2	3	1	2	3	1	2	3
sector	7.41	5.14	5.82	3	1.91	8.04	0	0.31	0.06
mean	<sup>3-1</sup> 1.60	<sup>3-2</sup> 0.68		<sup>3-1</sup> 5.04	<sup>3-2</sup> 6.13		<sup>3-1</sup> 0.06	<sup>3-2</sup> 0.25	
difference	<sup>2-1</sup> 2.28			<sup>2-1</sup> 1.09			<sup>2-1</sup> 0.31		

\*Denotes a statistically significant difference at the 95% confidence level.



**Fig. 9.** Bubbles maps of normalized mean recruits index (R/km<sup>2</sup>) in the studied area

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## ORIGINAL ARTICLE

# Reproductive biology of *Symphodus mediterraneus* (Teleostei, Labridae) in the Azores

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## Keywords

Azores; first sexual maturity; Labridae; spawning period; *Symphodus mediterraneus*.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

A description of the colour patterns, reproductive behaviour, spawning season and size at first maturity is given for an Atlantic population of *Symphodus mediterraneus*, based on underwater observations and animals collected by spear-fishing and photographed. Gonad histology was used to determine the sex and maturity stages. Size at first maturation was estimated as the length at which 50% of the individuals are mature. General appearance was a brown or brownish-red body, with a series of large brown transverse stripes, a conspicuous yellow-ringed black spot on the base of the pectoral fins and a black spot on the caudal peduncle. Terminal males could be distinguished from females by the blue tones on the extremities of the median fins and on the throat. Differences from the colour pattern of the Mediterranean population are noted. This is probably a gonochoric species: the larger males are territorial, building algal nests and defending them from conspecific males and from other micro-benthic carnivorous fishes; the smaller mature males did not show sneaking or satellite behaviour. Reproduction takes place in late spring and summer, after the spring productivity peak, suggesting that the feeding biology of the adults may be the main determinant factor of the timing of spawning. Total length at first maturity is 12.1 cm for males and 9.6 cm for females.

## Introduction

*Symphodus mediterraneus* is a shallow water wrasse, one of the nine species of Labridae represented in the Azorean coastal waters (Porteiro *et al.* 1996). Originally described from the Mediterranean, *S. mediterraneus* is known to occur also in the Northeastern Atlantic, from the Bay of Biscay to Northern Morocco, and also in Madeira and the Canaries (Quignard & Pras 1986; Westneat 2008). Some aspects of its biology in the Mediterranean were first studied by Quignard (1966), who published data on diet, age and growth, and reproduction, the latter including first sexual maturation and spawning period. The behaviour of this species, particularly in connection with its reproductive cycle, was subsequently described by Helas *et al.* (1982) and Lejeune (1985), again based on Mediterranean specimens. These authors describe the col-

our patterns associated with the different life cycle stages, based on field observations.

There is no published information on the biology of the species in the Atlantic. Moreover, there are contradictions in the available literature. Quignard (1966) stated that about 3% of the animals with a female colour pattern are in fact males, based on the presence of well developed testicles. He went on to suggest that all *Symphodus* were protogynous hermaphrodites, although only a small proportion of the females actually changed sex. Lejeune (1985), however, found no evidence of sex change in a group of four species from this genus, with the possible exception of *Symphodus tinca*. This was further supported by the work of Warner & Lejeune (1985), who looked at the effect of parental care on sex change and concluded that the two factors are negatively related. In species like *Symphodus melanocercus*, the terminal males defend territories and monopolize

access to the females. Smaller males, unable to compete, have a much lower reproductive success than females of the same size. Thus natural selection should favour the individuals that are born females and change for males above a certain size, maximizing their lifetime reproductive output (Warner *et al.* 1975). The opposite situation was found in species such as *Symphodus ocellatus* or *Symphodus roissali*: the larger males build complex nests and display elaborate parental care, ventilating and defending the nest. The smaller males parasitize the spawnings on the nests of the territorial males, leading to a reproductive success similar to that of the females. In these cases there is no sex change.

The present paper presents data from a study of the reproductive biology of an Atlantic population of *S. mediterraneus*, aimed at describing the colour patterns of the different life-cycle phases, determining the reproductive season and size at first maturation, looking for evidence of sex, and giving a description of the reproductive behaviour.

## Material and Methods

A total of 169 specimens were obtained by spear-fishing in São Miguel Island from May 2001 to April 2002. Collected fish were placed in buckets with seawater and transported to the laboratory where they were measured (total length, Lt, to the nearest mm), weighed (total weight, Wt, with 0.01 g of precision). To analyse the relationship between colour patterns, sex and gonad development stage, each animal was photographed using a technique modified from Emery & Winterbottom (1980) and Svoboda (1992). The fish were then dissected and the gonads removed, weighed (Wg, with 0.01 g of precision) and processed histologically: fixed in 10% buffered formalin for 24 or 48 h (Hopwood 1996), dehydrated and embedded in paraffin (Anderson & Gordon 1996). Longitudinal sections 7 µm thick were stained with haematoxylin and eosin (Stevens & Wilson 1996).

The gonadosomatic index (GSI) was calculated to complement the determination of the reproductive season using the formula given by Sparre & Venema (1992):

$$GSI = \frac{Wg}{(Wt - Wg)} * 100.$$

Thesize at first maturity was estimated from L50, the length at which 50% of the individuals are mature, calculated from a logistic function that relates the proportion of mature individuals with the fish length (Ghorbel *et al.* 2002),

$$P = \frac{1}{1 + e^{-(b+aLt)}}$$

where P = proportion of mature individuals; a and b = constants. The proportion of mature individuals in

each length class of 1 cm was calculated based on the microscopic examination of the histological preparations. The adjustment between the observed and predicted maturity proportions was assessed using the chi-squared test.

Reproductive behaviour was studied with underwater observations conducted while SCUBA-diving. Observations were recorded on polyester paper attached to an acrylic clipboard, and by underwater photography. Approximately 40 h of observations were made.

## Results

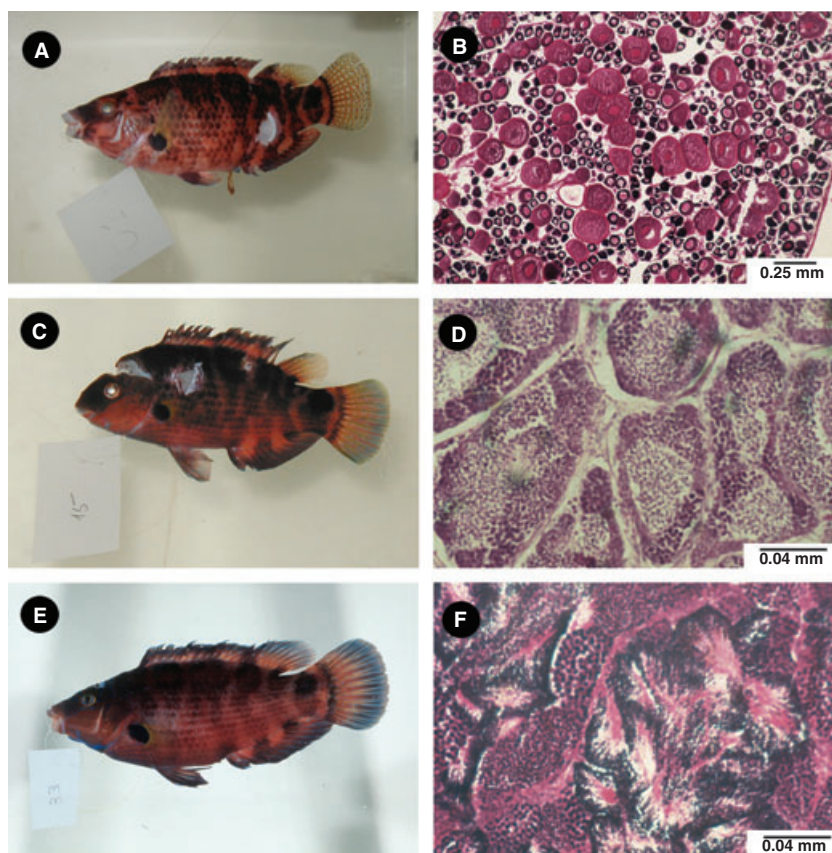
### Colour patterns

The colour patterns are described based on histologically confirmed ripe specimens (Fig. 1). General appearance was a brown or brownish-red body with yellow-brownish eyes and white lips. All specimens presented a conspicuous yellow-ringed black spot on the base of the pectoral fins and a black spot on the caudal peduncle, above the lateral line. A series of large brown transverse stripes was also present, darker in the dorsal region and continuing and interconnecting down the body. There were five under the dorsal fin, one on the caudal peduncle, sometimes almost masking the dark spot.

Terminal males could be distinguished from females by the blue tones on the extremities of the median fins and the throat, and by reduced size of the urogenital papilla. Initial phase males have less marked blue tones on the median fins and the throat is the same colour as the body. No evidence for sex change was seen in any of the specimens observed.

### Reproductive behaviour

Only the terminal phase males were observed building algal nests. Nests were built on crevices or on small gullies between adjacent boulders. The algae were either ripped off the substrate or collected from the water column with the mouth. In the beginning of the nest construction the male collected the algae usually not farther than 6–7 m away. By the end of the construction the algae were collected up to 20 m away. The algae were pushed amongst the nest material with the mouth, aided by lateral movements of the body using short and quick caudal fin movements. Shell fragments, sand and small stones were visible over the nest. The nests were composed of several species of algae, mainly *Stypocaulon scoparia*, *Dictyota* spp., *Halopteris filicina*, *Pterocladia capillacea* and *Asparagopsis armata*. They were globular in shape (width 20–36 cm, length 20–56 cm) and had a reddish general coloration. The nesting male was always seen at or near the nest. When the nest was partially



**Fig. 1.** Colour patterns and life cycle stages in *Symphodus mediterraneus*: mature female (A), initial phase male (C) and terminal phase male (E). Corresponding gonad histology in (B, D, and F).

constructed the males started to show aggressive behaviour, chasing and biting other fish that approached the nest. This behaviour was directed towards conspecifics and other species, such as *Coris julis*, *Symphodus caeruleus*, *Thalassoma pavo*, *Labrus bergylta* and *Chromis limbata*.

Males would leave the nest to meet passing females, with dorsal and anal fins distended, and try to direct them towards the nest. Courtship consisted of the two fishes circling in and out of the nest. Eventually, the female entered the nest, pressing her belly against it, closely followed by the male. Possibly, this was when spawning and fertilization took place. After this the female would swim away. The whole process took less than 2 min. On some occasions, initial phase males hovered around the nest. These were never seen helping with nest construction or defence.

#### Reproductive season

*Symphodus mediterraneus* reproduces in late spring and summer, as recorded in underwater observations of nest building, courtship and spawning behaviours from April to August. The highest values of GSI were recorded in May and June (Fig. 2), a period when the seawater temperature was between 17 and 18 °C and then starts to

decrease from the spring peak (Fig. 3). Histologically mature gonads were observed from May to August. The first juveniles were observed in September.

#### Size at first maturity

Figure 4 shows the evolution of sexual maturation with size. The adjustment of the logistic function to the data is highly significant ( $\chi^2$ ,  $P > 0.99$ ). The size at first maturity is estimated to be 9.6 cm for females and 12.1 cm for males. Terminal phase males were at least 15 cm in total length (Fig. 5). A class of mature initial phase males was therefore present.

#### Discussion

The fact that no histological evidence of sex change was found in the present study supports the predictions of the Warner & Lejeune (1985) model, given the high intensity of parental care in this *Symphodus* species, where terminal males build and maintain elaborate nests. We conclude that *Symphodus mediterraneus* is a gonochoric species. The initial phase males were never seen sneaking on the spawning of the territorial males, as recorded by Lejeune (1985) in the Mediterranean, where the frequency of this

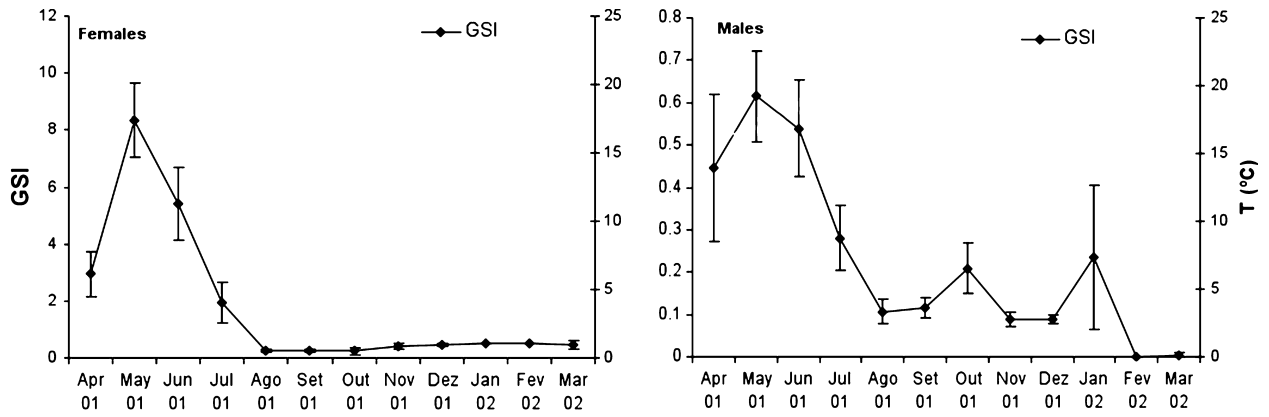


Fig. 2. Mean monthly values of GSI ( $\pm$ SE). Note different GSI scale on graphs.

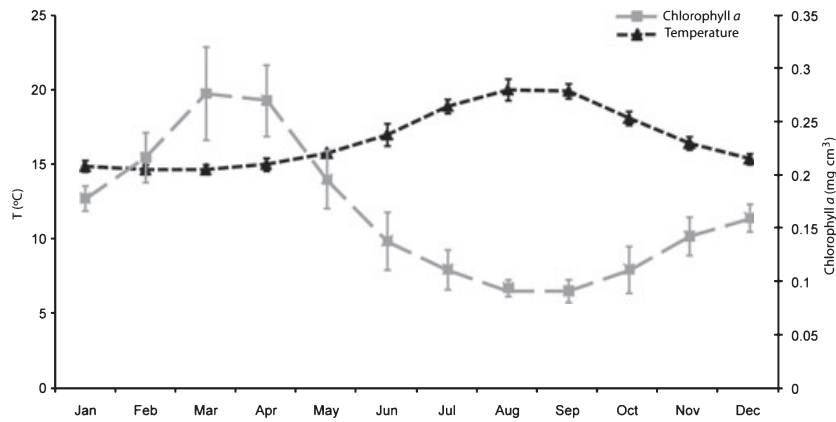


Fig. 3. Mean monthly values of seawater surface temperature and chlorophyll a concentration ( $\pm$ SD). Data from the DETRA Project (<http://ocean.horta.uac.pt/detra>), 2002–2007.

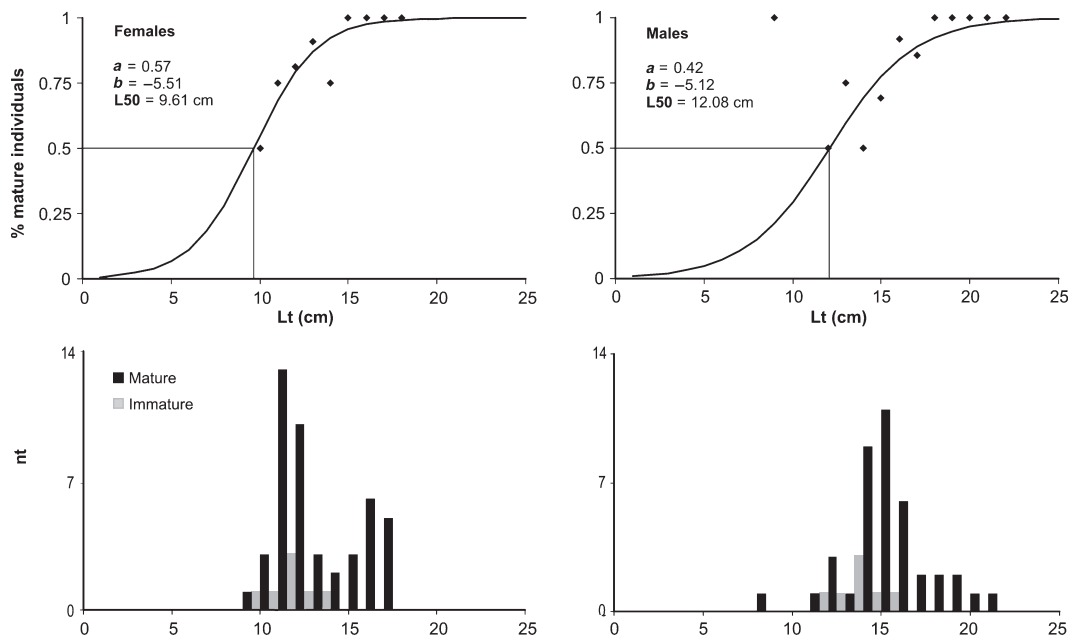
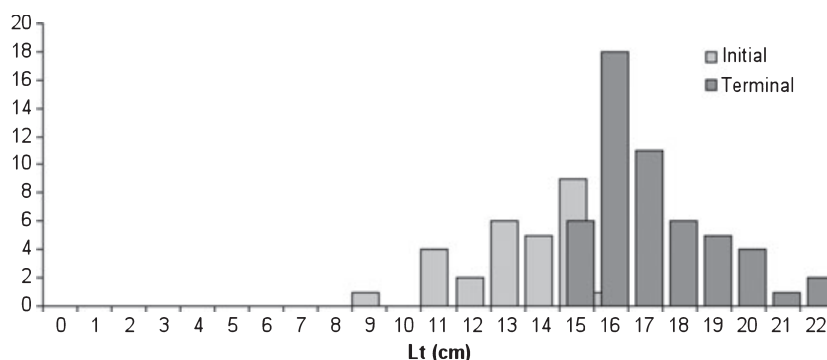


Fig. 4. Proportion of mature individuals per length class and adjusted logistic function (A) and size frequency by sexual maturity (B).





**Fig. 5.** Length frequency distribution of initial and terminal phase males.

kind of behaviour may be high in certain periods. This difference may be linked to differences in density at each location, as it can be expected that at low densities the large dominant males can control most of the spawning (Warner *et al.* 1975).

Differences were found between the fresh colouration of the Mediterranean and Atlantic specimens. The latter have a strong reddish background, with a series of dark brown vertical bars, whereas the Mediterranean specimens have a lighter coloration, with marked dorso-ventral contrast. The colour differences between the territorial males of both regions are also clear, with the Azorean specimens lacking most of the distinct lines of blue dots clearly seen on their Mediterranean counterparts. The taxonomic importance of these differences is being investigated, taking into account the work of Macpherson & Raventos (2006). These authors have determined the pelagic larval duration of several Mediterranean littoral fishes and noted a correlation between this predictor of a species' dispersal potential and its distribution range. It may be that the demersal eggs and short larval duration of *S. mediterraneus* (13.6 days on average) are reducing the gene flow between the island and mainland populations of this species, thereby favouring morphological differentiation.

The reproductive biology of the two populations, on the other hand, was rather similar. In both the Azores and the European mainland, females matured to a smaller size than males, which is typical from European wrasses as a consequence of the slower growth rate of the females (Quignard 1966; Treasurer 1993). The reproductive season is the same for both populations (May to August, Quignard 1966) and starts in both areas when the seawater temperature is rising from the winter low and ends when the summer peak is reached. However, there are differences in the absolute values. The highest GSI values, for instance, were observed in the Azores in May and June, when the surface seawater temperature was between 17 and 18 °C, a much narrower interval than that

reported by Quignard (1966) for the corresponding period (May to July): 12 to 23 °C.

Macpherson & Raventos (2006) link the spawning in spring and summer of most species of littoral Mediterranean fish with the higher phyto- and zooplanktonic productivities in those seasons. An alternative hypothesis, based on the adult biology, has also been proposed to explain the reproductive seasonality (Robertson 1991). In the present study, the spawning starts immediately after the spring chlorophyll *a* peak, and the larvae are released when the primary productivity is decreasing. Therefore, the theory of a spawning period adapted to maximize larval survival seems unlikely. The higher productivity of spring should give adults more opportunities of feeding, increasing the energy available for gonad development. Thus, the feeding biology of the adults may be the main factor determining the timing of spawning.

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## ORIGINAL ARTICLE

# Validation of macroscopic maturity stages according to microscopic histological examination for European anchovy

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## Keywords

European anchovy; histology; macroscopic identification; maturity scale.

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## Abstract

The identification and classification of macroscopic maturity stages plays a key role in the assessment of small pelagic fishery resources. The main scientific international commissions strongly recommend standardizing methodologies across countries and scientists. Unfortunately, there is still a great deal of uncertainty concerning macroscopic identification, which remains to be validated. The current paper analyses reproductive data of European anchovy (*Engraulis encrasicolus* L. 1758), collected during three summer surveys (2001, 2005 and 2006) in the Strait of Sicily, to evaluate the uncertainty in the macroscopic maturity stage identification and the reliability of the macroscopic adopted scale. On board the survey vessels, the maturity stage of each fish was determined macroscopically by means of an adopted maturity scale subdivided in six stages. Later, at the laboratory, the gonads were prepared for histological examination. The histological slides were analysed, finally assigning the six maturity stages for macroscopic examinations. A correspondence table was obtained with the proportion and number of matches between the two methods. The results highlight critical aspects in the ascription of macroscopic maturity stages, particularly for the present research aim. Different recommendations were evaluated depending on the scope of the study conducted on maturity (e.g. daily egg production, fecundity and maturity ogive computation). The most interesting results concern the misclassification of stage IV and stages III and V (the most abundant), which confirms their macroscopic similarity. Although the results are based on a small number of samples, the advantages and disadvantages of macroscopic and histological methods are discussed with the aim to increase the accuracy of correct identification and to standardize macroscopic maturity ascription criteria.

## Problem

The small pelagic species, especially sardines and anchovies, represent an important resource worldwide. In particular, the European anchovy (*Engraulis encrasicolus*) is widely distributed along the European Atlantic coast from South Africa to North Atlantic, and over the whole of the Mediterranean and Black Sea. This species was the one most fished by the Italian fleet in the last 4

years (IREPA – Istituto Ricerche Economiche per la Pesca e l'Acquacoltura, 2008). These resources undergo wide interannual biomass fluctuation mainly due to the effects of environmental variability on recruitment. Sustainable management and exploitation of fish resources are linked to the Stock Reproductive Potential concept (SPR; Trippel 1999). The value of reproducible potential for stock assessment appears to be very important for several commercially important demersal or semi-demersal

sal species (Murua & Saborido-Rey 2003; Murua *et al.* 2003).

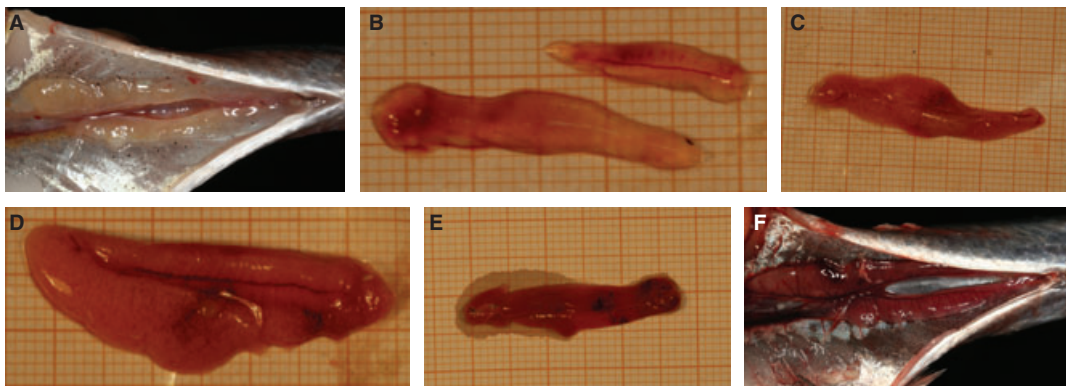
In recent decades, the refinement of knowledge on the growth and development of fish oocytes (Yanamoto & Yoshoka 1964; Wallace & Selman 1981) and reproductive biology (Begenal 1973; Hunter & Goldberg 1980) has permitted more precise definitions of fish fecundity. The Daily Egg Production Method (DEPM) was developed in the late 1970s by the Coastal Division of the Southwest Fisheries Center, La Jolla, CA, USA (Parker 1980) as a response to the growing need to devise a suitable direct method for the assessment of Northern anchovy (*Engraulis mordax*), an indeterminate spawner with pelagic eggs (Hunter & Goldberg 1980). In the case of indeterminate spawners, the DEPM is based on the number of oocytes released per fish in each spawning event (batch fecundity) and the proportion of females reproducing daily (spawning fraction). Estimation of spawning fraction for Northern anchovy is possible because of the identification of post-ovulatory follicles (POFs; cellular remnants in the ovary after ovulation; Hunter & Goldberg 1980). Estimates of relative fecundity (batch fecundity divided by mean female weight) and spawning fraction give the number of eggs released per unit weight of mature females, which, multiplied by the sex ratio in the population expressed in weight, provides daily fecundity (number of eggs released per unit weight of stock). In the 1980s, applications rapidly extended to several important anchovy stocks worldwide. DEPM revisions in the 1990s (Alheit 1993; Hunter & Lo 1997) and 2000s (Stratoudakis *et al.* 2006) confirmed its wide scope and potential, its robustness in estimating spawning biomass and indicated possible challenges for better future applications.

In multiple-spawning fishes the determination of macroscopic maturity stages is difficult to achieve without the support of microscopic examination. Because of its subjectivity and variability, macroscopic examination of ova-

ries plays a key role in the assessment of fishery resources. The identification and classification of maturity stages are used for the determination of spawning period according to different geographical and environmental areas and for studying the relationship between length at maturity and fishery exploitation (Picquelle & Hewitt 1983; Armstrong *et al.* 1988; Pérez *et al.* 1989; Millan 1999). Several international commissions for fisheries studies (*i.e.* ICES – International Council for the Exploration of the Sea, PICES – North Pacific Marine Science Organization, GFCM, – General Fisheries Commission for the Mediterranean) strongly recommended standardizing methodologies across countries and researchers. In the present study the gonads maturity data from the Strait of Sicily European anchovy (*E. encrasicolus* L. 1758) were examined. The results of macroscopic and microscopic methods were compared to verify the correspondence or the discordance of these two classification methods. Critical aspects in the ascription of macroscopic maturity stages were investigated. An improvement in macroscopic classification performance during the three study years can be seen. The obtained results underline the importance of adopting the histological analysis when the investigation required the highest possible accuracy.

## Material and Methods

The anchovy samples were collected during the peak spawning period in the Strait of Sicily (July–August), onboard a research vessel equipped with a mid-water pelagic trawl (experimental design). DEPM surveys were made by IAMC – CNR during 2001, 2005 and 2006. From each trawl a subsample of 50 specimens was randomly collected and processed, measuring total and standard length ( $\pm 1$  mm) and total and somatic weight ( $\pm 0.1$  g). The sex was determined and the fish gonads were macroscopically classified (Fig. 1).



**Fig. 1.** Six macroscopic maturity stages of ovary. A: immature ovary in body cavity. B: developing ovary. C: imminent spawning. D: spawning. E: partial post-spawning. F: spent ovary in body cavity.

**Table 1.** Description of macroscopic and microscopic characteristics of each maturity stage.

stage	stage name	macroscopic characteristics	microscopic characteristics
I	Immature or rest	Invisible or very small ovaries (cord shaped), translucent or slightly coloured (when resting)	In the case of immature females, all the oocytes in the ovary were in primary growth stage and oocytes well packaged. Resting females might contain late atretic oocytes
II	Developing	Wider ovaries occupying 1/4 to 1/3 of body cavity; pinkish or yellow colour. Visible oocytes are not present	Occurrence of cortical alveoli and no signs of advanced spawning process such as thick ovary wall, high vascularization of gonad
III	Imminent spawning	Ovaries occupying 3/4 to almost fitting body cavity; opaque with yellow or orange colour. Opaque oocytes are visible.	Occurrence of vitellogenic oocytes, but post-ovulatory follicles are not present and some oocytes in nucleus migration phase could be observed
IV	Spawning	Large ovaries occupying the full body cavity; fully or partially translucent with gelatinous aspect. Hyaline oocytes are visible	This stage starts with the nucleus migration phase following by oocyte hydration, without post-ovulatory follicles
V	Partial post-spawning	Size from 1/2 to 3/4 of abdominal cavity; not-turgid ovaries with haemorrhagic zones. Blood coloured	Post-ovulatory follicles are observed together with vitellogenic oocytes in different stages. Signs of advanced spawning process could be detected. Atresia could also be present.
VI	Spent	Reddish ovary shrunked; Size <2/3 of abdominal cavity Flaccid ovary. Some small opaque oocytes	High level of POF and atresia, disorganization of ovary structures, numerous blood vessels, absence of yolked oocyte groups

The maturity stage for each fish was determined using the six-degree macroscopic maturity scale shown in Table 1 (Holden & Raitt 1974, modified for six stages; ICES 2004, modified by authors). The ovaries were fixed in 4% buffered formalin. For species of the genus *Engraulis* it is possible to exploit the method of Harris Hematoxylin and Eosin (H & E). The preparation of samples with the H & E method includes five steps (Hunter & Macewicz 1985): (i) fixation; (ii) infiltration; (iii) sectioning; (iv) mounting; (v) staining. The microscopic data were also arranged according to the six maturity stages from the macroscopic scale (Fig. 2). The six maturity stages could be split into immature (stages I and II) and mature (stages III to VI).

The operator was the same during the study period. For samples collected during the acoustic survey the identification of the six microscopic stages was based on the characteristics of the most advanced oocyte stage (West 1990). The microscopic and macroscopic maturity data were compared to assess the correspondence levels achievable by an advanced operator using macroscopic examination. The proportion of a robust classification and its variability was also evaluated.

## Results

The comparison results contain the following observations: stage I is not widely represented in the samples (around 3%), most probably because the surveys were conducted during the peak anchovy spawning season. Stages I and II represent immature ovaries. The main staging mistake resulted in the misclassification of stage V as stage I in 6/14 examined ovaries (43%, Table 3).

Macroscopic examination correctly classified stage II in only 7/39 ovaries (18%, Table 3). However, the bulk of misclassification occurred between stages II and V (44%, Table 3).

Stage III is the most abundant in the samples (Table 3), and shows the highest matching proportion between micro- and macroscopic classifications (Table 3). After a rather low matching rate in 2001, the correspondence between the two classification methods

**Table 2.** The percentage of correspondence between macroscopic (MACRO) and microscopic (MICRO) examination in three study years; the number of specimens is given in parentheses.

survey	MACRO	MICRO						
		I	II	III	IV	V	VI	III + V
2001	I	0	–	–	–	100 (1)	–	
	II	–	0	–	–	–	–	
	III	–	9 (2)	35 (8)	39 (9)	17 (4)	–	52
	IV	–	–	24 (5)	24 (5)	52 (11)	–	
	V	–	–	55 (6)	18 (2)	27 (3)	–	82
	VI	–	–	100 (2)	–	–	0	
2005	I	0	–	–	–	100 (1)	–	
	II	33 (1)	0	67 (2)	–	–	–	
	III	–	5 (8)	71 (108)	6 (10)	18 (28)	–	89
	IV	–	–	24 (5)	24 (5)	52 (11)	–	
	V	–	6 (8)	50 (69)	4 (5)	39 (54)	1 (1)	89
	VI	11 (1)	23 (2)	33 (3)	–	33 (3)	0	
2006	I	15 (2)	15 (2)	23 (3)	–	39 (5)	8 (1)	
	II	17 (6)	19 (7)	8 (3)	6 (2)	47 (17)	3 (1)	
	III	–	1 (1)	66 (57)	5 (4)	28 (24)	–	94
	IV	–	–	62 (5)	38 (3)	–	–	
	V	2 (1)	2 (1)	41 (25)	–	52 (25)	3 (2)	93
	VI	33 (1)	–	–	–	33 (1)	33 (1)	



**Table 3.** The overall percentage of correspondence between macroscopic (MACRO) and histological examinations (MICRO) for each maturity stage in the whole study period (2001, 2005 and 2006); the number of specimens is given in parentheses.

MACRO	MICRO						
	I	II	III	IV	V	VI	III + V
I	14 (2)	14 (2)	21 (3)	–	43 (6)	7 (1)	
II	18 (7)	18 (7)	13 (5)	5 (2)	44 (17)	3 (1)	
III	–	4 (9)	69 (165)	6 (14)	22 (52)	–	91
IV	–	–	34 (10)	28 (8)	38 (11)	–	
V	1 (1)	5 (9)	48 (94)	3 (5)	43 (85)	2 (3)	91
VI	17 (2)	17 (2)	25 (3)	–	33 (4)	8 (1)	

increased about 70% in 2005 and 2006 (Table 2). The most common misclassification occurred from stage III to stage V because these stages are different for only a short period after spawning, when POFs have not yet been reabsorbed. In the multiple-spawner species there is a continuous transition from stage III to V and from V to III during the spawning season. Macroscopically, these two stages are very similar and several external factors can make stage III appear as stage V (*i.e.* trawl time, trawl type, presence of damaging object with the sample). The percentage of stage V that was classified as stage III macroscopically was 22%, (52/240 samples) (Table 3). When the two stages were merged, the per-

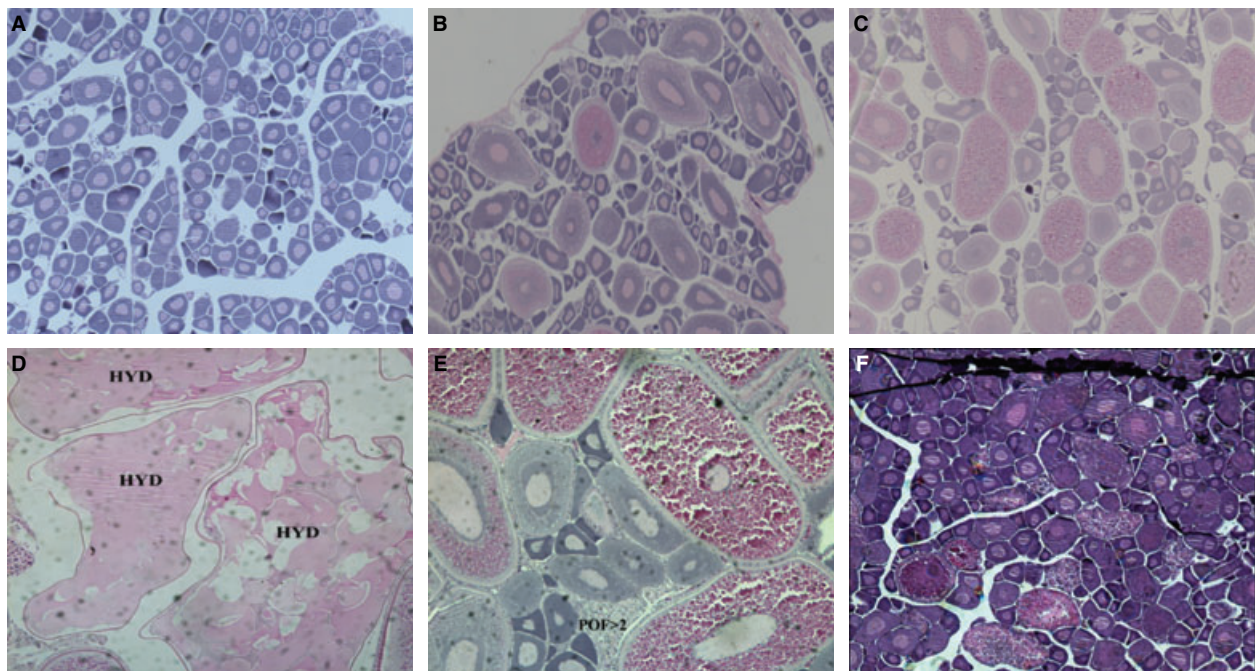
centage of agreement between macroscopic and microscopic classifications increased from 52% and 82% in 2001 (Table 2) and 89% in 2005 (Table 2), to over 90% in 2006 (Table 2).

Stage IV (hydrated) is recognizable only during the daily peak of spawning, for few hours. That explains the low presence of these stages in the samples (Table 3), although it is well known from the literature that this stage is easily recognized by its evident attributes (Fig. 1; Holden & Raitt 1974 modified for six stages; ICES 2004). The bulk of ovaries (21 of 29), macroscopically identified as stage IV, were microscopically assigned to stage III (10, Table 3) and stage V (11, Table 3), 34% and 38%, respectively (Table 3). The correspondence was weak for stage IV in all surveys (Table 2).

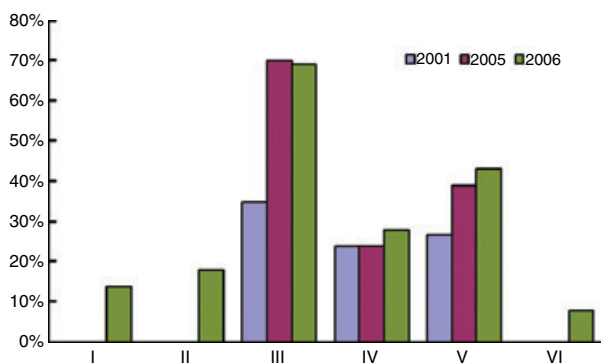
During the study period, the correspondence between macroscopic and microscopic maturity classifications increased mainly for stages IV and V.

The abundance and percentage of correspondence was highest for stage III, followed by stage V (Table 3, Fig. 3). The bulk of misclassification was between these stages: 94/197 checked ovaries (Table 3), equal to 48% (Table 3).

The percentage of correct classification for stage VI was very low (8%, Table 3) because it was confused mainly with stage V (4/12 ovaries; Table 3). Analysing data by year, it is clear that misclassification is always present,



**Fig. 2.** Six microscopic maturity stages of ovary. A: immature oocytes. B: developing ovary with oocytes in nucleus migration. C: imminent spawning ovary with vitellogenic oocytes. D: spawning ovary with hydrated oocytes (HYD). E: partial post-spawning ovary with old POF (POF >2). F: spent ovary.



**Fig. 3.** Bar chart of percent correspondence between macroscopic and microscopic classifications.

despite an improvement during 2006: however, there is a correspondence between macro- and microscopic classification (Fig. 3), even if low: only 1/12 samples (Table 3).

## Discussion

Generally, it was observed that for anchovy, as for many other species, it is not possible to distinguish immature and resting females macroscopically (Trippel & Morgan 1997; Saborido-Rey & Junquera 1998; Domínguez-Petit 2007). In both stages the oocytes are not visible. Only by means of histological analyses is it possible to identify each stage accurately. This misclassification has an impact on the estimation of the mature proportion of the stock because resting females have already contributed to the spawning biomass of that year and are macroscopically considered immature.

Stages I and II are not widely represented in the samples, probably due to the sampling periods, which occurred during the peak of the anchovy spawning season. The large misclassification of stages I and II with stage V is remarkable, especially when the aim of analysis is to discriminate mature and immature specimens.

By comparing the results with literature data (ICES, 2004), it is possible to see that the misclassification between III and V stages is a recurring problem and that the match rate between macroscopic and microscopic classification increases when the two stages are merged. This is mainly because these stages differ for only a short time after spawning, when the POFs are not yet reabsorbed, which, in the studied area, with high mean water temperature, represents a very fast process (a few hours). In the multi-spawner species, the change between stages III and V is a continuous process during the spawning season until the last batch has been spawned. During the study period, there is an increasing proportion of correct ascription, but it appears lower than the values reported in the literature, e.g. the anchovies from Portugal (100%

matching in Stage III; ICES, 2004). In any case, there appears to be a clear need for a training period onboard the fishing vessels to be able to discriminate the maturity stages of very fresh gonads. Freezing, formalin or alcohol preservation is known to produce deep alterations at a macroscopic level, mainly in the colour and consistency of ovaries. As reported in Lasker (1980) the gonad tissues have to be sampled within 2 h of the catch, before degradation becomes significant. Bearing in mind that Lasker used the hydrated stage for histological preparation, some hours more could be a reasonable time within which to carry out the macroscopic examination.

Further interesting comments concern stage IV. Despite this stage being generally easily recognizable by visual inspection of fresh individuals, the bulk of ovaries macroscopically identified as stage IV became stage III and V when later microscopically examined. Stage IV could easily become stage V when the spawning begins. So if only a few eggs are spawned, the macroscopic aspect does not differ compared to the entire hydrated oocyte. However, it should be noted that the percentages for stage IV are based on only a few observations, and for this reason their representativeness is proportionally low.

During the whole study period, stage VI was rarely correctly identified. The misclassification between stage VI and stages I and II is particularly problematic, because stage VI represents mature fish and stages I and II immature fish. In addition, the proportions of stage VI are based on a small number of observations.

Maturity ogives should only be based on data collected during the peak of the spawning season, taking into consideration geographical variation, as it is impossible to distinguish immature and resting females macroscopically. The proportion of resting females during the peak of the spawning season is lower than during the rest of the year. If possible, a gonad subsampling for histological analysis should be carried out to obtain a correction factor to reduce the impact of misclassification between immature and resting females.

Furthermore, the samples should be analysed as soon as caught, when the ovaries are in their best condition, allowing the right macroscopic maturity stage to be assigned more reliably. However, samples are generally analysed after a preservation period in ice or formalin, which changes the colour and texture. Also, frozen gonads are not appropriate for histological examination. When fecundity has to be estimated for stock assessment purposes, it is necessary to validate the macroscopic classification with a microscopic one, to reduce mistakes.

The results from macroscopic and microscopic maturity classifications could be used for several applications. In the case of the estimation of size at first maturity of the population ( $L_{50}$ ) the macroscopic examination would

be enough to discriminate immature from mature specimens, as follows: immature = stage I and II; mature = III to VI. Histological analysis would not be necessary if macroscopic classification were applied, especially when a high number of individuals have to be processed. Also, in the latter case, a higher level of accuracy may be achieved when a representative subsampling, for further histological examinations, can be done. When macro- and microscopic examinations are compared, it is possible to build a table, as in the present study (Table 3), where the correspondence proportion for each macroscopic maturity stage can be used as a correction factor for all the sampled specimens classified macroscopically. Finally, because maturity ogives are generally correlated to the length of the fish, a correction table has to be obtained for size classes. From the present study the misclassification between stage I versus V, stage II versus V and stage VI versus I and II, has to be corrected to avoid a significant source of mistake. With regard to the fecundity estimation, the misclassification rate from macroscopic examination is very large when determining consistent variations in batch fecundity (F) and in spawning fraction (S) estimation; consequently, there is a greater uncertainty in the stock assessment abundance estimations (e.g. DEPM applications). As known from the literature (Hunter & Macewicz 1985) and as supported by the present data, in multiple-spawning fishes, histological analysis is essential when the DEPM is applied.

During the three study years there was an improvement in the macroscopic classification performance of the operator: clearly, experience helps to improve the ability to recognize different stages; however, it is also true that the level of misclassification in the last year was high enough to compromise the estimation of the DEPM parameter.

Finally, the interpretation of maturity scales and determination of maturity stages may vary considerably among people and labs because no exchanges or standardization meetings are conducted regularly among countries where similar resources are studied. Sometimes, when datasets from different countries are compared, this variability may be larger than the misclassification errors. The obtained results confirm the importance of common and standardized protocols for the identification and macroscopic classification of maturity stages among scientists from different country. As a future objective, to overcome this problem, we suggest that common training and intercalibration exercises be extended as much as possible to all the labs that study fish fecundity.

The correspondence analysis between macroscopic and microscopic classifications underlined the weakness of macroscopic classification even when conducted by the same operator with the best sampling conditions (direct on board) and following clear maturity scale criteria

widely accepted by the scientific community. Unfortunately, when the above recommendations about intercalibration exercises and samples freshness, are followed the misclassification proportion remains highly significant. The reasons for the discrepancies between macroscopic and histological observations are mainly as follows: operator subjectivity; unclear distinction between the descriptions of maturity stages in the reference tables; daytime sampling; the level of fish injury due to the trawl; and the elapsed time from the catch to examination of the samples. Therefore, histological preparation appears to be the only way to obtain an accurate classification (i.e. DEPM applications, fecundity studies, etc.). When the study aims do not require such high standards, it is possible to carry out the macroscopic examinations with the scale used here with satisfying results (i.e. maturity ogives computation).

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## ORIGINAL ARTICLE

# Community structure of bathyal decapod crustaceans off South-Eastern Sardinian deep-waters (Central-Western Mediterranean)

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## Keywords

Deep-sea; decapod crustaceans; faunal zonation; Sardinian deep-waters; Mediterranean.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Abstract

Community structure and faunal composition of bathyal decapod crustaceans off South-Eastern Sardinian deep-waters (Central-Western Mediterranean) were investigated. Samples were collected during 32 hauls between 793 and 1598 m in depth over the 2003–2007 period. A total of 1900 decapod specimens belonging to 23 species were collected. Multivariate analysis revealed the occurrence of three faunistic assemblages related to depth: (i) an upper slope community at depths of 793–1002 m; (ii) a middle slope community at depths of 1007–1212 m and (iii) a lower slope community at depths greater 1420 m. In the upper and middle slopes the benthic (*Polycheles typhlops*) and epibenthic–endobenthic feeders (mainly *Aristeus antennatus* and *Geryon longipes*), which eat infaunal prey, were dominant, followed by the macroplankton–epibenthic feeders such as *Acantheephyra eximia* and *Plesionika acanthonotus*. In the deepest stratum, the most remarkable feature was the prevalence of macroplankton–epibenthic feeders (*A. eximia* and *P. acanthonotus*). A small percentage of the benthic deep-sea lobster *Polycheles sculptus* was also present. The biomass presented higher values in the middle slope and declined strongly in the lower slope. There was no general pattern of mean individual weight/size *versus* depth among decapods, and the changes seemed to be species-specific with different trends.

## Problem

The study of the distribution of species along environmental gradients has traditionally been important for characterising the organisation of animal communities in aquatic systems (Wenner & Boesch 1979). In particular, in deep-sea marine environments, species and communities often change with increasing depth rather than along horizontal gradients (Gage & Tyler 1991; Cartes *et al.* 2004, 2007a), suggesting the idea of depth bands of high faunal homogeneity separated by boundaries of faunal renewal. Moreover, the structure of megafaunal assemblages in the continental slope is largely determined by a combination of both abiotic (the structure and type of the bottom and oceanographic conditions) and biotic (resource availability and food web structure) factors (Moranta *et al.* 1998, 2008), which may also play a funda-

mental role in the local zonation pattern (Maynou & Cartes 2000; Carbonell *et al.* 2003).

In this context, bathyal decapod crustaceans represent, after the ichthyofauna, a dominant faunal component in the benthic communities of the Mediterranean Sea (Abelló & Valladares 1988; Cartes & Sardà 1992; Sardà *et al.* 1994; Maynou & Cartes 2000; Company *et al.* 2004) and are a key taxon linking lower and higher trophic levels (Wenner & Boesch 1979; Cartes 1998). Their high abundance in the Mediterranean in comparison with other oceans, in which the echinoderms predominate among invertebrates (Tyler & Zibrowius 1992; Sardà *et al.* 1994), is probably due to the oligotrophic nature of Mediterranean (Company *et al.* 2004).

Community assemblages, distribution and abundance of decapod crustacean fauna have been described in detail in the Western Mediterranean basin (Abelló *et al.* 1988,



2002; Cartes & Sardà 1993; Mura & Cau 1994; Maynou *et al.* 1996; Maynou & Cartes 2000; Moranta *et al.* 2000; Cartes *et al.* 2001; Morales-Nin *et al.* 2003; Company *et al.* 2004; Fanelli *et al.* 2007; García Muñoz *et al.* 2008) and in the Central and Eastern Mediterranean (Pipitone & Tumbiolo 1993; Ungaro *et al.* 1999, 2005; Kallianotis *et al.* 2000; Colloca *et al.* 2003; Company *et al.* 2004; Galil 2004; Politou *et al.* 2005).

In Sardinian waters (Central-Western Mediterranean), the knowledge available on the deep-sea decapod crustaceans concerns the bio-ecology (mainly reproduction and trophism) of a few deep-sea species (Mura *et al.* 1993; Follesa *et al.* 2007; Cabiddu *et al.* 2008). In fact, the studies in this area are limited to the epibathyal and the mesobathyal levels (mostly at depths of less than 750 m) generally subject to intense trawl fishing. Mura (1987) and Mura & Cau (1992, 1994) described the faunistic composition and bathymetric distribution of the decapod crustaceans present in the lower part (down to 1050 m) of the mesobathyal zone of the Sardinian Channel.

The object of this paper was to update the data on bathyal decapod crustaceans (faunal composition, bathymetric distribution, zonation, abundance, biomass and length frequency distribution) in Sardinian waters (Central-Western Mediterranean). Despite the limitations of bathyal sampling, this study represents the first attempt to identify the deep-sea crustacean assemblages down to 1000 m and to improve knowledge of this area, considered the link between the Western and Eastern Mediterranean (Hopkins 1988).

## Methods

The data analysed in the present work came from 32 hauls carried out during experimental trawl survey cruises intended to study the bottom-living community in the continental slope. Sampling was conducted on compact mud bottoms off South-Eastern Sardinian deep-waters (Sardinian Channel, Central-Western Mediterranean) (Fig. 1). All hauls were performed at main depths of 793–1598 m over the 2003–2007 period.

On board, trawl data (date, position and duration) were recorded (Table 1). The duration of each haul (bottom time) varied between 64 and 164 min. The towing speed was about 2.2 knots for all hauls. The otter trawl used was equipped with a 20-mm stretch mesh size cod end. Gear selectivity was assumed to be constant because the same fishing gear for each trawl was used.

Usually, the monitoring of crustacean assemblages is confined to the economically important depths (<750 m). For this reason, due to the lack of long-time series of data on deep-sea assemblages, catches from

different years and seasons have been pooled in our analysis.

For each haul, crustaceans were sorted by species and abundance (N) and biomass (g) data were noted and standardised to 1 h of haul ( $N \cdot h^{-1}$ ). In the laboratory, the carapace length (CL, in mm, from the posterior margin of the eye socket to the posterior end of the carapace), individual weight (g) and sex of each species were also determined. Moreover, the crustaceans were classified as mesopelagic species, nektobenthic species or benthic species on the basis of their relative location in the water column (Maynou & Cartes 2000).

Standardised abundance data of decapod crustaceans were pooled in a matrix of species abundance with the PRIMER (v6) package. Cluster analysis was performed using the Bray–Curtis similarity index and group linkage was used for the dendrogram (Bray & Curtis 1957; Field *et al.* 1982). Prior to analysis, standardisation and fourth-root transformation were applied.

Analysis of similitude (ANOSIM) was used to test the significance of species assemblages between trawl surveys (Clarke 1993) and similarity percentage (SIMPER) was also applied to detect bathymetric differences.

The ecological parameters such as mean abundance ( $N \cdot h^{-1}$ ) and mean biomass ( $g \cdot h^{-1}$ ), and the measures of species diversity, such as species richness (S) (DIVERSE routine), Shannon–Wiener index ( $H'$ ) (Shannon & Weaver 1949) and evenness ( $J'$ ) (Pielou 1977) were calculated for all hauls of the main groups obtained by prior classification.

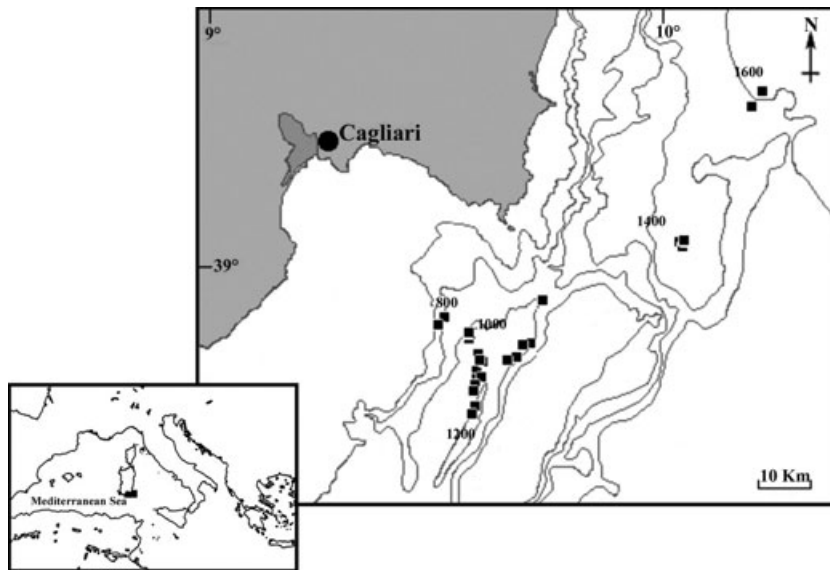
Between-assemblage differences in species richness, diversity indexes, abundance and biomass spectra were tested using repeated ANOVA measures (Zar 1999). Each haul was considered an observation and the data of abundance and biomass were normalized [ $\ln(x + 1)$ ] to adjust residuals to normality.

A bubble scatterplot was applied to the individual mean weight (g) of the most frequent species to detect the abundance tendencies with depth.

To show the bathymetric distribution of the main species, the overall length frequency distribution (2 mm size class) by sex for each depth interval was determined.

## Results

During the trawl surveys, a total of 1900 individuals (total weight 11,919 g), subdivided in 23 species of bathyal decapod crustaceans, were collected and are listed in Table 2. Within the investigated depth range, *Plesionika acanthonotus* was present in all hauls and *Polycheles typhlops*, *Sergia robusta* (97% frequency of occurrence) and *Aristeus antennatus* (91% frequency of occurrence) were present at nearly all stations. A high frequency of occur-



**Fig. 1.** Map of the study area and positions of trawl stations (black squares) off the South-Eastern Sardinian deep-waters (Central-Western Mediterranean).

haul code	date	mean depth (meters)	starting position		haul duration (minutes)
			latitude (N)	longitude (E)	
1	16/06/2003	1007	38°53'94"	9°27'04"	106
2	16/06/2003	991	38°50'99"	9°31'93"	103
3	10/11/2003	996	38°57'39"	9°37'06"	112
4	10/11/2003	995	38°52'65"	9°34'07"	108
5	11/11/2003	793	38°54'83"	9°22'58"	90
6	11/11/2003	1002	38°45'36"	9°27'22"	97
7	12/11/2003	1035	38°53'95"	9°26'79"	90
8	12/11/2003	842	38°55'58"	9°23'45"	65
9	17/01/2005	961	38°52'75"	9°34'99"	73
10	18/01/2005	1166	38°49'92"	9°27'78"	64
11	23/05/2005	1033	38°53'79"	9°26'75"	74
12	27/05/2005	1145	38°51'02"	9°28'21"	76
13	27/05/2005	1188	38°46'16"	9°27'60"	79
14	19/01/2006	1147	38°51'34"	9°28'25"	111
15	19/01/2006	974	38°51'34"	9°33'16"	109
16	15/05/2006	1020	38°53'35"	9°26'81"	146
17	15/05/2006	1163	38°50'82"	9°28'61"	142
18	15/05/2006	1099	38°49'10"	9°27'74"	126
19	16/05/2006	1037	38°53'79"	9°26'88"	164
20	16/05/2006	1086	38°48'39"	9°27'62"	131
21	18/07/2006	1420	39°03'27"	9°56'04"	135
22	21/11/2006	1212	38°47'84"	9°27'45"	96
23	21/11/2006	1421	39°03'49"	9°56'07"	103
24	21/11/2006	1598	39°19'37"	10°06'93"	89
25	06/03/2007	1133	38°49'74"	9°28'02"	107
26	06/03/2007	1151	38°48'45"	9°27'47"	154
27	07/03/2007	1420	39°03'38"	9°55'52"	138
28	24/04/2007	1044	38°53'74"	9°26'95"	111
29	24/04/2007	1115	38°51'66"	9°27'93"	130
30	24/04/2007	1105	38°49'31"	9°28'48"	88
31	03/09/2007	1573	39°17'82"	10°05'53"	105
32	03/09/2007	1421	39°02'90"	9°55'88"	125

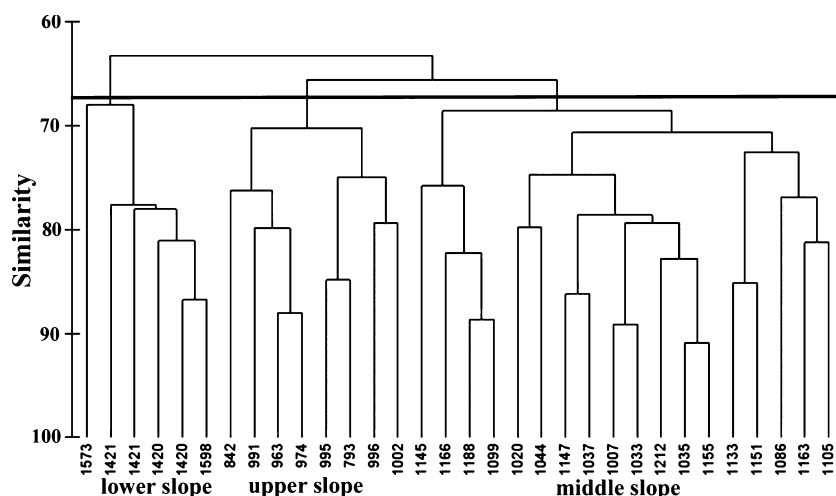
**Table 1.** Characteristics of hauls (date, mean depth, position, time period of hauls) carried out off South-Eastern Sardinian deep-waters (Central-Western Mediterranean).

rence (%F > 50) of *Acantheephyra eximia*, *Acantheephyra pelagica*, *Ponthophilus norvegicus* and *Geryon longipes* was observed (Table 2). In terms of total abundance, the predominant species were found to be *A. antennatus* (23.6%) and *P. typhlops* (18.4%). In terms of biomass, *A. antennatus* (31.6%), and *G. longipes* (21.05%) were the species with the highest percentage of contribution (Table 2).

The similarity dendrograms of the trawls revealed the presence of three main groups (Fig. 2), which can be clearly identified along the bathymetric gradient. The first group consisted of the deepest stations covering a depth range of 1420–1598 m (six hauls, mean depth 1475.5, SD 85.57) (lower slope) (Fig. 2). A second group was made up of stations investigated at 793–1002 m depth (eight

**Table 2.** Bathyal decapod crustacean species collected off South-Eastern Sardinian deep-waters (Central-Western Mediterranean) between 793 and 1598 m with their bathymetric range, occurrence's frequency (%F), percentage of abundance (%Abundance) and biomass (%Biomass).

species	depth range (m)	%F	%abundance	%biomass
<i>suborder Dendrobranchiata</i>				
superfamily Penaeoidea				
family Aristeidae				
<i>Aristaeomorpha foliacea</i> (Risso, 1827)	793–1037	3	0.1	0.57
<i>Aristeus antennatus</i> (Risso, 1816)	793–1598	91	23.6	31.6
<i>Gennadas elegans</i> (S. I. Smith, 1882)	1163–1421	13	0.3	0.01
family Penaeidae				
<i>Parapenaeus longirostris</i> (H. Lucas, 1846)	793	3	0.2	0.88
superfamily Sergestoidea				
family Sergestidae				
<i>Sergestes arcticus</i> Krøyer, 1855	842–1188	19	0.3	0.01
<i>Sergia robusta</i> (S.I. Smith, 1882)	793–1598	97	11.2	2.8
<i>suborder Pleocyemata</i>				
infraorder Caridea				
family Oplophoridae				
<i>Acantheephyra eximia</i> S. I. Smith, 1884	793–1598	88	12.3	16.93
<i>Acantheephyra pelagica</i> (Risso, 1816)	996–1573	59	3.4	3.03
family Pasipheidae				
<i>Pasiphaea multidentata</i> Esmark, 1866	793–1573	44	1.4	1.48
family Pandalidae				
<i>Plesionika acanthonotus</i> (S. I. Smith, 1882)	793–1598	100	13.2	1.6
<i>Plesionika martia</i> (A. Milne-Edwards, 1883)	793	3	0.1	0.03
family Crangonidae				
<i>Pontocaris lacazei</i> (Gourret, 1887)	793	3	0.1	0
<i>Ponthophilus norvegicus</i> (M. Sars, 1861)	1020–1598	63	3.5	0.25
infraorder Thalassinidea				
family Axiidae				
<i>Calocaris macandreae</i> Bell, 1853	1421	3	0.1	0.01
infraorder Palinura				
family Polychelidae				
<i>Polycheles sculptus</i> S. I. Smith, 1880	1037–1598	50	2.6	1.08
<i>Polycheles typhlops</i> Heller, 1862	793–1598	97	18.4	13.2
infraorder Anomura				
family Paguridae				
<i>Pagurus alatus</i> (Fabricius, 1775)	793	3	0.1	0.03
family Galatheididae				
<i>Munida tenuimana</i> G. O. Sars, 1871	793–1598	34	2.4	0.42
infraorder Brachyura				
family Xanthidae				
<i>Monodaeus couchii</i> (Couch, 1851)	793	3	0.1	0.02
family Homolidae				
<i>Paromola cuvieri</i> (Risso, 1816)	974–1212	38	1	4.54
family Geryonidae				
<i>Geryon longipes</i> A. Milne Edwards, 1881	842–1421	69	5.6	21.05
family Portunidae				
<i>Bathynectes maravigna</i> (Prestandrea, 1839)	793–1105	6	0.2	0.32
<i>Macropipus tuberculatus</i> (Roux, 1830)	995	3	0.1	0.1



**Fig. 2.** Dendrograms of hauls using group-average clustering from Bray-Curtis similarity by depth strata in terms of standardised abundance ( $N \cdot h^{-1}$ ) of total catches off South-Eastern Sardinian deep-waters (Central-Western Mediterranean). Mean depth of each sample is given. The upper line indicates groups at the 66% level of similarity.

hauls, mean depth 944.5, SD 80.48) (upper slope). The third aggregation consisted of six hauls (mean depth 1107, SD 64.06) carried out at middle depths of 1007–1212 m (middle slope).

The pair-wise test comparisons (ANOSIM) showed, in terms of abundance, a low level of overlap between the hauls ( $R = 0.534$ ,  $P < 0.01$ ).

The results of the SIMPER routine showed a high percentage of similarity for the assemblages identified by clustering (Table 3). In the upper slope (793 and 1002 m), the species which took part in the assemblage appeared to be *P. typhlops*, *A. antennatus* and *S. robusta* (59.06%). *Aristeus antennatus*, *P. typhlops*, *P. acanthonotus* and *S. robusta* contributed 58.74% of the middle slope assemblage (1007–1212 m). The most typical species of the deepest bottoms (1420–1598 m) were *A. eximia* (19.10%), *P. acanthonotus* (14.83%) and *A. antennatus* (14.13%). Moreover, in the lower slope, *Polycheles sculptus* was a common species (13.65%).

Mean values of the ecological parameters of each assemblage are reported in Table 4. Significant differences in species richness ( $S$ ) were observed ( $F_{11,86}$ ;  $P < 0.05$ ).

The highest value (an average of 2.47 species) was found on the middle slope, followed by the lower (2.32 species) and the upper slope (1.79 species). The highest diversity ( $H'$ ) was obtained for the lower ( $H' = 1.89$ ,  $J' = 0.86$ ) and the middle slope ( $H' = 1.8$ ,  $J' = 0.81$ ), with significant differences between assemblages ( $F_{5,25}$ ;  $P < 0.05$ ).

The mean values of abundance showed an increase with depth (24, 36, 37 individuals per hour, respectively), with significant differences between assemblages ( $F_{3,43}$ ;  $P < 0.05$ ), whereas no significant difference ( $F_{1,95}$ ;  $P > 0.05$ ) was observed between the mean biomass that showed a maximum value in the middle stratum.

The relative abundance analysis of the species caught in each identified group (Fig. 3A,C,E) generally highlighted a species' dominance similar to that obtained from the SIMPER analysis (Table 3). Moreover, in terms of relative biomass, *A. antennatus*, *P. typhlops* and *G. longipes* were the most abundant species in the upper and middle slopes (Fig. 3B and D). In the deepest strata, *A. eximia* and *A. antennatus* were the predominant species (Fig. 3F).

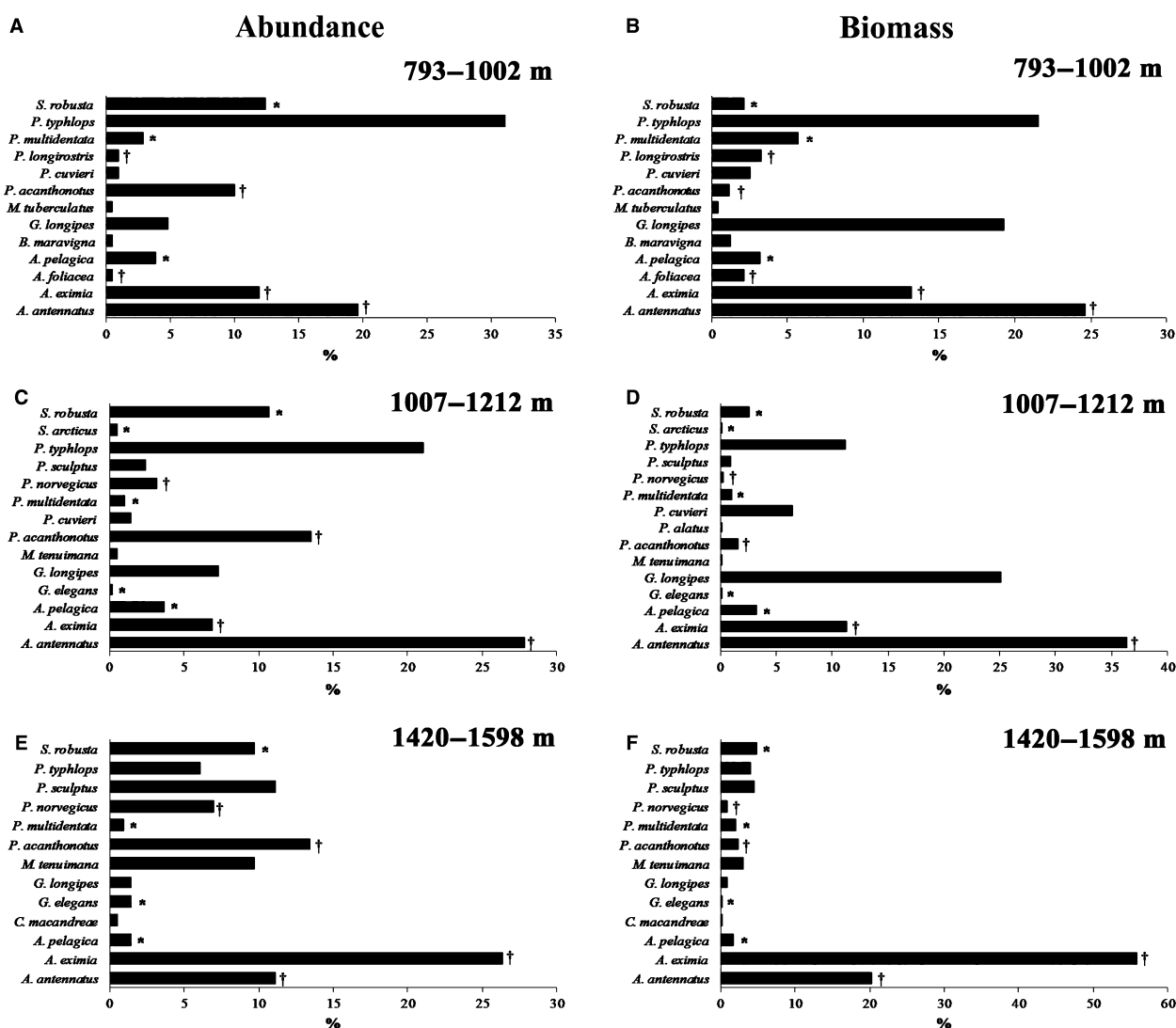
Regarding their relative depth distribution, in the shallowest stations the crustacean decapods could be charac-

**Table 3.** Results of the SIMPER routine to analyse the percentage contribution of typifying species (over 7%) to within-group similarity resulting from cluster analysis for crustacean samples during trawl surveys off the South-Eastern Sardinian deep-waters (Central-Western Mediterranean).

793–1002 m		1007–1212 m		1420–1598 m	
average similarity: 73.74		average similarity: 72.41		average similarity: 75.59	
species	contrib%	species	contrib%	species	contrib%
<i>Polycheles typhlops</i>	21.35	<i>Aristeus antennatus</i>	16.42	<i>Acantheephyra eximia</i>	19.10
<i>A. antennatus</i>	18.93	<i>P. typhlops</i>	16.16	<i>Plesionika acanthonotus</i>	14.83
<i>Sergia robusta</i>	18.78	<i>P. acanthonotus</i>	14.41	<i>A. antennatus</i>	14.13
<i>A. eximia</i>	17.44	<i>S. robusta</i>	11.75	<i>Polycheles sculptus</i>	13.80
<i>P. acanthonotus</i>	15.07	<i>Geryon longipes</i>	8.97	<i>S. robusta</i>	13.65
–		<i>A. eximia</i>	8.03	<i>Ponthophilus norvegicus</i>	9.11
–		<i>Acantheephyra pelagica</i>	7.88	<i>Munida tenuimana</i>	7.93
–		<i>P. norvegicus</i>	7.83	–	

**Table 4.** Some ecological parameters (mean and deviation standard) in the three groups resulting for the cluster analysis.

ecological parameters	upper slope	middle slope	lower slope
	793–1002 m	1007–1212 m	1420–1598 m
mean abundance (N·h <sup>-1</sup> )	24 ± 10.78	36 ± 13.31	37 ± 12.03
mean biomass (g·h <sup>-1</sup> )	205.48 ± 141.38	224.25 ± 106.27	121.81 ± 42.80
number of species	11	17	13
mean species richness (S)	1.79 ± 0.28	2.47 ± 0.34	2.32 ± 0.32
diversity (H')	1.56 ± 0.26	1.81 ± 0.20	1.89 ± 0.20
evenness (J')	0.83 ± 0.11	0.81 ± 0.08	0.86 ± 0.03



**Fig. 3.** Relative abundance and biomass of the most abundant deep-sea decapods collected off South-Eastern Sardinian deep-waters (Central-Western Mediterranean). \*Mesopelagic species; †nektobenthic species; all other species are benthic.



terised mainly by nektobenthic and benthic species (43% and 38% in number and 44% and 45% in weight, respectively) (Fig. 5). In the middle slope, nektobenthic species again dominated both in weight and number, while the benthic species were also present with a high percentage in biomass (44%), probably due to the occasional presence of large-bodied species such as the brachyuran crab *G. longipes*. The deepest range was inhabited by nektobenthic species (principally *A. eximia* and *A. antennatus*) but also by benthic decapods (29%), which became significant in number probably as consequence of the high abundance of the small deep-sea lobster *P. sculptus*.

The bubble plot for the bathyal decapod crustaceans showed different mean weight tendencies with depth depending on the species (Fig. 4). *Aristeus antennatus*, *A. eximia*, *A. pelagica*, *P. typhlops* and *G. longipes* showed a negative correlation between individual mean weight and depth, probably due to the recruitment of small individuals in the deepest waters. Otherwise, *Munida tenuimana* and *S. robusta* presented a 'bigger-deeper' trend, with mean weight increasing with depth. On the lower slope, the deepest species *P. sculptus* and *P. norvegicus*, which were captured for the first time below 1000 m, were characterized by small to medium sized individuals.

Figure 5 shows the size frequencies and sex distribution by depth interval of the main bathyal decapod species. Juveniles of *A. antennatus* (CL < 20 mm; Sardà *et al.* 2004) were most representative in the deepest part of range (below 1420 m), with a high percentage of females; between 793 and 1212 m (middle slope), the adults appeared well represented, with an elevated proportion of males. On the upper and middle slopes, *A. eximia* showed a range in size of 20–38 mm CL, with males mainly represented only by the smallest size class, whereas on the lower slope, juveniles (CL 14–18 mm) were also present. *Plesionika acanthonotus* showed a range in size of between 4 and 22 mm CL and a sex-ratio in favour of females increasing with depth. An inverse sex-ratio was observed in *S. robusta* (range 8–26 mm CL). It was difficult to find a clear pattern for the population structure of *P. norvegicus* because the individuals were present exclusively below 1000 m and were relatively scarce in all depth intervals.

## Discussion

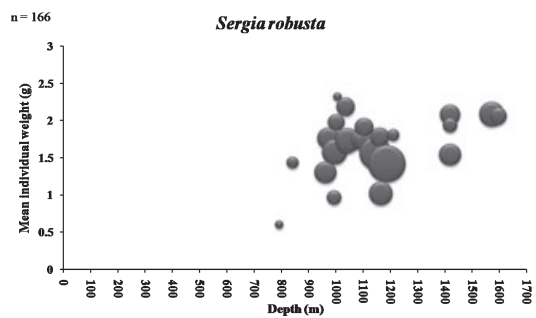
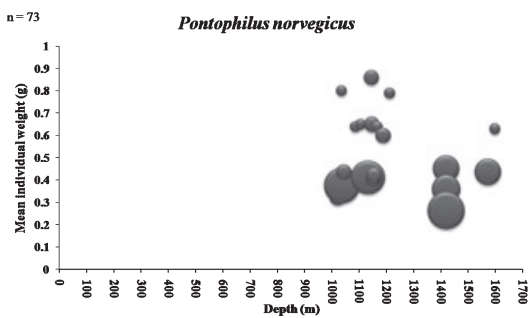
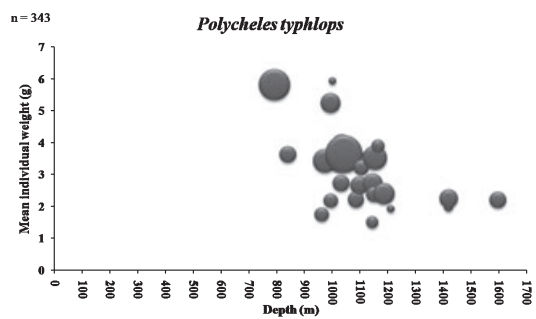
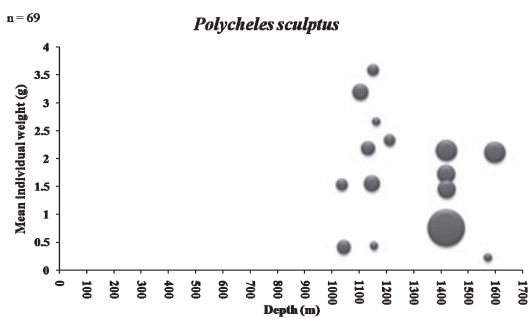
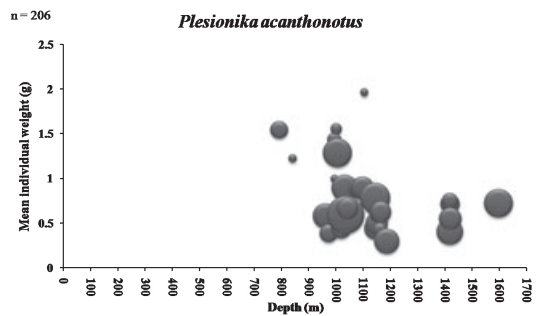
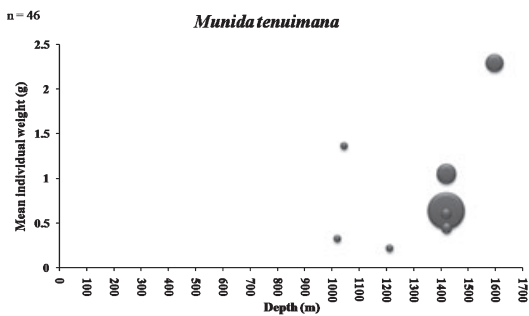
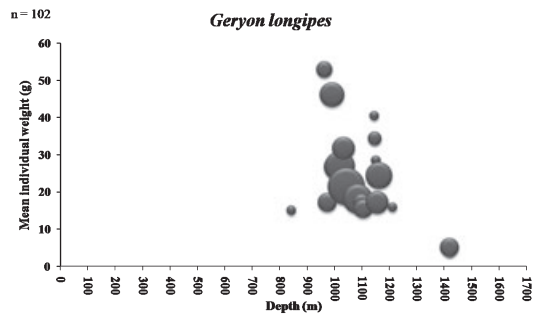
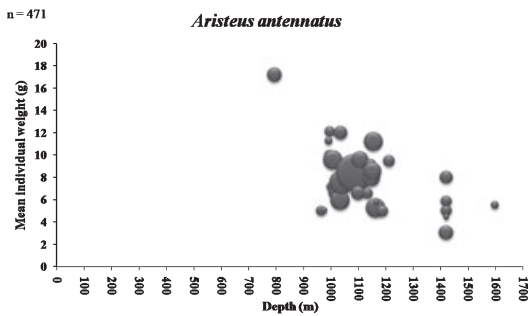
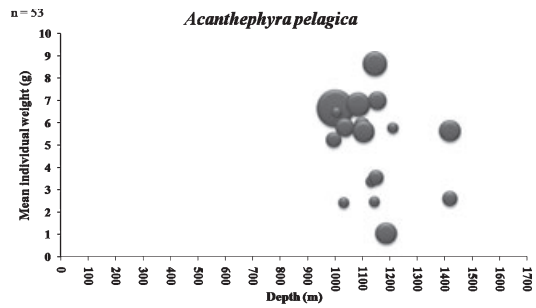
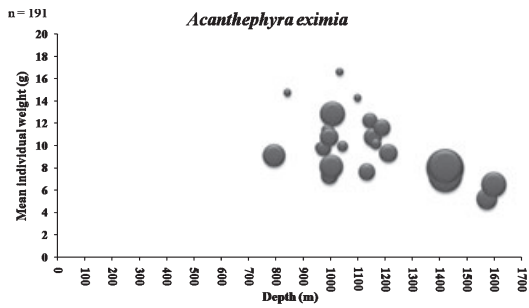
Our results confirm the importance of decapod crustaceans in Mediterranean deep-sea benthic communities because they may be more competitive than other invertebrate groups, in contrast to more productive oceans like the Atlantic (Tyler & Zibrowius 1992).

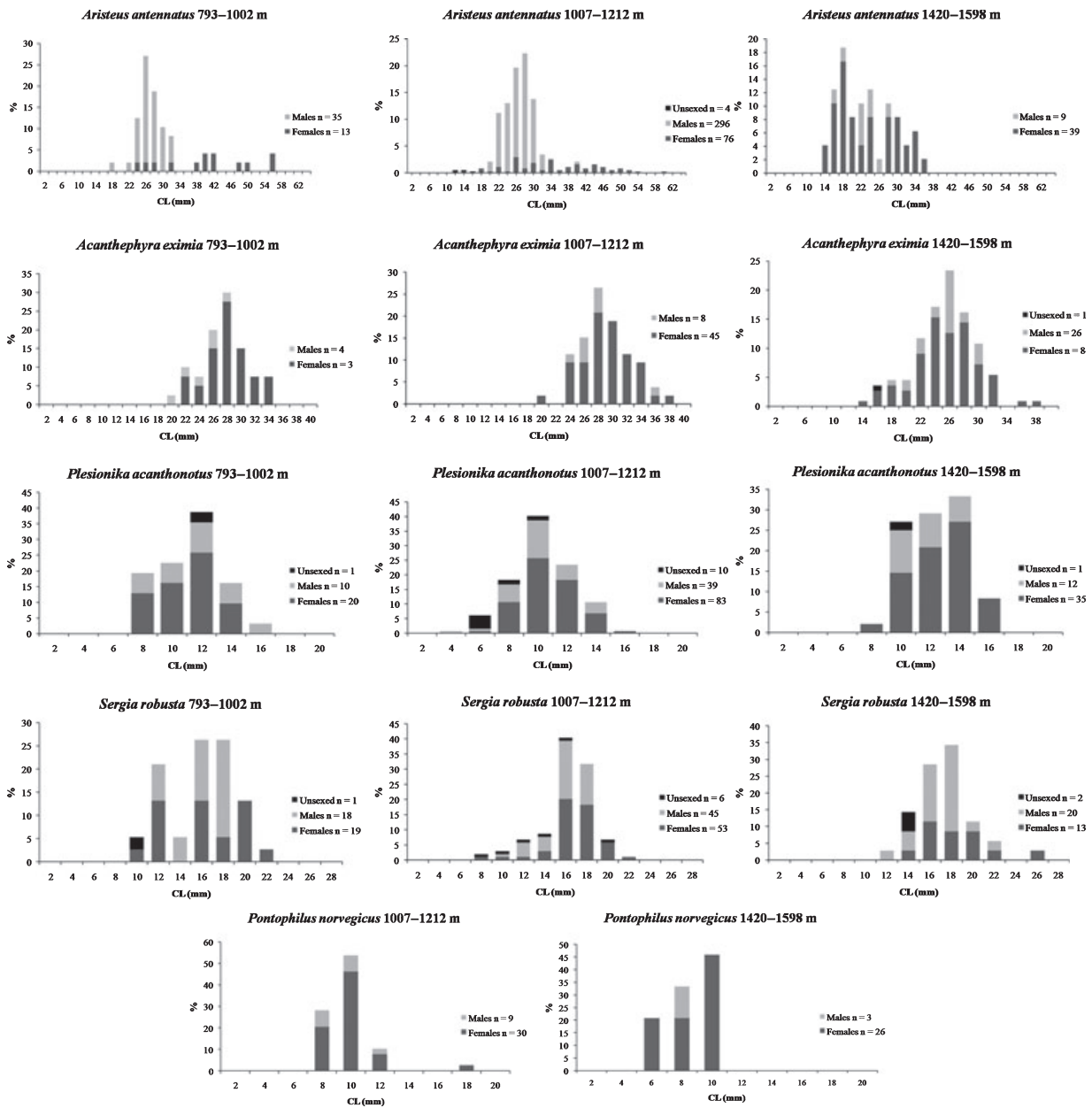
Depth represents the main structuring factor in many areas of the Mediterranean Sea (Abelló *et al.* 1988, 2002; Cartes & Sardà 1993; Ungaro *et al.* 1999; Kallianotis *et al.*

2000; Morales-Nin *et al.* 2003; Gaertner *et al.* 2005; Masuti & Reñones 2005; Abad *et al.* 2007; Fanelli *et al.* 2007), although it has often been argued that marine organisms may respond to a combination of depth-related factors such as food availability, light, temperature and pressure (Cartes *et al.* 2004). Trophic issues have often been used to explain community organisation at different spatial and temporal scales (Gage & Tyler 1991) and seasonally averaged phytoplankton pigment concentration has also been utilised to describe the organic vertical flux and food supply for demersal megafauna (Rex *et al.* 1993).

The bathyal decapod crustacean community of the South-Eastern Sardinian deep-waters (Central-Western Mediterranean) presented a clear zonation effect, with a series of well-defined bathymetric boundaries that seemed to be connected to depth-related factors. Three faunistic assemblages along the continental slope were identified by means of cluster analysis. The lowest values of species richness (S) were found in the upper and lower slope, which could be explained best by a decrease in food supply enhancing competitive exclusion of the species; the highest mean value of species richness was found in the mid-bathyal interval (1002–1212 m), probably implying low trophic pressure or diminished competition with fish (Maynou & Cartes 2000). This phenomenon, also reported in various taxa among macrofauna (*e.g.* gastropods; Rex 1973), generates a typical bell-shaped response along depth or other environmentally mediated gradients (Gage & Tyler 1991).

Following the feeding classification of Cartes (1998) for the main bathyal decapod species in the Catalan Sea, our study area showed in the upper and middle slope (between 793 and 1212 m), a prevalence of benthic (*P. typhlops*) and epibenthic–endobenthic feeders (mainly *A. antennatus* and *G. longipes*) that eat infaunal prey, with a low percentage of macroplankton–epibenthic feeders such as *A. eximia* and *P. acanthonotus*. In contrast, in the deepest stratum [the main boundary similar to the lower subzone defined by Pérès (1985), Cartes & Sardà (1993) and Stefanescu *et al.* (1993)] the most remarkable feature was the prevalence of macroplankton–epibenthic feeders (*A. eximia* and *P. acanthonotus*), followed by the benthic deep-sea lobster *P. sculptus*. A similar distribution of feeders in the continental slope was found by Maynou & Cartes (2000) off the South-West Balearic Islands (Western Mediterranean), where the distance from the mainland and the absence of submarine canyons justify the low values of superficial primary production and the consequent dominance of the macroplankton–epibenthic feeders. This result differs greatly from what was registered in the Catalan Sea (Cartes *et al.* 1994), where advective inputs of organic carbon via submarine canyons represent an additional contribution to deposit feeders and epibenthic–endobenthic feeders





**Fig. 5.** Size frequencies and sex distribution of any bathyal decapod species collected off South-Eastern Sardinian deep-waters (Central-Western Mediterranean).

(Maynou & Cartes 2000). This phenomenon, supporting the food availability and local geographic conditions as responsible for species distribution, also highlighted the presence of a remarkable west–east productivity gradient in the Mediterranean, probably mainly due to the variability

in the vertical fluxes of organic carbon to the sea floor (Danovaro *et al.* 1999). Many studies have compared the phytoplankton pigment concentrations (PPC) in the Mediterranean Sea, highlighting differences of an order of magnitude between the west and eastern basin, and con-

**Fig. 4.** Bubble plot showing the relationship between depth strata and mean individual weight (g) of the decapod crustacean species predominant off South-Eastern Sardinian deep-waters. N = number of analysed individuals. The diameter of the bubble is proportional to the number of individuals.

firming the increase of oligotrophy in the west *versus* east (Maynou & Cartes 2000; Cartes *et al.* 2004; Company *et al.* 2004; Tselepidis *et al.* 2004; Politou *et al.* 2005).

The decrease of total decapod biomass with depth has been established in oceans worldwide (Haedrich *et al.* 1980; Lampitt *et al.* 1986). The data available in the Mediterranean are consistent with the general decrease down to 2200 m in the Western Mediterranean (Cartes & Sardà 1992) and down to 1000 m in the Cretan Sea (Kallianotis *et al.* 2000). On the whole, this trend was confirmed for the South-Eastern Sardinian deep-waters, where the biomass showed a strong decrease in the deepest slope (1420–1598 m), probably due to the small size of specimens caught (principally *A. eximia*, *P. acanthonotus* and *P. sculptus*). The highest value of biomass was found between 1007 and 1212 m, due to the presence of big size species (*A. antennatus* and *G. longipes*).

The relationship between mean individual weight/size and depth has been the subject of a considerable number of studies in deep-sea biology, basically focused on fish (Stefanescu *et al.* 1992; Moranta *et al.* 2000, 2004; Morales-Nin *et al.* 2003) rather than on decapods (Polloni *et al.* 1979; Cartes & Sardà 1993; Morales-Nin *et al.* 2003; Company *et al.* 2004). In our study there was no general pattern of mean individual weight/size *versus* depth among decapods, and the changes seemed to be species-specific. *Aristeus antennatus*, *A. eximia* and *P. typhlops*, according to Abelló & Cartes (1992), Company (1995), Company *et al.* (2004), Sardà *et al.* (2004), Follesa *et al.* (2007) and Guijarro *et al.* (2008), showed a significant 'smaller deeper trend' (Stefanescu *et al.* 1992), with juvenile specimens mainly distributed in the deepest part of the continental slope (below 1420 m). Instead, a 'bigger-deeper pattern' was only found for *S. robusta* and *M. tenuimana*, which showed a progressive increase of size or mean individual weight below 1200 m, as reported by Morales-Nin *et al.* (2003) and Cartes *et al.* (2007b). The bigger-deeper pattern, described also for fish such as *Phycis blennoides* (Massutí *et al.* 1996), *Trachyrhynchus scabrus* (Massutí *et al.* 1995), *Lepidion lepidion* and *Mora moro* (Rotllant *et al.* 2002), has been attributed to the fact that the metabolic demands per unit weight of a large animal are less than for a small one (Haedrich *et al.* 1980). Therefore, in our results the simultaneous existence of 'smaller and bigger deeper trends' in the whole fauna highlighted the co-existence of small and large-size specimens at increasing depth. Fishery activity might also be considered a factor that could affect individual characteristics as mean size and species size structure (Mytilineou *et al.* 2001).

In conclusion, this study provides useful information about the composition, distribution and structure of bathyal decapod crustaceans in the Central-Western Mediterranean, considered the link between the western and

eastern basin. Further investigations should be devoted to increasing the bathymetric range of the research to improve knowledge of the Mediterranean Sea fauna.

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## John Gray (1941–2007): a tribute



John Stuart Gray was born in Bolsover (near Chesterfield, UK) in 1941 and went to Bangor University [then the University College of North Wales (UCNW), Bangor] in 1959. UCNW was then a very small but rapidly growing constituent college of the University of Wales, with a highly regarded Zoology department and a rather new Marine Biology Station (now incorporated into the School of Ocean Sciences) in a rather old building close by Menai Bridge pier. F. W. Rogers Brambell, the Professor of Zoology, was a mammalian embryologist but had the breadth of interest and foresight to recognize the marine biological potential of the Menai Strait and surrounding area. He ran a wonderful Easter Marine Vacation Course and had been instrumental in setting up the Marine Biology Station. With marine biologists contributing to teaching, it provided a splendid opportunity for aspiring marine biologists.

After graduating in Zoology (1962), John went to work under the supervision of Dennis Crisp at the Marine Biology Station. Crisp, with a background partly in physical chemistry, specialized in barnacles and larval settlement behaviour, and encouraged a numerical and experimental approach to ecological problems. Visitors to the Marine Biology Station commonly shared Crisp's particular interests, but in 1957 included Bertil and Martha Swedmark. Bertil was one of the founder researchers on meiofauna, who found the area to be excellent for it, and in the following year Crisp took on his first doctoral student in this field, Pat Boaden. John Gray followed in 1962. John is remembered by his contemporaries as a rather dashing young man, with a sports car (any car was unusual in those days!) and plenty of girlfriends; he enjoyed music and was also a good hockey player. Not allowing himself to be overly distracted by his social life, John achieved an

outstanding PhD thesis 'Behaviour and ecology of meso-sammic archiannelids', which was awarded the Zoological Society of London's T.H. Huxley Prize for the best thesis of 1965.

After finishing at Menai Bridge, John was appointed in 1965 to an Assistant Lectureship (Lectureship in 1977) at the Wellcome Marine Laboratory (part of Leeds University's Department of Zoology, and now closed) at Robin Hood's Bay, North Yorkshire. The then active laboratory was under the direction of Jack Lewis, well known as a rocky shore ecologist, and he and John had some outstanding graduate students in the late 1960s. John continued with his studies on meiofauna but expanded his interest to include the River Tees estuary (then with the unenviable reputation as Britain's most polluted river). John, as throughout his life, made regular working visits abroad but particularly to the Kristineberg Marine Laboratory in Sweden, where Swedmark was director. Kristineberg served as a special summer meeting place for several of the 1960s' generation of meiofauna workers, who regarded Bertil Swedmark as 'le Patron' in this research field. It was a great place for the exchange of ideas which helped push European meiofaunal studies forward on a broad front. It was at Kristineberg that John met Anita, who became his wife.

During the early 1960s. Otto Kinne had instituted annual marine biological conferences at the Biologische Anstalt, Helgoland. At first essentially German, Kinne felt the need to broaden the participation. So, following consultation with marine biologists in other European countries, the first European Marine Biology Symposium (EMBS) took place in Helgoland in September 1966, launching the successful format that continues to this day. For some relaxation it had been proposed to hold a light-hearted competition between the participating nations, but for what prize? It happened that during the summer of 1966 the Beatles had released an album containing the song (written for children!) 'The Yellow Submarine', which had rapidly gone to the top of the charts. When a few of the participants, including John, saw a toy, plastic yellow submarine in a local shop window, they had the inspiration to buy it for the prize. So was born the tradition of the Yellow Submarine competition, which has enlivened every EMBS for over 40 years. It also provides an insight into why John was not just a distinguished scientist but good company and a popular colleague and companion. Within the EMBS, John's contribution was recognized when he was elected President for the 1985–87 triennium.

John was awarded a DSc degree by the University of Wales in 1975. Then, the following year, he left Robin

Hood's Bay to take up the chair of Marine Biology and Zoology at Oslo University, where he remained until his premature death on 21st October 2007. He continued to work in various parts of the world, including the Antarctic (from his appointment in Norway he naturally developed an interest in polar ecosystems). On one Antarctic cruise, when gear failure interrupted the sampling programme, John used the time to draft out his book *The ecology of marine sediments: an introduction to the structure and function of benthic communities* published in 1981 (and subsequently translated into German by Heye Ruhmohr, and published as *Ökologie mariner Sedimente. Eine Einführung* in 1984). Not long after the book was published John became a member of the NATO working group on bacteria and bacterivory in the sea, which met in Bourbannes, France, in May 1982. The outcome of that was the famous MEPS paper by Farook Azam and five others, which introduced the concept of the 'microbial loop' in marine ecology. This highly cited paper is inevitably referred to as Azam *et al.*, which conceals the names of those, including John, who were the intellectual inspiration of this seminal work.

John's early work on meiofauna was among the first to establish the preference of meiofaunal species for particular substratum parameters, and his work with *Protodrilus* (one of the little polychaetes once known – misleadingly – as 'archiannelids') and sand bacteria was particularly innovative. John's other major interests included marine pollution. Among the characteristics of his work were his experimental approach (including his use of mesocosms at Oslo) and intellectual rigour in planning and in analysing data. The latter is exemplified by his use of the log-series and log-normal distribution functions in benthic and pollution studies. It had been known since the 1940s and 1950s that the diversity and abundances of species often provided a remarkably close fit to one or other of these distributions. John applied the log-normal as a means of demonstrating whether a fauna was impoverished but – as his papers show – he was meticulous in how it should be applied and critical of misuse.

John's view of science was geographically very broad, with visits to labs all over the world. He was a vigorous supporter of young scientists, and there are many worldwide who have reason to be grateful for his encouragement. He had a remarkable ability to spot a potentially significant area of research slightly ahead of everyone else (the microbial loop, statistical approach to pollution studies, high-latitude ecosystems, and marine biodiversity being examples) and a characteristically forthright approach to 'good' and 'poor' science. He served on the Marine Sciences committee of the Natural Environment Research Council (NERC) in the UK at a time when bio-

logical input seemed undervalued. Through his premature death, all of us (including the EMBS) have lost an inspirational colleague and wise counsellor, marine science has lost one of its most innovative and distinguished biologists, and many of us have lost a good friend. Many recall John and Anita as welcoming hosts. Typically, during the year of his illness, John not only fulfilled educational commitments overseas but revised the text of his book [now *Ecology of marine sediments: science to management* (November 2008)] to the state in which Mike Elliot could complete it and see it through the press. It will be a fitting legacy.

Pat Boaden, Andrew Clarke, Robin Gibson, Heye Rumohr, Bangor University alumni office and Leeds University have contributed information for this article.

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