VECTORS of Change in Oceans and Seas Marine Life, Impact on Economic Sectors

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VECTORS Overview

"VECTORS seeks to develop integrated, multidisciplinary research-based understanding that will contribute the information and knowledge required for addressing forthcoming requirements, policies and regulations across multiple sectors."

Marine life makes a substantial contribution to the economy and society of Europe. In reflection of this VECTORS is a substantial integrated EU funded project of 38 partner institutes and a budget of €16.33 million. It aims to elucidate the drivers, pressures and vectors that cause change in marine life, the mechanisms by which they do so, the impacts that they have on ecosystem structures and functioning, and on the economics of associated marine sectors and society. VECTORS will particularly focus on causes and consequences of invasive alien species, outbreak forming species, and changes in fish distribution and productivity. New and existing knowledge and insight will be synthesized and integrated to project changes in marine life, ecosystems and economies under future scenarios for adaptation and mitigation in the light of new technologies, fishing strategies and policy needs. VECTORS will evaluate current forms and mechanisms of marine governance in relation to the vectors of change. Based on its findings, VECTORS will provide solutions and tools for relevant stakeholders and policymakers, to be available for use during the lifetime of the project.

The project will address a complex array of interests comprising areas of concern for marine life, biodiversity, sectoral interests, regional seas, and academic disciplines and especially the interests of stakeholders. VECTORS will ensure that the links and interactions between all these areas of interest are explored, explained, modeled and communicated effectively to the relevant stakeholders. The VECTORS consortium is extremely experienced and genuinely multidisciplinary. It includes a mixture of natural scientists with knowledge of socio-economic aspects, and social scientists (environmental economists, policy and governance analysts and environmental law specialists) with interests in natural system functioning. VECTORS is therefore fully equipped to deliver the integrated interdisciplinary research required to achieve its objectives with maximal impact in the arenas of science, policy, management and society.

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Executive Summary

- Although there is a substantial body of evidence linking changes in biodiversity to changes in ecosystem functioning, research to test effects of specific invasive species is limited. Observational data can reveal associations between the arrival of invaders and changes in ecosystem structure and functioning (e.g. Deliverables 3.1.1 and 3.1.2). Experimental research is needed to directly assess causal links between invaders and particular changes in native assemblages and ecosystem processes.

- This Deliverable reports on a set of five experimental case studies completed in the VECTORS regional seas to test effects of selected invasive species on native ecosystems and, in some cases, to examine the extent to which those effects vary with the density of invaders and the environmental context.

- Studies focused on the impacts of invasive algae and mussels in the Mediterranean, Chinese mitten crabs in the North Sea and amphipods, mud crabs and cladocerans in the Baltic.

- Methods included field, laboratory and mesocosm-based experiments and response variables included changes in native assemblages, community interactions, food webs, trophic pathways, sediment stability and bioturbation.

- Findings are summarized in the Core Activity section and the work is reported in more detail in Appendices 1 to 5. Key findings include:
  
  - Invasive algae in the Mediterranean have greater impact on native assemblages in pristine areas than in areas affected by human activities. It is therefore recommended that management focus on preventing spread of the species to pristine areas.
  
  - In the Gulf of Oristano, Sardinia, the flow of Carbon from the first to the second trophic levels of the benthic food web is at risk from the invasion of the Asian mussel with potential consequences for the entire coastal food web.
  
  - Chinese mitten crabs maintain their bioturbatory activity at low temperatures, which may enable them to expand further northwards than previously considered likely. They burrow more deeply than the average depth of other species in the region and may cause changes in erosion, turbidity, nutrient release and siltation rates with possible impacts on fish spawning grounds.
  
  - The mechanism by which G. tigrinus replaces native amphipods in the Baltic is related to high fecundity and strong predation pressure of adult gammarids on native juveniles. Specifically, G. tigrinus reproduces earlier in the season, has more broods per year and matures faster and thereby Gammarus. tigrinus exerts strong predation pressure on the juveniles of native gammarids.
  
  - Mud crabs in the Baltic reduce biomasses of all bivalve and gastropod species regardless of sediment or vegetation type. The arrival of crabs in the Baltic marks a significant transition for the system.
  
  - Although the cladoceran Cercopagis pengoi has evidenced more than one abrupt shift in abundance since its invasion in the early 1990s and significantly modified cladoceran seasonal dynamics in general, its impact as a generalist predator on pelagic communities should be considered as more structural and functional rather than causing a major shift of the invaded ecosystems into a new regime.
  
  - The predation impact of C. pengoi on copepod nauplii, which are important food for small herring larvae, might be substantial, but is likely to be of relatively limited importance for the larval fish (as it occurs substantially later than the first-feeding herring larvae appear).
This work has drawn on information collated and reviewed in WP2.1 and Task 3.1.1. Its findings will feed directly into WPs3.2 and 4 and are relevant for WPs 3.3 and 5.

Introduction
Changes in biodiversity, including those due directly and indirectly to outbreaks of indigenous species, invasions by alien species and changes in distribution, can have major implications for the functioning of marine ecosystems (Stachowicz et al. 2007). Changes in ecosystem functioning can, in turn, impact strongly on services and direct economic benefits to society, such as productive fisheries, aquaculture and tourism (Worm et al 2006, Beaumont et al. 2007). For example, invasive gelatinous zooplankters such as *Mnemiopsis* may be changing foodwebs, reducing survival of larval fish and damaging fisheries (Piraino et al 2002).

In global terms, a substantial research effort is underway to develop a body of ecological theory to characterise and explain relationships between biodiversity and ecosystem functioning (Naeem et al. 2009). Much of this work has been done in terrestrial habitats, but research in marine systems has expanded in recent years (Stachowicz et al 2007, Solan et al. 2012). Much of the research in this field has been motivated by development of ecological concepts and theory rather than practical application. Study systems have been selected on the basis of tractability rather than relevance to society (Raffaelli 2006). This does not offer an intuitive, quantifiable link to ecosystem functioning and services which would provide clearer direction for scientific-based management of marine ecosystems.

Although extensive datasets are available establishing changes in biodiversity and ecosystem functioning coincident with the arrival of invasive species (e.g. see AquaNIS database (WP 2.1), Deliverables 3.1.1, 3.1.2), understanding the mechanisms by which such changes arise and linking them directly to the invader requires experimental research. In this deliverable, we report on a series of experimental case studies designed specifically to assess the impact of invasive species on native assemblages and ecosystem processes. The target species have been selected in consultation with WP2.1. Response variables include changes in biodiversity and trophic structure, as indicated by stable isotope analysis and functional measures such as sediment stability and bioturbation, which plays a key role in nutrient cycling. Most of the case studies are based in shallow coastal systems due to their tractability, productivity, vulnerability and strong socio-economic importance. Coastal ecosystems are also linked functionally to offshore systems, particularly in terms of the import and export of nutrients and organic material, as well as their role as nursery and breeding areas for important offshore species. Thus impacts to these systems can have far ranging effects for society and economy, such that the findings are of direct relevance to WPs3.2 and 3.3 as well as WP4.

In some cases, the studies have examined how the activities of invaders and their impacts vary with environmental context, e.g. in terms of temperature and existing human impacts. These findings will help in developing predictions of impacts under different scenarios of future change (WP5). By selecting prominent species, with high potential for ecosystem impacts with relevance to society, the work is also targeted towards maximal applicability to management and policy, e.g. by helping to inform priorities and approaches for management to minimise impacts of invasive species.
Core Activity

In the sections below, the rationale, methodology, results and recommendations are summarised for each experimental case study in turn. More extensive, detailed reports of the work are included as Appendices (whose numbers correspond to the numbers below).

Case study 1 - Invasive algae in the Tuscany Archipelago (UPISA)

This work was completed by Laura Tamburello, Elena Maggi, Lisandro Benedetti-Cecchi, Chiara Ravaglioli, L Rindi and Fabio Bulleri.

Exotic seaweeds severely threaten marine biodiversity. To promote a better understanding of their impacts on native assemblages, we experimentally evaluated whether and how the direction and magnitude of their effects varies in relation to local human pressures.

We compared the effects of the exotic alga, Caulerpa racemosa, on resident assemblages among locations exposed to different human pressures within the Tuscany Archipelago (Western Mediterranean Sea).

C. racemosa did not affect the structure of native assemblages in urban sites, where abiotic, stressful conditions likely limited the performance of both native and invasive species. In contrast, C. racemosa altered the structure of native assemblages at sites exposed to intermediate or low anthropogenic disturbance, where biotic interactions are presumably the driving force structuring native communities.

Hence, efforts for controlling the impacts of exotic seaweeds should be mostly directed to prevent their spread into relatively pristine areas.

Case study 2 - Invasive mussels in the Oristano Gulf (CNR-IAMC)

This work was completed by Serena Como and Paolo Magni.

In the marine environment, the introduction and spread of non-indigenous mussels may cause major modifications to native assemblages. Yet, how invasive mussels alter the trophic flow within the food web remains unknown.

We analysed the impacts of the Asian date mussel Arcuatula (=Musculista) sehnousia on native benthic assemblages and sedimentary features and, for the first time, on the transfer of carbon from suspended microalgae to native primary benthic consumers.

We compared benthic assemblages, sedimentary features and the carbon and nitrogen isotopic composition of deposit feeders (DF) and Sedimentary Organic Matter (SOM) in areas where the Asian mussel was present and those where it was absent in two coastal lagoons of the Gulf of Oristano system (Western coast of Sardinia, Italy).

In the laboratory, using the stable isotope enrichment approach, we compared the impacts of the Asian mussel on the amount of suspended microalgae taken up by target species such as the polychaete deposit feeder Cirrhiphormia tentaculata, to the impacts of the native clam Ruditapes decussatus.

The results showed that the Asian mussel did not affect benthic assemblages; neither did it change the isotopic signatures of SOM and benthic deposit feeders in the two lagoons of the Oristano gulf system that it invaded.
The results of our laboratory experiment revealed that the microalgae C-uptake of *C. tentaculata* was lower in the presence of the Asian mussel than in the presence of the native clam, indicating that the Asian mussel has a negative effect on the transfer of carbon from suspended microalgae to the deposit feeder.

The results also showed an interactive effect of the Asian mussel and the native clam on the microalgae C-uptake of *C. tentaculata*, indicating that the Asian mussel can also control the carbon transfer from suspended microalgae to deposit feeders indirectly through the interaction with clams.

We showed the C flow from the first to the second trophic levels of the benthic food web to be at risk from the invasion of the Asian mussel. The consequences on functioning of the entire coastal food webs should be taken into account.

**Case study 3 - Invasive Chinese mitten crabs in NE UK (USTAN)**

*This work was completed by Andrew Blight, David Paterson, Stephan Gollasch and Tasman Crowe (in collaboration with the National Oceanography Centre, University of Southampton; Natural History Museum, London; Royal Holloway, University of London and Newcastle University).*

*Eriocheir sinensis* (Chinese mitten crab) is an aggressive invader spreading around the UK coast. It is on the IUCN list of the 100 most damaging invasive species in the UK and was also included in the handbook of the most invasive alien species in Europe (Gollasch 2009). The mitten crab is known for its burrowing activity. Organisms which modify their physical environment and regulate the availability of resources for other species through burrowing activities and bioturbation are recognised as ecosystem engineers (Jones et al 1994). Bioturbators can have significant influences on ecosystem processes which can vary depending on a range of factors.

Targeted experiments were carried out to (a) examine the influence of the crabs at different densities on ecosystem functioning (sediment destabilisation, sediment erosion and primary production) in synthetic assemblages to reveal the effect on the ecosystem as the invader becomes established. Synthetic assemblages simulated densities of 17.6, 52.7 & 105.4 individuals m⁻² which represent mean and maximum possible densities observed in the wild and (b) to test how bioturbatory activity varies with temperature and size of the crabs.

Bioturbatory activity was examined for two size classes across three temperature levels. Fluorescent sediment profile imaging (f-SPI, Solan et al 2004) was used to quantify the bioturbatory activity of mitten crabs at 10°C, 15°C and 19°C. Replicate aquaria containing a single individual of *Eriocheir sinensis* (mean ± SD, n = 15: Size Class A, biomass = 8.85 ± 0.94 g, carapace length 26.77 ± 1.03 mm; Size Class B, biomass = 5.35 ± 0.39 g, carapace length 22.30 ± 0.49 mm) were assembled.

The experiments have shown:

1. There was a significant reduction in sediment stability (expressed as equivalent horizontal shear stress, Nm⁻²; Tolhurst et al 1999) of all crab treatments when compared to controls. Invaded habitats therefore have reduced resistance to sediment erosion.
2. There was no significant difference in the bioturbation depth profiles between the three temperature treatments, which suggests the mitten crabs are equally as active across the temperatures examined.
3. There was no significant effect of Size Class, however the presence of individuals of *Eriocheir sinensis* with larger biomass and carapace length (Size Class A) was responsible for slightly higher levels of particle redistribution relative to individuals with smaller biomass and carapace length (Size Class B).
4. The mean depth of vertical displacement of fluorescent particles initially placed on the surface of the sediment ranged from 0.621 cm (Size Class B) to 1.533 cm (Size Class A), the median depth ranged from 0.590 cm (Size Class B) to 1.510 cm (Size Class A), and the maximum depth ranged from 1.600 cm (Size Class B) to 3.265 cm (Size Class B).

5. The surface rugosity of the sediment-water interface (Surface Boundary Roughness) ranged from 0.970 cm to 2.960 cm.

6. The maximum penetration depth of fluorescent particles in experiments with larger *Eriocheir sinensis* is greater than the average observed for other species in the North Sea region (mean ± SD of 2.7 ± 2.3 cm; Teal et al. 2008). The implications are that this would increase turbidity, increase nutrient release from sediments and potentially increase siltation of gravel beds, including in fish spawning grounds.

7. Reduced sediment stability will impact invaded depositional habitats resulting in increased erosion and therefore changes in the ecology and dynamics, as well as the services provided by these ecosystems.

**Case study 4 - Invasive amphipods and mud crabs in the NE Baltic Sea (EMI-UT)**

This work was completed by Jonne Kotta, Holger Jänes, Kristjan Herkül, Külli Lokko, Helen Orav-Kotta, Kristiina Nurkse, Merli Pämoja, Thomas Wemberg, Ilmar Kotta and Ivan Kuprijanov

**A. Invasive amphipods**

The distribution of the invasive gammarid amphipod *Gammarus tigrinus* has rapidly expanded during the last decade in the northeastern Baltic Sea. Decrease in the diversity and density of native amphipods has been observed concurrent with the invasion of *G. tigrinus*.

Two mesocosm experiments (*in situ* enclosures and flow-through tanks) and numerical modeling were carried out to address the following questions:

- what are the mechanisms underlying the increase of *G. tigrinus* and decrease of native gammarids?
- what are the effects of *G. tigrinus* on local benthic communities?
- do the niche spaces and distribution of local and invasive gammarids coincide or diverge?

We found that the mechanism by which *G. tigrinus* replaces the native amphipods is related to high reproductivity of invasive species and strong predation pressure of adult gammarids on native juveniles. Specifically, *G. tigrinus* reproduces earlier in the season, has more broods per year and matures faster and thereby *G. tigrinus* exerts strong predation pressure on the juveniles of native gammarids.

Other than predation on juveniles of native gammarids, *G. tigrinus* had no specific effects on benthic communities.

*G. tigrinus* had smaller niche space (i.e. higher habitat specialization) than local gammarids and the overlap of distributions was larger among native gammarids than between native species and *G. tigrinus*. 


B. Invasive mud crabs (work additional to that planned in the proposal)

In the course of the VECTORS project, the North American mud crab *Rhithropanopeus harrisii* invaded into the northern parts of the Baltic Sea. This recent invasion has provided a unique opportunity to quantify rigorously the transition from no crab to crab domination in the Baltic Sea ecosystem.

Two mesocosm experiments (*in situ* enclosures and flow-through tanks) were carried out to address the following questions:

- what are the effects of *R. harrisii* on local benthic communities?
- do habitat characteristics modulate the effect of mud crab on benthic communities?

In the outdoor mesocosm experiments crabs increased the mortality of all bivalves and amphipods on soft sediment only. Crabs had no significant effects on invertebrates on mixed sediments. Crabs preferentially ate larger clams. Algae modulated the crabs’ impact on gammarids with significant effects being observed only on unvegetated soft sediment. Thus, algae and rocks provide mobile amphipods refuge from crab predation.

Mud crabs modified meiofaunal communities primarily on unvegetated soft bottom sediments. The effects were mostly due to changes in taxonomic composition rather than abundances. Nevertheless, the mud crab decreased the number of settling bivalves and increased the abundance of turbellarians.

In a field experiment, crabs significantly reduced benthic invertebrate biomass but had no effect on macroalgal biomass. Including all bivalve and gastropod species. The effect was the same regardless of sediment and/or vegetation type.

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<th>RESULTS</th>
<th>ADDED SPECIES</th>
<th>SOFT SEDIMENT</th>
<th>MIXED SEDIMENT</th>
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<td>Individuals per mesocosm</td>
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<tr>
<td>Macoma balthica</td>
<td>Unvegetated</td>
<td>Mortality ↑</td>
<td>Unvegetated</td>
</tr>
<tr>
<td></td>
<td>Chara horrida</td>
<td>Mortality ↑</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Length ↓</td>
<td></td>
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<tr>
<td>Cerastoderma glaucum</td>
<td>Unvegetated</td>
<td>Mortality ↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chara horrida</td>
<td>NO IMPACTS</td>
<td></td>
</tr>
<tr>
<td>Gammarus sp.</td>
<td>Unvegetated</td>
<td>Mortality ↑</td>
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<td></td>
<td>Chara horrida</td>
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Figure 1. Schematic diagram of the habitat-specific effects of mud crab on biomasses of native species in an outdoor mesocosm experiment.
These two case studies demonstrated that invasive alien species can have similar consequences for benthic communities regardless of the mechanisms involved. Both a non-selective predation of juvenile amphipods by *G. tigrinus* coupled with their high fecundity and a direct consumption of bivalves and gastropods by *R. harrisii* lead to dramatic shifts in species composition and domination structure of native communities. Consequently, when predicting future consequences of invasive species manipulative experiments are needed as correlative field data can not reveal mechanisms of impacts and thereby lack predictive power.

Figure 2. Schematic diagram showing direct and indirect effects of the invasive mud crab in the Baltic Sea ecosystem.

**Case study 5 - Invasive predatory cladoceran in the NE Baltic Sea (EMI-UT)**

This work was completed by Henn Ojaveer, Mart Simm and Maria Põllupüü

Multiple sources of field based evidence suggest that changes in zooplankton community composition are associated with invasion by pelagic invertebrate predators. It has been suggested that the invasive predatory cladoceran *Cercopagis pengoi* may affect local native zooplankton communities via direct predation, especially causing declines of small sized cladocerans and the dominant copepods. This suggestion has been supported by evidence of impacts of another predatory cladoceran of similar morphology and feeding strategy – *Bythotrephes longimanus*. However, very few laboratory experiments have been performed to investigate the underlying processes. In the current work we have studied the feeding habits of *C. pengoi* by offering various single and mixed prey diets, under laboratory conditions, at densities that largely correspond to the natural conditions.

**Methods**

- *Cercopagis pengoi* and its prey were obtained from Pärnu Bay (Gulf of Riga, Baltic Sea) and acclimatised in the laboratory before feeding trials began;
- Altogether 177 successful experiments were performed by using the following prey: *Balanus improvisus* larvae, *Bosmina* spp., nauplii and copepodids of *Eurytemora affinis* and *Acartia* spp.;
Experimental runs were performed with *C. pengoi* individuals which were free of attached detritus or other material and healthy, i.e., freely swimming during the 24-hours period prior to the experiment; Healthy prey, at densities largely corresponding to that in the field conditions, were used; *C. pengoi* individuals were visually checked at the beginning of the experiment to ensure that they were intact. Also, swimming behaviour of the predator was checked several times during the experiment and just prior to the termination of the trial; Experiments were conducted in 1.0-L vessels at the temperature of 20 ± 2 °C, allowing only diffuse overhead light; To prevent potential sedimentation of prey, vessels were gently stirred a few times during the experiment; The duration of experiments was usually eight hours; Experiments were terminated by adding formalin into all vessels. The contents of experimental vessels were concentrated by reverse filtration (using 60 μm mesh) and investigated under a dissecting microscope to assess prey mortality, as well as their condition.

**Results**

- It appeared that the invasive alien predatory cladoceran *Cercopagis pengoi* is an aggressive predator capable of consuming almost any provided prey, with the exception of newly born young being unable to consume copepodids, probably because of size limits and swimming capabilities.
- According to the estimates, one individual of *C. pengoi*, juvenile or adult, may consume on average as many as 9.9 *Bosmina* spp., 9.6 copepod *nauplii*, 8.1 *Balanus improvisus* larvae and 4.5 copepodids 12 h⁻¹.
- Copepod *nauplii* and the small-sized cladoceran *Bosmina* spp. were, in the case available, the major and preferred prey for *C. pengoi*.
- Higher densities of the small-sized prey generally resulted in elevated consumption rates, through predation on copepodids by both young and adult *C.*
- Copepod *nauplii* were highly preferred over copepodids.
- Out of the total 202 experiments performed, 91.8% were successful. The reasons for failure were: i) mortality and/or non-healthy condition of predator which became evident during the course of the experiment; ii) reproduction of the predator and/or prey during the experiment; iii) contamination of sample with other/additional prey during the pipetting.

**Recommendations**

- Significant interannual and seasonal abundance alterations of the selected small-sized zooplankton taxa, associated with *Cercopagis pengoi* invasion, are very likely to be caused by direct predation by this invasive pelagic predator;
- The predation impact of *C. pengoi* on copepod *nauplii*, which are an important food for small herring larvae, might be substantial, but it is likely to be of relatively limited importance for larval fish (as occurs substantially later than the appearance of the first-feeding herring larvae);
- Although *C. pengoi* has evidenced more than one abrupt shift in abundance since its invasion in the early 1990s and significantly modified cladoceran seasonal dynamics in general, its impact on pelagic communities should be considered as more structural and functional rather than causing a major shift of the invaded ecosystems into a new regime.

**References**
D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning


Tolhurst TJ et al 1999. Measuring the in situ Erosion Shear Stress of Intertidal Sediments with the Cohesive Strength Meter (CSM). *Estuarine, Coastal and Shelf Science*, **49**(2), 281-294

Appendices – full reports

Appendix 1

Case study 1 – Invasive algae in the Tuscany Archipelago

Variation in the impact of exotic seaweeds along gradients of anthropogenic disturbance: a meta-analysis and an experimental test

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Abstract

Aim Exotic macroalgae severely threaten marine biodiversity. To promote a better understanding of exotic impacts on native assemblages, we evaluated whether and how the direction and magnitude of exotic effects varies in relation to local human pressures.

Location Global and regional (coasts of Tuscany, North-Western Mediterranean Sea).

Methods Through a meta-regression, we globally estimated how the effects of exotic seaweeds on both native species and communities vary according to cumulative anthropogenic impact levels. In addition, by means of a field experiment, we compared the effects of the exotic alga, Caulerpa racemosa, on resident assemblages among locations exposed to different human pressures.

Results The relationship between human disturbance and exotic impact emerged only for a reduced number of variables examined. The effects of exotic seaweeds on the abundance of consumer species shifted from negative, in relatively intact environments, to neutral or slightly positive in locations heavily impacted by humans. Likewise, there was a trend for the effects of exotics on community and primary producer diversity to become less negative at degraded sites. Experimental results showed that C. racemosa did not affect the structure of native assemblages in urban sites. In contrast, C. racemosa altered the structure of native assemblages at sites exposed to intermediate or low anthropogenic disturbance.

Main conclusions Our results suggest that impacts of exotic species on native assemblages can be more negative in less degraded or pristine sites, where biotic interactions are presumably the driving force structuring native communities. On the other hand, minor to no effects were observed in heavily degraded environments, where abiotic, stressful conditions likely limited the performance of both native and invasive species. Hence, efforts for controlling the impacts of exotic seaweeds should be mostly directed to prevent their spread into relatively pristine areas.
Keywords biological invasions, seaweeds, meta-analysis, trophic level, effect size, urbanization, habitat degradation, Caulerpa racemosa, anthropogenic disturbance, benthic assemblage.

Introduction
Biological invasions represent a major threat to marine and terrestrial biodiversity (Mack et al. 2000, Halpern et al. 2007). Exotic species can severely impair the structure and functioning of natural ecosystems, with dramatic, ecological, social and economic consequences (Parker et al. 1999, Grosholz 2002). A large research effort has, thus, been produced to assess the role of resident community properties in regulating their susceptibility to invasion (Elton 1958, Levine and D’Antonio 1999, Stachowicz et al. 2002) and to identify which life-traits may confer exotics greater invasiveness (Mack et al. 2000).

Comparably less studies have attempted to assess generalities in the mechanisms determining the ecological impacts of exotics and, in particular, to identify exotic species traits or features of the recipient community that magnify the intensity of impacts (Ricciardi et al. 2013). The goal is extremely challenging, as the consequences of invasion by different species are highly variable in magnitude, direction of effect (either negative or positive, e.g. Crain and Bertness 2006, Parker et al. 1999) and underlying mechanisms (Levine et al. 2003). Even when considering a single exotic species, impacts can vary greatly across space and time (Jokela and Ricciardi 2008, Strayer et al. 2011), as the abundance and performance of the exotic are modulated by local biotic and abiotic conditions (Jokela and Ricciardi 2008, Neira et al. 2005). Yet, to date, there is a paucity of studies that explicitly examine how the impact of an exotic varies in relation to environmental factors, including the resident biota, resource levels or abiotic conditions (Thomsen et al. 2011, but see Ceccherelli and Sechi 2002, Piazzi et al. 2005, Dassonville et al. 2008, Richardson et al. 2012).

According to the definition of ecological impact proposed by Parker et al. (1999), the effect of an exotic on native species or communities is proportional to its total abundance, calculated as the product of its range of distribution and its average abundance per unit area. Thus, mechanistic hypotheses predicting the success of an invasion are, to some degree, capable also to explain variations in its ecological consequences (Ricciardi et al. 2013). For example, a positive relationship between disturbance and ecosystem invasibility has been widely documented (Hobbs and Huenneke 1992, Bulleri et al. 2011a, Tamburello et al. 2014), suggesting that more degraded habitats are likely to experience stronger impacts (Ricciardi et al. 2013). Conversely, more pristine habitats inhabited by species-rich assemblages, according to the biotic resistance hypothesis, should be more resistant to invasion, likely experiencing weaker impacts than highly invaded species-poor assemblages (Maron and Marler 2008, Carey and Wahl 2010).

Yet, the magnitude of impacts is often non-linearly proportional to exotic abundance (Yokomizo et al. 2009). According to the "interaction modification effect" proposed by Didham et al. (2007), habitat-modifying stressors can interact with the exotics and generate impacts disproportionate to their abundance, due to alterations of the interaction strength between native and invasive species. Thus, the effects of an exotic colonizing a new ecosystem depend not only on its invasive success, but also on the direction and intensity of its interaction with the native assemblage, which are strongly modulated by environmental conditions.

Climatic and environmental conditions can drive variations in the strength and nature of biological interactions, including competition, grazing and predation (Menge and Lubchenco 1981, Bertness and Ewanchuk 2002, Pennings and Stillman 2005). Community assembly and structure are, in fact, more likely to be regulated by physical factors in harsh environments, with the strength of biotic interactions increasing in more benign environments (Connell 1961). In addition, the direction and magnitude of species interactions is predicted to vary along gradients of abiotic stress, with negative interactions being more frequent and intense under physically
benign conditions (Bertness and Callaway 1994). This tenet has been tested in the framework of biological invasions, showing that interactions between exotic and native plants can switch from competitive to facilitative along a gradient of increasing environmental harshness (MacDougall et al. 2006, Von Holle 2013).

Here, we explore how the effects of exotics on the resident assemblage vary across gradients of anthropogenic stress. Exotic species can be expected to strongly affect native assemblages in pristine habitats which, due to favorable environmental conditions, are mainly structured by negative interactions (Connell 1961, Bertness & Callaway 1994). In contrast, weaker impacts are expected in degraded habitats, where physical conditions would limit the performance of both native and invasive species. Alternatively, if the impacts of exotic species are proportional to their invasive success, we can expect that anthropogenically stressed areas would show stronger impacts of the exotics, when compared to pristine, more diverse assemblages (Richardson et al. 2013).

We focused on the effects of exotic seaweeds, a taxonomical group that enumerate a rich literature of correlative and experimental studies estimating the effects of invasions worldwide (Smith and Williams 2007, Thomsen et al. 2009). We tested our predictions 1) at a global scale through a meta-analytical approach and 2) on a regional scale, by means of a field experiment. We evaluated whether and how the direction and magnitude of effects of several exotic species varies in relation to local human pressures. While the effects of exotics on primary producers are mainly negative and driven by competitive interactions, effects on consumers can vary from negative to positive and include several different mechanisms, such as habitat or food provision (Vilà et al. 2011, Thomsen et al. 2014). Therefore, we estimated separate relationships to compare the effects of exotics on native primary producers to those exerted on native consumers (i.e., across trophic levels).

In addition, we tested these hypotheses experimentally, by comparing the effects of the exotic green alga, Caulerpa racemosa, on resident assemblages among locations exposed to different human pressures (namely urban, extra-urban and pristine sites).

Methods

Meta-analysis

Literature search and inclusion criteria
The ‘ISI Web of Science’ database was searched in June 2012, for relevant literature, with no restriction on publication year, using the search term combinations reported in Annex 1 in Supporting Information. Reference lists from all the retrieved articles were then screened to identify further relevant publications. Evaluation of references obtained through the search was performed through a 3-step process: 1) scanning of article titles, 2) reading of the abstract and 3) reading of full text. We included studies that quantitatively compared relevant response variables (see below) between invaded vs. non-invaded units (with the term ‘unit’ meaning organisms / individual / plots / treatments / areas / sites / locations / regions), invaded vs. exotic removal units or control (no exotic) vs. exotic transplanted units. This resulted in an initial set of 144 papers that were evaluated against the following criteria:
1) Papers suffering lack of replication or appropriate controls were excluded. Determining whether replication and choice of controls had been carried out appropriately was somewhat subjective. We evaluated whether controls and invaded units were sufficiently similar but spatially and temporally independent.
2) Studies were excluded when the manipulation (generally the removal) of the exotic seaweed was not carried out independently from that of native species.
3) Studies were excluded when non-invaded sites were characterized by the presence of other exotic species.
4) Studies were excluded when variations in response variables could not be unambiguously interpreted as positive or negative effects (e.g., relative proportion of individuals exhibiting a certain coloring or behavior).
5) In the case of correlative studies reporting time series, the first and last time of sampling were considered, in order to account for variation through time. In contrast, for experimental studies, only the last time of sampling was extracted, assuming that the effects of experimental manipulations (removal or addition of the exotic) were more likely to manifest on the longer temporal scale (Strayer et al. 2006).
6) When data could not be directly extracted from papers, the authors of the original study were asked to provide either raw data or relevant information (e.g. means, standard deviation/variance, sample size). Studies were not included when this procedure did not allow us to obtain estimates of variation in the effect sizes, necessary for weighted analyses.

**Data extraction and response ratios**

We extracted means, measures of variability (standard errors, standard deviations, confidence limits) and sample sizes for units where the invasive species was present or absent. Data extraction from graphs was carried out by means of the image analysis software ImageJ (Schneider et al. 2012). When data could not be directly extracted from papers, the authors of the original study were asked to provide either raw data or relevant information (e.g. means, standard deviation/variance, sample size). We retained variables accounting for the response to invasion of either single species or communities. Our operative definition of community includes the presence of more than one species or any taxonomic group higher than species, including morphological or functional groups. We analyzed data on variables related to abundance, biomass, growth and survival of single species and abundance, biomass, diversity and evenness of communities (Table S1 in Annex S1). A total of 96 studies were finally included in the analyses (see Annex S1 for the complete list of retained papers). All variables were analyzed separating native primary producers (macroalgae and phanerogams) from consumers, including all animal species. In addition, we extracted geographical coordinates of the location where the study was conducted. For each study site, we extracted the cumulative human impact score estimated by Halpern et al. (2008) with ArcGIS 10.1. This score combines 17 anthropogenic drivers into a single comparable estimate of cumulative human impact on 20 ecosystem types across the entire globe. Several shortcomings of this index have been identified: for example, regions with overlapping human activities cannot spatially match up with areas of major impact (Heath 2008) or the impact score cannot account for interactive effects that frequently display when multiple stressors co-occur (Crain et al. 2008). However, to date it represents the only synthetic measure of human impact available for comparisons between sites at a global scale.

We calculated Hedges’ $g^*$, which measures the unbiased, standardized mean difference between invaded and non-invaded means (Borenstein et al. 2009). Negative and positive Hedges’ $g^*$ values indicate negative and positive effects of exotic species on native communities or species, respectively. Hedges’ $g^*$ was calculated as:

$$ g^* = g/J $$

where

$$ g = \frac{x_{\text{invaded}} - x_{\text{Not invaded}}}{SD_{\text{pooled}}} $$

and

$$ J = 1 - \frac{3}{4(n_{\text{invaded}} + n_{\text{Not invaded}} - 2)} $$

$$ SD_{\text{pooled}} = \sqrt{\frac{(n_{\text{invaded}} - 1)SD_{\text{invaded}}^2 + (n_{\text{Not invaded}} - 1)SD_{\text{Not invaded}}^2}{n_{\text{invaded}} + n_{\text{Not invaded}} - 2}} $$

Effect sizes were estimated from the dataset and its subsets (experimental data only) by means of a random-effect model (Borenstein et al. 2009). Analyses were performed including all the data available in the dataset, for experimental data only and then separately for primary producers and consumers, including multiple estimates from each study, when available. However, this procedure does not take into account potential autocorrelation among observations within studies, thus violating the assumption of independence in the data (Borenstein et al. 2009). In addition, when computing summary effects across studies, it assigns more weight to studies with multiple outcomes. One approach to solve this issue is to average within studies across sources of independence (e.g.
multiple sites, different times of sampling, comparison of the same invasive species with several native species, etc.) in order to generate one single effect size per response variable per study. However, unless the degree of autocorrelation among observations being averaged is known and explicitly incorporated into calculations, the estimates of variances associated to the means are based on the assumption of a zero correlation (Borenstein et al. 2009). This is likely to lead to over- or under-estimation of variance and underestimation of the precision of the difference (Borenstein et al. 2009). An alternative approach would be that of extracting at random a single test for each study. The drawback of this approach is that of losing a large amount of information. For this reason, following Gibson et al. (2011) and He et al. (2013), median effect sizes and 95% confidence intervals were calculated by resampling one observation per publication, using 10000 bootstrap samples, generated with replacement. To evaluate whether the impact of exotic seaweeds varies according to environmental conditions, effect size on the different native community and species response variables were regressed against Halpern’s cumulative human impact score.

The null hypothesis (slope of the regression equal to zero) was tested through a meta-regression based on the Q statistic, a weighted sum of squares following a $\chi^2$ distribution which describes variation in the effect size across environmental conditions (Borenstein et al. 2009, Viechtbauer 2010). The slope was deemed as significantly different from zero when the median $Q_M$, generated by data permutations, exceed the critical value (corresponding to $\alpha = 0.05$) obtained from the null distribution generated by permutations with re-shuffling of labels. The exact significant level was estimated as the number of times out of 10000 permutations in which the median $Q_m$ was smaller than the critical $Q$ value from the null distribution (i.e., $(Q_{MNull} > medianQ_M)/10000$).

Tests for publication bias
To assess publication bias we visually examined funnel plots of effect size standard errors against residuals (based on mixed-model effect size calculations) and tested their asymmetry through a rank correlation test (Viechtbauer 2010). Asymmetry in funnel plots emerged for community abundance ($\tau=-0.21$, $P<0.0001$) and diversity ($\tau=-0.34$, $P<0.01$), but not for the other variables (Table S2, Figure S1). Asymmetry emerged as a consequence of some data with large residual values and high variances. Following He et al. (2013), these data were removed to adjust the publication bias (Figure S1). Adjusting for publication bias did not change the outcomes of the analyses on both native community abundance and diversity, suggesting that publication bias was not severe. Analyses were conducted using the metafor package (Viechtbauer 2010), in R v2.15 (R Development Core Team 2013).

Experimental study

Study site
According to prevailing human activities, we identified three levels of decreasing human pressure along the coasts of Tuscany (North-Western Mediterranean Sea): 1) urban reefs, 2) extra-urban reefs, and 3) relatively pristine sites on islands (Tamburello et al. 2012). Two locations were randomly selected for each of these conditions (Fig. S3 in Annex S3).

Urban reefs were sampled in front of the cities of Livorno and Rosignano Solvay, which are located about 25 km apart along the coast of Tuscany and are considered pollution hotspots in European coastal waters (OECD 2002). Livorno has about 160000 inhabitants and hosts an oil refinery and one of the largest commercial ports of NW Italy. Rosignano Solvay is a smaller urban area, but industrial plants for the production of baking soda have been operating in the area since 1914.
Extra-urban areas (Quercianella and Fortullino) were selected at least 8 km far away from main sources of pollution and disturbance. These locations are characterized by lower urbanization and lower values of organic, nutrient and inorganic pollution indexes (Tamburello et al. 2012). Pristine locations (Capraia and Capo S. Andrea on Elba Island, respectively 27.8 and 32 km from the coast), having the majority of the territory occupied by natural parks and low population densities, are nearly free from organic, inorganic and nutrient pollution. Detailed descriptions of native assemblages and of patterns of distribution of the exotic alga C. racemosa in the region are reported in Bulleri et al. (2011a) and Tamburello et al. (2012).

Experimental design
In June 2012, twenty 20x20 cm plots were marked with epoxy putty (Veneziani S subcoat) in each of the 6 experimental locations (i.e. urban, extra-urban and pristine environments). Five plots were randomly assigned to each of the following treatments: 1) removal of C. racemosa from extant assemblages, 2) total removal of extant assemblages preventing the re-invasion by C. racemosa, 3) total removal of extant assemblages allowing the re-invasion by C. racemosa and 4) controls invaded by C. racemosa.

Our experimental design included a treatment of mechanical disturbance, applied once at the beginning of the experiment, which consisted of the removal of all erect organisms with a metal brushe. In degraded systems, the concomitant action of human stressors and invasive species can trigger shifts to alternative ecosystem states, which become dominated by stress-tolerant species. Although the exotic can act just as a passenger, not representing the primary driver of the shift, it can facilitate the persistence and dominance of specific native species. In these "alternative ecosystem states", the removal of the exotic may be not sufficient to trigger the recovery of the original native assemblage. A disturbance removing both the exotic and a significant amount of extant biomass has been, in contrast, shown to promote the effective recovery of native populations, allowing a better estimate of the impact of invasion (Bulleri et al. 2010).

Re-colonization of plots by C. racemosa was prevented by manually removing the exotic fortnightly, paying attention not to damage native macroalgae. On these occasions, we visually sampled the percentage cover of C. racemosa in the experimental plots, to assess the effectiveness of manual removal treatment (Fig. S4 in Annex S3).

Experimental plots were visually sampled after 15 months, in September 2013, during the seasonal peak of maximum abundance of C. racemosa. The percentage cover of macroalgae and sessile invertebrates was visually estimated by superimposing a grid of 25 sub-quadrats onto each plot. A score from 0 to 4% was given to each taxon or morphological group in each sub-quadrat and the percentage cover was obtained by summing over the entire set of subquadrats.

Statistical analyses
Variations in the effects of C. racemosa on extant and recovering assemblages among areas subjected to different human influences were tested by means of a permutational multivariate analysis of variance (PERMANOVA, Anderson 2001), performed on a Bray-Curtis dissimilarity matrix of untrasformed data. Pairwise a posteriori comparisons were performed to assess differences among factor levels. The model included 4 factors: Condition (fixed, with 3 levels: Urban, Extra-Urban, Pristine), Location (random, nested within Condition, with 2 levels), Disturbance (fixed, with 2 levels: Disturbed and Undisturbed) and C. racemosa (fixed, with 2 levels: Present and Removed). In order to account for variations in exotic abundance among locations, this analysis was repeated including C. racemosa cover in each plot as a covariate. Since C. racemosa cover did not significantly contribute to explain variability in assemblage structure, the analysis is not reported. Permutational analysis of multivariate dispersion (PERMDISP, Anderson 2006) and pairwise a posteriori comparisons were performed to evaluate
whether *C. racemosa* caused, among other effects, differences in dispersion and, hence, heterogeneity of assemblages.

Species richness and the percentage cover of the most abundant species or morphological groups were analyzed using a 4-factor ANOVAs, with the same design described for PERMANOVA. Cochran’s C-test (Winer 1971, Underwood 1997) was used to check for the homogeneity of variances and, when necessary, data were log- or square root transformed. Pooling procedures were also used when appropriate, according to Winer (1971). Student-Newman-Keuls (SNK) tests were used for *a posteriori* comparison of the means.

**Results**

**Meta-analysis**

There was no significant relationship between Halpern’s cumulative human impact index and the effect size of non-indigenous seaweeds on community abundance, biomass, diversity or evenness (Tables S3-S4 in Annex S2). However, confidence intervals of positive median slopes of the relationship between Halpern’s index and community diversity did not overlap zero when all (Median=0.02, CI=0.003/0.03), experimental (Median=0.01, CI=0.01/0.03) or primary producers only data (Median=0.03, CI=0.003/0.05) were analyzed (Fig. 1A-C, Tables S3-S4), suggesting that the negative effects of exotics on community diversity are reduced at increasing levels of human impact. The results of the analyses on community abundance and diversity data did not change after correcting for publication bias.

There was no relationship between Halpern’s index and the effects of seaweeds on species biomass, growth and survival (Table S5). In contrast, there was a significant positive relationship between Halpern’s index and the effects of seaweeds on the abundance of consumer species (*P* = 0.037) (Table S6). The effect size shifted from negative at low values of Halpern’s index, that is in environments little impacted by human activities, to slightly positive values when the index reached values at the upper end of the gradient, that is in heavily impacted environments (Fig 1D).

**Experimental study**

The effect of *C. racemosa* on the structure of native assemblages varied across the gradient of anthropogenic stress (Table 1). Invaded and uninvaded assemblages differed in extra-urban sites, where the invasion by *C. racemosa* promoted greater heterogeneity between plots (average dissimilarity with *C. racemosa* present = 45.23 ± 1.48, without *C. racemosa* = 36.69 ± 2.61) (Table 1). Although no net effect of invasion was detected in urban or pristine sites, the structure of the assemblages became more different between the two conditions following the removal of *C. racemosa* (Table 1). Yet, the removal of *C. racemosa* did not affect either the heterogeneity among replicates at pristine and urban sites (i.e., deviation from centroids in invaded vs. uninvaded plots, within each condition), or the difference in dispersion between the two conditions (i.e., deviation from centroids in urban vs. pristine sites, for invaded and uninvaded plots) (Table 1).

*C. racemosa* did not affect total species richness, but it impacted the most abundant macroalgal groups (Table 2). At pristine sites, *C. racemosa* reduced the cover of articulated corallines to values comparable to those observed in extra-urban assemblages (Table 2, Fig. 2). Also, *C. racemosa* had a negative effect on filamentous algae at extra-urban sites, reducing their cover to values comparable to those observed in pristine sites (Table 2, Fig. 2). There was no difference in the cover of encrusting corallines between invaded and uninvaded extant assemblages. However, following the disturbance, encrusting corallines were positively affected by the seaweed at extra-urban sites (Table 2, Fig. 2). Finally, *C. racemosa* reduced the abundance of canopy forming-species (mean cover with *C. racemosa* present = 10.91 ± 2.51, without *C. racemosa* = 7.95 ± 1.85).
Discussion

The effects of exotic seaweeds on the abundance of consumer species shifted from negative, in relatively intact environments, to neutral or slightly positive in locations heavily impacted by human activities. In addition, there was a trend for the effects of exotic seaweeds on community diversity to become less negative when moving from relatively pristine to heavily disturbed environments. This trend persisted also when only experimental data were analyzed and was mostly due to the response of primary producers.

These results only partially support our hypothesis that the severity of effects of exotic species may be negatively related to environmental disturbance. In fact, such a relationship emerged only for a reduced number of the response variables examined. This may indicate that the degree of environmental degradation influences some ecological processes, but not others. For instance, the effects of seaweeds on community biomass and abundance did not vary along the disturbance gradient, suggesting that loss of biomass due to species loss in more pristine environments could be compensated for by enhanced abundance of invader-tolerant species. In addition, variations in the impacts of seaweeds along a human disturbance gradient would be more marked for primary producers than consumers. This pattern is consistent with the results of two recent meta-analyses suggesting that, in general, effects of plant invaders are more negative on communities at the same than at higher trophic levels (Thomsen et al. 2014, Maggi et al. in press).

The seminal work of Connell (1961) showed that the lower limit of distribution of barnacles in rocky intertidal systems was strongly regulated by inter-specific competition, whilst the upper distribution limit was determined by severe physical conditions (e.g. extreme heat and desiccation). Ecologists have tested the generality of this “environmental stress model” in different terrestrial plant assemblages (Kadmon 1995, Huckle et al. 2002, Brose and Tielborger 2005) and identified a variety of potential stressors, including environmental conditions, resource availability and grazing pressure (He et al. 2013 and references therein). In addition, the validity of the environmental stress model is not limited to interactions between species at the same trophic level, but there is empirical evidence suggesting that also herbivore-plant interactions can shift from negative to positive under severe environmental conditions (Daleo and Iribarne 2009). Our analysis extends this evidence to the context of biological invasions, documenting a tendency of exotic species to exert more negative and intense effects on primary producers and native consumers in benign environmental conditions.

Negative effects of exotics on the diversity of native macroalgae and phanerogams showed a tendency to reduce in intensity along the disturbance gradient. This result is in accordance with previous findings from an experimental study on plant interactions by Richardson et al. (2012), documenting variations in community diversity along a latitudinal gradient due to a weakening of competitive interactions and the onset of facilitative effects under increasing environmental harshness. A similar shift from negative to slightly positive impacts of exotic seaweeds under stressful environmental conditions was observed on consumer’s abundance. In contrast to plant-plant interactions, which can shift from competitive to facilitative, interactions between an exotic plant and the animal assemblage include a wider window of mechanisms of action, likely responsible for the high variability in direction and intensity of effects documented by previous meta-analysis (Maggi E. et al. submitted, Thomsen et al. 2014). Exotic seaweeds can exert direct positive effects on animals, by providing habitat/refuge from predation or a palatable food resource (Siddon and Witman 2004, Schmidt and Scheibling 2007), or negative direct effects, through the production of toxic chemical compounds (Box et al. 2009, Terlizzi et al. 2011) or food of low nutritional value (Scheibling and Anthony 2001, Tomas et al. 2011). Also, exotic seaweeds can indirectly exert negative effects, by reducing habitat complexity or food availability to native animals (i.e. grazers and detritivores) through reduction in native seaweed biomass or diversity, or by decreasing herbivores availability to their predators. In
highly degraded systems, where the structure of native assemblages is simplified and dominated by few, stress-
tolerant species, positive effects on animal species would prevail over negative interactions, as the exotic species
will represent an additional resource of food or habitat for native consumers.

Experimental results confirmed, to some extent, the patterns shown by the meta-analysis. The exotic species, C.
racemosa, did not affect the structure of native assemblages in highly degraded urban sites. On the contrary, C.
racemosa altered the structure of native assemblages in sites exposed to intermediate or low anthropogenic
disturbance (extra-urban and pristine sites). Although no net effect of invasion was detected, assemblages at
pristine sites became more dissimilar to urban, highly degraded habitats, following the removal of C. racemosa.

The lack of effects at urban sites suggests that C. racemosa would act as a passenger rather than a driver of
change at degraded sites (MacDougall and Turkington 2005, Bulleri et al. 2010). The “passenger” model predicts
that exotic, stress-tolerant species can take advantage of habitat degradation and become dominant in weakly
interactive communities, where environmental stressors highly limit native species (MacDougall and Turkington
2005). In this context, alterations of native assemblages are directly driven by abiotic stressors and the exotic itself
exerts little or no effect on the structure or diversity of native assemblages, as evidenced by several experimental

Sites exposed to different levels of anthropogenic stress selected in our study nearly coincided with areas
investigated in a previous survey, aimed at estimating patterns of distribution of C. racemosa (Bulleri et al. 2011).
The exotic seaweed was more widely spread in urban areas compared to extra-urban or off-shore reefs,
evidencing a positive effect of habitat degradation on invasion success (Bulleri et al. 2011). However, our results
underline that a better performance (i.e., greater abundance) of the invader does not coincide with stronger
negative impacts. Not only exotic abundance, but also the direction and intensity of interactions between the exotic
and the native assemblages seems to determine the occurrence or lack of invasion effects. Thus, models capable
to predict the success of invasion may prove ineffective in predicting the ecological effects of exotics.

Native assemblages in extra-urban and pristine sites were affected by the invasion of C. racemosa. This seaweed
caused the decline of canopy-forming species, Cystoseira spp. and Halopithys incurva, respectively dominating
pristine and extra-urban assemblages. Direct inhibition of growth of the native canopies has been observed as a
consequence of shading by Sargassum muticum, an exotic canopy-forming species of large size (Britton-Simmons
2004). More frequently, exotic species have been shown to prevent the recovery of juvenile native canopies after a
racemosa might have inhibited the recruitment of native canopy-forming species, by reducing substratum and
resource availability.

C. racemosa had a negative effect on filamentous algae and articulated corallines, respectively in extra-urban and
pristine sites, likely due to its competitive superiority in exploiting available resources and space. On the contrary,
C. racemosa had a positive effect on the cover of encrusting corallines in extra-urban sites. In the presence of the
exotic, encrusting corallines efficiently recolonized bare substratum after disturbance, likely taking advantage of
the reduced growth of filamentous algae. Although encrusting algae have been described as poor competitors for
space (Breitburg 1984), they are highly tolerant to overgrowth by erect algal forms (i.e. C. racemosa) and to
sediment burial (Bulleri 2006). C. racemosa creates a mat of stolons that trap and retain sediments, generating
unfavorable physical conditions for other erect macroalgae (Piazza et al. 2007), but less so for encrusting
corallines.
In summary, our results suggest that environmental degradation due to human activities is likely to modulate the direction and magnitude of the impacts of exotic species on native assemblages. In contrast to other anthropogenic sources of stress (Crain et al. 2008), invasion impacts seem not to interact synergistically with the effects of human-generated abiotic stress. Indeed, impacts are more negative in less degraded or pristine sites, where biotic interactions are presumably the driving force structuring native communities. Hence, efforts for controlling the impacts of exotic seaweeds should be mostly directed to prevent their spread into relatively pristine areas. An effective way of achieving this goal could be that of preserving the integrity of extant communities. On the other hand, efforts to control non-indigenous seaweeds in heavily degraded environments may be not warranted.

**Literature**


22


Table 1. PERMANOVA, PERMDISP and pairwise tests, comparing assemblages in plots exposed to different combinations of experimental treatment (invaded or uninvaded by *Caulerpa racemosa*, after a disturbance event or in mature assemblages) in different locations across a gradient of human conditions (i.e. urban, extra-urban and pristine sites). * P < 0.05, ** P < 0.01

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<th>Pseudo-F</th>
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Pairwise test between conditions

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<td>Extra-urban</td>
<td>Invaded vs. Uninvaded</td>
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<tr>
<td>Islands</td>
<td>Invaded vs. Uninvaded</td>
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| For pairs of levels of factor Condition
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Deviations from centroid

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| For pairs of levels of factor Condition
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D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning

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<th>Value</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Urban vs. Pristine</td>
<td>7.785</td>
<td>0.001**</td>
</tr>
<tr>
<td>Extra-urban vs. Pristine</td>
<td>1.984</td>
<td>0.068</td>
</tr>
</tbody>
</table>
### Table 2. ANOVAs on the effects of Condition, Location, Disturbance and *Caulerpa racemosa* Treatment on the Species richness (number of taxa) and on the cover of morphological algal groups. Pooling procedures were used according to Winer *et al.* (1991). * P < 0.05, ** P < 0.01, *** P < 0.001

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Artifacted corallines</th>
<th>Encrusting corallines</th>
<th>Filamentous algae</th>
<th>Canopy-forming macroalgae</th>
<th>Species richness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MS</td>
<td>F</td>
<td>df</td>
<td>MS</td>
</tr>
<tr>
<td>Condition = C</td>
<td>2</td>
<td>60.85</td>
<td>3.34</td>
<td>30.65</td>
<td>8.19</td>
</tr>
<tr>
<td>Location = Lo(C)</td>
<td>3</td>
<td>18.22</td>
<td>13.92***</td>
<td>3.74</td>
<td>4.58</td>
</tr>
<tr>
<td>Disturbance = D</td>
<td>1</td>
<td>0.19</td>
<td>0.16</td>
<td>2.02</td>
<td>8.62</td>
</tr>
<tr>
<td><em>C. racemosa = Cr</em></td>
<td>1</td>
<td>3.40</td>
<td>20.16</td>
<td>0.98</td>
<td>5.66</td>
</tr>
<tr>
<td>C x D</td>
<td>2</td>
<td>0.28</td>
<td>0.24</td>
<td>0.36</td>
<td>1.53</td>
</tr>
<tr>
<td>C x Cr</td>
<td>2</td>
<td>4.02</td>
<td>23.86*</td>
<td>0.53</td>
<td>3.06</td>
</tr>
<tr>
<td>D x Lo(C)</td>
<td>3</td>
<td>1.19</td>
<td>0.91</td>
<td>0.23</td>
<td>0.29</td>
</tr>
<tr>
<td>Cr x Lo(C)</td>
<td>3</td>
<td>0.17</td>
<td>0.13</td>
<td>0.17</td>
<td>0.21</td>
</tr>
<tr>
<td>D x Cr</td>
<td>1</td>
<td>0.14</td>
<td>0.14</td>
<td>4.16</td>
<td>178.05***</td>
</tr>
<tr>
<td>C x D x Cr</td>
<td>2</td>
<td>0.23</td>
<td>0.22</td>
<td>2.40</td>
<td>102.51**</td>
</tr>
<tr>
<td>Cr x D x Lo(C)</td>
<td>3</td>
<td>1.06</td>
<td>0.81</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Residual = Res</td>
<td>96</td>
<td>1.31</td>
<td>0.82</td>
<td>8.24</td>
<td>24.66</td>
</tr>
</tbody>
</table>

Cochran’s test  0.15,ns  0.15,ns  0.15,ns  0.11,ns  0.25,**
Transformation  Sqrt(x+1)  Ln(x+1)  none  Ln(x+1)  none
Legend to Figures:

**Figure 1.** Regression of effect size on community diversity against Halpern’s index for a) all data, b) experimental data and c) primary producers. Panel d) shows the regression of effect size on consumer species abundance against Halpern’s index.

**Figure 2.** Mean percentage cover (± SE) of (i) articulated corallines, (ii) encrusting corallines and (iii) filamentous algae across a gradient of anthropogenic stress encompassing urban, extra-urban and pristine locations (n = 20). For encrusting corallines, we show mean percentage cover values for each combination of Condition x Disturbance x *Caulerpa racemosa* Treatment separately (n = 10). Black bars represent plots invaded by *Caulerpa racemosa*, grey bars represent plots where *Caulerpa racemosa* was removed. Letters above columns illustrate the outcome of SNK tests; different letters indicate significant differences at P < 0.05, capital letters show comparisons between invaded and uninvaded plots within each condition, lower case letter show the comparison across Conditions within each level of *Caulerpa racemosa* Treatment.
D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning

Figure 1

a) Community diversity: all data

Effect size vs. Halperrn index

b) Community diversity: experimental data

Effect size vs. Halperrn index

c) Community diversity: primary producers

Effect size vs. Halperrn index

d) Species abundance: consumers

Effect size vs. Halperrn index
Figure 2.
Annex S1: Meta-analysis additional methods

Search criteria:
(alga* OR macroalga* OR seaweed*) AND (alien*OR invasi*OR introduced OR allochthonous OR nonindigenous OR non-indigenous OR “non native” OR non-native* OR exotic*) AND (marine OR brackish OR estuar*OR coastal OR shallow OR sea*OR aquatic OR maritime OR lagoon* OR pelagic OR benth* OR demersal OR shore* OR intertidal OR subtidal OR ocean* OR bay OR cove) AND (impact* OR effect* OR influence OR consequence* OR food-web* OR “food web”* OR ecosystem* OR biomass OR biodiversity OR “biological diversity” OR communit* OR richness OR diversity OR abundance OR evenness OR cover OR density OR “reproductive capacity” OR mortality OR growth OR assemblage* OR producti* OR decomposition OR “nutrient cycl”* OR oxygen OR carbon OR flux OR respiration OR “ecosystem metabolism” OR “sediment stabilisation” OR epiphyte* OR “sediment mixing” OR resilience OR stability OR resistance OR invasibility)

Table S1. Summary of the ecological impacts due to exotic seaweeds classified by ecological levels, impact types and response variables analyzed.

<table>
<thead>
<tr>
<th>Level</th>
<th>Impact type</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophyte community (e.g. macroalgae or phanerogams)</td>
<td>Abundance</td>
<td>cover or density of adults/recruits</td>
</tr>
<tr>
<td></td>
<td>Biomass</td>
<td>biomass of plants, biomass production</td>
</tr>
<tr>
<td></td>
<td>Diversity</td>
<td>number of species/taxa, Shannon diversity index</td>
</tr>
<tr>
<td></td>
<td>Evenness</td>
<td>Pielou’s evenness</td>
</tr>
<tr>
<td>Macrophyte species</td>
<td>Abundance</td>
<td>cover or density of adults/recruits</td>
</tr>
<tr>
<td></td>
<td>Biomass</td>
<td>above/belowground biomass of leaves/roots/rizhomes; biomass primary productivity/production</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>size/change in size of whole organisms or parts</td>
</tr>
<tr>
<td></td>
<td>Survival</td>
<td>% survival, mortality, longevity</td>
</tr>
<tr>
<td>Consumer community (animal community)</td>
<td>Abundance</td>
<td>cover or density of individuals/colonies</td>
</tr>
<tr>
<td></td>
<td>Biomass</td>
<td>biomass</td>
</tr>
<tr>
<td></td>
<td>Diversity</td>
<td>number of species/taxa, diversity indices (Shannon, Margalef)</td>
</tr>
<tr>
<td></td>
<td>Evenness</td>
<td>Pielou’s evenness</td>
</tr>
<tr>
<td>Consumer species (animal community)</td>
<td>Abundance</td>
<td>cover or density of adults/recruits/juveniles/colonies</td>
</tr>
<tr>
<td></td>
<td>Biomass</td>
<td>biomass of whole organisms or parts</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>size/change in size of whole organisms or parts</td>
</tr>
<tr>
<td></td>
<td>Survival</td>
<td>% survival, mortality rates, predation rates</td>
</tr>
</tbody>
</table>

Table S2. Results of rank correlation tests for the asymmetry of the funnel plots from the global analysis of community abundance, biomass, diversity and evenness and species abundance, biomass, growth and survival.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Kendall’s τ</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community abundance</td>
<td>-0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Community biomass</td>
<td>0.01</td>
<td>0.789</td>
</tr>
<tr>
<td>Community diversity</td>
<td>-0.34</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Community evenness</td>
<td>0.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Species abundance</td>
<td>0.01</td>
<td>0.835</td>
</tr>
</tbody>
</table>
**D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning**

<table>
<thead>
<tr>
<th>Species</th>
<th>Effect Size</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species biomass</td>
<td>-0.00</td>
<td>0.956</td>
</tr>
<tr>
<td>Species growth</td>
<td>-0.04</td>
<td>0.533</td>
</tr>
<tr>
<td>Species survival</td>
<td>0.14</td>
<td>0.129</td>
</tr>
</tbody>
</table>

**Figure S1.** Funnel plots for community A) abundance, B) biomass, C) diversity and D) evenness. Data included in squares represent those that have been removed to correct for publication bias.
D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning

**Figure S2.** Funnel plots for species A) abundance, B) biomass, C) growth and D) survival.

**List of the papers included in the meta-analysis:**


Lyons DA & Scheibling RE (2007) Differences in somatic and gonadic growth of sea urchins (Stronglyocentrotus droebachiensis) fed kelp (Laminaria longicuris) or the invasive alga Codium fragile ssp tomentosoides are related to energy acquisition. Marine Biology, **152**, 285-295.


D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning


**Annex S2: Results of the meta-analysis**

Table S3. Summary of the regressions of effect size on community variables (including both primary producers and consumers) against Halpern's index for the entire dataset or only for experimental studies. Effect sizes ("Median" and "95% CI") were estimated based on 10000 bootstrap samples generated by resampling with replacement (N number of studies included).

<table>
<thead>
<tr>
<th>Analysis</th>
<th>N</th>
<th>Median</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Community Intercept</td>
<td>53</td>
<td>-0.69</td>
<td>-1.21/-0.17</td>
<td>0.405</td>
</tr>
<tr>
<td>Community Slope</td>
<td>21</td>
<td>0.01</td>
<td>-0.01/0.03</td>
<td></td>
</tr>
<tr>
<td>Community Intercept</td>
<td>44</td>
<td>-1.28</td>
<td>-1.73/-0.89</td>
<td>0.25</td>
</tr>
<tr>
<td>Community Slope</td>
<td>6</td>
<td>0.02</td>
<td>0.003/0.03</td>
<td></td>
</tr>
<tr>
<td>Community Intercept</td>
<td>16</td>
<td>-0.97</td>
<td>-1.23/-0.7</td>
<td>0.563</td>
</tr>
<tr>
<td>Community Slope</td>
<td>0.01</td>
<td>0.01/0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experimental studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Community Intercept</td>
<td>22</td>
<td>-0.71</td>
<td>-1.28/-0.05</td>
<td>0.576</td>
</tr>
<tr>
<td>Community Slope</td>
<td>7</td>
<td>-0.27</td>
<td>-0.59/0.01</td>
<td></td>
</tr>
<tr>
<td>Community Intercept</td>
<td>16</td>
<td>-0.97</td>
<td>-1.23/-0.7</td>
<td>0.217</td>
</tr>
<tr>
<td>Community Slope</td>
<td>0.01</td>
<td>0.01/0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table S4. Summary of the regressions of effect size on community variables against Halpern’s index for primary producers or consumers. Effect sizes (“Median” and “95% CI”) were estimated based on 10000 bootstrap samples generated by resampling with replacement (N number of studies included).

<table>
<thead>
<tr>
<th>Analysis</th>
<th>N</th>
<th>Median</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary producers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Community abundance</td>
<td>24</td>
<td>-1.09</td>
<td>-1.97/-0.24</td>
<td>0.653</td>
</tr>
<tr>
<td>Community biomass</td>
<td>18</td>
<td>-0.38</td>
<td>-0.68/-0.08</td>
<td>0.697</td>
</tr>
<tr>
<td>Community diversity</td>
<td>21</td>
<td>-2.15</td>
<td>-2.85/-1.58</td>
<td>0.295</td>
</tr>
<tr>
<td><strong>Consumers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Community abundance</td>
<td>32</td>
<td>-0.25</td>
<td>-0.83/0.34</td>
<td>0.508</td>
</tr>
<tr>
<td>Community biomass</td>
<td>6</td>
<td>0.29</td>
<td>-1.63/1.53</td>
<td>0.219</td>
</tr>
<tr>
<td>Community diversity</td>
<td>26</td>
<td>-0.4</td>
<td>-0.92/0.08</td>
<td>0.695</td>
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</tbody>
</table>
Table S5: Summary of the regressions of effect size on species variables (including both primary producers and consumers) against Halpern's index for the entire dataset or only for experimental studies. Effect sizes (“Median” and “95% CI”) were estimated based on 10000 bootstrap samples generated by resampling with replacement (N number of studies included).

<table>
<thead>
<tr>
<th>Analysis</th>
<th>N</th>
<th>Median</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species abundance</td>
<td>46</td>
<td>-0.78</td>
<td>-1.5/-0.13</td>
<td>0.25</td>
</tr>
<tr>
<td>Species biomass</td>
<td>13</td>
<td>0.02</td>
<td>-0.85/0.53</td>
<td>0.512</td>
</tr>
<tr>
<td>Species growth</td>
<td>13</td>
<td>-1.24</td>
<td>-2.62/0.58</td>
<td>0.551</td>
</tr>
<tr>
<td>Species survival</td>
<td>9</td>
<td>-1.35</td>
<td>-2.19/-0.6</td>
<td>0.79</td>
</tr>
<tr>
<td>Experimental studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species abundance</td>
<td>22</td>
<td>-0.47</td>
<td>-1.2/0.27</td>
<td>0.567</td>
</tr>
<tr>
<td>Species biomass</td>
<td>6</td>
<td>-0.44</td>
<td>-0.98/0.02</td>
<td>0.738</td>
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<tr>
<td>Species growth</td>
<td>12</td>
<td>-1.16</td>
<td>-2.7/1.13</td>
<td>0.641</td>
</tr>
<tr>
<td>Species survival</td>
<td>7</td>
<td>-0.57</td>
<td>-1.49/0.29</td>
<td>0.725</td>
</tr>
</tbody>
</table>
Table S6. Summary of the regressions of effect size on species variables against Halpern’s index for primary producers or consumers. Effect sizes (“Median” and “95% CI”) were estimated based on 10000 bootstrap samples generated by resampling with replacement (N number of studies included).

<table>
<thead>
<tr>
<th>Analysis</th>
<th>N</th>
<th>Median</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary producers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species abundance Intercept</td>
<td>23</td>
<td>-0.3</td>
<td>-1.18/0.65</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-0.01</td>
<td>-0.04/0.02</td>
<td>0.528</td>
<td></td>
</tr>
<tr>
<td>Species biomass Intercept</td>
<td>9</td>
<td>-0.06</td>
<td>-0.45/0.33</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-0.01</td>
<td>-0.04/0.004</td>
<td>0.553</td>
<td></td>
</tr>
<tr>
<td>Species growth Intercept</td>
<td>8</td>
<td>-2.18</td>
<td>-2.66/-1.75</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.09</td>
<td>0.06/0.11</td>
<td>0.551</td>
<td></td>
</tr>
<tr>
<td><strong>Consumers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species abundance Intercept</td>
<td>29</td>
<td>-0.94</td>
<td>-1.78/-0.34</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.03</td>
<td>0.01/0.06</td>
<td><strong>0.037</strong></td>
<td></td>
</tr>
<tr>
<td>Species biomass Intercept</td>
<td>4</td>
<td>1.02</td>
<td>-3.36/2.62</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-0.03</td>
<td>-0.08/0.08</td>
<td>0.635</td>
<td></td>
</tr>
<tr>
<td>Species growth Intercept</td>
<td>8</td>
<td>-0.2</td>
<td>-3.94/5.82</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-0.01</td>
<td>-0.16/0.1</td>
<td>0.611</td>
<td></td>
</tr>
<tr>
<td>Species survival Intercept</td>
<td>7</td>
<td>-0.57</td>
<td>-1.49/0.29</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-0.02</td>
<td>-0.06/0.03</td>
<td>0.725</td>
<td></td>
</tr>
</tbody>
</table>
Annex S3: Study area and effectiveness of *C. racemosa* removal treatment

Figure S3. Experimental sites in the North-Western Mediterranean Sea: coast of Tuscany (upper right panel), Capraia and Elba Island (lower panels, respectively left and right). Squares = Urban sites, Triangles = Extra-urban sites, Circles = Pristine sites.
Effectiveness of *C. racemosa* removal treatment

![Figure S4](image_url)

Figure S4. Average percentage cover of *C. racemosa* in different experimental conditions (black bars = controls, dark grey bars = invaded disturbance, light grey bars = *C. racemosa* removed, white bars = uninvaded disturbances) for each location (U-1 = Livorno, U-2 = Rosignano, E-1 = Quercianella, E-2 = Fortullino, P-1 = Capraia, P-2 = Elba). Data were averaged across 5 to 7 sampling times and between the 5 replicates per experimental treatment.
Appendix 2

Case study 2 - Invasive mussels in the Oristano Gulf

S. Como, P. Magni

Consiglio Nazionale delle Ricerche – Istituto per l’Ambiente Marino Costiero (CNR-IAMC), Località Sa Mardini, Torregrande, 09170 Oristano, Italy

Abstract

Background. In the marine environment, the introduction and spread of non-indigenous mussels may cause major modifications to native assemblages. Yet, how invasive mussels alter the trophic flow within the food web remains unknown. We analysed the impacts of the Asian date mussel *Arcuatula (=Musculista) sehnousia* on native benthic assemblages and sedimentary features and, for the first time, on the transfer of carbon from suspended microalgae to native primary benthic consumers.

Methods. We compared areas where the Asian mussel was present and those where it was absent in two coastal lagoons of the Gulf of Oristano system (Western coast of Sardinia, Italy). We looked at the changes in benthic assemblages and sedimentary features in Santa Giusta lagoon, and at the changes in the carbon and nitrogen isotopic composition of deposit feeders (DF) and Sedimentary Organic Matter (SOM) in Marceddì lagoon. We compared the impacts of the Asian mussel on the amount of suspended microalgae taken up by target species, such as the polychaete deposit feeder *Cirrhophoria tentaculata* to those of the native clam *Ruditapes decussatus* by using the stable isotope enrichment approach in the laboratory.

Results and discussion. The results showed that the Asian mussel did not affect benthic assemblages; neither did it change the isotopic signatures of SOM and benthic deposit feeders in the two lagoons of the Oristano gulf system that it invaded. Instead, the results of our laboratory experiment revealed changes in the amounts of carbon derived by the suspended microalgae taken up by *C. tentaculata* due to the Asian mussel. The microalgae C-uptake of *C. tentaculata* was lower in the presence of the Asian mussel than in the presence of the native clam, indicating that the Asian mussel has a negative effect on the transfer of carbon from suspended microalgae to the deposit feeder. In addition, the results showed an interactive effect of the Asian mussel and the native clam on the microalgal C-uptake of *C. tentaculata*, indicating that the Asian mussel can control the carbon transfer from suspended microalgae to deposit feeders also indirectly through the interaction with clams. In association with mussels and clams, *C. tentaculata* had values of microalgal C-uptake that were intermediate between those found in the presence of mussels only (the lowest values) and those found in the presence of clams only (the highest values). We also found that the association between mussels and clams increased the microalgal C-uptake of mussels by a factor 2 as compared to the values found in the presence of mussels only while it reduced the microalgal C-uptake of clams below the values found in presence of clams only. These results indicated that (i) mussels can benefit by an association with clams, increasing the food intakes while reducing those of clams and that (ii) by impairing feeding, incorporation and biodeposition of clams, mussels reduce the availability of suspended microalgae for deposit feeders.

Conclusions

This study provides the first evidence of the impact of the Asian mussel *Arcuatula sehnousia* on the C flow within the food web with two-fold implications. Firstly, the Asian mussel can benefit by the association with native suspension feeders, increasing the food intakes. By doing this, it reduces the transfer of food from the water column to the native suspension feeders. Secondly, by lessening the benthic-pelagic coupling, it also reduces the food availability for deposit feeders.
These effects of the Asian mussel were found to be independent of mussel densities, indicating the C flow from the first to the second trophic levels of the benthic food web are at risk from this invasion. The consequences on functioning of the entire coastal food webs should therefore be taken into account.

2 Background

Invasive mussels are often introduced accidentally into marine and freshwater environments. Over the past century, the Asian date mussel *Arcuatula (=Musculista) senhousia* (Benson in Cantor, 1842) has been introduced from Asia to the Pacific coast of North America, Australia, New Zealand and in the Mediterranean Sea (Willan 1987, Crooks 1996, Creese et al. 1997, Mistri 2002). The Asian mussel typically lives semiburied in the intertidal and shallow subtidal soft sediments of bays, estuaries and lagoons and secretes byssal threads to attach to conspecifics (Crooks 1996, Mistri 2002, Magni et al 2006). The Asian mussel is a small, short-lived bivalve growing rapidly (about 20 mm in 12 months) and showing dramatic population fluctuations (Crooks 1996, Mistri 2002, Munari 2008). The invasion of the Asian mussel to bays, estuaries and lagoons worldwide is considered to be one of the most important threats to local bivalves including several species with a high economic value (i.e., *Ruditapes decussatus* and *Ruditapes philippinarum*) (Rilov and Crooks 2009).

Because of its life history and ecological characteristics, predisposing it to spread rapidly and form large mats on the seabed, the Asian mussel can impact the resident fauna through many different effects. When the Asian mussel occurs at very high densities (up to 5000 to 10 000 ind m$^{-2}$), the number of species and the total abundances of benthic invertebrates decrease in mussel mats compared to the surrounding sediment (Creese et al. 1997, Munari 2008). Instead, the impact of the Asian mussel is more variables at lower densities, ranging from negative to positive effects on species richness and total abundances of local infauna (Crooks 1998, 2001, Mistri 2002). The Asian mussel reduces the abundances of co-occurring suspension feeding bivalves (Creese et al. 1997, Crooks 2001), whereas it favours small deposit feeders (Crooks and Khim 1999). In addition, it is also known to impair the growth and survival of other bivalves (Crooks 2001, Kushner 2005) and to modify the sediment properties (Crooks 1998, Reuch and Williams 1998, Crooks and Khim 1999, Mistri et al. 2003). Yet, how the Asian mussel alters the trophic flow within the food web remains unknown.

In general, suspension feeding bivalves couple pelagic and benthic processes because they filter suspended particles from the water column and the undigested remains, ejected as mucus-bound feces and pseudofeces, sink to the sediment surface (Kausky and Evans 1987, Han et al, 2001, Newell 2004). This biodeposition is extremely important because biodeposits are mixed into the sediment and consumed by deposit-feeders (Amouroux et al. 1990). The Asian mussel is characterized by high ingestion and excretion rates because of the high growth rate and high metabolic requirements (Inoue and Yamamuro 2000, Magni et al 2000). Thus, suspended food sources are expected to be more available to deposit feeders in the presence of the Asian mussel than in the presence of other bivalves, because of the higher quantity and quality of biodeposits ejected. In addition to biodeposition, there could be structural changes in the benthic habitat resulting from the presence of the shells (Crooks and Khim 1999). Natural mussel mats typically raise a few millimeters above the surface sediments, and this biogenic structure may potentially alter the hydrodynamic features on flat bottoms favouring the trapping and deposition of fine sediments and labile detritus (Committ et al. 2005, 2008). As the Asian mussel is a densely-living suspension feeder, it can exert important effects on the depositional environment with respect to other suspension feeders (Crooks and Khim 1999; Mistri et al. 2003). Another characteristics is that the Asian mussel may indirectly reduce the availability of suspended food sources to deposit feeders through the interaction with other bivalves. Although biological interactions between the Asian mussel and native bivalves are well acknowledged (see Rilov and Crooks 2009 for reviews and examples), little attention has been given to understanding the consequences of such interactions on the other benthic organisms. If the Asian mussel locally depletes food supplies and limits living space for larger clams, the Asian mussel may affect the feeding and biodeposition of clams with direct consequences for the availability of food sources for the co-occurring deposit feeders.
A way to understand how an invasive species changes food supplies for co-occurring animals is the use of stable isotopes (Peterson and Fry 1987). This analyses is based on the fact that the carbon and nitrogen stable-isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in the tissues of an organism reflect those in its diet (Post 2002). As an alternative, enrichment experiments have been proposed as a useful tool to analyse the transfer of material from a food source to a consumer (Pasotti et al. 2012, Leroy et al. 2012, Pascal et al. 2013) so that it can be applied in studies of the impacts of invasive species. In isotope enrichment experiments, the abundance of the rare isotope (e.g. $^{13}C$) of a food source is heavily increased so that the isotopic label can be traced in the organisms of interest (Herman et al. 2000, Rossi 2007). Incorporation of label is defined as excess above background values and is expressed as an amount of label incorporated by the individuals (Herman et al. 2000, Middleburg et al. 2000, Rossi 2007). It has also shown that it is possible to convert the amounts of label incorporated in the amount of food source taken up by that individual and expressed as total carbon assimilated (individual uptakes; Pascal et al. 2008).

3 Objective of the study

The objective of this study was to further the understanding of the impacts of the invasive Asian mussel in coastal systems. We looked at the impacts of A. senhousia on the spatial distribution of native benthic assemblages and sedimentary features and, for the first time, on the transfer of carbon from the first to the second trophic levels of the benthic food webs. We focused our activities on two coastal lagoons of the Oristano lagoon-Gulf system (Western Sardinia) where the Asian mussel has recently been recorded (Cannas 2009).

Firstly, we tested the effect of the Asian mussel on benthic assemblages and sedimentary features. To attain this, we analysed the species composition and structure of benthic assemblages as well as grain sizes, organic matter (OM), water content and percentage of bicarbonate in the sediment in stations with and without the Asian mussel, in Santa Giusta lagoon.

Secondly, we tested for differences in the carbon and nitrogen isotopic composition of deposit feeders (DF) and sedimentary OM (SOM) where the Asian mussel is present (hereafter called Impact, I) and where it is absent (hereafter called Control, C). If the Asian mussel increases the “benthic-pelagic coupling”, we expected that the isotopic values of DF and SOM would be closer to the isotopic values of particulate OM and suspension feeders in I than in C. Animals, sediment and water were collected at two impacted and two control sites interspersed along the coastline 1 km apart, in Marceddi lagoon.

Thirdly, we analyzed the effects of the Asian mussel on the amount of suspended microalgae (phytoplankton) taken up by a target species, the surface deposit feeder polychaete Cirriphormia tentaculata, by using the stable isotope enrichment approach. The effects of the Asian mussel were compared to the effects of the native clam Ruditapes decussatus. In a mesocosm with natural sediment, we added living microalgae previously labelled with $^{13}C$. The carbon derived by suspended microalgae (microalgae C) taken up by C. tentaculata (individual uptake) was then quantified at the end of feeding experiments in presence/absence of these suspension feeders. The Asian mussel may undergo drastic fluctuations in abundance and biomass in an area (Yamamuro et al. 2000; Crooks 2001; Magni et al. 2006). In addition to the presence/absence of suspension feeders, we also manipulated their biomass to control for density-dependent effects (Benedetti-Cecchi 2004, 2006). We expected (i) the microalgae C-uptake by C. tentaculata to be higher with the Asian mussel than with the native clam, independent of the biomass of the suspension feeders. Because the Asian mussel can have interactive effects with clams (Crooks 2001, Mistri, 2004, Rilov and Crooks, 2009), we expected that the effects of the presence of one single species of suspension feeders (i.e. Asian mussel or native clam) differed from those of the combination of the Asian mussel and the native clam.
4 Methods

4.1 Effect of the Asian mussel on macrobenthic assemblages and sediment variables

4.1.1 Study area

The Santa Giusta lagoon (Western Mediterranean, Italy) is a polyhaline basin located in the Gulf of Oristano (Fig. 1). The lagoon is included in the Ramsar convention (1971) and belongs to a complex system of transitional waters of high natural and economic value. The Santa Giusta lagoon is approximately circular in shape with an area of 7.9 km² and a mean depth of 1 m; it is located near the town of Oristano and Santa Giusta, in the plain of Pesaria, an agricultural area that is intensively cultivated with rice. The lagoon has no natural attributes and is separated from the sea by a longshore bar, it is also connected with two inner small basins called Pauli Maiori and Pauli Figu (Fig. 1). Central and peripheral canals have been dredged about 2 m deep in order to facilitate seawater flow into the lagoon. As a consequence, waters of Santa Giusta lagoon are now well mixed as regards circulation and stratification (Tataranni et al. 2009). Salinity ranges from 25‰ to 42‰, with a mean annual value of 30‰ (Tataranni et al. 2009). There is a prevalent sandy-muddy bottom, with small patches of both macroalgae and angiosperms (e.g. Enteromorpha sp., Gracilaria sp., Ruppia cirrhosa, Zostera sp.), which are distributed all over the lagoon. The presence of A. sehnousia in Santa Giusta lagoon was recorded for the first time in 2009 (unpublished data).

4.1.2 Sample collection and data analyses

Sediment samples were collected in Santa Giusta lagoon in 2009 at three sites, according to the sampling design described in Tataranni et al. (2009). There were twenty-seven samples at each site.

Samples of macrofauna were collected from the bare bottom with a box-corer (10 × 17 cm²), sieved through a 0.5 mm mesh and preserved in 4% formaldehyde. As additional data, at each sampling station, sediment cores (30 mm inner diameter) were collected for the grain size analyses (mud, <64 µm and sand, >64 µm fractions) and the determination of the percentages of organic matter (OM), water and bicarbonate in the sediment. In the laboratory, macrofauna were then sorted, identified to the species level (when possible), counted and preserved in 70% ethanol. The surface layer (0–2 cm) of sediments was carefully sliced off each core. A sub-sample of ca. 4 g was suspended in 500 ml of distilled water and treated with hydrogen peroxide (H₂O₂, 4% solution) in order to eliminate organic matter before being wet sieved through a net of 64 µm. The sand fraction [Sand (>64 µm)] remaining in the sieve was dried and weighed. Ten milliliters of suspension with the mud fraction (<64 µm) were then treated with Na-Hexametaphosphate 0.6% to avoid particle flocculation after a dilution to obtain a sediment concentration of ≈0.5 mg ml⁻¹. The organic matter (OM) content in the sediments was determined from a sub-sample (about 1 g) by loss of ignition at 500 °C for 3 h. Water content in the sediment was quantified from a sub-sample (about 1 g) by loss of weight at 60°C for 48 h. Carbonates (CaCO₃, dry wt%) were determined by dissolution in 1 M HCl for 4 h after being filtered through Whatman GF/C filters, the residue was dried and weighed.

Differences in macrozoobenthic assemblages and sediment variables were analyzed using a canonical discriminant analysis (CDA). Six groups of stations were obtained as a combination of the three sites and presence/absence of A. sehnousia were taken into account in the CDA analysis.

4.2 Effect of the Asian mussel on δ¹³C and δ¹⁵N of deposit feeders and SOM

4.2.1 Study area and sample collection

The Marceddì lagoon is a brackish basin located in the southern part of the Gulf of Oristano (Fig. 1). Marceddì lagoon has an area of 8 km² and a mean depth of 1.5 m; it is located near the plain of Arborea, an area that is intensively exploited by agricultural and zootechnical activities. Salinity ranges from 23‰ to 42‰, with a mean annual value of 33‰ (Cannas 2009). There is a prevalent sandy-muddy bottom, with small patches of both macroalgae and angiosperms (e.g. Enteromorpha sp., Gracilaria sp., and Zostera sp.), which are distributed all over the lagoon. The presence of A. sehnousia in Marceddì lagoon was recorded for the first time in 2008 (Cannas 2009).
At the end of October 2012, animals, sediment and water were collected at two impacted sites (I; characterized by the presence of *A. sehnousia*) and two control sites (characterized by the absence of *A. sehnousia*, C) interspersed along the coastline 1 km apart (Fig. 2). There were 3 sampling stations (100 m apart) at each site (Fig. 2). Samples were collected in less than 1.5 m water depth. The densities of *A. sehnousia* in the impacted sites were about 1000 ind. m⁻² and all individuals had a size lower than 20 mm.

At each station, sediment samples for benthic macroinvertebrates were collected using an Ekman-Birge grab and sieved on a 500 mm mesh-size on the shore. For sedimentary organic matter (SOM), the top few millimetres of the sediment layer were collected using a hand corer (30 mm inner diameter) at the same stations that were sampled for benthic macroinvertebrates. Seston were collected as a proxy for detritus and suspended microalgae (<55 µm). At each station, 2 L of lagoon water were collected for obtaining the <55 µm fraction of seston. At each station with *A. sehnousia*, samples were collected within or in proximity of patches of *A. sehnousia*. *A. sehnousia* was collected by using a clam rake for the quantification of the density (n. ind. m⁻²).

### 4.2.2 Data analyses

In the laboratory, benthic macroinvertebrates were sorted and identified to species level under a stereo-microscope. The individuals were then put in jars containing filtered lagoon water and left for 24 h to allow the gut contents to clear. Then, they were killed by freezing the flesh of molluscs, polychaetes and crustaceans, which had been separated from the calcareous shells, tubes or external cuticles and rinsed with distilled water. From fifteen to twenty individuals of each species collected from each station were pooled and treated as a whole.

Sediment samples for SOM were sieved using a 35 mm mesh size to remove large pieces of debris or animals. Lagoon water was filtered by a precombusted (450 °C, 4 h) Whatman GF/F filter after a pre-filtration through a 55 µm mesh for the small-sized seston determination. All samples were oven-dried to a constant weight at 70 °C, ground to fine powder and stored frozen (-20 °C) until isotopic analyses.

Prior to the isotopic composition analysis, all samples were acidified using a 1 N HCl solution to eliminate carbonates. Measurements of natural abundances of ¹³C and ¹⁵N in animals, SOM and sized-fractionated seston were taken with a mass spectrometer (Finnigan DeltaPlus). Isotopic values were expressed in the δ notation as deviations from standards (Vienna Pee Dee belemnite limestone for δ¹³C and nitrogen in air for δ¹⁵N), following the formula: $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where R in ¹³C/¹²C or ¹⁵N/¹⁴N. The precision of replicate analyses was 0.2‰ for both δ¹³C and δ¹⁵N. Average reproducibility based on replicate measurements of internal standards Sucrose (IAEA-CH-6) for δ¹³C and Ammonium sulphate (IAEA-N-2) for δ¹⁵N was about 0.15‰. For system checks, acetalnilde was used as the laboratory reference.

To test for the effects of *A. sehnousia* on the δ¹³C and δ¹⁵N values of benthic deposit feeders and SOM we used a 2-way model analysis of variance (ANOVA). The analysis took into consideration the factor *A. sehnousia* (two levels: Impact [I] and Control [C]; fixed) and the factor Site (two levels; nested in *A. sehnousia*) and three stations as replication units. Cochran’s C-test was used to check the assumption of homogeneity of variances using ANOVA in tests of hypotheses (Winer et al. 1991). When required, the Student-Newman-Keuls (SNK) tests were used as posteriori comparisons of the mean estimated by analysis of variance (Underwood 1997).

### 4.3 Effect of the Asian mussel on the carbon transfer to a target deposit feeder

#### 4.3.1 Experimental design and protocol

The Asian mussel *Arcuatula sehnousia* and the native clam *Ruditapes descussatus* were added to the experimental mesocosms, containing the surface deposit feeder *Cirrophormia tentaculata*, at different combinations of species and density, in order to simulate changes in the density of one or both species of suspension feeders (Table 1). This generated the following six treatments: 1) high density of *A. sehnousia* (17 g fresh weight, FW) that corresponded to about 4000 ind. m⁻²; 2) high density of *R. descussatus* (17 gFW;
about 600 ind. m\(^{-2}\) ; 3) presence of both \textit{A. sehnousia} and \textit{R. descussatus} at density (17 gFW); 4) low density of \textit{A. sehnousia} (7 gFW) that corresponded to about 1500 ind. m\(^{-2}\); 5) low density of \textit{R. descussatus} (7 gFW; about 250 ind. m\(^{-2}\) ; 6) presence of both \textit{A. sehnousia} and \textit{R. descussatus} at low density (7 gFW). For each treatment, there were three experimental replicates. The abundances of \textit{A. sehnousia} and \textit{R. descussatus} used in this experiment were ecologically realistic, as it is within the range of abundances of \textit{A. sehnousia} and \textit{R. decussatus} observed in marine coastal and transitional systems (Magni et al. 2006, Munari 2008, Cannas 2009).

Each replicate, held in a mesocom (0.4×0.3×0.3 m) filled with 15 liters of filtered sea water, was set-up in a room with controlled temperature under a natural photoperiod (39°48' N; Fig. 3). A plastic jar (14 cm inner diameter and 5 cm high; Fig. 3) with natural sediment was placed within each mesocosm. The day before the start of the experiment, six individuals of \textit{C. tentaculata} were added to each jar. \textit{A. sehnousia} and \textit{R. decussatus} were added to the jars only when the individuals of \textit{C. tentaculata} had sunk into the sediment. The animals and sediment were collected in Marceddi lagoon (Gulf of Oristano, Italy) and then transported to the University of Sassari, where the experiment was carried out. The animals of each species were maintained in 100 litre holding tanks (0.6×0.4×0.4 m), filled with re-circulated filtered sea water, where they were allowed to acclimatise to the experimental temperature (20°C) for at least one week (Fig. 3). Temperatures and salinity ranged between 19 and 21 °C (mean±S.E.: 20°C±0.2, n=30) and 28 and 33 psu (mean±S.E.: 30 psu±0.3, n=30), respectively. During this period, animals were under a natural photoperiod of the location (39°48’ N). \textit{A. sehnousia} and \textit{R. decussatus} were fed with living phytoplankton daily whereas \textit{C. tentaculata} were maintained in natural sediment. The mean total length (TL) ± SE of \textit{A. sehnousia} and \textit{R. decussatus} used in this experiment was 2.33±0.04 and 3.48±0.04 cm, respectively, whereas the mean fresh weight (FL) ± SE of \textit{C. tentaculata} was 0.14±0.00 g. The sediment was collected three days before the experiment, sieved through 5 mm screens to eliminate plant debris, kept on plastic plates (0.30×0.20×0.15 m) and then laid on the bottom of a holding tank filled with re-circulated filtered sea water under a natural photoperiod (39°48’ N).

At the beginning of the experiment, 13C-enriched microalgae (see paragraph below for the preparation method) were re-suspended in the water of the mesocosms and the feeding experiment lasted 6 days. Since only six mesocosms were available, one replicate of each treatment was run each week and the study lasted 3 consecutive weeks (between the end of November and the beginning of December 2013).

4.3.2 Isotope enrichment of phytoplankton
An axenic clone of the Haptophyte \textit{Isochrysis} sp. was cultured at 20°C in an artificial seawater medium. Cells in an exponential growth phase were then cultured in medium containing NaH\(^{13}\)CO\(_3\) (4mM) for three days, following a procedure described by Pascal et al. (2008). Microalgae were then concentrated by centrifugation (1500 g × 10 min, 20°C) and washed three times to remove the \(^{13}\)C-bicarbonate before being resuspended in seawater (0.5 L) of identical quality. Microalgae were supplied to animals less than 15 min after this step. Samples of \(^{13}\)C-enriched microalgae were frozen at -20°C and then storage at -80°C for further quantification of the fractional abundances of \(^{13}\)C. The mean values of \(^{13}\)C-enriched microalgae added to each treatment and the percentage of carbon (C) that was replaced with \(^{13}\)C in algal preparations are reported in table 2.

4.1.3 Data collection and data analysis
At the end of each replicate, animals were sorted and put in jars containing filtered sea-water and left for 24 h to allow the gut contents to clear. Then, they were killed by freezing the flesh of molluscs and polychaetes, which has been separated from the calcareous shells or tubes and rinsed with distilled water. All individuals of each species collected from each replicate were pooled and treated as a whole. For each experimental run, individuals of each species were also collected from the holding tanks for the determination of the background signals. All samples were freeze-dried, grounded using a ball mill and stored until isotopic analyses.

Isotopic measurements were taken as described in the paragraph above (4.2.2 Data analyses). Samples were not acidified. Incorporation of \(^{13}\)C (I) was calculated as the product of excess \(^{13}\)C (E) and the amount of
organic carbon (OC) of each sample. The excess (E) is the difference between the fraction of the heavy isotope in the experiment and in the background: \( E = F_{\text{Experiment}} - F_{\text{Background}} \), where \( F \) is the fraction of heavy isotope of C compared to the total carbon measured. \( F \) is computed from the isotope ratio \( R \) with \( F = R / (R+1) \) for \(^{13}\text{C}\). \( R \) was derived from the measured \(^{13}\text{C}\) as \( R = (\delta^{13}\text{C}/1000+1) \times \text{RVPDB} \), where \text{RVPDB} = 0.0112372. The amount of carbon (C) derived by microalgae (hereafter called microalgae C) taken up by \( C. \) tentaculata, \( A. \) sehnousia or \( R. \) decussatus in each replicate were calculated as the quotient of the amount of \(^{13}\text{C}\) incorporated (I) and the fractional abundance of \(^{13}\text{C}\) in the algal preparations (Pascal et al. 2008). This amount of microalgae C incorporated in animals was then divided by the fresh weight of all individuals in each replicate and is expressed as mg C per unit of biomass (mg C gFW\(^{-1}\)).

Microalgae C-uptake by \( C. \) tentaculata was analysed by means of a two-way ANOVA model including the factors: Treatments (3 levels; fixed) and Densities (fixed and crossed with Treatments) and three replicate mesocosms as replicates. The term Treatments was partitioned into two contrasts, resulting in an asymmetrical design (Underwood 1991). The terms in this contrast were: 1) presence of \( A. \) sehnousia versus presence of \( R. \) decussatus and 2) presence of \( A. \) sehnousia or \( R. \) decussatus (hereafter indicated as A/R) versus the presence of both \( A. \) sehnousia and \( R. \) decussatus (hereafter indicated as A+R). This was necessary in order to establish if the effects of the suspension feeders on the deposit feeder \( C. \) tentaculata differed between species (first contrast) and if the effects of the presence of one single species differed from those of the presence of both (second contrast). Microalgae C-uptake by \( A. \) sehnousia and \( R. \) decussatus was analysed separately by means of a two-ways ANOVA model including the factors: Treatments (2 levels; fixed) and Densities (fixed and crossed with Treatments) and three replicate mesocosms as replicates. Cochran’s C-test was used to check the assumption of homogeneity of variances using ANOVA in tests of hypotheses (Underwood, 1997). SNK tests were used for posteriori comparisons among the levels of factors when terms were significant (Underwood 1997).

5. Results

5.1 Effect of the Asian mussel on macrobenthic assemblages and sediment variables

A total of 15,325 specimens were collected. The crustaceans \( Tanaid\) dulongii and \( Gammarus\) aequicauda accounted for 49% of this amount. There were 59 taxa belonging to polychaetes (33 taxa), crustaceans (15 taxa), mollusks (11 taxa). The sand fraction (i.e., >64 µm) represented about 72% of the total sediment dry weight in Santa Giusta lagoon. Organic matter (OM) exceeded 10% of the total sediment weight whereas the content of water and carbonates represented 54% and 12% of the total sediment weight, respectively.

Canonical discriminant analysis (CDA) revealed differences in both macrofauna and sediment variables among the six groups of stations (permutation test: \( \delta^2=0.79, P=0.001 \) and \( \delta^2=0.45, P=0.001 \), respectively; Fig. 4). Cross-validation in the CDA analysis showed the highest number of misclassifications to occur in the groups of stations of the same site (values in bold in Table 3), indicating a major separation among sites, regardless of presence/absence of \( A. \) sehnousia.

5.2 Effect of the Asian mussel on \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) of deposit feeders and SOM

Organisms that were sufficiently abundant at sites for the analyses included three deposit feeders (\( Hediste\) diversicolor, \( Haminoea\) navicula and \( Cyclopec\) neritea) and one suspension feeder (\( Cerastoderma\) glaucum) (Table 4). The 2-way ANOVA detected significant variability between sites (P<0.05) but no significant effect of \( A. \) sehnousia on the \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) values of the deposit feeders \( H.\) diversicolor, \( C.\)neritea and \( H. \) navicula and SOM (P>0.05) (Table 5; Fig. 5), indicating that spatial variation between sites was more important than the effect of \( A. \) sehnousia.

Differences among sites were further analyzed by using a 1-way model of analyses of variance with site as factor (4 levels; fixed) and three replicates. The 1-way ANOVA and SNK test showed that the deposit feeders \( H.\) diversicolor, \( C.\)neritea and \( H. \) navicula and SOM had the highest \( \delta^{13}\text{C} \) and the lowest \( \delta^{15}\text{N} \) in the site located closest to the inlet (C2) (P<0.05 in Table 6; Fig. 5). As for the deposit feeders and SOM, the most
abundant suspension feeder *C. glaucum* and the seston were more $^{13}$C-enriched in C2 as compared to the other sites (Table 6; Fig. 5).

5.3 Effect of the Asian mussel on carbon transfer to a target deposit feeder

After 6 days of the experiment, no differences in the uptake rates of microalgae derived-C (microalgae C-uptake) by *C. tentaculata* were found in the main factor Densities or in the interaction term Treatments × Densities but significant differences were found among Treatments (Table 7). The microalgae C-uptake of *C. tentaculata* varied between the presence of *A. senhousia* and the presence of *R. decussatus* (contrast: A vs R; Table 1), being higher in presence of *R. decussatus* than in presence of *A. senhousia*, irrespective of the densities of the suspension feeders (Fig. 6). The microalgae C-uptake of *C. tentaculata* also varied according to the presence of one or both the species of suspension feeders (contrast: A vs R; Table 1). Visual inspection of the graphs suggests that the values of microalgae C-uptake of *C. tentaculata* where both *A. senhousia* and *R. decussatus* were present, were intermediate between those found in the presence of only *R. decussatus* and *A. senhousia* (i.e., the highest and lowest values, respectively) (Fig. 6).

Significant differences in the microalgae C-uptake of *A. senhousia* and *R. decussata* were found in the main factor Treatments, but not in Densities or in the interaction term Treatments × Densities (Table 8). The values of the microalgae C-uptake of *R. decussatus* were lower where both *A. senhousia* and *R. decussatus* were present than where *R. decussatus* was alone (R > A+R; Fig. 7). As an opposite trend, the values of the microalgae C-uptake of *A. senhousia* were higher where both *A. senhousia* and *R. decussatus* were present than where *A. senhousia* was alone (A < A+R; Fig. 7).

6 Discussion

The results show that the Asian mussel *A. senhousia* did not affect benthic assemblages; neither did it change the isotopic signatures of SOM and benthic deposit feeders, in the two lagoons of the Oristano gulf system that it invaded. Instead, the results of our laboratory experiment revealed changes in the amounts of carbon derived by the suspended microalgae taken up by the target deposit feeder *Cirriphormia tentaculata*, in response to the presence of the Asian mussel. Contrary to our expectations, however, the results showed that the microalgae C-uptake of *C. tentaculata* was lower in the presence of the Asian mussel than in the presence of the autochthonous clam *Ruditapes decussatus*, indicating that the Asian mussel has a negative effect on the transfer of carbon from suspended microalgae to this deposit feeder. In addition, the results showed an interactive effect of the Asian mussel and the native clam on the microalgae C-uptake of *C. tentaculata*, indicating that the Asian mussel can control the carbon transfer from suspended microalgae to the deposit feeders also indirectly through the interaction with clams.

6.1 Effects of the Asian mussel on benthic assemblages and isotopic signatures

Although the Asian mussel can impair species richness and the benthic structure at very high densities of mussels (e.g. > 5000 ind m$^{-2}$; Creese et al. 1997; Munari 2008), benthic assemblages can also respond to the Asian mussels at lower mussel densities (Crooks 1998, 2001; Mistri 2002), such as those found in our study areas (≈1000 ind m$^{-2}$). The lack of responses of benthic assemblages and isotopic signatures of different benthic comportments (i.e. deposit feeders and SOM) in our field studies may depend on the duration of the effects at any impacted sites or stations investigated (Crooks 2009). Contrary to other mussel species (Commoti et al. 2005, 2008), the Asian mussel does not form self-perpetuating structures (Creese et al. 1997, Mistri 2002). Rather, the mussel patches are short-lived and the lifespan of these patches is determined by the longevity of the mussels (1-2 years) (Creese et al. 1997, Mistri 2002). The Asian mussel has a rapid growth, reaching the size of 20 mm in 12 months and short life span, living for only 2 years (Crooks 1996). Creese et al. (1997) showed that about 60% of the original bed at Bucklands Beach (Auckland, New Zealand) was eroded away over a 12-month period, and the entire mat disappeared after about 2 years. Similarly, in Sacca di Goro, populations of the Asian mussels decreased in abundance by about 85% between July and September 1999 and, after one year, the 1999 cohort was about 10% of its former abundance (Mistri 2002). In Marceddi lagoon, the Asian mussels found in the impacted sites were numerically dominated by individuals of
similar size (<20 mm), suggesting that the mussel patches in these sites were the result of a single recruitment and colonization event that occurred just before our study started, less than 1 year.

The localized, short-term effects of mussel patches can be compensated by the high fecundity and good dispersal ability of the Asian mussel (Crooks 1996). For example, in Sacca di Goro, mussels recruit throughout the years and after only two years from their first detection in 1994, mussels formed large beds on the bottom of the entire lagoon (Mistri 2002). As for Sacca di Goro, the Asian mussel, which was absent in both the control sites chosen in Marceddì lagoon in 2012, was found in these control sites in autumn of the subsequent year (2013; data not shown), indicating a high ability of dispersal and recruitment.

6.2 Effects of the Asian mussel on the carbon transfer to a target deposit feeder

Our results provide the first evidence that the invasion of the Asian mussel can alter the transfer of food from suspended microalgae to the benthic deposit feeders within the food web. However, the mechanisms linking the presence of the Asian mussel to changes in the carbon uptake by deposit feeders are more complex than previously thought (Crooks 1998; Crooks and Khim 1999; Mistri et al. 2003). We had predicted positive effects of the Asian mussel on the microalgal uptake by deposit feeders because of the production of biodeposits, due to mussel feeding, and alteration of depositional environment, due to the shells, increases the availability of suspended microalgae into the sediment. Our results showed that the microalgae C-uptake by the surface deposit feeder Cirriphormia tentaculata was lower with the Asian mussel than with the native clam, Ruditapes decussatus, indicating that the Asian mussel has a negative effect on the transfer of carbon from suspended microalgae to this deposit feeder (Fig. 8).

Data from literature to compare with our values are scarce. However, our results are in contrast with other studies showing that the Asian mussel can increase organic matter into the sediment that is thus potentially available for deposit feeders (Crooks 1998, Reusch and Williams 1998, Crooks and Khim 1999, Magni et al. 2000, Mistri et al. 2003). For instance, Mistri (2003) showed microphytobenthic carbon to be at least twice higher with the Asian mussel than with the Manila clam, Ruditapes philippinarum after a few days before the start of an experiment in a mesocosm. Unlike structural effects (Crooks and Khim 1999, Mistri et al. 2003), changes in biodeposition due to the Asian mussel may account for the low transfer of carbon from suspended microalgae to the deposit feeder. Although mussels can rapidly increase the amount of fine sediment and organic matter in the sediment through feeding (Crooks 1998, Crooks and Khim 1999, Mistri et al. 2003), it is possible that the nutritional value of biodeposits ejected by the Asian mussel is very low because of a high efficiency in the extraction of nutrients from food (i.e. digestion) and nutrients absorption (i.e. assimilation). Due to the short life-cycle and the high growth rate (Crooks 1996, Creese et al. 1997, Mistri 2002), the Asian mussel has higher energetic and metabolic requirements for the accretion of tissues (i.e. growth) than the long living clams, possibly resulting in a more complete algal digestion. The efficiency of such a process is highlighted by the higher ammonium excretion rates of the Asian mussel as compared to those of the Manila clam (Magni et al. 2000), suggesting a more complete mineralization of organic matter.

In addition to the direct negative effect of the Asian mussel, our results showed that the Asian mussels can also control the carbon transfer from suspended microalgae to deposit feeders indirectly, through their interaction with clams (Fig. 8). In association with mussels and clams, C. tentaculata had values of microalgae C-uptake that were intermediate between those found in the presence of mussels only (the lowest values) and those found in the presence of clams only (the highest values). As additional results, we also found that the association between mussels and clams increased the microalgae C-uptake of mussels by a factor 2 as compared to the values found in the presence of mussels only, while it reduced the microalgae C-uptake of clams below the values found in the presence of clams only. These results indicated that (i) mussels can take be advantaged by an association with clams, increasing the food intakes while reducing those of clams and that (ii), by impairing feeding, incorporation and biodeposition of clams, mussels can reduce the availability of suspended microalgae for deposit feeders.
The negative effect of the Asian mussel on clam’s feeding was in accordance with other studies that showed the Asian mussel to decrease the densities, individual growth and survivorship of clams (Creese et al. 1997, Crooks 2001, Kushner 2005). The Asian mussel can interfere with the feeding of clam by both competition for food and/or space (Creese et al. 1997, Crooks 2001, Kushner 2005). Because the Asian mussel is gregarious and fast-growing, its filtration near-bottom water may locally deplete food supplies, thus affecting clam’s feeding (Crooks 2001, Kushner 2005). Although space is often not limiting in three dimensional, soft-sediment environments (Peterson and André 1980, Peterson 1991), the presence of mussel patches may also limit living space for clams. In our experimental settings and during field sampling it was observed that the Asian mussel can attach to living clams through the byssus and similar association has been also observed in other studies (Crooks 2001).

The association between invasive mussels and clams is very common in aquatic systems and an example is the association between the Zebra mussel and larger unionids in freshwater (Morales et al. 2006, Sousa et al. 2009). In the case of the Asian mussel, we assume that mussels use clam’s shells as substrata and that the mussel’s patches likely prevent the natural extension of the siphons at the water-sediment interface impairing food capture of the clams. Nevertheless, our results showed that the Asian mussel can be advantaged by an association with clams, increasing the microalgal intake, indicating that the role of clams in the association with the Asian mussel may be more complex than a simple structural role (i.e. the shells provide hard substrata for the byssal attachment). We anticipate that, in association with clams, the Asian mussel can exploit the water circulation generated by the clam’s feeding between the incurrent and the excurrent siphons. Exploiting the water circulation generated by the clam’s feeding may be an opportunistic strategy to save energy for other activities such as growth.

Conclusions.
This study provides the first evidence of the impact of the Asian mussel Arcuatula senhousia on the C flow within the food web with two-fold implications. Firstly, the Asian mussel can be advantaged by an association with native suspension feeders, increasing food intake. By doing this, it reduces the transfer of food from the water column to the native suspension feeders. Secondly, by lessening the benthic-pelagic coupling, it also reduces the food availability for deposit feeders.

These effects of the Asian mussel were found to be independent of mussel’s densities, indicating the C flow from the first to the second trophic levels of the benthic food web to be at risk from this invasion.

A. senhousia has a short lifespan and a high ability of dispersal and recruitment that ensures the periodic formation of the mussel’s patches in a system. For these reasons, it is possible that the effects of the Asian mussel on the C flow within the coastal food web would induce changes on biodiversity and abundances of native populations over long periods. We conclude that the alteration of the C flow imposed by the presence of the Asian mussel should be taken into account in future studies of management and conservation of coastal systems.

References
D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning


Crooks JA: The Role of Exotic Marine Ecosystem Engineers In: Biological Invasions in Marine Ecosystems Ecological, Management, and Geographic Perspectives (Rilov G and Crooks JA Eds.) 2009 Springer.


Rilov G and Crooks JA (Eds.): Biological Invasions in Marine Ecosystems Ecological, Management, and Geographic Perspectives. Springer


D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning

Tables

**Table 1. Experimental design.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Brief description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density of suspension feeders (SF)</td>
<td></td>
</tr>
<tr>
<td>High density of SF (H; 17 gFW core⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Presence of one species A</td>
<td>Mesocosms where <em>A. sehnousia</em> was added at high density (≈4000 ind. m⁻²)</td>
</tr>
<tr>
<td>Presence of one species R</td>
<td>Mesocosms where <em>R. decussatus</em> was added at high density (≈600 ind. m⁻²)</td>
</tr>
<tr>
<td>Presence of the two species A+R</td>
<td>Mesocosms where both <em>A. sehnousia</em> and <em>R. decussatus</em> were added at high density.</td>
</tr>
<tr>
<td>Low density of SF (L; 7 gFW core⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Presence of one species A</td>
<td>Mesocosms where <em>A. sehnousia</em> was added at low density (≈1500 ind. m⁻²)</td>
</tr>
<tr>
<td>Presence of one species R</td>
<td>Mesocosms where <em>R. decussatus</em> was added at low density (≈250 ind. m⁻²)</td>
</tr>
<tr>
<td>Presence of the two species A+R</td>
<td>Mesocosms where both <em>A. sehnousia</em> and <em>R. decussatus</em> were added at high density.</td>
</tr>
</tbody>
</table>
Table 2. The mean values (±SE) of the content of $^{13}$C in microalgae added to each treatment ($\mu$g$^{13}$C mgC), the percentage of carbon (C) that was replaced with $^{13}$C in algal preparations and the final microalgal concentration (mgC Microalgae L$^{-1}$). The mean values of the fractional abundance of $^{13}$C in the microalgal preparations (i.e. the fraction of heavy isotope of C compared to the total carbon measured) for each treatments is also reported.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>$\mu$g$^{13}$C mgC Microalgae</th>
<th>%</th>
<th>mgC Microalgae L$^{-1}$</th>
<th>$^{13}$C F Microalgae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SE</td>
<td>Mean ±SE</td>
<td>Mean ±SE</td>
<td>Mean ±SE</td>
</tr>
<tr>
<td>High density (17g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>11.14 0.00</td>
<td>1 0</td>
<td>146 22</td>
<td>0.01 0.00</td>
</tr>
<tr>
<td>R</td>
<td>11.14 0.00</td>
<td>1 0</td>
<td>138 22</td>
<td>0.01 0.00</td>
</tr>
<tr>
<td>A+R</td>
<td>11.14 0.00</td>
<td>1 0</td>
<td>152 26</td>
<td>0.01 0.00</td>
</tr>
<tr>
<td>Low density (7g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>11.13 0.01</td>
<td>1 0</td>
<td>163 27</td>
<td>0.01 0.00</td>
</tr>
<tr>
<td>R</td>
<td>11.12 0.00</td>
<td>1 0</td>
<td>107 14</td>
<td>0.01 0.00</td>
</tr>
<tr>
<td>A+R</td>
<td>11.13 0.00</td>
<td>1 0</td>
<td>135 33</td>
<td>0.01 0.00</td>
</tr>
</tbody>
</table>
Table 3. Results of leave-one-out allocation of observations to groups in CDA for macrofauna and sediment variables. Macrofauna were square-rooted transformed.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Macrofauna Classified into groups</th>
<th>Sediment variables Classified into groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6  Total  % correct</td>
<td>1 2 3 4 5 6  Total  % correct</td>
</tr>
<tr>
<td>Site 1–Pres. A. senhousia = 1</td>
<td>5 4 0 0 0 9 55.56%</td>
<td>4 3 0 0 2 0 9 44.44%</td>
</tr>
<tr>
<td>Site 1–Abs. A. senhousia = 2</td>
<td>4 13 1 0 0 0 72.22%</td>
<td>4 5 3 0 3 3 18 27.78%</td>
</tr>
<tr>
<td>Site 2–Pres. A. senhousia = 3</td>
<td>0 2 7 4 0 0 13 53.85%</td>
<td>0 1 4 6 0 2 13 30.77%</td>
</tr>
<tr>
<td>Site 2–Abs. A. senhousia = 4</td>
<td>0 0 5 9 0 0 14 64.29%</td>
<td>1 0 5 7 0 1 14 50.00%</td>
</tr>
<tr>
<td>Site 3–Pres. A. senhousia = 5</td>
<td>0 3 4 0 0 5 12 0.00%</td>
<td>2 0 0 0 5 12 41.67%</td>
</tr>
<tr>
<td>Site 3–Abs. A. senhousia = 6</td>
<td>0 2 0 0 4 9 15 60.00%</td>
<td>0 0 1 3 4 7 15 46.67%</td>
</tr>
</tbody>
</table>

Total correct = 43/81 = 53.09%
Mis-classification error = 46.91%

Total correct = 31/81 = 39.51%
Mis-classification error = 60.49%
## Table 4. Mean (±SE) δ\(^{13}\)C and δ\(^{15}\)N values in the tissues of *Hediste diversicolor*, *Haminoea navicula*, *Cyclope neritea*, *Cerastoderma glaucum*, two sizes of the Asian mussel *Arcuatula senhousia* (i.e., Juvenile [< 0.7 cm] and Adult [0.7-2.5 cm]), sedimentary organic matter (SOM) and seston (<55 µm) at each site.

<table>
<thead>
<tr>
<th></th>
<th>Sites with <em>A. senhousia</em> (I)</th>
<th></th>
<th>Sites without <em>A. senhousia</em> (C)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I1</td>
<td>I2</td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td><strong>δ(^{13})C</strong></td>
<td>Mean ±SE</td>
<td>Mean ±SE</td>
<td>Mean ±SE</td>
<td>Mean ±SE</td>
</tr>
<tr>
<td><em>H. diversicolor</em></td>
<td>-18.781 ± 0.159</td>
<td>18.685 ± 0.278</td>
<td>-19.625 ± 0.417</td>
<td>19.817 ± 0.334</td>
</tr>
<tr>
<td><em>H. navicula</em></td>
<td>-14.82 ± 1.25</td>
<td>18.02 ± 0.17</td>
<td>-18.73 ± 0.54</td>
<td>17.27 ± 0.35</td>
</tr>
<tr>
<td><em>C. neritea</em></td>
<td>-15.87 ± 0.48</td>
<td>19.90 ± 0.37</td>
<td>-17.26 ± 0.56</td>
<td>20.04 ± 0.19</td>
</tr>
<tr>
<td>SOM</td>
<td>-20.08 ± 0.59</td>
<td>6.03 ± 0.25</td>
<td>-22.53 ± 0.18</td>
<td>5.84 ± 0.81</td>
</tr>
<tr>
<td><em>C. glaucum</em></td>
<td>-19.97 ± 0.25</td>
<td>17.17 ± 0.29</td>
<td>-20.48 ± 0.18</td>
<td>17.75 ± 0.12</td>
</tr>
<tr>
<td>Seston[&lt;55µm]</td>
<td>-28.29 ± 0.10</td>
<td>10.35 ± 0.28</td>
<td>-24.82 ± 0.41</td>
<td>16.82 ± 0.20</td>
</tr>
<tr>
<td><em>A. senhousia</em>Juvenile [0.7-2.0 cm]</td>
<td>-22.50 ± 0.23</td>
<td>16.29 ± 0.27</td>
<td>-21.24 ± 0.02</td>
<td>17.00 ± 0.16</td>
</tr>
</tbody>
</table>
Table 5. Two-way analyses of variance on the δ¹³C and δ¹⁵N values of the deposit feeders *Hediste diversicolor*, *Haminoea navicula*, *Cyclope neritea* and SOM. The design included two sites within each level of factor *Arcuatula* (i.e. Impact and Control). Data were untransformed. The number of replicate stations within sites was three.

<table>
<thead>
<tr>
<th></th>
<th>Hediste diversicolor</th>
<th>Haminoea navicula</th>
<th>Cyclope neritea</th>
<th>SOM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ¹³C</td>
<td>δ¹⁵N</td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td><strong>Arcuatula = A</strong></td>
<td>1 14.26</td>
<td>1.55</td>
<td>0.34</td>
<td>21.79</td>
</tr>
<tr>
<td>Site(A) = S(A)</td>
<td>2 9.17</td>
<td>38.43</td>
<td>0.00</td>
<td>5.54</td>
</tr>
<tr>
<td>Residual</td>
<td>8 0.24</td>
<td>0.28</td>
<td>1.82</td>
<td>0.28</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cochran's C Test</strong></td>
<td>none</td>
<td>none</td>
<td>C=0.54, P&gt;0.05</td>
<td>none</td>
</tr>
<tr>
<td><strong>Transformation</strong></td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

|                   | δ¹³C | δ¹⁵N | MS | F | P | MS | F | P | MS | F | P | MS | F | P |
| **Arcuatula = A** | 1 22.01 | 1.85 | 0.31 | 11.64 | 1.77 | 0.32 | 32.47 | 3.29 | 0.21 | 0.41 | 0.05 | 0.85 |
| Site(A) = S(A)    | 2 11.91 | 18.25 | 0.00 | 6.59 | 23.22 | 0.00 | 9.88 | 18.21 | 0.00 | 8.73 | 7.31 | 0.02 |
| Residual          | 8 0.65 | 0.28 | 0.54 | 1.19 | 1.19 |
| Total             | 11  |  |  |  |  |  |  |
| **Cochran's C Test** | C=0.36, P>0.05 | C=0.36, P>0.05 | C=0.36, P>0.05 | C=0.48, P>0.05 | C=0.48, P>0.05 |
| **Transformation** | none | none | none | none | none | none |

Cochran's C Test C=0.54, P>0.05 C=0.37, P>0.05 C=0.64, P>0.05 C=0.55, P>0.05
Table 6. Analyses of variance for differences among sites and SNK test on the $\delta^{13}C$ and $\delta^{15}N$ values of *Hediste diversicolor*, *Haminoea navicula*, *Cyclope neritea*, SOM, *Cerastoderma glaucum* and Seston.

<table>
<thead>
<tr>
<th></th>
<th>$\delta^{13}C$</th>
<th></th>
<th>SNK test</th>
<th>$\delta^{15}N$</th>
<th></th>
<th>SNK test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_{3,8}$</td>
<td>$P$</td>
<td></td>
<td>$F_{3,8}$</td>
<td>$P$</td>
<td></td>
</tr>
<tr>
<td><em>H. diversicolor</em></td>
<td>45.53</td>
<td>0.00</td>
<td>I1=I2=C1&lt;C2</td>
<td>39.28</td>
<td>0.00</td>
<td>I1&gt;l2=C1&gt;C2</td>
</tr>
<tr>
<td><em>H. navicula</em></td>
<td>9.18</td>
<td>0.01</td>
<td>Nah (all&lt;C2)</td>
<td>139.30</td>
<td>0.00</td>
<td>I1=I2&gt;C1&gt;C2</td>
</tr>
<tr>
<td><em>C. neritea</em></td>
<td>23.40</td>
<td>0.00</td>
<td>I1=I2=C1&lt;C2</td>
<td>29.17</td>
<td>0.00</td>
<td>I1=I2&lt;C1&gt;C2</td>
</tr>
<tr>
<td>SOM</td>
<td>32.10</td>
<td>0.00</td>
<td>I1&lt;l2=C1&lt;C2</td>
<td>4.99</td>
<td>0.03</td>
<td>Nah (all&lt;C2)</td>
</tr>
<tr>
<td><em>C. glaucum</em></td>
<td>158.72</td>
<td>0.00</td>
<td>I1=I2&lt;C1&lt;C2</td>
<td>159.70</td>
<td>0.00</td>
<td>I1&gt;l2=C1&gt;C2</td>
</tr>
<tr>
<td>Seston (&lt;55µm)</td>
<td>89.83</td>
<td>0.00</td>
<td>I2&lt;C1&lt;I1&lt;C2</td>
<td>56.28</td>
<td>0.00</td>
<td>Nah (all&lt;C2)</td>
</tr>
</tbody>
</table>
Table 7. Asymmetrical ANOVA on the effects of the manipulation of suspension feeders on the microalgae C-uptake by the surface deposit feeder *Cirrphormia tentaculata* (μgC gFW⁻¹). Data were untransformed. *p*<0.05; Bold entries denote significant effects of factors or interactions in the analyses.

<table>
<thead>
<tr>
<th>Sources</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Densities = D</td>
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<td>1482946</td>
<td>1.31</td>
</tr>
<tr>
<td>Treatments = T</td>
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</tr>
<tr>
<td>A vs R</td>
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<td>11367560</td>
<td>6.80</td>
</tr>
<tr>
<td>A/R vs A+R</td>
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<td>828331</td>
<td>18.40</td>
</tr>
<tr>
<td>D × T</td>
<td>2</td>
<td>884728</td>
<td>0.78</td>
</tr>
<tr>
<td>D × A vs R</td>
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<tr>
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<tr>
<td>Residual</td>
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<tr>
<td>Residual (A vs R)</td>
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<td>1672180</td>
<td></td>
</tr>
<tr>
<td>Residual (A/R vs A+R)</td>
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<td>45007</td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td></td>
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</table>
Table 8. Analysis of variance and SNK test for differences in the algae-C uptake by *Arcuatula senhousia* and *Ruditapes decussatus* (mgC gFW⁻¹). Bold entries denote significant effects of factors or interactions in the analyses.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Arcuatula senhousia</th>
<th></th>
<th></th>
<th></th>
<th>Ruditapes decussatus</th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>MS</td>
<td>F</td>
<td>P</td>
<td>MS</td>
<td>F</td>
<td>P</td>
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<td>10.26</td>
<td><strong>0.01</strong></td>
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<tr>
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<td>0.18</td>
<td>0.68</td>
<td>107.24</td>
<td>0.96</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>T × D</td>
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<td>0.01</td>
<td>0.18</td>
<td>0.68</td>
<td>0.01</td>
<td>0.00</td>
<td>0.99</td>
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<td>Residual</td>
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<td></td>
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<td>C = 0.51(P &gt; 0.05)</td>
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<td>SNK test</td>
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<td></td>
<td></td>
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<td>R &gt; A+R</td>
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</table>
Figure 1. Location of the Gulf of Oristano (western Sardinia, W-Mediterranean Sea) and Santa Giusta and Marceddi lagoons where the study was conducted.
Figure 2. Sampling design and location of impacted (I) and control (C) sites in Marceddi lagoon. The sampling design included two factors, *A. senhousia* (2 levels; fixed) and Site (2 levels; nested in *A. sehnousia*) and 3 replicate stations.
Figure 3. Experimental set-up of the laboratory experiment in mesocosms with $^{13}$C-enriched microalgae to test the impact of *A. sehnousia* on the transfer of carbon to target deposit feeder (trophic flow).
Figure 4. Canonical discriminant analysis (CDA) for differences among stations in macrofauna and sediment variables. Symbols represent the three sites: Square, site 1; Circle, site 2; Diamond, site 3. Colours represent stations characterized by the presence/absence of *A. senhousia*: Black = Presence of *A. senhousia*; White = Absence of *A. senhousia*. 
Figure 5. Mean $\delta^{13}$C and $\delta^{15}$N values (±SE) in the deposit feeders *Hediste diversicolor* (open square), *Cyclope neritea* (open diamond), *Haminoea navicula* (open triangle), the suspension feeder *Cerastoderma glaucum* (full square), sedimentary organic matter (SOM; open circle) and seston (<55 µm) (full circle), in each site. The symbols indicate the mean $\delta^{13}$C and $\delta^{15}$N values (±SE) calculated among sampling stations within each site.
Figure 6. Mean (±SE) amount of microalgae derived-C taken up by the surface deposit feeder *Cirriphormia tentaculata* (µgC gFW⁻¹) for each treatment (n=3 replicate mesocosms). Treatments were mesocosms where only *Ruditapes decussatus* (R) or *Arcautula sehnousia* (A) were added and mesocosms where both *R. decussatus* and *A. sehnousia* were included (A+R), at each level of density of suspension feeders (i.e., low and high density).
Figure 7. Mean (±SE) amount of microalgae derived-C taken up by suspension feeder *Ruditapes decussatus* and *Arcuatula sehnousia* (µgC gFW⁻¹) for each treatment (n=3 replicate mesocosms). Treatments were mesocosms where only *Ruditapes decussatus* (R) or *Arcuatula sehnousia* (A) were added and mesocosms where both *R. decussatus* and *A. sehnousia* were included (A+R), at each level of density of suspension feeders (i.e., low and high density).
Figure 8. Schematic representation of direct and indirect effects of the invasive Asian mussel Arcuatula sehnousia on the transfer of carbon from primary producers to primary consumers in the benthic food web. The carbon uptake of specific consumers is represented by the dashed grey ovals and the transfer of carbon across trophic levels by dashed grey lines. Direct effects are represented by solid lines and indirect effects by dashed black lines. - = negative effects, + = positive effects. (1) The Asian mussel can directly reduce the microalgae C-uptake of the deposit feeder Cirrhophormia tentaculata, possibly decreasing the nutritional values of biodeposits. (2) The Asian mussel can reduce the transfer of carbon to C. tentaculata indirectly through the interaction with native clams. (3) The Asian mussel can reduce the microalgae C-uptake by the native clam, possibly because of competition for food and space. (4) Clams can increase the microalgae C-uptake of C. tentaculata directly, through production of biodeposits. (5) The Asian mussel can increase its microalgae C-uptake in presence of the native clam.
Appendix 3

Case study 3 - Invasive Chinese mitten crabs in NE UK

Impact of the invasive Chinese mitten crab (Eriocheir sinensis) on sedimentary ecosystems

VECTORS contributors: Andrew Blight (University of St Andrews), David Paterson (University of St Andrews), Stephan Gollasch (GoConsult) and Tasman Crowe (University College Dublin)

Other contributors: Martin Solan (National Oceanography Centre, University of Southampton), Paul Clark (Natural History Museum, London), David Morritt (Royal Holloway, University of London) and Matt Bentley (Newcastle University).

This document presents a somewhat more detailed description of the work and its findings and will be developed into a manuscript for publication.

Summary

Eriocheir sinensis (Chinese mitten crab) is an aggressive invader spreading around the UK coast and is on the IUCN list of the 100 most damaging invasive species in the UK and was also included in the handbook of the most invasive alien species in Europe (Gollasch 2009). Targeted experiments were carried out to (a) examine the influence of the crabs on ecosystem function (sediment destabilisation, sediment erosion and primary production) in synthetic assemblages to reveal the impact of the crabs on the ecosystem as the invader becomes established and (b) to test how bioturbatory activity varies with temperature and size of the crabs.

The experiments have shown:

1. There was a significant reduction in sediment stability (expressed as equivalent horizontal shear stress, Nm⁻²; Tolhurst et al 1999) of all crab treatments when compared to controls. Invaded habitats therefore have reduced resistance to sediment erosion.
2. There was no significant difference in the bioturbation depth profiles between the three temperature treatments, which suggests the mitten crabs were equally active across the temperatures examined.
3. There was no significant effect of Size Class, however the presence of individuals of Eriocheir sinensis with larger biomass and carapace length (Size Class A; mean ± SD, biomass = 8.85 ± 0.94 g, carapace length 26.77 ± 1.03 mm) were responsible for slightly higher levels of particle redistribution relative to individuals with smaller biomass and carapace length (Size Class B; biomass = 5.35 ± 0.39 g, carapace length 22.30 ± 0.49 mm).
4. The mean depth of vertical displacement of fluorescent particles initially placed on the surface of the sediment ranged from 0.621 cm (Size Class B) to 1.533 cm (Size Class A), the median depth ranged from 0.590 cm (Size Class B) to 1.510 cm (Size Class A), and the maximum depth ranged from 1.600 cm (Size Class B) to 3.265 cm (Size Class B).
5. The surface rugosity of the sediment-water interface (Surface Boundary Roughness) ranged from 0.970 cm to 2.960 cm.
6. The maximum luminophores penetration depth of the larger Eriocheir sinensis is greater than the average observed for other species in the North Sea region (mean ± SD of 2.7 ± 2.3cm; Teal et al 2008). This has implications for increased turbidity, nutrient release from sediments and potentially increased siltation of gravel beds, including fish spawning grounds.
7. Reduced sediment stability will impact invaded depositional habitats resulting in increased erosion and therefore changes in the ecology and dynamics, as well as the services provided by these ecosystems.

Introduction

The Chinese mitten crab (*Eriocheir sinensis*) is native to eastern Asia, ranging in occurrence from temperate Russia to tropical southern China. It is a well-known nuisance species in Europe and is found in all North and Baltic Seas countries with well-established populations in the UK, Germany, the Netherlands and France (Fig. 1). Introductions are likely to have been through ship movements and the discharge of ballast water in ports and river systems (Bentley, 2011). The mitten crab has moved up the eastern coast of the UK to establish its most northerly population in the river Tyne (Newcastle) (Herborg et al. 2002). The current range extension is unlikely to be due to coastal shipping movements alone, but is probably also supported by larval drift with regional water currents. We must consider that given past migration and an increase in global temperatures (as reviewed in WP1 and scenarios in WP5.1) the potential for the further northward movement of *E. sinensis* in European coastal waters is high.

Successful larval development occurs in water temperatures greater than 12°C, with survival rates increasing as temperatures increase (Anger, 1991). Optimal growth conditions occur between 20 and 30°C, with the cessation of growth below 7°C (Hymanson et al. 1999). Mitten crabs are known to threaten native species in freshwater, brackish and marine ecosystems and impact invaded habitats through bank erosion and collapse (Panning 1939; Rudnick et al. 2005). Key negative socio-economic impacts include damage and blockage of fishing gear, and predation upon inland aquaculture target species while a potential positive impact is the development of a fishery for mitten crabs in Germany for Chinese restaurants (Gollasch 2011).

Organisms which modify their physical environment and regulate the availability of resources for other species through burrowing activities and bioturbation are recognised as ecosystem engineers (Jones et al. 1994). The mitten crab is known for its burrowing activity and VECTORS researchers have been examining bioturbatory activity under the varying temperatures experienced by the crabs within their invaded habitats.

Figure 1. European distribution of *Eriocheir sinensis* with coastal records (solid red) and freshwater/brackish records (hatch red) (Image Gollasch 2009).
The erosion and transport of sediments is a key process in the ecology and dynamics of many aquatic ecosystems particularly estuaries and rivers. Depositional habitats such as mud banks, sandbanks, saltmarshes and mudflats develop where sediments accumulate. These systems provide a habitat and resource to many organisms as well as a variety of direct and indirect ecosystems services for the local environment (Paterson et al 2009). As mitten crabs invade new habitats they may have a significant impact on the erosional dynamics of the system and this impact is worthy of further investigation.

Materials and Methods

Density Experiment

We used a mesocosm approach to examine the progressive effect of increasing numbers of *Eriocheir sinensis* on sediment stability. Replicate aquaria (n = 5) containing varying densities of mitten crabs (0 crabs/controls; 1 crab/17.6 m$^{-2}$; 3 crabs/52.7 m$^{-2}$; 6 crabs/105.4 m$^{-2}$) were assembled. The experiment was conducted twice; once with males and once with females. Each aquarium (Exoterra Standard Faunaria, large; internal dimensions, LWH: 37 × 22 × 25 cm) was filled to a depth of 5 cm with sediment homogenate overlain by 10 cm of ambient water. Overlying ambient water was replaced after 24 h to remove excess nutrients associated with assembly. Aquaria were maintained under a 12:12h light cycle (AquaBeam 1000 HD Ultra Reef White; 7 × 14,000k white LED’s and 3 × 50,000k blue LED’s) and were continually aerated.

Using a cohesive strength meter (CSM, Paterson 1989) VECTORS researchers examined sediment stability in experimental mesocosm under varying densities of mitten crabs. The erosion threshold (psi) was established for each mesocosm by exposing the sediment to erosive water jets of gradually increasing pressure. The pressure at which sediment erodes is then expressed as an equivalent horizontal shear stress (Nm$^{-2}$; Tolhurst et al 1999).

Bioturbation Experiment

We used a mesocosm approach to quantify the contributions that individual *Eriocheir sinensis* make to the bioturbation of surface sediments. As body size is known to be a strong determinant of a species bioturbation potential (Solan et al. 2004a) and infaunal activity is affected by temperature (Ouellet et al. 2004), two size classes were investigated across three temperature levels (10°C, 15°C and 19°C) that reflect the range experienced at the study site across the annual cycle. Replicate (n = 5) aquaria containing a single individual of *Eriocheir sinensis* (mean ± SD, n = 15: Size Class A, biomass = 8.85 ± 0.94 g, carapace length 26.77 ± 1.03 mm; Size Class B, biomass = 5.35 ± 0.39 g, carapace length 22.30 ± 0.49 mm) were assembled. Each aquarium (transparent Perspex square core, internal dimensions, LWH: 12 × 12 × 33 cm) was filled to a depth of 12 cm with sediment homogenate overlain by 18 cm of ambient water. Overlying ambient water was replaced after 24 h to remove excess nutrients associated with assembly. Aquaria were maintained under a 12:12h light cycle (Aqualine T5 Reef White 10 K fluorescent light tubes, Aqua Medic) and were continually aerated.

Faunal mediated sediment particle reworking was estimated non-invasively using a sediment profile imaging camera (Canon 400D, 10s exposure, aperture f5.6 and ISO 400; 3888 × 2592 pixels, i.e. 10.1 megapixels, effective resolution 56 × 56 µm per pixel), optically modified to allow preferential imaging of fluorescently labelled sand-based particulate tracers under UV light (f-SPI, Solan et al. 2004b, Fig. 2). The redistribution of these tracers (90 g aquarium$^{-1}$, pink colour, size class ≤500 µm; Brian Clegg Ltd., UK) was determined from stitched composite images (RGB colour, JPEG compression) of all four sides of each aquarium obtained in a UV illuminated imaging box after 3 days using a custom-made semi-automated macro that runs within ImageJ (Version 1.47), a java-based public domain program...
developed at the US National Institutes of Health (available at http://rsb.info.nih.gov/ij/index.html). From these data, the median ($f^{SPI}_{med}$, typical depth of mixing), maximum ($f^{SPI}_{max}$, maximum extent of mixing) and mean ($f^{SPI}_{mean}$, indication of mixing) mixed depth of particle redistribution were calculated. In addition, the maximum vertical deviation of the sediment-water interface (upper – lower limit of mixing = surface boundary roughness, SBR) provided an indication of surficial activity.

Figure 2. $f$-SPI with luminophore tracers and a mitten crab on the sediment surface at the beginning of the experiment (top photo) and luminophores tracers mixed into the sediment at the end of the experiment (bottom photo) (Images by A Blight).
Results

Density Experiment

There was a significant reduction in sediment stability (expressed as equivalent horizontal shear stress, Nm⁻²; Tolhurst et al 1999) of all crab treatments when compared to controls for both males and females (ANOVA: males, $F_{3,16} = 10.88$, $p < 0.001$; females, $F_{3,8} = 6.54$, $p < 0.05$; Tukey HSD; Fig. 4).

![Figure 4](image)

Figure 4. Sediment stability (mean equivalent horizontal shear stress, Nm⁻²) under different densities of Chinese mitten crabs; (a) males and (b) females.

Bioturbation Experiment

Overall, vertical particle redistribution revealed a slightly non-significant effect of Size Class, but we found no evidence for the effects of “Temperature” or the interactive effects of “Size Class × Temperature” for any of our response variables. The presence of individuals of *Eriocheir*...
Eriocheir sinensis with larger biomass and carapace length (Size Class A) were responsible for somewhat higher levels of particle redistribution relative to individuals with smaller biomass and carapace length (Size Class B), although these differences were not quite significant (ANOVA: f-SPIL\textsubscript{mean}, F\textsubscript{1,28} = 3.82, p=0.0606, Fig. 3a; f-SPIL\textsubscript{med}, F\textsubscript{1,28} = 3.1777, p=0.0855, Fig. 3b). The mean depth of vertical displacement (f-SPIL\textsubscript{mean}, n = 30; Fig. 3a) ranged from 0.621 cm (Size Class B) to 1.533 cm (Size Class A), the median depth (f-SPIL\textsubscript{med}, n = 30; Fig.3b) ranged from 0.590 cm (Size Class B) to 1.510 cm (Size Class A), and the maximum depth (f-SPIL\textsubscript{max}, n = 30) ranged from 1.600 cm (Size Class B) to 3.265 cm (Size Class B). Despite this range of activity, it was not possible to determine any effect of Size Class, Temperature, or their interaction, on surface boundary roughness (intercept only model, F=651.6, d.f. = 1, p<0.0001). Interestingly, however, the model for surface boundary roughness did require a variate-covariate structure to account for the influence of both Temperature and Size Class (GLS: F = 651.61, L-ratio = 0.699, d.f. = 1, p<0.0001). The vertical displacement of the sediment-water interface (SBR, n = 30) ranged from 0.970 cm to 2.960 cm.

![Figure 3](image-url)

**Figure 3.** Sediment reworking associated with *Eriocheir sinensis*. Individual effects are depicted for Size Classes A and B for the particle reworking metrics (a) f-SPIL\textsubscript{mean} and (b) f-SPIL\textsubscript{med}.

**Discussion**

The presence of mitten crabs significantly reduces sediment stability, with little difference observed between the densities examined. The simple occurrence of the crabs in invaded habitats therefore reduces the resistance of that habitat to sediment erosion. A consequence of this will be the transport of sediment from the river to the estuary and the change in conformation and diversity of depositional habitats.
The lack of any difference in the bioturbation depth profiles between the temperature treatments suggests the mitten crabs are equally active across the temperatures examined. Exposure to lower temperatures within invaded habitats could pose an energetic cost to growth and development in mitten crabs. Some limited research has indicated, however, that there is no such cost at lower temperatures (Jakubowska & Normant 2011). The marginal difference in bioturbation depth profiles between size classes suggest that body size is a determinant of a species bioturbation potential (Solan et al. 2004a). The maximum luminophores penetration depth of the larger *Eriocheir sinensis* is greater than the average observed for other species in the North Sea region (mean ± SD of 2.7 ± 2.3cm; Teal et al. 2008).

It is clear that lower temperatures do not seem to impede the bioturbatory activity of the Chinese mitten crab which has ecosystem-wide implications for invaded habitats, with increasing turbidity, nutrient release from sediments and potentially increased siltation of gravel beds, including fish spawning grounds. It also has implications under current climate warming predictions for the further northward movement of mitten crabs in European coastal waters. Reduced sediment stability will impact invaded depositional habitats resulting in increased erosion and therefore changes in the ecology and dynamics, as well as the services provided by these ecosystems.

**Main contributing partners:** USTAN, UCD, GoConsult

**References**


Appendix 4A-E

Case study 4 - Invasive amphipods in the Baltic Sea

Appendix 4A. High fecundity and predation pressure of non-indigenous *G. tigrinus* cause a decline of indigenous gammarids.

Jonne Kotta, Holger Jänes, Kristjan Herkül
Estonian Marine Institute, University of Tartu, Estonia

Material and methods

In situ mesocosm experiment

In situ mortality of juvenile amphipods was estimated in a factorial field experiment in Kõiguste Bay in 2011. The studied factors and factor levels were as follows: presence of alga (present, absent) and adult gammarid community (no adults, *G. tigrinus*, *G. duebeni*, *G. oceanicus*, *G. zaddachi*, a mixture of *G. tigrinus* and *G. duebeni*, a mixture of *G. tigrinus* and *G. oceanicus*, a mixture of *G. tigrinus* and *G. zaddachi*). The freshly collected macroalga *Ulva intestinalis* was instantly cleaned from macroepibionts, weighed, placed in nylon meshbags. The meshbag had the following dimensions: diameter 5.5 cm, height 20 cm, 1 mm mesh size. Each meshbag had a plastic frame inside in order to avoid collapsing of the meshbag. The other half of the meshbags included no algae. Then gammarid adults were added to meshbags at a density of 6 individuals per bag. Mixed community treatments had 3 individuals of each species. The mesocosms were incubated at 1 m depth (at 1 m above the sea bottom) for two weeks from 26 July to 10 August 2011. Each combination of treatment levels had four replicates (total n = 64). Our design allowed water exchange between algae and the ambient environment as well as free movement of juvenile gammarids.

Prior to the experiment the wet weight of algae was determined to the nearest 0.01 g. Before weighing the algae were gently dried on blotting paper until the paper did not become wet any more. An additional five replicates of each macroalgal treatment served as the control to obtain the ratio of wet to dry weight (the algae were dried at 60 °C during 48 h; the ratio differed among replicates < 0.3%). At the end of the incubation period the dry weight of the remaining algal material was measured. On average the algal biomass within the meshbag was estimated at 0.31 g dw.

Outdoor mesocosm experiment

Fecundity and mortality of gammarid amphipods were studied in flow-through mesocosms with a dimension of 39 × 48 × 59 cm on the coast of Kõiguste Bay in 2012. Water flow (filtered natural seawater) kept the mesocosm temperature similar to the field conditions. In order to mimic a natural habitat a boulder (15 cm diametre) with a well-developed community of *Ulva intestinalis* was placed into each mesocosm. Care was taken to remove all benthic invertebrates from the algal communities. Each mesocosm was then seeded with adult gammarid amphipods. The mesocosm experiment included one factor (adult gammarid community) with the following factor levels: low dense *G. tigrinus* (8 individuals), high density *G. tigrinus* (16 individuals), low dense *G. duebeni* (8 individuals), high density *G. duebeni* (16 individuals), a mixture of *G. tigrinus* and *G. duebeni* (8 individuals each, total 16 individuals), no gammarids. The number of individuals in the two species treatment was equal to that of a
single-species high-density treatment enabling comparison of species and density effects on mortality and reproduction. The experiment lasted from 11 June to 5 July 2012.

Appendix 4B. Spatial predictive modeling reveals niche separation between native and invasive gammarid species (Crustacea, Amphipoda) in the Baltic Sea

Kristjan Herkül, Jonne Kotta, Estonian Marine Institute, University of Tartu, Estonia

1. Introduction

Invasions of nonindigenous species (NIS) are one of the most serious threats to global biodiversity (Costello et al 2010). This human-aided process has led to significant unpredictable and irreversible changes to both the abiotic and biotic environment and has caused severe economic damage in a variety of waterbodies worldwide (e.g. Carlton, 1996; Vitousek et al, 1997; Sala et al, 2000). Several non-indigenous amphipods have been found in the northern Baltic Sea in recent years (Herkül et al 2006a, Herkül et al 2009).

Six sympatric species of the amphipod genus *Gammarus* can be found in the brackish water of the northern Baltic Sea. There are five native *Gammarus* species: *G. zaddachi*, *G. salinus*, *G. oceanicus*, *G. duebeni* and *G. locusta*, and an invasive species *G. tigrinus* that originates from North America. *G. tigrinus* was found for the first time in the northern Baltic Sea in 2003 and its range has been continuously expanding (Herkül et al 2006b, Herkül et al 2009, Kotta et al 2013). A decrease in the diversity and density of native amphipods has been observed concurrent with the invasion of *G. tigrinus* (Grabowski et al 2006, Kotta et al 2006, Orav-Kotta et al 2009).

Species distribution models (SDMs) are numerical methods that relate observations of species occurrences or abundances to environmental variables (Elith & Leathwick 2009). These relationships are further used to predict species distribution across different spatial and/or temporal scales (Elith & Leathwick 2009). Unlike in the case of simple plotting of species occurrences on the map, modeled distribution maps enable assessment of (1) surface area of habitats, (2) distribution of species in the areas that were not sampled or sampled sparsely, (3) spatial overlap of distributions of different species.

Approaches for estimating environmental niche are mainly based on either ordination methods or species distribution models (SDMs) (Broennimann et al 2012). Ordination enables assessment of a niche in multidimensional environmental space by constructing synthetic axes from measured environmental variables (e.g. principal component analysis, canonical correspondence analysis, outlying mean index; ter Braak & Verdonschot 1995, Doledec et al 2000). Using SDMs enables assessment of spatial distribution overlap. We used both approaches in this study.

By applying multivariate ordination and species distribution modeling we addressed the following questions:
- do environmental niche spaces differentiate between the gammarid species?
- how many of the distribution areas of gammarid species overlap?
2. Material and methods

2.1. Study area

The Baltic Sea is a tideless brackish water sea. This study was conducted in the coastal water of western Estonia, northern Baltic Sea (figure 1). The area is characterized by complex topography with numerous islands, islets, bays, and peninsulas. Most of the study area is very shallow with water depths seldom exceeding 25 m. Strong gradients of wave exposure and salinity exist in the area. The sea areas west of the islands Saaremaa and Hiiumaa are exposed to the open Baltic Proper and have a wave fetch of hundreds of kilometers. Contrastingly, the inner reaches of the bays of the mainland (e.g. Matsalu and Haapsalu bays, see figure 1) are very sheltered both by the mainland and islands. Similarly to the wave exposure, the salinity gradient generally follows the E-W-direction. Salinity reaches 7 in the Baltic Proper while it falls almost to zero in the inner parts of bays with riverine inflow in the eastern study area. Water transparency is highest in the Baltic Proper. Hard limestone substrate and granite boulders dominate in the most exposed areas. Different mixed sediments of sand, gravel, and pebbles can be found in the mid-range of the exposure gradient. Fine sand and mud dominates in the most sheltered bays. Scattered single boulders or boulder fields can be found throughout the area in shallow waters.

Variability in the abiotic environment is also reflected in the structure of macrobenthic communities. Species of marine origin dominate in the areas of medium and high salinity while fresh water species dominate in the eastern bays with riverine inflow. Among algal species the brown alga *Fucus vesiculosus* and the red alga *Furcellaria lumbricalis* are the most important perennial key species on hard substrate seabed. Several annual and perennial filamentous green, brown, and red algae like *Ulva intestinalis*, *Cladophora glomerata*, *Pilayella littoralis*, *Ceramium tenuicorne*, *Polysiphonia spp* are very common. Many species of vascular plants are common on soft substrate: *Zostera marina*, *Stuckenia pectinata*, *Potamogeton perfoliatus*, *Zannichellia palustris*, *Myriophyllum spicatum*, *Ruppia maritima*. Charophytes (*Chara spp.*, *Tolypella nidifica*) are common on sandy and muddy sediments in the shallow waters of the most sheltered bays. Extensive growth of ephemeral filamentous algae and formation of drift algal mats, as a result of eutrophication, are common phenomena in the area (Kotta et al 2008).

Among the invertebrates, the bivalve *Mytilus trossulus* and the barnacle *Amphibalanus improvisus* prevail on hard bottoms. Gammarid amphipods, idoteid isopods, the snails *Peringia ulvae*, *Radix balthica*, and *Theodoxus fluviatilis* are common in vegetated areas. Common infunal species in soft sediments are the bivalves *Macoma balthica* and *Cerastoderma glaucum* and polychaetes *Hediste diversicolor* and *Marenzelleria neglecta*. Various insect larvae are common in shallow coastal areas with low salinity.

All five species of native gammarids are present in the study area but *G. duebeni* and *G. locusta* are very rare in benthic samples compared to the other native species (*G. salinus*, *G. oceanicus*, *G. zaddachi*) and the non-indigenous *G. tigrinus*. Therefore, only *G. salinus*, *G. oceanicus*, *G. zaddachi*, and *G. tigrinus* were included in this study.

2.2. Benthos samples

Macrobenthos database of the Estonian Marine Institute, University of Tartu was used as a data source on the distribution of gammarids. Only quantitative biomass samples from the...
period of 2005-2012 were used. Data was averaged when several samples were collected from the same sampling station. Biomass samples from soft bottoms were predominantly collected using Ekman type or Van Veen type bottom grab samples. Samples from hard substrate were collected by divers by harvesting all material inside a 20×20 cm metal frame. Samples were sieved through a 0.25 mm mesh and all retained material was stored deep frozen (-18º C) until analysis. In the laboratory, all samples were sorted under a binocular microscope (20–40 × magnification). All macrobenthic organisms were identified to species level except for oligochaetes, chironomids, and juveniles of gammarid amphipods (length < 5 mm). Juvenile gammarids were not included in the further data analyses. Biomass sampling and analysis followed the guidelines developed for the HELCOM COMBINE programme (HELCOM 2014). The locations of sampling sites are shown in Figure 1.

![Figure 1. Study area. Locations of sampling stations and species distribution modeling area are shown.](image)

2.3. Environmental niche analysis

Niche breadth and separation of habitat niche between gammarid species was assessed using analysis of outlying mean index (OMI). OMI, or species marginality, measures the distance between the mean habitat conditions used by species (niche center), and the mean habitat conditions of the sampling area (Dolédec et al 2000). The higher the value of the OMI index of a species, the higher its habitat specialization. OMI analysis is a multivariate coinertia analysis that, unlike canonical correspondence analysis (CCA) and redundancy analysis (RDA), can handle non-unimodal and nonlinear species–environment relationships. Compared to the traditional multivariate methods, CCA and RDA, OMI gives a more even weight to all sampling units even if they exhibit a low number of species or individuals. Thus, OMI more adequately captures the multivariate environmental space represented by sampling units (Dolédec et al
A detailed description of OMI can be found in Dolédec et al (2000). The package “ade4” (Dray & Dufour 2007) was used for running OMI analysis in the statistical software R 2.15.1 (R Core Team 2012). A permutation test with 9999 permutations was used to calculate the statistical significance of the values of OMI of each gammarid species. The environmental niche space of gammarid species was visualized based on the OMI ordination of sampling points on two synthetic axes and by drawing a convex hull over the points where the species was present. 5 % of species occurrences that were most distant from niche center were considered as outliers and were excluded when drawing the border of niche space.

Abiotic and biotic georeferenced environmental data was used for environmental niche analysis and for species distribution modelling. The abiotic environmental variables included different bathymetrical (depth, slope of seabed), topographic (distance to land), hydrodynamic (wave exposure), geological (seabed substrate), and physico-chemical (temperature, salinity, transparency, ice conditions) variables. The biotic variables included chlorophyll content and modeled probability of occurrence of key phytobenthic species (Fucus vesiculosus, Furcellaria lumbricalis, and charophytes). Altogether 20 environmental variables were used that were all available as raster layers in a geographical information system. The full list of variables with additional information is presented in table 1.
Table 1. Variables used in the environmental niche analysis and species distribution models. Five variables with the highest importance in the final random forest (RF) models are indicated for each gammarid species (higher rank indicates higher importance).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>G. oceanicus</th>
<th>G. salinus</th>
<th>G. zaddachi</th>
<th>G. tigrinus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Average depth in 500 m radius</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Average depth in 2000 m radius</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Slope of seabed</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope of seabed in 500 m radius</td>
<td>1</td>
<td>3</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Slope of seabed in 2000 m radius</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance to land</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance to 20 m depth isoline</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of soft sediment (modeled)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Salinity</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wave exposure</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll a content based on satellite imagery; average over 2009-2010</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water transparency estimated as attenuation coefficient based on satellite imagery; average over 2010-2012</td>
<td>2</td>
<td></td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Water temperature based on satellite imagery; average over 2009-2010</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of ice days per year; average over 2009-2011</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice coverage; average over 2009-2011</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice thickness; average over 2009-2011</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability of occurrence of Furcellaria lumbricalis (modeled)</td>
<td>2</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability of occurrence of Fucus vesiculosus (modeled)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Probability of occurrence of charophytes (modeled)</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sources:
1 - Bathymetric raster, developed in the Estonian Marine Institute
2 - Databases of the Estonian Marine Institute
3 - Wave exposure calculations for the Estonian coast (Nikolopoulos & Isæus 2008)
4 - Finnish Meteorological Institute

2.4. Species distribution models (SDMs)

Generalized additive models (GAM), boosted regression trees (BRT) and random forests (RF) were used as modelling algorithms for predicting the spatial distribution of gammarid species. All distribution modelling exercises were done in the statistical software R 2.15.1 (R Core Team 2012). Several candidate models were built for each gammarid species to find the one with the best predictive performance. 80% of the input data was randomly selected and used as model training data while 20% of the data was reserved for validation. The predictive performance of the candidate models was validated by calculating the area under the receiver operating curve (AUC, Fielding & Bell 1997). The algorithm that produced models with the highest overall AUC value was then used to build the final model using 100% of the input data. The approach of selecting a single best-performing algorithm to produce the final models for all species was
chosen in order to get comparable distribution predictions and to achieve a more balanced interpretation of differences in distributions.

GAM is a semi-parametric extension of generalized linear models that enables the user to fit complex non-linear relationships and handle different types of error distributions (Hastie & Tibshirani 1990). Due to these characteristics, GAM has been one of the most widely used methods for SDM (Elith et al 2006). The package “mgcv” was used for building GAMs (Wood 2006). The models were built using penalized regression splines as the smoothing function, binomial error distribution, and automatic calculation of smoothing parameters. The maximum degree of freedom was set to four for each variable. At first, single predictor models were built. Predictors were then added into the final model in the decreasing order of explained deviances of single variable models.

BRT is an ensemble method that combines the strength of two algorithms: regression trees and boosting (Elith et al 2008). Regression trees are good at selecting relevant predictor variables and can model interactions. Boosting enables a building of a large number of trees in a way that each successive tree adds small modifications in parts of the model space to fit the data better (Friedman et al 2000). The algorithm keeps adding trees until finding the optimal number of trees that minimizes the predictive deviance of a model. The predictive performance of BRT has been shown to be superior to most other modeling methods (Elith et al 2006, Revermann et al 2012). The BRT modelling was performed using packages “gbm” (Ridgeway 2012) and “dismo” (Hijmans et al 2012). Important parameters in building BRT models are learning rate, tree complexity, and bag fraction (Elith et al 2008). Learning rate determines the contribution of each tree to the growing model and tree complexity defines the depth of interactions allowed in a model. Bag fraction determines the proportion of data to be selected randomly at each iteration. Different combinations of these parameters may yield variable predictive performance but generally a lower learning rate and inclusion of interactions gives better results. For each species, two groups of BRT models were built that had tree complexity of 1 and 5, respectively. A tree complexity of 1 fits a model without interactions between predictors while a tree complexity of 5 fits a model with up to five-way interactions. In both groups models with learning rates of 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, and 0.1 were built. The bag fraction was set at 0.5 which is the recommended default value for presence-absence models (Elith et al 2008). This design resulted in 14 models for each species.

RF is a machine learning method that generates a large number of regression trees, each calibrated on a bootstrap sample of the original data (Breiman 2001). Each node is split using a subset of randomly selected predictors and the tree is grown to the largest possible extent without pruning. For predicting the value of a new data point, the data is run through each of the trees in the forest and each tree provides a value. The model prediction is then calculated as the average value over the predictions of all the trees in the forest (Breiman 2001). The package “party” (Hothorn et al 2006) was used to run RF models in R. Two parameters must be set in RF models: the number of predictor variables to be randomly selected at each node (mtry) and the number of trees in a forest (ntree). mtry was set to one third of the number of predictor variables as suggested by Liaw & Wiener (2002). ntree was set to 1000 as 500 trees usually yield stable results. The importance of predictor variables was assessed by using the AUC-based permutation (n=1000) routine (Janitza et al 2012) in the package “party” (Hothorn et al 2006)

Schoener’s D was used to assess the overlaps of distributions of gammarid species based on the results of SDMs. There are many metrics for measuring the distribution overlap but
Schoener’s D was chosen because of its simplicity, long history of use and good performance (Rödder & Engler 2011, Warren et al. 2008). Schoener’s D varies between zero and one. Zero indicates no overlap while one indicates full distribution overlap between species.

3. Results

3.1. Environmental niche analysis

According to OMI analysis *G. salinus* had the largest environmental niche space followed by *G. zaddachi, G. oceanicus,* and *G. tigrinus*. The niche space of invasive *G. tigrinus* was notably less than that of the native species *G. salinus* and *G. zaddachi* (Figure 2). The centers of niche positions of native species almost coincided while that of *G. tigrinus* laid notably farther apart (Figure 2). Based on the values of OMI, *G. tigrinus* had the highest habitat specialization among the studied gammarid species while *G. salinus* had the lowest specialization (Figure 2). OMI values of all species were statistically significantly (p < 0.001) related to environmental variables.

![Figure 2. Environmental niche spaces and niche centers of gammarid species based on OMI analysis. Dots represent sampling sites in the environmental space. 5 % of species occurrences that were most distant from niche center were excluded when drawing the border of niche space. The value of OMI is shown in square brackets. Letters on the plot indicate the centers of niche space of the species.](image)
3.2. Species distribution models (SDMs)

The accuracy of models was good, as the AUC values of all models exceeded 0.8. GAM showed somewhat lower accuracy than BRT and RF while the AUC values of BRT and RF were very similar (Table 2). Among the models of native species, RF had the highest AUC values. BRT was only fractionally better than RF in the case of *G. tigrinus*. As RF produced the best models among three out of four species and gave essentially equally good result in the case of one species, RF was chosen as the method for modeling the spatial distribution of gammarid species. The predicted distributions clearly differed between gammarid species in terms of both the distribution area and distribution pattern (Figure 3). Based on SDMs, *G. salinus* had the most extensive distribution area. The distribution pattern of the invasive *G. tigrinus* clearly distinguished from the distributions of the native gammarids: the probability of occurrence of *G. tigrinus* was highest in sheltered bays where the probability of occurrence of native species was very low (Figure 3).

Table 2. AUC values of SDMs. GAM – generalized additive model, BRT – boosted regression trees, RF – random forest. RF was chosen to model the spatial distribution of gammarid species.

<table>
<thead>
<tr>
<th>Species</th>
<th>GAM</th>
<th>BRT</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. tigrinus</em></td>
<td>0.884</td>
<td>0.898</td>
<td>0.896</td>
</tr>
<tr>
<td><em>G. oceanicus</em></td>
<td>0.824</td>
<td>0.847</td>
<td>0.857</td>
</tr>
<tr>
<td><em>G. salinus</em></td>
<td>0.816</td>
<td>0.858</td>
<td>0.865</td>
</tr>
<tr>
<td><em>G. zaddachi</em></td>
<td>0.815</td>
<td>0.821</td>
<td>0.829</td>
</tr>
</tbody>
</table>

Figure 3. Modeled distributions of the gammarid species. The color gradient indicates the probability of occurrence of a given species. The central area of the study area is zoomed in for easier comparison.
Based on the SDMs, the overlap of distribution was larger among native species than between \textit{G. tigrinus} and native species (Table 3). Among distribution overlaps between native species and invasive \textit{G. tigrinus}, the overlap was largest between \textit{G. zaddachi} and \textit{G. tigrinus} and smallest between \textit{G. oceanicus} and \textit{G. tigrinus}.

Table 3. Schoener’s D values indicating distribution overlaps between gammarid species. Higher value of Schoener’s D indicates larger overlap.

<table>
<thead>
<tr>
<th></th>
<th>\textit{G. tigrinus}</th>
<th>\textit{G. oceanicus}</th>
<th>\textit{G. salinus}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{G. oceanicus}</td>
<td>0.277</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{G. salinus}</td>
<td>0.265</td>
<td>0.670</td>
<td></td>
</tr>
<tr>
<td>\textit{G. zaddachi}</td>
<td>0.380</td>
<td>0.596</td>
<td>0.526</td>
</tr>
</tbody>
</table>

4. Discussion

OMI index, that shows the habitat specialization, varied between the studied species. There was a more than three-fold difference between the value of OMI index of the most specialized species \textit{G. tigrinus} and the least specialized species \textit{G. salinus}. Earlier studies have demonstrated that \textit{G. tigrinus} is a very euryoecious species in its native distribution range (Steele & Steele 1972, Bousfield 1973) while the habitat niches of \textit{G. oceanicus}, \textit{G. salinus}, and \textit{G. zaddachi} are narrower than that of \textit{G. tigrinus} in North America. Contrastingly, based on the results of this study, the habitat niche of \textit{G. tigrinus} is significantly narrower than that of the studied three common native gammarids in the Baltic Sea. A recent study in the Gulf of Finland, northern Baltic Sea, also suggests that \textit{G. tigrinus} is more selective in the invasive range than in the native range (Kotta et al 2013). The reason for a narrower niche in the invasive range in Europe than in the native range may be related to the lower genetic diversity of the species in the invasive range as the number of genetically distinct clades has found to be much lower in Europe than in the native range (Kelly et al 2006).

The species distribution models (SDMs) produced predictions with high accuracy and thus proved to be useful for comparing the distribution of gammarid species. The high prediction accuracy can be explained by several reasons: (1) the input dataset of the presence and absence of gammarid species was very representative including thousands of records and covering all important environmental gradients; (2) modern modeling algorithms like RF and BRT have proved to produce highly accurate predictions (e.g. Elith et al 2006, Lindegarth et al 2014); (3) the elaborate set of environmental variables (including biotic predictors) for model building reflected well the natural gradients and heterogeneity in the study area. Most studies on species distribution modeling have focused only on abiotic drivers of species distribution (Zimmermann et al 2010). However, inclusion of biotic predictors, like competitors or facilitators, may increase the predictive power of models (Araújo & Lutu 2007, Pellissier et al 2010). In this study, the distribution of key phytobenthic species were highly important predictors in the distribution models of gammarids: the probability of occurrence of \textit{Fucus vesiculosus}, \textit{Furcellaria lumbricalis} and charophytes were among the top four predictors in the models of every gammarid species (Table 1). This proves that available species distribution maps can be a rewarding input for species distribution modeling. As gammarid amphipods inhabit the phytobenthic zone, the distribution of key macrophytes reflects the habitat distribution of gammarids. The three key macrophytes represent different habitat conditions. Charophytes grow on soft sediments in the most sheltered bays. \textit{F. vesiculosus} and \textit{F. lumbricalis} grow on hard substrate but in different depths and wave exposure levels. Based on the importance of predictor variables in the SDMs, \textit{G. tigrinus} was most related to charophytes.
and native gammarids to *F. vesiculosus* and *F. lumbricalis* indicating habitat separation between the invasive and native species.

The modelled distribution maps (Figure 3) clearly improved the understanding of the spatial distribution of gammarids in the study area. Additionally, the distribution maps enabled assessment of the spatial overlap between species. Based on the pair-wise comparisons of distribution overlap (Schoener’s D), the distribution of *G. tigrinus* had the smallest overlaps with all the other species. This result complies well with the values of OMI that indicated that *G. tigrinus* had the highest habitat specialization. Among native gammarids, the distribution of *G. zaddachi* had the largest overlap with the distribution of *G. tigrinus*. Based on that we can assume that among native gammarids the competitive pressure of *G. tigrinus* is strongest on *G. zaddachi*.

5. Conclusions

The environmental niche space of the non-indigenous *G. tigrinus* was narrower than those of the native gammarids. Additionally, the centers of niche positions of native species almost coincided while that of *G. tigrinus* laid notably farther apart. This differentiation was also well reflected in the modeled distributions: highest probabilities of occurrence of *G. tigrinus* were spatially restricted to the most shallow and sheltered areas that are predominantly of lower salinity. These findings indicate that the success of invasion of *G. tigrinus* has been facilitated by specific habitats that are of lower suitability for the native gammarid species. Considering the intraguild competition and predation between the invasive and native gammarids, *G. tigrinus* most likely has the strongest effect on *G. zaddachi* as the distribution overlap between the two was the largest.
References


Herkül K, Kotta J, Kotta I, Orav-Kotta H. 2006b. Effects of physical disturbance, isolation and key macrozoobenthic species on community development, recolonisation and sedimentation processes. Oceanologia 48 (S), 267-282


and facilitative effects of a dominant species on the distribution of tundra plants. Ecography 33, 1004-1014


Appendix 4C.

Effects of the invasive crab *Rhithropanopeus harrisii* (Gould, 1841) on shallow water meiofauna in the Baltic Sea

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Abstract

Recently the Harris mud crab *Rhithropanopeus harrisii* expanded into large parts of the Baltic Sea. Providing the appearance of novel functions (top-down predation, efficient burrowing) this invasion is expected to cause significant repercussions on the structure and functioning of benthic communities. In this study we experimentally evaluated the effect of the invasive crab on the species composition and dominance structure of the shallow water meiofauna community of the Baltic Sea. Among the studied environmental variables *R. harrisii* had by far the strongest effects on meiofauna. The effects of *R. harrisii* varied among different habitats with the mud crab mostly modifying meiofaunal communities on unvegetated soft bottom sediments. The effects were mostly due to changes in taxonomic composition rather than abundances. Nevertheless, the mud crab decreased the number of settling bivalves and increased the abundance of turbellarians.

Introduction

The Baltic Sea hosts no native crabs and therefore local benthic communities are largely shaped by the availability of food and space (Pallo et al. 1998; Giere 2009) rather than the presence of a strong top-down predation pressure in the oceanic waters (Menge 1976). It was only recently when the Harris mud crab *Rhithropanopeus harrisii* (Gould, 1841) expanded into large parts of the Baltic Sea. The mud crab is the first crab species capable of reproducing in the diluted Baltic Sea and forming high-density populations (Kotta and Ojaveer 2012). The invasion of the crab will therefore provide a new functional trait in the Baltic Sea range, which is expected to have significant repercussions for the structure and functioning of soft and hard-bottom benthic macrophyte and invertebrate communities. Besides being an efficient burrower, the mud crab intensively reworks sediment and through such bioturbation activity can also modify sediment granulometry and chemistry (Gilbert et al. 1998; Botto and Iribarne 2000; Fanjul et al. 2007).

The introduction of an efficient predator is expected to result in changes in the abundance of organisms across many trophic levels (e.g. Carpenter and Kitchell 1993). For example, crabs may control the abundance of grazers (Silliman et al. 2004) and thereby modify the growth patterns of micro- and macroalgae. Crabs may also prey on deposit and suspension feeding bivalves and thereby affect the trophic state of sediment (Norkko et al. 2001; Giles et al. 2006; Cranford et al. 2009). Burrowing crabs can inhibit the growth of snails (Armitage and Fong 2006) and disturb bivalves feeding and damage their shells (Lomovasky et al. 2006). Moreover, chemical cues caused by crab predation may induce prey populations to change their
behaviours. Such shifts may also have dramatic consequences for benthic ecosystem functioning (Premo and Tyler 2013).

*R. harrisii* can influence meiofauna through different mechanisms. Specifically, the diet of *R. harrisii* consists of detritus to a considerable extent (e.g. Czerniejewski and Rybczyk 2008) and thereby the species may exert a strong food competition on some taxa of meio- and macrofauna. Moreover, the reduction of macrofauna may have severe repercussions to meiofauna, as macrofauna is known to have an important structuring effect on meiobenthic communities (Ólafsson 2003). Moreover, the reduction of abundance of deposit feeding bivalves or other macrobenthos may reduce the competitive interactions for food among detritus feeding meiofauna. Also the burrowing activity of crabs can cause physical disturbance that directly alter the abundance and species composition of meiofauna (Warwick et al. 1990; Rosa and Bemvenuti 2005). However, the effects are known to vary among the taxa. For example, crab burrowing reduces the abundance of ostracods, copepods and turbellarians (Rosa and Bemvenuti 2005) but not nematodes (Warwick et al. 1990) and in some instances their abundance may even be increased (Shimanaga et al. 2012). Alternatively, crab faecal pellets together with associated bacteria might serve as a new food resource for some taxa of meiofauna. The evidence above indicates that the potential effects of the crab on meiofauna are diverse and the introduction of the mud crab is likely to disrupt natural meiobenthic communities by establishing new ecological relationships, displacing or completely disassembling native communities but will also likely facilitate some native species through the provision of alternative food to native consumers.

Due to the harsh environment meiobenthic species inhabiting the Baltic Sea are expected to have a high tolerance capacity to not only variable salinity conditions, but also to other environmental variables (Giere 2009). There also exists an alternative view that the species of the Baltic Sea live at their tolerance limit (Lozan et al. 1996) and therefore an additional strong stress due to e.g. the invasive mud crab may completely destabilize the meiobenthic assemblages. Although the Baltic Sea harbours species from both marine and freshwater environments, the actual species richness is low, reflecting the mesohaline conditions. Species poor communities are expected to be more susceptible to invasions because they use less of the available resources (Paavola et al. 2005), however, empirical evidence exists that indicates the lack of relationships between species introductions, diversity and disturbance (Klein et al. 2005). Considering the vast array of potential impacts and the lack of crabs in the Baltic Sea basin, the effects of invasive mud crab on meiofauna are difficult to predict. Moreover, there are virtually no studies on the effects of invasive species on the Baltic meiofauna (but see Urban-Malinga et al. 2013).

In its native range *R. harrisii* prefers habitats that provide some type of shelter either in the form of plants or debris (Ryan 1956). In its invasive range, however, the species is even more flexible occurring from exposed hard bottoms to sheltered soft bottoms either unvegetated or vegetated (Fowler et al. 2013). Within these habitats *R. harrisii* has a very broad diet including detritus, macroalgae and various invertebrates, often bivalve clams (Czerniejewski and Rybczyk 2008). The diet composition is largely driven by prey availability (Hegele-Drywa and Normant 2009). It is possible that the mud crab can even directly feed on meiofauna. To our knowledge, this is not known for mud crabs; however, the soldier crab *Mictyris longicarpus* possibly feeds on meiofauna (Spilmont et al. 2009). To date, the actual impacts of *R. harrisii* on meiofauna is unknown. It is plausible though, that sediment type and the availability of algal food/shelter influence the feeding and sediment reworking activity of crabs and thus their impacts on meiofauna.
Based on the above the aim of this study was to experimentally investigate the effect of *R. harrisii* on the taxonomic composition and abundance of a shallow water meiofauna community in the Baltic Sea area. We expect that (1) *R. harrisii* enriches sediment with organic matter and thereby their presence favours nematodes, ciliates and disfavours oligochaetes, turbellarians and Gastrotricha. We also expect that (2) the mud crab has a larger effect on meiobenthos species composition than species abundances. This is because the majority of meiobenthic species are expected to be highly tolerant to various types of disturbances. The increasing number of meiobenthic species, however, is expected to be related to the provision of new niches of the crab habitat. (3) Finally, we expect that the mud crab has stronger effect on unvegetated soft bottoms than on other types of environments as such habitat is characterised by low availability of food and microhabitats.

**Materials and Methods**

**Experiment design and set-up**

In order to investigate the effect of *R. harrisii* on meiofauna the manipulative experiment was conducted in Kõiguste Bay in the north-eastern Baltic Sea in June 2013 (58°22.10' N, 22°58.69' E) using 10 l plastic buckets with a diameter of 24 cm. The sediment (fine sand) together with meiofauna was collected from a shallow water embayment (0.5–1 m depth) adjacent to the experiment site. In order to obtain similar starting conditions, sediment was homogenized prior to the experiment. The buckets were then filled with a 6 cm layer of homogenized sand and natural seawater at 6 PSU and were allowed to settle for 6 hours. The filled buckets were placed into an outdoor mesocosm facility, assuring ambient light and seawater temperature for all experimental buckets.

The experiment consisted of the following treatments and treatment levels: crab presence (present, absent), sediment (soft, mixed) and vegetation (vegetated, unvegetated); resulting in 8 treatment combinations, each replicated five times. In order to avoid intraspecific competition between crabs only one crab was added per bucket. This value also corresponds to the natural density of crabs in eutrophic bays of the study area. Soft sediments contained homogenized sand only, whereas mixed sediments were generated by adding two similar-sized small stones on the homogenized sands. For vegetated soft sediments the charophyte *Chara aspera* was planted whereas on vegetated mixed sediments stones overgrown with the green alga *Cladophora glomerata* was used instead of unvegetated stones.

In order to mimic natural invertebrate communities we also added bivalves *Macoma balthica*, *Cerastoderma glaucum* and *Mya arenaria* (55, 25 and 6 specimens, respectively) to each bucket containing soft sediment only and *Mytilus trossulus* and *Theodoxus fluviatilis* (60 and 51 specimens respectively) to buckets containing mixed sediment. In addition, 6 specimens of *Gammarus* spp. were added per bucket. The seeded invertebrates were caught from the Kõiguste Bay area. In addition, the homogenized sediment contained Chironomids and *Bathyporeia pilosa* at natural densities.

**Sampling and sample analyses**

As a starting control, five samples of meiofauna were taken from 5 cm down into the homogenized sand using a sharp-edged corer with an inner diameter of 2.7 cm. After incubation for 4 days, one core sample was taken from each bucket using the above
procedure. Care was taken that samples did not contain benthic macroalgae and invertebrates. Each sample was transferred to a plastic container, immediately rinsed three times with carbonated drinking water (each time the supernatant was poured off into plastic bottle), and then fixed with Lugol’s solution.

The samples were condensed to 100 ml by sedimentation over 4 days and the excess water was siphoned off. From each sample, five 2 ml subsamples were taken and all organisms were counted under a microscope in a Bogorov chamber. For the identification, the specimens were slide-mounted in glycerol and determined down to the genus level, where possible, using an Olympus BX 50 microscope.

Additional core samples for sand grain size, chlorophyll \(a\) and organic content of the sediment were collected from each bucket. Sediment grain size was determined using sieving techniques. The following sieves were used: 0.5, 0.25 and 0.125 mm. The software Gradistat, version 8 (Blott and Pye 2001) was used to calculate sorting and to obtain the mean percentages of different sediment fractions. The content of sediment organic matter was measured as a loss of ignition at 500 °C during 3 hours. Chlorophyll \(a\) samples were extracted in 96% ethanol overnight. Chlorophyll \(a\) was quantified fluorometrically correcting for phaeopigments (Strickland and Parsons 1972).

Data analysis

In order to describe the effects of environmental variables on the taxonomic composition and dominance structure of the meiofauna community a canonical correspondence analysis (CCA) was carried out on the complete environmental data set. The analysis was carried out in the statistical software R (The R Foundation for Statistical Computing 2013).

A crossed three-factor non-parametric permutational analysis of variance (PERMANOVA) was then performed to investigate the separate and interactive effects of the Harris mud crab, sediment and vegetation on the abundance of each meiofauna taxa. In addition, PERMANOVA was used to study the effect of the mud crab on sediment properties i.e. the content of sediment organic matter, chlorophyll \(a\) and silt. Prior to analysis, a Bray–Curtis similarity matrix was calculated using raw data (untransformed) and presence/absence transformation to detect whether the potential differences between the meiofauna assemblages were due to differences in relative abundances or species composition. When an interaction was identified as significant (\(p < 0.05\)), post-hoc PERMANOVA pair-wise tests were conducted to detect which levels were responsible for significant interactions. Taxa responsible for observed differences were identified by similarity percentages (SIMPER). PERMANOVA and SIMPER analyses were conducted using PRIMER 6 PERMANOVA+ software (Clarke and Gorley 2006).

Results

The sediment used in the experiment was moderately sorted fine sand. The content of fine sand varied from 80 to 89 % and that of silt from 8 to 16 %. The organic content of the sediment was similar in all mesocosms, estimated at 0.17–0.61 %, while chlorophyll \(a\) content varied from 0 to 10.78 \(\mu g\) corer\(^{-1}\). The mud crab had no significant effect on any measured sediment properties (Permanova \(p > 0.05\)); however, the chlorophyll \(a\) and silt content were slightly higher in buckets containing a crab.
Meiofauna community in the buckets consisted of rotifers, nematodes, ciliates, testaceans, small crustaceans, gastrotrichs, turbellarians, settled bivalves and insect larvae. Nematodes were the most abundant taxa in all treatment groups.

The CCA analysis showed that the mud crab contributed more to variability in the taxonomic composition and dominance structure of meiofaunal communities than any other studied environmental variable (Fig. 1). Other key variables for meiofauna were the content of sediment silt and organic matter. As crabs increased sediment silt content, these two variables largely co-varied in the analysis. The effects of sediment type and vegetation were largely independent of that of the mud crab. Crabs increased the abundance of testaceans, turbellarians and rotifers and reduced the abundance of copepod nauplii and settled bivalves.

PERMANOVA showed that the mud crab had no independent effect on the taxonomic composition and dominance structure of meiofaunal communities ($p = 0.163$ and $p = 0.215$ respectively). However, in interaction with sediment type and vegetation the crab interactively affected taxonomic composition ($p = 0.026$, presence/absence data), but not dominance patterns of meiofauna ($p = 0.239$, abundance data).

The mud crab modified the meiofauna community on soft bottoms but not on mixed bottoms. Vegetation also modulated the crab impact on meiofauna. Specifically, the crab had a significant effect on meiofauna in unvegetated bottoms but not in vegetated areas. In unvegetated soft bottoms the presence of the mud crab increased the abundance and probability of occurrence of ciliates, rotifers and insect larvae, but decreased nematode abundance and probability of occurrence of gastrotrichs, copepods and settled bivalves. In vegetated soft bottoms, however, the mud crab increased nematode abundance and the probability of occurrence of copepods, gastrotrichs, turbellarians, testaceans and reduced the number of ciliates, rotifers, insect larvae and the probability of occurrence of bivalves and copepod nauplii (SIMPER analysis).

PERMANOVA analyses performed separately for different taxa showed that the presence of R. harrisii significantly increased the abundance of turbellarians (PERMANOVA, $p=0.017$, Fig. 2a) and reduced the abundance of juvenile bivalves ($p=0.014$) (Fig. 2b). In interaction with vegetation the mud crab significantly affected the abundance of settled bivalves (PERMANOVA, $p=0.014$), copepods ($p=0.036$) and insect larvae ($p=0.045$) with the effect direction varying among taxa and habitats. The mud crab reduced copepods abundance in unvegetated sediments but increased abundance in vegetated sediments (Fig. 3b). The mud crab had little effect on the abundance of juvenile bivalves in unvegetated sediment. However, in vegetated habitats the crab basically eliminated bivalves (PERMANOVA, interactive effect of crab and vegetation $p=0.014$, Fig. 3a).

The mud crab had no effect on nematodes. There was a significant interaction between sediment and vegetation on nematodes ($p =0.053$) with their abundances being highest in the vegetated mixed sediment. Crabs, sediment and vegetation interactively affected ciliates ($p=0.008$). Crabs increased the abundance of ciliates in unvegetated soft sediment and vegetated mixed sediment whereas they reduced the abundance of ciliates in unvegetated mixed sediment and slightly also in vegetated soft sediment (Fig. 4).

Discussion
The results of this experiment demonstrate that the invasive *R. harrisii* has a significant and strong effect on meiofauna communities in the Baltic Sea. As seen from the CCA results (Fig. 1), the mud crab shaped the community of meiofauna more than any other studied environmental variable. Only sediment silt content had a comparable effect on meiofauna. This is because silt content is the key environmental factor for meiofauna and sediments with a high silt content are often considered as unfavourable for many meiobenthic organisms (Nogrady et al. 1993; Giere 2009).

As compared to the mud crab, bottom type and vegetation had only marginally separate effects on meiofauna. However, these factors in interaction with mud crab strongly contributed to the variability of the meiofaunal community. The experiment conformed to our expectation that the mud crab significantly altered the taxonomic composition of meiofauna in unvegetated soft bottom but had no effects in mixed bottoms or vegetated habitats. Mixed and/or vegetated sediments provide more microhabitats for meiofauna. With an increasing number of microhabitats the elevated diversity of meiofauna, including stress tolerant species, is expected (Gingold et al. 2010). Consequently, such elevated species diversity may provide an insurance against various stressors including those associated with the presence of mud crab. In addition to such a compensatory mechanism, small stones in the mixed bottom treatment as well as vegetation may provide shelter for *R. harrisii* and hence reduce its burrowing activity, hence the effects on meiofauna.

The mud crab had no effect on sediment properties, though silt content was somewhat increased in the presence of crab. In general, burrowing crabs are known to modify sediment properties (i.e. Botto and Iribarne 2000; Fanjul et al. 2007) and increase the content of organic matter in the sediment (Botto and Iribarne 2000). It is possible though, that the experimental period was far too short and/or the density of crabs was too low for such significant changes to occur. As the mud crab did not increase sediment organic content, did not affect nematodes and ciliates but instead increased the abundance of turbellarians, our first expectation was not confirmed.

PERMANOVA analysis showed that the mud crab modified meiofaunal taxonomic composition rather than abundance. While *R. harrisii* had an effect on the abundance of turbellarians and settled juvenile bivalves, no effects were observed for nematodes, a dominant component of the studied community. In this study we did not identify nematodes to lower taxonomic levels. It is plausible, though, that the mud crab also induces significant shifts in the species composition of nematodes. In a previous study, Warwick et al. (1990) showed that a burrowing crab may reduce nematode species richness, diversity and evenness without actually changing the overall nematode densities. This suggests that in our experiment pristine nematode species were likely replaced by stress tolerant species. Nevertheless, future studies should resolve the effects of Harris mud crab on the taxonomic composition of nematodes.

The mud crab significantly reduced the abundance of settling bivalves. In unvegetated habitats the mechanisms behind the observed decline may be related to direct predation, mechanical damage due to crab burrowing and/or the transport of organic matter in the deeper layers of sediment i.e. a reduction of food availability in the sediment surface (Strasser and Günther 2001; Lomovasky et al. 2006; Whitton et al. 2012). However, the effects were most severe in vegetated habitats. In such habitat the juvenile bivalves are settling on the top of algae. The crab often forages within the algal carpet and a high mortality of the bivalves is likely a side effect of the crab grazing on filamentous macroalgae.
The crab significantly increased the abundance of turbellarians. Turbellarians are known to prefer well oxygenated fine sandy sediments rich in food supply (Giere 2009) and the majority are predacious, but are seldom preyed upon (Martens and Schockaert 1986). Thus, crab burrowing and a consequent increase in organic matter in sediment can be beneficial to turbellarians. Also Urban-Malinga et al. (2013) found that bioturbation by macrofauna increases turbellarians abundance, however a burrowing crab may also have negative impact on turbellarians (Rosa and Bemvenuti 2005) or no impact at all (Olafsson and Ndaro 1997). It is most likely that sediment properties and prey availability defines the abundance of turbellarians. This is also supported by a recent finding that sand structure is the key environmental variable for turbellarians (Lokko et al. 2014).

In vegetated habitats, *R. harrisii* increased the abundance of copepods. This may be due to improved feeding conditions i.e. when crab diet is supplemented by algae the faecal material together with associated bacteria may provide a good food source for copepods. Werry and Lee (2005) found that faecal material of crab feeding on mangrove leaves significantly improved copepods survival compared to other diets. In unvegetated habitats, the slight decrease in copepods abundance in the presence of crab may be caused by physical disturbance. Also Olafsson and Ndaro (1997) found that burrowing crabs significantly lowered the abundance of harpacticoid copepods.

The effect of crab on the abundance of ciliates is complex and habitat specific. Our previous study showed that ciliates are favoured by elevated content of sediment organic matter (Lokko et al. 2014). So, in unvegetated soft sediments crabs are expected to improve feeding conditions for ciliates by enriching sediment with uneaten food particles and faeces. However, in vegetated soft sediments crabs actually reduced the abundance of ciliates, but the difference was not statistically significant. The positive effect of crab on ciliates in vegetated mixed sediment possibly relates to the provision of an extra food source (i.e. similar mechanism as for copepods).

Our experiment demonstrated that within only a few days the mud crab had many direct and interactive effects on meiofauna. Long-term exposure to the mud crab is expected to be even more severe but is likely to be less predictable due to the presence of many indirect effects. For example, the mud crabs are known to feed on adult bivalves (Hegele-Drywa and Normant 2009) and therefore the abundance of bivalves is expected to be reduced in the presence of the mud crab. The decrease of deposit-feeding bivalves in benthic communities may relax interspecific competition for food between meio- and macrofauna and ultimately boost up the density of meiofauna. This mechanism is supported by some experimental evidence as the native bivalve *Macoma balthica* is known to decrease the abundance of meiobenthic ostracods both by physically disturbing ostracods and by using the same food source (Nascimento et al. 2011). The presence of *M. balthica* also reduces the abundance of nematodes, copepods and oligochaetes by preventing microalgal mats development on the sediment surface (Olafsson et al. 2005). Thus, meiobenthic taxa that are not disfavoured by the crab burrowing activity may become more abundant. Alternatively, some suspension-feeding bivalves enhance meiofauna by organic-rich biodeposits that provide an excellent food source (Castel et al. 1989; Kotta et al. 2009). Thus, the actual outcome depends on the synergistic effects of the mud crab on meio- and macrofauna as well as the indirect effects of macrofauna on meiofauna. As the distribution of the mud crab is not limited by reduced salinity (Boyle et al. 2010; Roche et al. 2009; Patton et al. 2010), the mud crab may invade the whole Baltic Sea including adjacent inland waterbodies and rivers. Some specimens are already reported from Rhine, hundreds of kilometres upstream from the sea (Freyhof and Steinmann 1998). Providing a large habitat
range and a lack of natural enemies in the invaded waterbodies (e.g. Fowler et al. 2013), the mud crab has the potential to affect the communities of meiofauna not only in the Baltic Sea but likely in the adjacent fresh-water basins.

References


Fig. 1 Results of CCA ordination demonstrating differences in responses of meiofaunal taxa to crab and environmental variables. Only taxa which occurred in three or more samples are shown.
Fig. 2 Average abundance (± SE) of turbellarians and settling bivalves with and without Harris mud crab.
Fig. 3 Average abundance (± SE) of settling bivalves (A), copepods (B) and insect larvae (C) with and without Harris mud crab in unvegetated and vegetated habitats.
Fig. 4 Average abundance (± SE) of major meiofaunal taxa with and without Harris mud crab at different sediments in unvegetated and vegetated habitats.
Appendix 4D

Introduced crab drives switch from bottom-up to top-down control of community structure in the Baltic Sea

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Abstract

Aim In most marine coastal ecosystems species occupying the highest trophic level exert a controlling influence on species at lower trophic levels whereas bottom up regulation is less important. Crabs are the key top predators worldwide and through bioturbation, grazing and predation, they modulate the availability of resources for multiple trophic levels. The Baltic Sea, however, naturally lacks such top-down control. The North American mud crab Rhithropanopeus harrisii has recently become the first crab known to reproduce throughout the Baltic Sea. This invasion has provided a unique opportunity to quantify rigorously the transition from no crab system to crab dominated system in the Baltic Sea.

Location Baltic Sea

Methods ‘Before-and-after’ field observations, complemented with field- and outdoor mesocosm experiments tested the impacts of the invasive crab on benthic macrophyte and invertebrate communities.

Results Within 2–3 years of R. harrisii's arrival, the density of native bivalves (Macoma balthica) declined significantly in soft bottom habitats but not in mixed bottom habitats. Experiments showed the observed decline in bivalves was consistent with crab predation. Specifically, in a short-term outdoor artificial community competition experiment the mud crabs increased the mortality of bivalves and amphipods on soft sediment but not on mixed sediments. Algae modulated the impact of crabs on amphipods with significant effects being observed only on unvegetated soft sediment. Thus, algae and rocks provide mobile amphipods with refuge from crab predation. In a longer in situ enclosure experiment the mud crabs significantly reduced overall benthic species richness as well as the density of all bivalve and gastropod species regardless of sediment and/or vegetation type.

Main conclusions Our study demonstrates how the invasion of a mud crab has introduced a new ecological function replacing a strong bottom-up regulation by top-down control several key invertebrate species.
Key words: Baltic Sea, phase-shift, invasive species, species interaction, predation, ecological function,

Introduction

Primary production is the groundwork for all the life on the Earth. But it is the relative contribution of different regulation mechanisms that yield to fundamentally different expressions of ecosystem functioning (e.g. Elser et al., 2007; Hillebrand et al., 2007; Gruner et al., 2008; Hereu et al., 2008). Bottom-up regulation is driven by energy moving up the food web from plants to herbivores to carnivores. Such controlling mechanisms are common in open oceans where anthropogenic impact on top of the food chain is negligible (e.g. Barton et al., 2013). In contrast, top-down control refers to situations when species occupying the highest trophic level (top carnivores) exert a controlling influence on species at the next lower level and so forth down to the trophic ladder. This is very common in coastal seas with a classic example of kelp forest ecosystems (Estes et al., 1998).

Crabs are the key top predators worldwide and they play an important ecological role in marine coastal food webs. They exert strong control over benthic communities via bioturbation, grazing and predation. They also clean up the bottom of the sea by collecting decaying plant and animal substance and on the other hand many fish, birds and sea mammals rely on crabs as a food source. Thus, crabs modulate diversity, species abundances and community structure and thereby the availability of resources for multiple trophic levels in a variety of marine systems (Grosholz et al., 2000; Hollebone & Hay, 2008).

Natural barriers separate different biogeographic provinces (Elton, 1958) whereas human-mediated invasions of non-indigenous species reduce such isolation, thereby posing a serious threat to the integrity of a natural ecosystem (Gurevitch & Padilla, 2004). The arrival of a new organism may lead to new ecological relationships e.g. through competition and predation, modifying ecosystem functioning or causing the appearance of an entirely new function (Savidge, 1987; Callaway & Aschehoug, 2000; Sanders et al., 2003). Hence, the biological consequences to the community structure, species interactions, energy flow and evolution can be profound (Mills et al., 1993; Daehler & Strong, 1996; Parker et al., 1999).

In the geologically young Baltic Sea ecosystem no native crab species are found and until recently a brackish water of the Baltic Sea was an efficient barrier to non-indigenous decapod crabs. The non-indigenous *Eriocheir sinensis* is the only crab dispersing into the inner margins of the Baltic Sea, but the species causes no real threat to the ecosystem as it is found only occasionally and cannot reproduce in low salinity waters (Ojaveer et al., 2007). As a consequence the majority of local benthic communities are largely bottom-up regulated with the availability of food and space defining species densities (Kotta et al., 2008ab). This contrasts to true oceanic waters where large predators including crabs constitute an essential element of the nearshore benthic ecosystems where they exert a strong top-down grazing and predation pressure on lower trophic levels (e.g. Menge, 1976; Lee, 1998).

The North American mud crab *Rhithropanopeus harrisii* is rapidly expanding its distribution area in Europe and is the first crab species known to reproduce throughout the entire Baltic Sea (Kotta & Ojaveer, 2012; Hegele-Drywa & Normant,
2014, and references therein). As this non-indigenous species performs an entirely novel function in the recipient community and it has become a dominant element in many shallow water ecosystems, dramatic shifts in structure and functioning of soft and hard-bottom benthic macrophyte and invertebrate communities are expected (Simberloff, 1991; Ruesink et al., 1995). Considering the vast array of potential direct and indirect impacts and the historical lack of crabs in the Baltic Sea basin, the effects of invasive mud crab on benthic communities are difficult to predict. However, the introduction of an efficient predator is expected to result in changes in the abundance of organisms across many trophic levels (e.g. Carpenter & Kitchell, 1993). In general crabs have a broad diet range, nevertheless bivalves make up the largest part of their diet (Reise, 1978; Gee et al., 1985; Raffaelli et al., 1989). As bivalves are currently by far the most important element in the Baltic Sea benthos, a severe decimation of bivalve populations by crabs may relax subordinate invertebrates from competitive interaction leading to completely new dominance hierarchies. Such a dramatic reduction in bivalve densities may also affect sediment nutrients (Norkko et al., 2001; Cranford et al., 2009) and algal growth (Kotta et al., 2009). Besides, crabs may also control the abundance of grazers (Silliman et al., 2004) and thereby modify the growth patterns of micro- and macroalgae. Burrowing crabs intensively rework sediment and through such bioturbation activity can also modify sediment granulometry and chemistry (Gilbert et al., 1998; Botto & Iribarne, 2000; Fanjul et al., 2007). As an indirect consequence of such burrowing activity, crabs may inhibit the growth of snails (Armitage & Fong, 2006) and disturb bivalves feeding and damage their shells (Lomovasky et al., 2006). Moreover, chemical cues caused by crab predation may induce prey populations to change their behaviours and such shifts may also have dramatic consequences to the benthic ecosystem’s functioning (Premo & Tyler, 2013).

This recent invasion has provided a unique opportunity to quantify the ecological transition from no crab to crab dominated ecosystem in the Baltic Sea. In this study we combined long-term data observational and manipulative mesocosm and field experiments to identify changes in benthic communities following the mud crab invasion. Using this approach, we quantified the magnitude of impact of crab predation on macroalgae and invertebrates and established the time scale required for those changes. We expected that (1) mud crabs would reduce the abundance of invertebrate prey, especially bivalve molluscs. We also expected that (2) mud crabs would graze on benthic filamentous macroalgae but not on algae with coarser thalli. Finally, we expected (3) that mud crabs would have stronger effects in unvegetated soft bottom habitats than in other habitat types as such environments are characterised by low availability of food and microhabitats for refuge.

Methods

Study area

This study took place in the north-eastern Baltic Sea within Estonian territorial waters. The study area is characterized by a wide coastal zone with diverse bottom topography and underwater habitats. The prevailing substrate is a thin layer of slightly silted sand mixed with pebbles, gravel or boulders (Kotta et al., 2008ab).
The Baltic Sea is a geologically young semi-enclosed temperate sea. Due to its short evolutionary history, low salinity and strong seasonality in temperature and light conditions, the number of benthic macrophyte and invertebrate species is small (Hällfors, *et al.*, 1981). While overall species richness is low, those species that inhabit the area often form abundant populations. Among the invertebrates amphipods, isopods, insect larvae and burrowing bivalves have the highest biomass, and the macrophytes are dominated by ephemeral green and brown filamentous macroalgae and perennial bladderwrack (*Fucus vesiculosus*) (Bonsdorff & Pearson, 1999; Kotta, *et al.*, 2008ab). Until recently there were no permanent populations of any crab species anywhere in the Baltic Sea proper (Hällfors, *et al.*, 1981??).

The mud crab *Rhithropanopeus harrisii* has a native distribution range from New Brunswick (Canada) to Veracruz (Mexico). This invasive species was first found in Europe in 1874 in the Netherlands. Despite occasional observations in the Baltic Sea area as early as 1936 (Nikolaev, 1951), it was only in the late 1990’s and early 2000’s that the crab suddenly expanded into all water basins of the Baltic Sea. The species was first discovered in Estonian waters in Pärnu Bay in August 2011 and in 2012 virtually the entire bay was already populated by a dense population. The Estonian coastal sea provides *R. harrisii* with a favourable habitat – a rich feeding ground with very few natural enemies. This has promoted a rapid dispersal of the mud crab after its initial invasion (Kotta & Ojaveer, 2012). Due to the high fishing pressure, commercially valuable predatory fishes are nowadays depressed in the study area (e.g. Saat & Ojaveer, 2005). Water birds might consume some mud crabs but due to high turbidity and low number of birds this control is not important in the study area (Tomczak *et al.*, 2009). Therefore, predation risk for the crab is currently low and top-down control of the food web should be considered as weak.

**Field observations**

To determine possible changes in benthic community structure as a consequence of the mud crab invasion, we compared benthic invertebrates and macrophytes in two comparable areas, with (Pärnu Bay) and without (Gulf of Riga) crabs, respectively (Figure 1). Every summer from 2002 to 2013, three replicate samples of benthic invertebrates and macrophytes were sampled in each bay on soft and mixed bottom habitats. On soft bottom habitats an Ekman-Lenz type bottom grab (0.02 m²) was used to collect samples at 3-4m depth. On mixed bottom habitats, transects perpendicular to the seashore was used and samples collected by a diver with a metal frame (20 × 20 cm) in three random replicates from depths 0.1, 0.3, 0.5, 1, 2, 3 and 5 m.

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 macrophyte and invertebrate species were performed in the laboratory using a stereomicroscope. All species were determined to the species level except for oligochaetes, juvenile gammarids, and insect larvae. The dry weight of species was obtained after drying the individuals at 60 °C for 2 weeks.
Outdoor mesocosm experiment

To investigate the effect of crabs on other invertebrates an outdoor mesocosm experiment was conducted in the Gulf of Riga sampling site in June 2013. Sediment (fine sand) and natural seas water was collected from a shallow embayment adjacent to the experimental site. The mesocosms (10 L containers with a diameter of 24 cm) were filled with a 6 cm layer of sediment and seawater, and were allowed to settle for 6 hours. The mesocosms were placed in an outdoor facility, assuring ambient light and temperature for all experimental units.

The experiment consisted following treatments and treatment levels: crab presence (present, absent), sediment (soft, mixed) and vegetation (vegetated, unvegetated); resulting in 8 treatment combinations, each replicated five times. In order to avoid intraspecific competition between crabs only one crab was added per bucket. This value also corresponds to their natural density in the crab infested area. Soft sediments contained homogenized sand only, whereas mixed sediments were generated by adding two similar-sized small stones on the homogenized sands. For vegetated soft sediments the charophyte Chara horrida were seeded and on mixed sediment stones overgrown with the green alga Cladophora glomerata were placed on sand instead of unvegetated stones. Stones and macroalgae were collected adjacent to the experiment site.
In order to mimic natural invertebrate communities we also added bivalves *Macoma balthica*, *Cerastoderma glaucum* and *Mya arenaria* (55, 25 and 6 specimens, respectively) to each bucket containing soft sediment only and the bivalve *Mytilus trossulus* and the gastropod *Theodoxus fluviatilis* (80 and 51 specimens respectively) to buckets containing mixed sediment. In addition, 6 specimens of the amphipod *Gammarus* spp., predominantly *Gammarus zaddachi* and *Gammarus locusta*, were added per bucket of either sediment. The seeded invertebrates were caught from the Kõiguste Bay area. Besides, the homogenized sediment contained Chironomid larvae and the amphipod *Bathyporeia pilosa* at natural densities (50 and 11 specimens). Prior to the experiment, 25 individuals of each studied species (representing the same cohort as used in the experiment) were randomly selected and their length (±0.1 mm) and dry weight (48 h at 60 °C ±0.001 g) determined.

As a starting control, five samples for chlorophyll *a* and organic content of the sediment were taken 5 cm down into the homogenized sand using a sharp-edged corer with an inner diameter of 2.7 cm. Care was taken that samples did not contain benthic macroalgae and invertebrates. After incubation for 4 days, additional core samples were taken from each bucket for chlorophyll *a* and organic content of the sediment using the above procedure. The content of sediment organic matter was measured as a loss of ignition at 500 °C during 3 hours. Chlorophyll *a* samples were extracted in 96% ethanol overnight. Chlorophyll *a* was quantified fluorometrically correcting for phaeopigments (Strickland & Parsons, 1972). Remaining sediment was sieved through a 0.25 mm mesh net and test organisms were counted. The length values of all experimental animals were determined in order to assess size-specific predation by the mud crab.

**Field experiment**

To investigate if *R. harrisii* affects benthic macrophyte and invertebrate communities, when exposed to migration from adjacent non-invaded areas either due to active movement or dislodgement by waves, the manipulative in situ mesocosm experiment was conducted in the northern Gulf of Riga, the north-eastern Baltic Sea in August–September 2012 using 10 l plastic buckets with a diameter of 24 cm. Experimental timing reflects the season when benthic algal and invertebrate communities are the best developed. Similar to the outdoor mesocosm experiment the buckets were filled with a 6 cm layer of homogenized sand and natural seawater at 6 PSU and were allowed to settle for 6 hours. To half of the mesocosms *R. harrisii* (one crab per bucket) specimens were added. The buckets were closed with a mesh net (1 cm mesh size) to avoid emigration of the crabs and at the same time assuring sufficient water exchange in the buckets as well as immigration of benthic invertebrates from adjacent benthic communities. Experimental buckets were randomly placed on the seafloor at a depth of 1 m i.e. a typical depth of the mud crab in the invaded areas. The experiment had same treatments, treatment levels and replication as the outdoor mesocosm experiment. We also had procedural controls i.e. those assessing the effect of mesh net. Comparison of screened and unscreened communities showed no significant effects of mesh on benthic communities (PERMANOVA, *p* > 0.05; for further details on the PERMANOVA analysis see a subsection of data analysis).

As a starting control, five samples for chlorophyll *a* and organic content of the sediment were taken. After incubation for 49 days, additional core samples were taken
from each bucket for chlorophyll $a$ and organic content of the sediment. The sampling and sample analyses were done as described above. Remaining sediment was sieved through a 0.25 mm mesh net, all macrophyte and invertebrates were sorted to the species level except for insect larvae, invertebrates were counted and both macrophytes and invertebrates were weighed after drying the material at 60 °C for 2–3 weeks.

**Data analysis**

**Field observations.** Multivariate data analyses were conducted using PRIMER 6 PERMANOVA+ software (Clarke & Gorley, 2006). The non-parametric permutational analysis of variance (PERMANOVA) was performed to compare the studied sea areas (with and without the invasive crab) and study periods (before and after invasion) in terms of macrophyte and invertebrate communities. Prior to analysis, a Bray–Curtis similarity matrix was calculated using raw (untransformed) biomass data. When a factor was identified as significant ($p < 0.05$), post-hoc PERMANOVA pair-wise tests were conducted to detect which levels were responsible for significant interactions. Taxa responsible for observed differences were identified by similarity percentages (SIMPER). Non-metric multidimensional scaling analysis (nMDS) was used to visualize the dissimilarities between areas and times.

**Outdoor mesocosm experiment.** Analyses of variance (ANOVA) were performed to separate the effects of the mud crab, sediment and vegetation on the density and size of the seeded invertebrates. Three-way analyses were performed on those species seeded in both sediment types and two-way analyses on those species specific to either type of sediment, respectively. Bartlett’s test was carried out prior to the analyses and the results confirmed the assumption of homoscedasticity (Sokal & Rohlf, 1981). Post-hoc Bonferroni tests were used to analyse which treatment levels were statistically different from each other.

**Field experiment.** PERMANOVA was performed to investigate the separate and interactive effects of the mud crab, sediment and vegetation on the species richness, composition and biomass dominance structure of macrophyte and invertebrate communities. PERMANOVA was also used to study the effect of the above factors on sediment properties i.e. the content of sediment’s organic matter and chlorophyll $a$ level. Post-hoc PERMANOVA pair-wise tests were conducted to detect which levels were responsible for significant interactions. Taxa responsible for observed differences were identified by similarity percentages (SIMPER).

**Results**

**Field observations**

Altogether 51 taxa of benthic invertebrates were identified in soft bottom habitats over the period of ten year. Among the most common were several species of bivalve molluscs such as *Macoma balthica*, *Mya arenaria*, *Cerastoderma glaucum*, *Dreissena polymorpha* the gastropods *Peringia ulvae*, *Potamopyrgus antipodarum*, the crustacean *Amphibalanus improvisus*, *Corophium volutator*, the polychaetes *Hediste*
diversicolor, Marenzelleria neglecta, Laonome armata and oligochaetes. Rare species were mostly different amphipod, isopod and insect larval species.

On mixed bottoms altogether 124 macrophyte and benthic invertebrate taxa were found. The ephemeral algae Cladophora glomerata, Pilayella littoralis, Polysiphonia fucoides and the perennial Fucus vesiculosus, Furcellaria lumbricalis and the higher plants Stuckenia pectinata, Myriophyllum spicatum dominated in the study area. Among benthic invertebrates mostly bivalves and gastropods prevailed including Mytilus trossulus, Cerastoderma glaucum, Macoma balthica, Dreissena polymorpha, Theodoxus fluviatilis, Radix balthica, Peringia ulvae. Occasionally chironomidae larvae also had high biomasses.

The study period (2000–2010 vs. 2011–2013) significantly contributed to the variability in species composition and biomasses of invertebrate communities on crab-infested soft bottom habitats (PERMANOVA, $p < 0.001$) but not in control area ($p = 0.573$). Since the establishment of R. harrisii in 2011, biomass of the dominant native bivalve M. balthica has significantly declined and biomasses of the invasive D. polymorpha and Amphibalanus improvisus have increased. On mixed habitats, however, no such differences were found between crab-infested and control areas. Over the period of ten years perennial macrophytes, especially F. vesiculosus and charophytes have increased their biomasses, no systematic changes in the biomasses of invertebrate species were found (Figure 2).

**Outdoor mesocosm experiment**

In the outdoor enclosures over the 4 days period, crabs did not have any significant effects on chlorophyll $a$ levels and the organic content of the sediment (three-way ANOVAs, effect of crab $p > 0.05$). Moreover, none of the interactions between factors were significant for the studied sediment parameters ($p > 0.05$).

On the other hand, crabs significantly increased the mortality of all bivalves and amphipods on soft sediments (two and three-way ANOVAs, effect of crab $p < 0.01$). Crabs preferentially ate larger M. balthica demonstrated by a significant reduction of average size of clams in mesocosms seeded with crab ($p = 0.035$). On soft sediment algae modulated the effect of crab on gammarids with elevated mortalities being measured on unvegetated areas. Such interaction was not observed for other species.
Unlike in soft sediment environments, crab had no separate or interactive effects on any benthic invertebrate species in mixed sediment ($p > 0.05$) (Figure 3).

Field experiment

In the in situ experiment over the 49 days period, the mud crab did not affect either directly or combined with other studied factors chlorophyll $a$ and organic content of the sediment (three-way PERMANOVA, all factors and interactions $p > 0.05$). There was a significant interaction of sediment and presence of crab on the phaeopigment content (three-way PERMANOVA, effect of crab $p = 0.004$) with the crab significantly reducing the sediment phaeopigments from 8.6 to 4.0 $\mu g$ core$^{-1}$.

Direct effects of crab predation on invertebrates largely prevailed over their interactive effects. Regardless of sediment type or availability of algae, crabs significantly reduced species richness of benthic invertebrates by 20% (three-way PERMANOVA, effect of crab $p = 0.025$, other factors and interactions were not significant). Crab also reduced the total invertebrate biomass (three-way PERMANOVA, effect of crab $p = 0.003$, other factors and interactions were not significant) and modified structure of the species domination in the benthic invertebrate communities (three-way PERMANOVA, effect of crab $p = 0.002$, effect of sediment = 0.003, other factors and interactions were not significant). When doing so, the mud crabs significantly reduced all bivalve and gastropod species richness and their biomasses in the sediment (Table 1, Figure 4). Crabs had no effect on the total biomass, species composition and biomasses of macrophyte species (three-way PERMANOVA, all factors and interactions were not significant).
Figure 4. Non-metric multidimensional scaling (nMDS) plots showing the effect of the crab on the species biomasses in benthic macrophyte and invertebrate communities. Labels above symbols refer macroalgal communities as follows: Cladophora – Cladophora glomerata dominated community, Chara – Chara horrida dominated community, No – unvegetated community.

Table 1. Results of the SIMPER analysis giving the average biomasses of benthic invertebrate species per mesocosm with and without mud crab together with their share in the statistical dissimilarities.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Biomass without crab</th>
<th>Biomass with crab</th>
<th>Contribution %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macoma balthica</td>
<td>0.51</td>
<td>0.18</td>
<td>12.80</td>
</tr>
<tr>
<td>Cerastoderma glaucum</td>
<td>0.27</td>
<td>0.06</td>
<td>9.62</td>
</tr>
<tr>
<td>Theodoxus fluviatilis</td>
<td>0.25</td>
<td>0.10</td>
<td>8.63</td>
</tr>
<tr>
<td>Gammarus oceanicus</td>
<td>0.19</td>
<td>0.23</td>
<td>5.81</td>
</tr>
<tr>
<td>Radix balthica</td>
<td>0.15</td>
<td>0.05</td>
<td>5.67</td>
</tr>
<tr>
<td>Gammarus salinus</td>
<td>0.13</td>
<td>0.09</td>
<td>5.41</td>
</tr>
<tr>
<td>Gammarus zaddachi</td>
<td>0.16</td>
<td>0.17</td>
<td>5.34</td>
</tr>
<tr>
<td>Gammarus juv.</td>
<td>0.17</td>
<td>0.12</td>
<td>4.87</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>0.71</td>
<td>0.65</td>
<td>4.51</td>
</tr>
<tr>
<td>Idotea baltica</td>
<td>0.05</td>
<td>0.09</td>
<td>3.97</td>
</tr>
<tr>
<td>Gammarus tigrinus</td>
<td>0.58</td>
<td>0.58</td>
<td>3.32</td>
</tr>
</tbody>
</table>

Discussion
Crabs are important predators that exert strong top-down control of community structure in many marine ecosystems. Until recently, there were no crabs in the entire Baltic Sea ecosystem. Here, combining long-term observations and experiments, we have shown how the invasion by a mud crab caused a switch in ecosystem organisation from bottom-up to top-down control of community structure. Our mesocosm experiment showed that mud crabs can effectively prey on bivalves on soft bottom habitats. Similarly, our field observations demonstrated a clear reduction in the abundance of many numerically dominant bivalve species in the soft bottom areas infested by mud crabs whereas no such changes were observed in the control areas. Although in situ and outdoor mesocosm experiments may magnify predation effects, field and experimental evidence together support the hypotheses that the mud crabs significantly reduce the abundance of invertebrate prey, especially for bivalve molluscs. Providing to the lack of such function in the coastal ecosystem of the Baltic Sea and a significant reduction of dominant invertebrates in the area, the introduction of the mud crab is likely disrupting the natural benthic invertebrate communities by establishing completely new ecological relationships, displacing or disassembling native communities by relaxing competitive interactions between some native species by the removal of competitively superior species.

Before the invasion of the mud crab, community structure in the study area was controlled by bottom-up processes (Kotta, et al., 2008b, 2009b). Bivalves constituted the majority of benthic biomass (Lauringson et al. 2009) with changes in habitat and food conditions largely affecting their population sizes (Tomczak, et al., 2009). After the establishment of the mud crab, however, this strong bottom-up regulation has been replaced by top-down control. The long-term consequences of this invasion are uncertain, but as demonstrated here, benthic communities have already changed and further changes are likely to involve many direct and indirect effects such as shifting community baselines and amplifying the effects of eutrophication. In the longer run it is expected that the mud crab takes a role similar to other crabs in the true oceanic waters (Reise, 1978; Gee, et al., 1985; Raffaelli, et al., 1989).

We also expected that the mud crabs are effective grazers of filamentous algae, however, this was not supported either by the results of enclosure experiments or field observations. Coastal eutrophication is a high concern in the Baltic Sea region and high nutrient levels are coupled with high proliferation of macroalgae. As a consequence of such blooms algal mats more than a meter thick occasionally form and cover an area of several hectares. These mats usually become anoxic at later stages and have a strong structuring force of the coastal ecosystems of the Baltic Sea (Conley, et al., 2011; Lyons, et al., 2014). As the macroalgal blooming starts immediately after the retreat of ice when the population densities and activity of mesoherbivores and crab are the lowest (Turoboyski, 1973), top-down control of benthic macrophytes is unlikely in the Baltic Sea region (Kotta, et al., 2006).

Finally, we expected that mud crabs would have stronger effects on unvegetated soft bottoms than on other types of environments as such habitats are characterised by low availability of food and microhabitats for refuge. In experimental conditions the mud crabs were able to decimate the population of gammarid amphipod on unvegetated soft bottom habitats. However, our long-term field data did not support this observation. This is could be because completely unvegetated bottoms are rare in the
D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning

coastal ecosystems of the Baltic Sea (Martin, et al., 2013) and in field conditions herbivores often find shelter within benthic vegetation. Owing to high fishing pressure and low density of commercially valuable predatory fishes, the mud crab lacks natural enemies in its invaded range. Thus, the mud crab is expected to increase its habitat occupancy in the near future (Fowler, et al., 2013) with subordinate crabs inhabiting low-quality habitats in order to avoid being eaten (Iribarne, et al., 1994). In this high crab density environment gammarid amphipods are likely forced out of their preferred macrophyte habitats and consumed either by crabs or small benthic fish such as sticklebacks (Kotta, et al., 2010; Järv, et al., 2011).

Large motile predators play an important role in marine ecosystems by controlling from top-down the abundance and distribution of benthic species for several trophic levels (Silliman, et al., 2004). We considered it likely that the introduction of an efficient predator into the diluted Baltic Sea results in changes in the abundance of organisms across many trophic levels (e.g. Carpenter & Kitchell, 1993). The current study showed otherwise and the mud crab was the causal factor in the declining abundances of dominating bivalves and gastropods only. This is in agreement with recent metaanalyses showing that invaders’ effects on resident biodiversity are generally more negative within the same or adjacent trophic levels than across multiple trophic levels (Thomsen, et al., 2014, Maggi et al., in press).

Our enclosure experiments demonstrated that the mud crab has many separate strong effects but only a few interactive effects on the resident invertebrates. This result is interesting in the Baltic Sea environment. Often the experimental studies yielded into complex patterns and context-dependent results (e.g. Kotta, et al., 2010; Wernberg, et al., 2013). This is because benthic invertebrate species inhabiting the Baltic Sea are used to cope with harsh environment and are expected to have high tolerance capacity to not only variable salinity conditions, but also to other environmental variables such as hypoxia, ice scour and exposure. Moreover, physically driven fluxes often override the effects of biological interactions in shallow water systems of the northern Baltic Sea (e.g. Herkül, et al., 2006). Albeit, the mud crab is not large, it represents a completely new function in the coastal ecosystem with strong bioturbation, grazing and predation effects involved. The Baltic Sea harbours species from both marine and freshwater environments but the actual species richness is low. Species poor communities are expected to be more susceptible to invasions because they use less of the available resources (Paavola, et al., 2005). Providing low species redundancy of the Baltic Sea and multiple effects of the invasive mud crab, strong direct impacts are likely.

The invasive species is tolerant to a broad range of environmental conditions including low salinity of the Baltic Sea. In its invasive range mud crab may occur from exposed hard bottoms to sheltered soft bottoms either unvegetated or vegetated (Fowler, et al., 2013). Within these habitats the mud crab has a very broad diet potentially including macroalgae and benthic invertebrates (Czerniejewski & Rybczyk, 2008) and the diet composition is largely driven on prey availability (Hegele-Drywa & Normant, 2009). Our experiments and field observations showed that mud crab predation is the causal factor in the declining abundances of bivalves and gastropods in these parts of the Baltic Sea infested by the mud crabs. As bivalves are currently by far the most important element in the Baltic Sea benthos, a severe decimation of bivalve populations by crabs may relax subordinate invertebrates from
competitive interaction leading to completely new dominance hierarchies. Providing
the fast expansion of crab distribution area, high crab density and the lack of such
functional trait in the north-eastern Baltic Sea range, this invasion is expected to cause
significant repercussions on the structure and functioning of soft bottom invertebrate
communities in the near future. This study also serves as a baseline study of this
iconic biological invasion.

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Biosketch

Jonne Kotta is a Lead Researcher in Marine Ecology at the Estonian Marine
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dependent relationships between environmental forcing and biotic patterns.

Author contributions: JK developed the concept of the study; JK, HO-K, MP, K. and
IvK collected the data; JK IIK, MP, KN and IvK analysed the data; JK led the writing
and all authors commented on drafts of the manuscript.

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Appendix 5

Case study 5 Invasive cladocerans in the Baltic Sea

Potential trophic impact of the predatory pelagic invertebrate *Cercopagis pengoi*: evidence from laboratory studies

Henn Ojaveer, Mart Simm and Maria Põllupüü

Abstract

Multiple field based studies provide evidence that suggests that changes in zooplankton community composition are associated with the invasion of pelagic invertebrate predators. However, very few laboratory experiments have been performed to investigate the underlying processes. In our current work we have studied the feeding habits of the invasive predatory cladoceran *Cercopagis pengoi* by offering various single and mixed prey under laboratory conditions at densities that largely correspond to the natural conditions. It appears that *C. pengoi* is an aggressive predator capable of consuming almost any provided prey, with the exception of newly born young, which are unable to consume copepodids, probably because of size limits and swimming capabilities. According to estimates, one individual of *C. pengoi*, juvenile or adult, may consume on average as many as 9.9 *Bosmina* spp., 9.6 copepod nauplii, 8.1 *Balanus improvisus* larvae and 4.5 copepodids 12 h⁻¹. Copepod nauplii and the small-sized cladoceran *Bosmina* spp. were, in the case available, the major and preferred prey for *C. pengoi*. Higher densities of the small-sized prey generally resulted in elevated consumption rates, while predation on copepodids by both young and adult *C. pengoi* appeared to be relatively stable and therefore independent of the provided prey densities. This supports our earlier field work based hypothesis that significant interannual and seasonal abundance alterations of the selected small-sized zooplankton taxa, associated with *C. pengoi* invasion, are very likely caused by the direct predation of this invasive pelagic predator.

Introduction

Predation is an important factor for influencing the structure and functions of aquatic ecosystems. There are multiple studies that show that organisms with a variety of foraging strategies exert a significant influence on nutrient recycling, taxonomic composition, size structure, abundance and the biomass of plankton (bacterio-, phyto and zooplankton) populations and force prey organisms to change their behaviour and initiate adaptations (e.g., Post and McQueen 1987, Durbin and Durbin 1998, Almond et al. 1996, Lehman and Cáceres 1993, Chang and Hanazato 2004). Whilst predatory effects are better known for vertebrates, large predatory invertebrates may also cause measurable and significant impacts. These are mainly trophic cascading effects, which include declines of prey taxa and the associated abundance increase of other species, which have been released from the previously occurring predation pressure (Stewart et al. 2010, Strecker et al. 2006, Warner et al. 2006).

*Cercopagis pengoi* is a predatory cladoceran which invaded new ecosystems – the Baltic Sea and the North American Great Lakes – in the 1990s (McIsaac et al. 1999, Ojaveer and Lumberg 1995). Based on field observations (Lehtiniemi and Gorokhova 2008, Ojaveer et al. 2004, Benoit et al. 2002) it has been suggested that *C. pengoi* may affect local native zooplankton communities, such as by causing declines in small sized cladocerans and the dominant copepods, via direct predation. This suggestion has been supported by evidence of
the impacts of other predatory cladoceran of similar morphology and feeding strategy – *Bythotrephes longimanus* – (Barbiero and Tuchman 2004).

*C. pengoi* suck the fluid of their prey, their gut content therefore resembles unidentified material (Rivier, 1998). This means that the gut contents of *C. pengoi* doesn’t provide any information on the taxonomic composition of its prey. Therefore, investigations on the feeding habits of the species require predation experiments. As with another large predatory cladoceran, *Leptodora kindtii* (Pichlová et al. 2004), experimental work with *C. pengoi* is complicated because it is sensitive to handling and suffers high mortality after being caught from the wild. Because of these complications, and despite already being present in the invaded ecosystem for nearly two decades, there are very few studies that have investigated the diet composition and prey preferences of *C. pengoi* in laboratory conditions (Pichlová-Ptáčníková and Vanderplug 2009, Lehtiniemi and Gorokhova 2008, Lehtiniemi and Lindén 2006, Laxson et al. 2003).

The current laboratory investigations were carried out with the intention of studying the feeding habits and prey selection of the predatory cladoceran and to assess the predation impact of *C. pengoi* on the zooplankton community in the Gulf of Riga, Baltic Sea. We have provided single and mixed prey with different size and escape reactions (Viitasalo et al. 2001, Viitasalo and Rautio 1998, Browman et al. 1989) at densities which largely correspond to the natural levels in the Gulf of Riga. The manifested prey included several very abundant zooplankton taxa, and/or their developmental stages, during the mass occurrence of this invertebrate predator: *nauplii* and copepodids of *Eurytemora affinis*, the cladoceran *Bosmina* spp. and larvae of the alien cirriped *Balanus improvisus*.

**Materials and methods**

**Plankton collection and storage**

We collected *Cercopagis pengoi* with a Juday net (mesh size 100 μm, mouth diameter 0.1 m²) from the upper layers of Pärnu Bay (Gulf of Riga, Baltic Sea) and placed it into 3.0-L containers. In order to reduce the stress, the collected individuals were transported to the laboratory within one hour, where they were immediately separated by means of a wide mouth pipette and placed as single individuals into 1.0-L containers containing filtered seawater. The containers were aerated. For the feeding experiments, we only selected *C. pengoi* individuals which were free of attached detritus or other material and healthy, i.e., freely swimming during the 24-hour period prior to the experiment. Zooplanktonic prey was collected from Pärnu Bay simultaneously with *C. pengoi*, pipetted out of the water and placed into filtrated seawater in 2.0-L containers where they were kept in aerated conditions until the start of the experiments.

**Laboratory experiments**

Experiments were conducted in 1.0-L vessels at the temperature of 20 ± 2 ºC, allowing only diffuse overhead light. There was no aeration during the experiment. Each container was filled with 0.8 L filtrated seawater and one individual of *C. pengoi* was added. Instar 1 individuals (newly born young) and instar 3 parthenogenetic females (adult animals) were used in the study.. Healthy prey of 10, 15, 20, 25 and 30 individuals per vessel were added to the containers (corresponding to 12.5, 18.8, 25.0, 31.3 and 37.5 ind. l⁻¹). The provided prey density largely corresponded to that in the field conditions in Pärnu Bay. We visually checked *C. pengoi* individuals at the beginning of the experiment to ensure that they were intact. Also, swimming behaviour of the predator was checked several times during the experiment and just prior to the termination of the trial. To prevent potential sedimentation of prey, vessels were gently stirred a few times during the experiment.
The duration of the experiments was usually eight hours. After the incubation period, experiments were terminated by adding formalin into all vessels. The contents of experimental vessels were concentrated by reverse filtration (using 60 μm mesh) and investigated under a dissecting microscope to assess prey mortality, as well as their condition. Prey which was found trapped within the water surface film after the experimental period was considered to be alive.

Altogether 177 successful experiments were performed (Table 1) by using the following prey: *Balanus improvisus* larvae, *Bosmina* spp., nauplii and copepods of *Eurytemora affinis* and *Acartia* spp. The experiments were run with single and mixed prey combinations. Control experiments, without predators, were carried out in exactly the same manner as with *C. pengoi*. These were performed with all prey items separately and the experimental results were corrected accordingly. Results of the predation experiments – consumption rate of *C. pengoi* – are expressed as the number of prey *C. pengoi* 12 h⁻¹ consumed. Prey density in experiments is shown per litre (i.e., ind. l⁻¹).

**Statistical analysis**

Statistical software “Statistica” was used for data analyses. The significance of differences in the consumption rates of *C. pengoi* on single and mixed prey combinations at different density levels was estimated by one-way analysis of variance (ANOVA) and the post-hoc Bonferroni test in repeated measures ANOVA. The differences were considered significant at *P* < 0.05.

**Results**

Experiments on the consumption of *C. pengoi* on single prey provided at several different density levels indicate that the consumption rate of *C. pengoi* on several small-sized prey increases along with an increase in prey density while predation on copepodids seems to be independent from their density levels (Figure 1). A comparison of consumption rates of small-sized prey by juvenile and adult *C. Pengoi* on *Bosmina* spp. provided at several different density levels (12.5-37.5 prey l⁻¹) indicated that higher densities resulted in significantly higher consumption rates only on predation of *Bosmina* spp. by adult *C. Pengoi* (ANOVA, *P*=0.02). *B. improvisus* larvae and copepod nauplii were eaten at elevated prey densities by juvenile *C. Pengoi* at higher rates, but the difference was insignificant (ANOVA, *P*>0.05). Consumption of juvenile *C. Pengoi* on copepodids was about two times lower than that of adult *C. Pengoi* (about 2 and 4 ind. 12 h⁻¹). Adult *C. Pengoi* consumed copepod nauplii at rates which were relatively stable, independent of the provided prey density levels and appeared to be about two times higher than that of copepodids (Figure 1). Within the manifested prey density levels (12.5-37.5 ind. l⁻¹), *C. Pengoi* juveniles were able to consume as many as 9.6 copepod nauplii, 8.1 larval *Balanus improvisus*, 6.7 *Bosmina* spp. and 2.4 copepodids 12 h⁻¹. Adults of *C. Pengoi* consumed as many as 8.2 copepod nauplii, 9.9 *Bosmina* spp. and 4.5 copepodids 12 h⁻¹ (Figure 1).

Comparative experiments on the consumption of juvenile and adult *C. Pengoi* on single prey provided at two (25.0 and 37.5 prey l⁻¹) different density levels indicate that there were no significant differences observed in the consumption of small-sized less motile prey (ANOVA, *P* > 0.05) by *C. Pengoi* juveniles and adults. At the same time, copepodids were consumed at substantially lower rates than small-sized and less motile prey species with significant differences in the case of adult *C. Pengoi* (ANOVA, *P* < 0.05) (Table 2). It also appeared that at higher prey densities, *Bosmina* spp. were utilised at a higher rate by adult and copepod nauplii than by juvenile *C. Pengoi*. The above suggests that not only copepod nauplii and *Bosmina*...
spp., but also larvae of the alien cirriped Balanus improvisus are, in case available, the major prey resource for C. pengoi.

The consumption rates of C. pengoi juveniles to different prey densities in mixed diets was studied for B. improvisus larvae and copepod nauplii. It was clearly evident that higher prey density in the mixed diet resulted in an elevated consumption rate for that prey without any species-specific differences (Figure 2). When copepod nauplii and B. improvisus larvae were provided at equal densities (18.8 prey l⁻¹), B. improvisus larvae were consumed at slightly higher rates than copepod nauplii. When provided with mixed prey items at a 2:1 density ratio, the dominating prey was consumed at a higher rate. In experiments with copepod nauplii and B. improvisus larvae at a significantly higher rate than copepod nauplii (4.5 ± 0.4 and 1.1 ± 0.0 12 h⁻¹, mean ± se respectively; P < 0.000; ANOVA). In case of mixture of copepod nauplii and larval B. improvisus at density ratio of 2:1, copepod nauplii were strongly preferred (5.0 ± 0.6 and 0.5 ± 0.3, respectively; P < 0.000; ANOVA).

The consumption rates of C. pengoi adults to different prey densities in mixed treatments was studied for Bosmina spp. and copepod nauplii. In contrast to C. pengoi juveniles, higher prey density did not necessarily result in an elevated consumption rate. Overall, it appeared that Bosmina spp. was clearly preferred over copepod nauplii (Figure 3). While this preference was insignificant for equal prey densities (18.8 prey l⁻¹), provision of unequal densities resulted in significantly higher consumption of Bosmina spp. In experiments that provided Bosmina spp. and copepod nauplii with ratio of 1:2 (i.e., 12.5:25.0 ind. l⁻¹), C. pengoi consumed Bosmina spp. at significantly higher rate than copepod nauplii (3.9 ± 1.2 and 0.6 ± 0.6 12 h⁻¹, respectively; P < 0.041; ANOVA). In cases of densities of 25.0 Bosmina spp. and 12.5 copepod nauplii l⁻¹, Bosmina spp. were again significantly preferred (8.7 ± 1.3 and 1.2 ± 0.7, respectively; P < 0.001; ANOVA).

In addition, we have provided adult C. pengoi with mixed food at equal densities (18.8 prey l⁻¹) containing copepod nauplii and copepodids. It appeared that copepod nauplii were highly preferred over copepodids (3.1 ± 0.4 and 0.3 ± 0.2; P < 0.000; ANOVA).

Out of the total 202 experiments performed, 91.8 were successful. The reasons for failure were: i) mortality and/or non-healthy condition of predator which became evident during the course of experiment; ii) reproduction of the predator and/or prey during the experiment; iii) contamination of sample with other/additional prey during the pipetting.

Discussion
Predatory cladocerans are considered as very efficient predators having a major direct impact on the structuring of small-sized cladocerans (Strecker et al. 2006, Warner et al. 2006; Barbiero and Tuchman 2004). They are also capable of consuming relatively fast-moving copepods, but generally seem to avoid rotifers (this study, Hovius et al. 2006, Lehtiniemi and Gorokhova 2008). However, under a predation risk, they may switch to eat rotifers (Lehtiniemi and Lindén 2006).

In general, results of the current study are in agreement with those of the most recent comprehensive laboratory exploration of predation of the dominating zooplankton taxa by C. pengoi in the North American Great Lakes (Pichlová-Ptáčníková and Vanderploeg 2009). Both studies support evidence that C. pengoi is an aggressive predator able to prey upon a variety of zooplankton taxa. Our study provides further evidence that individuals with different instar stages of C. pengoi did consume all provided prey with only one exception: the newly born
individuals were unable to prey on adult copepods. This is probably because adult copepods are too large in body size and their escape response is too rapid for the young C. pengoi to catch. Our experimental results also support the recent conclusion made by Lehtiniemi and Gorokhova (2008) that direct predation by C. pengoi could be considered as a reason why copepod E. affinis abundance has drastically decreased in the Gulf of Finland (Baltic Sea). Our study further suggests that this is essentially very likely to be achieved through consumption of the early life-history stages of copepods – nauplii.

Large pelagic predatory cladocerans are extremely sensitive to handling and experimental manipulations, and survive poorly in captivity (Pichlová et al. 2004, our own experience). This is very different compared to the behaviour of all other native zooplankters in the Baltic Sea. Therefore, only a few experiments to study feeding habits of the species have been carried out to date. One type of study has been to investigate what native single prey taxa and in which rates C. pengoi is exploiting (Lehtiniemi and Gorokhova 2008, Pichlová-Ptáčníková and Vanderploeg 2009, Laxson et al. 2003). Another type of approach has been to investigate potential predator-prey interactions between C. pengoi and the other invasive predatory cladoceran Bythotrephes longimanus to test the effect of the container size on the predator-prey interactions (Witt and Cáceres, 2004). Thus, most studies evaluating the trophic impact of C. pengoi upon the plankton communities (both invertebrates and fish) are based on field data, simple comparisons/likelihood assessments and/or by using different modelling approaches (Warner et al. 2006, Gorokhova et al. 2005, Laxson et al. 2003, Benoit et al. 2002, Uitto et al. 1999). As data availability for feeding, energetics and physiology of C. pengoi is still very limited, knowledge of the other predatory cladoceran – B. longimanus – was applied in the modelling exercises (e.g., Yurista and Schulz 1995). Thus, despite two decades of invasion history, our knowledge on trophic interactions of and related cascades caused by C. pengoi are not very advanced and there is a significant gap in understanding feeding behaviour of the species.

Our experience suggests that some feeding experiments with C. pengoi may be unsuccessful for three major reasons: i) mortality and/or non-healthy condition of predator; ii) reproduction of predator and/or prey during the experiment; iii) methodological mistakes – contamination of sample with other/additional prey during the pipetting. Earlier studies have also commented on failed trials, due to both mortality of a predator as well as because C. pengoi gave birth during the incubations (Lehtiniemi and Gorokhova 2008).

In their lab experiments Laxson et al. (2003) have obtained similar consumption rates of C. pengoi for Daphnia retrocurva and Bosmina longirostris: 2.8 C. pengoi\(^{-1}\) day\(^{-1}\). Our results (when calculated to the same time interval) vary between 4.8 (for copepodoids) and 11.3 (for copepod nauplii) C. pengoi\(^{-1}\) day\(^{-1}\) and are, thus remarkably higher than those obtained by Laxson et al (2003). However, the same authors have also used bioenergetic modelling and obtained the following consumption rates: 2.1–4.7 for D. retrocurva and 7.1–7.5 for B. longirostris. Considering also differences in prey size, it is concluded here that the latter consumption rates are in good agreement with the results of the current study. However, container size can significantly impact the results of the predation experiments with predatory cladocerans, although species (both predator and prey) involved and container/species interactions are also important (Witt and Cáceres 2004, Abrusan 2003). In this respect, our results should be comparable with those of Pichlová-Ptáčníková and Vanderploeg (2009) and Laxson et al. (2003), but not with the C. pengoi/copepods predation experiments carried out by Lehtiniemi and Gorokhova (2008) who used several times smaller jars than in the current study.
Predatory invertebrates can cause various changes to their prey, both at the individual and population level. These include, amongst others, prey abundance decline (Pichlová and Brandl 2003), regulation of prey species succession (Chang and Hanazato 2004), change in the vertical distribution (Põllumäe and Kotta 2007) and alterations in the external morphology (Lagergren and Stenson 2000). The ultimate mechanism behind these modifications is direct predation. It has been shown for several predatory invertebrates on several occasions, that body shape and escape response are important rather than prey size (e.g., Browman et al. 1989; Sakamoto and Hanazato 2008). In our study, prey with a strong escape response (i.e., copepodids) was less preferred than copepod nauplii, *B. improvisus* larvae and *Bosmina* spp., while the body shape characteristics probably led *C. pengoi* to prefer *Bosmina* spp. over the other offered less-evasive prey.

Different developmental stages of copepods (*nauplii*, copepodites and adults), dependent on the larval size and environmental conditions, strongly dominate (over 95%) in the diet of larval herring, which is the far most important commercial fish in the Gulf of Riga (Arula et al. 2012). Our previous studies have shown that compared to the pre-invasion time of *C. pengoi*, seasonal abundance of copepod *nauplii*, was significantly lower during the post-invasion time when there was no *Bosmina* spp. available in the plankton community, or when its abundance was very low. One such time-period occurs in June (Ojaveer et al. 2004), which is high spawning season of the local herring population and its larval abundance is therefore very high (Ojaveer et al 2010). The current study suggests that significantly temporarily lower abundance of copepod *nauplii* after the invasion of *C. pengoi* might be caused by direct predation by *C. pengoi*. However, it should be noted that the impact of *C. pengoi* on the abundance of copepod *nauplii* is rather limited in terms of seasonal duration and therefore the overall effect was not seen on the multi-annual scale (Ojaveer et al. 2004).

Larvae of the cirriped *Balanus improvisus* were relatively actively consumed by *C. pengoi*, especially when the prey was offered in high quantities. *B. improvisus* is an alien species of North American origin that invaded the Baltic in the 1800s. There is no evidence that either planktonic larvae or demersal sessile adults of *B. improvisus* are actually consumed by fish in the Baltic Sea. Thus, at least theoretically, the invasion of *C. pengoi* may have introduced new links in the system that transfer previously unutilised biological production to upper trophic levels accessible to commercial fish production. It is also likely, that *B. improvisus* was facilitating the invasion of *C. pengoi*, as has been suggested for other alien species elsewhere (Ricciardi 2001). As larval *B. improvisus* is also important food for the overwintering population of the alien ctenophore *Mnemiopsis leidyi* in the southern Baltic Sea (Javidpour et al. 2009), it could be concluded that trophic interactions between invertebrate alien species become more and more important in the pelagic foodwebs of the Baltic Sea and this information is just starting to be accumulated.

There seems to be a general consensus reached amongst scientists on the overall major type of impact that *C. pengoi* exerts on pelagic communities: modifications of pelagic foodwebs and trophic interactions in invaded ecosystems (e.g., Gorokhova et al. 2005, Warner et al. 2006, Bushnoe et al. 2003, Benoit et al. 2002). While the role of *C. pengoi* in fish diet is relatively well-studied, the absolute quantification of the predatory effect of *C. pengoi* on native plankton communities is still problematic and remains a substantial challenge (e.g., Witt et al. 2005, Brown and Balk 2008). Although the invasive species has been present in the Baltic Sea and the North American Laurentian Great Lakes for over a decade, very few feeding experiments have been carried out. A different experimental setup (container size, duration, etc.) makes it
difficult to fully compare results of these feeding experiments (see also above). Therefore, a
combination of different approaches, by including field observations on spatio-temporal
dynamics of zooplankton abundance and biomass, continuation of feeding experiments,
performing bioenergetic modelling and studying the invaded foodwebs by using stable isotopes
will most likely help to provide quantitative estimates on the impact of C. pengoi on native
plankton communities.

The Black Sea Mnemiopsis-experience suggests that a switch of a large marine ecosystem to a
totally invader-dominated state requires extremely strong environmental perturbations. More
often, environmental disturbances create a suitable niche for an alien planktonic invader to
become a member of the food web structure, and to share food resources with the native small
pelagic fish community (Oguz et al. 2008). Although C. pengoi has evidenced more than one
abrupt shift in abundance since its invasion in the early 1990s (Ojaveer et al. 2011) and
significantly modified cladocerans seasonal dynamics (Pollupuu et al. subm.) in general, its
impact to pelagic communities should be considered as more structural and functional rather
than causing a major switch of the invaded ecosystems into a new stable regime.

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new prey item for alewife (Alosa pseudoharengus) and rainbow smelt (Osmerus mordax)
in Lake Ontario. J. Great Lakes Res., 29, 205-212
(Leptodora kindtii, Thermocyclops taihokuensis, and Mesocyclops sp.) in a shallow,
eutrophic lake. Hydrobiologia, 528, 249-259
in Narragansett Bay, Rhode Island. Estuaries, 21, 449-465


Table 1. Number of feeding experiments of *Cercopagis pengoi* with various single and mixed diet items

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total</th>
<th>Successful</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Balanus improvisus</em> larvae</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td><em>Bosmina</em> spp.</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>Copepod <em>nauplii</em></td>
<td>41</td>
<td>39</td>
</tr>
<tr>
<td>Copepodids</td>
<td>45</td>
<td>37</td>
</tr>
<tr>
<td><em>B. improvisus</em> larvae + copepod <em>nauplii</em></td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td><em>Bosmina</em> spp. + copepod <em>nauplii</em></td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Copepod <em>nauplii</em> + copepodids</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>202</strong></td>
<td><strong>177</strong></td>
</tr>
</tbody>
</table>
D3.1.3 Novel experimental evidence for impacts of biodiversity on ecosystem functioning

Table 2. Consumption of *Cercopagis pengoi* juveniles and adults for various single prey taxa (ind. 12 h⁻¹) at densities (l⁻¹) of 25.0 and 37.5 prey. * - differences are significant at 0.05 level.

<table>
<thead>
<tr>
<th>C. pengoi / prey</th>
<th>Prey density</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25.0</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P</em></td>
<td><em>P</em></td>
<td></td>
</tr>
<tr>
<td><strong>C. pengoi juveniles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bosmina</em> spp. – copepod nauplii</td>
<td>0.456</td>
<td>0.840</td>
<td></td>
</tr>
<tr>
<td><em>Bosmina</em> spp. – <em>B. improvisus</em> larvae</td>
<td>0.911</td>
<td>0.865</td>
<td></td>
</tr>
<tr>
<td><em>Bosmina</em> spp. – copepodids</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Copepod nauplii</em> – <em>B. improvisus</em> larvae</td>
<td>0.594</td>
<td>0.906</td>
<td></td>
</tr>
<tr>
<td><em>Copepod nauplii</em> – copepodids</td>
<td>0.057</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C. pengoi adults</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bosmina</em> spp. – copepod nauplii</td>
<td>0.492</td>
<td>0.582</td>
<td></td>
</tr>
<tr>
<td><em>Bosmina</em> spp. – copepodids</td>
<td>0.005*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Copepod nauplii</em> – copepodids</td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Consumption of *Cercopagis pengoi* a) juveniles (stage I) and b) adults (females) for various prey taxa (average ± SE; ind. 12 h⁻¹) at different prey densities (l⁻¹) in single treatments.
Figure 2. Consumption rate of *Cercopagis pengoi* juveniles for copepod *nauplii* and *Balanus improvisus* larvae (ind. 12 h⁻¹) at three different prey densities (l⁻¹) in mixed treatments. Vertical bars denote 95% confidence intervals.
Figure 3. Consumption rate of *Cercopagis pengoi* adults for *Bosmina* spp. and copepod *nauplii* (ind. 12 h⁻¹) at three different prey densities (l⁻¹) in mixed treatments. Vertical bars denote 95% confidence intervals.