Response of shallow sediment ecosystems to environmental alterations

CHRISTIAN ALSTERBERG

UNIVERSITY OF GOTHENBURG

FACULTY OF SCIENCE
DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap med inriktning mot Marin Ekologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 8:e mars 2013 kl. 10.00 i Hörsalen, Institutionen för biologi och miljövetenskap, Carl Skottsbergsgata 22B, Göteborg

Examinator: Professor Per Jonsson, Institutionen för biologi och miljövetenskap, Göteborgs Universitet

Fakultetsopponent: Professor Graham Underwood, School of Biological Sciences, University of Essex, Colchester, United Kingdom

ISBN: 91-89677-55-2

Front cover photography: Christian Alsterberg & Fredrik Larson
Printed by Kompendiet
© Christian Alsterberg 2013
ISBN 91-89677-55-2

ABSTRACT

Shallow-water sediment systems are continuously exposed to a range of anthropogenic stressors, including increased nutrient loading, physical disturbance and toxicants. Superimposed on these local stressors are stressors related to the on-going climate change. Interacting stressors may strengthen or weaken effects of individual stressors, resulting in – often unexpected – non-additive effects. Thus, the combined effect from several simultaneous stressors in shallow-water sediment systems is the main topic of this thesis. Individual and combined stressor effects were studied on intact sediment cores placed in an outdoor flow-through facility and in the laboratory. Both functional (primary and bacterial production, community respiration, meiofaunal grazing, denitrification, nitrogen mineralization and sediment-water fluxes of oxygen and nutrients) and structural (biomass and composition of microphytobenthos and meiofauna) were studied in order to assess effects of multiple stressors.

It has been suggested that global warming can shift the trophic status of ecosystems from net autotrophy to net heterotrophy. A spring experiment (**Paper I)** showed, however, that the presence and activity of a well-developed microphytobenthic mat already in early spring sustained net autotrophy during simulated warming. The effects of increased temperature on the structure and function of the sediment systems were generally rather moderate. Consequently, it is possible that the presence and activity of microphytobenthos can buffer changes induced by seawater temperature. An autumn experiment (Paper II) suggested that warming combined with nutrient enrichment can induce shorter, intensive, heterotrophic periods that can be followed by longer autotrophic periods because initially increased mineralization induces a shortage of labile organic matter. Even though warming increased nutrient availability through increased mineralization, warming did not exacerbate the stimulatory effects of nutrient enrichment. The lack of interactive temperature-nutrient effects was mainly explained by a sustained filter function by microphytobenthos.

It has also been suggested that future warming will exacerbate the effect of toxicants. However, in **paper III** the combined effects of an antifouling compound (Cupper pyrithione) and warming resulted in antagonistic rather than synergistic effects. The two types of microalgal communities present at the end of the experiment, a typical benthic mat and a floating periphyhtic mat, were both significantly affected by the toxicant, but in opposite ways; the biomass of the benthic mat was stimulated by the toxicant, whereas the floating periphytic mat was negatively affected. Thus, autotrophic communities within the same ecosystem can respond differently. Also the nutrient status of an ecosystem can change toxicant effects. This was shown in **paper IV,** where the combination of pyrene (a polycyclic aromatic hydrocarbon) and nutrient enrichment synergistically reduced the grazing pressure of meiofauna on microphytobenthos, exerting a cascading effect on the primary producers.

In **paper V** we studied whether herbivores can mediate effects of ocean warming and acidification on microphytobenthos in a seagrass meadow, and how these effects were related to the biomass of *Zostera marina*, filamentous macroalgae and sediment-associated fauna. Analysis of variance and structural equation modeling (SEM) were used to partition net effects of warming and ocean acidification into direct and indirect effects. This statistical approach showed that the absence of stressor effects on microphytobenthos was actually a combination of direct and indirect effects mediated via grazers on filamentous macroalgae and sediment associated fauna.

Overall, the main results in my thesis is that even though changes in temperature, pH, toxin and nutrient availability, will occur in present time as well as the near future, the main function of shallow-water sediment ecosystems will be sustained given that microphytobenthos are present. My results also show that environmental alterations need to be studied together on an ecosystem level rather than on individual species level and that indirect effects always need to be taken into consideration when interpreting experimental results.

KEYWORDS

Shallow-water sediments | microphytobenthos | meiofauna | mesograzers | *Zostera marina* | multiple stressors | global warming | eutrophication | Copper pyrithione | pyrene | ocean acidification | grazing | trophic interactions | biogeochemistry | nutrient fluxes | primary production | bacterial production | structural equation modeling

POPULÄRVETENSKAPLIG SAMMANFATTNING

Ett ökat nyttjande av haven som födoresurs, soptipp, transportsträcka och turistattraktion resulterar i enorma störningar på de organismer som lever i, eller angränsande till haven. Vidare förutspås stora förändringar i det globala klimatet, varav vissa av dessa förändringar redan nu börjar bli märkbara. Hittills har många av dessa störningar studerats en och en, och inte sällan på enskilda arter. I verkligheten sker det många störningar samtidigt. Effekten av en störning då är svår att förutsäga eftersom den kan bli förstärkt eller försvagad av andra störningar. Även om antalet studier som kombinerar störningar ökar så är det fortfarande få som inkluderar flera arter och nivåer i näringskedjan samtidigt.

Jag har studerat hur förändringar i klimatet, tillsammans med lokala störningar påverkar organismer och dess funktion i grunda mjukbottnar. Grunda mjukbottnar kan vara allt ifrån sandstränder till leriga vikar och de fungerar ofta som uppväxt- och födoområden för många djur och kommersiellt viktiga fiskarter. Det är också dessa områden som är ”filtret” mellan land och hav, och får således ta emot en hel del skräp, gifter och näringsämnen från jordbruk och industrier. En förutsättning för att grunda mjukbottnar ska fungera som ett filter är att bottenlevande mikroalger är närvarande. Dessa mikroalger bildar ofta en tunn hinna på sediment botten där de producerar syre genom fotosyntesen och bidrar med över 50 procent av den totala primärproduktionen. Produktionen av syre styr flera biogeokemiska processer i sedimentet och avgör bland annat om näringsämnen tas upp eller släpps ut i den överliggande vattenmassan. Skulle de bottenlevande mikroalgerna försvinna, kommer flödet av näringsämnen och organismer som befinner sig på en högre trofinivå (fisk, krabbor etc) riskera att bli utan föda. Detta skulle få förödande konsekvenser för haven som livsmiljö och som en resurs för människa.

I mina studier fann jag att en förhöjning av vattentemperaturen i grunda mjukbottnar har en effekt på både bottenlevande mikroalger och djur. Denna effekt är dock inte tillräckligt starkt för att skapa några större förändringar i hur systemet fungerar strukturellt och funktionellt. En förhöjd vattentemperatur i kombination med tillförsel av näringsämnen som exempelvis kväve och fosfor så kommer det att ske förändringar i kvävets kretslopp. Dessa effekter kan på sikt bidra till en ökad övergödningseffekt, men återigen så är dessa effekter på ekosystem-nivå relativt svaga. I dessa fall är det närvaron av bottenlevande mikroalger som reglerar effekterna av störningar genom att bibehålla systemet autotroft (självförsörjande). På så sätt kan de grunda mjukbottnarnas funktion bibehållas relativt intakta. Det mest överraskande är att effekten av ett båtbottengift på processer styrda av bottenlevande mikroalger (framförallt primärproduktion) helt och hållet försvinner när vattentemperaturen höjs med fyra grader. I just detta fall hade närvaron av bottenlevande mikroalger en motverkande effekt mot toxinet. Toxin-effekter är specifika och styrs ofta av andra miljöfaktorer. Exempelvis så kan toxiner resultera i oförutsägbara indirekta effekter genom att påverka betningstrycket av organismer som livnär sig på bottenlevande mikroalger. Eftersom betydelsen av indirekta effekter är lika viktiga som de direkta, undersöktes den relative betydelsen av dessa genom att använda primärproducenter i en ålgräsäng som modellsystem. Resultaten från denna studie visar att styrkan av de indirekta effekterna kan bli lika stor som de direkta. Således kan direkta och indirekta effekter ta ut varandra så att nettoeffekten blir noll.

Resultaten av mina experiment visar att även om stora förändringar kommer att ske i vår havsmiljö de närmaste årtionden så kan den huvudsakliga funktionen av de grunda mjukbottnarna bestå, under förutsättning att bottenlevande mikroalger är närvarande. En viktig slutsats är också att kombinerande miljöstörningar bör studeras samtidigt på systemnivå, och att både direkta och indirekta effekter tas i beaktande för att inte fel slutsatser dras om vår framtida havsmiljö.

LIST OF PAPERS

I. Alsterberg, C., Hulth, S., Sundbäck, K. 2011. Response of a shallow-water sediment system to warming. *Limnology and Oceanography*, 56(6), 2147–2160.

II. Alsterberg, C., Sundbäck, K., Hulth, S. 2012. Functioning of a shallow-water sediment system during experimental warming and nutrient enrichment. *PLoS ONE 7(12): e51503.*

III. Alsterberg, C. and Sundbäck, K. Experimental warming and toxicant exposure can result in antagonistic effects in a shallow-water sediment system. *Revised manuscript submitted to Marine Ecology Progress Series*.

IV. Sundbäck, K., **Alsterberg, C**., Larson, F. 2010. Effects of multiple stressors on marine shallow-water sediments: Response of microalgae and meiofauna to toxicant–nutrient exposure. *Journal of Experimental Marine Biology and Ecology*. 388, 39-50.

V. Alsterberg, C., Eklöf, J.S., Havenhand, J.N., Gamfeldt, L., Sundbäck, K. Consumers mediate the effects of experimental warming and ocean acidification on primary producers. *Submitted Manuscript.*

Related publications not included in this thesis:

Alsterberg, C., Larson, F., Sundbäck, K. 2007. Direct and indirect effects of an antifouling biocide on benthic microalgae and meiofauna. *Journal of Experimental Marine Biology and Ecology*. 351, 56-72.

Eklöf, J.S., **Alsterberg, C.**, Havenhand, J.N., Sundbäck, K., Wood, H.L., and Gamfeldt, L., 2012. Experimental climate change weakens the insurance effect of biodiversity. *Ecology Letters* 15(8), 864–872.

Papers I, II and VI reprinted with kind permissions from; I: Copyright (2011) by the Association for the Sciences of Limnology and Oceanography, II: published in an open access journal and is reprinted with permission from co-authors, VI: Copyright (2010) by Elsevier B.V.

CONTENTS

BACKGROUND

FUNCTION OF SHALLOW-WATER SEDIMENT SYSTEMS

At the interface between land and sea, sandy beaches and muddy bays are important parts of the coastal marine ecosystem. On the sediment surface there is a thin, brown layer of benthic microalgae, also known as microphytobenthos (MPB). Microphytobenthos consist mainly of pennate diatoms, however, cyanobacteria, green algae and dinoflagellates are also present in large numbers (Colijn & Dejonge 1984). In estuarine ecosystems, MPB has been estimated to contribute with over 50 % of the total primary production (Underwood & Kromkamp 1999), thus its presence is not trivial. Shallow-water sediment systems (SWS) are also the home of seagrass meadows forming one of the most productive, yet threatened coastal ecosystems (Waycott et al. 2009). The presence of microphytobenthos and *Zostera marina* meadows in shallow-water sediment systems therefore constitute an important function for both the primary and secondary production of marine ecosystem.

In unvegetated sediment systems microphytobenthos are at the base of the food web (Admiraal 1984, Middelburg et al. 2000). When suspended by water movements, MPB likewise contributes to the pelagic primary and secondary production (de Jonge et al. 2012). The presence of microphytobenthos can therefore support an entire food-web, both benthic and pelagic, in SWS (Middelburg et al. 2000) and several meio- and macrofaunal species are even known to exert a strong top-down control on MPB biomass (Coull 1999, Burkepile & Hay 2006, Weerman et al. 2011). Through the production of oxygen via photosynthesis, microphytobenthos also 'indirectly' controls important biogeochemical processes in the sediment (Sundbäck et al. 2000, Dalsgaard 2003). Oxygen is the most energy favorable electron acceptor. Hence, the presence or absence of oxygen is an important determinant for the many redox sensitive biogeochemical processes occurring in the sediments (nitrification, denitrification, mineralization, sulfate reduction etc.) (Froelich et al. 1979). Usually the scale between oxic and anoxic layers in shallow sediments is within a few millimeters. Thus, any change in the trophic state (the balance between autotrophy and heterotrophy) can have large effects on nutrient cycling and mineralization processes (Engelsen et al. 2008). Because of the tight coupling between nutrient cycling and the activity of microphytobenthos, shallow-water sediment systems constitute an important functional component of the coastal filter by transforming, storing and removing nutrients from the environment (Sundbäck & Miles 2000, McGlathery et al. 2007, Cook et al. 2009). Microphytobenthos therefore have the capacity to buffer against abiotic nutrient discharges and hence counteract coastal eutrophication (McGlathery et al. 2007). In vegetated sediment systems such as *Zostera* meadows, microphytobenthos also constitute an important food source for crustacean and gastropod herbivores (Jaschinski et al. 2011).

Zostera marina is a clonal angiosperm that forms large underwater meadows and in shallow-water sediment systems they are typically found at depths around 2-4 meters on the Swedish west coast (Baden et al. 2010). Seagrass meadows support a wide array of trophic interactions between herbivores feeding on epiphytic and free-floating macroalgae, but they also function as nursery and feeding grounds for fish (Moksnes et al. 2008, Baden et al. 2012, Eklöf et al. 2012). There also seems to be a strong coupling between microbial activity and seagrass productivity, mainly through the oxygenation of sediments by seagrass rhizomes and roots (Pedersen et al. 1998). Thus, the microbial activity are higher in seagrass meadows when compared to unvegetated sediments (Lee & Dunton 2000). Regardless if the sediment system is unvegetated or vegetated, seasonal aspects need to be taken into consideration when studying structural and functional processes in temperate SWS. In general, SWS in Sweden turns net heterotrophic in late autumn and net autotrophic in spring. Thus, nutrients are taken up during spring and early summer, whereas in autumn nutrients are directed out of the sediment (Sundbäck et al. 2000). *Zostera marina* is a perennial plant, but during winter only roots and rhizomes are present in the sediments. Also, all the biogeochemical processes and trophic interactions are modified by the seasonal presence of macro- and meiofauna (Kristensen et al. 2012, Nascimento et al. 2012).

MULTIPLE STRESSORS AND COMMUNITY RESPONSES

Today, shallow-water sediment systems are increasingly exposed to local anthropogenic stressors like nutrient loading, toxicants, physical disturbance and overharvest of organisms (Halpern et al. 2008, Defeo et al. 2009). Superimposed on these local stressors is climate change such as elevated CO_2 concentrations and warming (IPCC 2007, Crain et al. 2008). The problem with any stressor, singly or in combination, is that they often cause both direct and indirect effects, making it very difficult to draw any firm conclusions from the results without an appropriate approach (Folt et al. 1999, Vinebrooke et al. 2004). Thus, climate change may interact with local stressor and cause unexpected ecological 'surprises' like ecosystem shifts and trophic cascades (Folt et al. 1999, Christensen et al. 2006, Crain et al. 2008).

In order to understand how several stressors simultaneously influence shallow-water marine sediment systems, stressors have to be studied in combination (Folt et al. 1999, Crain et al. 2008). However, evaluating effects of multiple stressors is difficult because of the complexity of marine ecosystems and often a multidisciplinary approach is needed (Adams 2005). The combined effects of stressors often depend on the specific stressors, the ecosystem studied, species diversity and trophic level (Breitburg et al. 1999, Vinebrooke et al. 2004, Larson et al. 2007). Depending on several ecological factors, stressors – singly or together – affect marine ecosystems on different scales, which lead to the question: Are stressors more harmful in combination than alone? Folt et al. (1999) highlight this question and discuss three models: Simple *comparative* (the effect of several stressors equals the strongest single stressor), *multiplicative* (the effect of one stressor controls the effect of another stressor) and *additive* (the combined effect of two stressors are smaller or larger than the sum of effect from those two stressors) to describe synergism and antagonism among multiple stressors. Folt et al. (1999) also emphasize the importance of describing which of the models is used during the experiment, because otherwise results might be difficult to interpret. The additive model is most frequently used, and was also used in this thesis, simply because it is easy to test with variance of analysis (ANOVA) and the model also discriminates between synergistic and antagonistic effects. Thus the model depends on the combined effect of stressors, i.e. whether it is less or more than the sum of the individual stressors (Folt et al. 1999). For example, temperature–toxicant interactions are complicated and the effects of toxins on aquatic and terrestrial organisms are very toxin and species specific, but in general a synergistic interactions would be expected, thus strengthening the toxicant effect beyond the individual effects (Heugens et al. 2001). This example highlights the fact that we need to complement the single-species and single-stressor tests, though they also are of great importance.

Microphytobenthos have been studied in several multiple-stressor experiments and despite the observed changes in diversity and primary production, MPB seem to be less sensitive to stressors compared to sediment bacteria and meiofauna (Alsterberg et al. 2007, Larson et al. 2007, Sundbäck et al. 2007). Among bacteria, it is especially those involved with nitrogen cycling that appears to be sensitive when exposed to multiple stressors (Petersen et al. 2004), and in general heterotrophs are more sensitive than autotrophs (Christensen et al. 2006, Sundbäck et al. 2007, Alsterberg et al. 2011). The aim of my research was therefore to clarify the combined effects of several environmental alterations and also to gain further basic knowledge about the biology and ecology of shallow-water sediment systems, and microphytobenthos in particular.

ENVIRONMENTAL ALTERATIONS INCLUDED IN THIS THESIS

Even though the number of environmental alternations on shallow-water sediment systems is numerous (Defeo et al. 2009), I have chosen to include, in this thesis, stressors that are predicted to have major impacts on the marine environment (IPCC 2007, Crain et al. 2008, Halpern et al. 2008).

Global warming. One of the major stressors associated with climate change is environmental warming, which is expected to increase the growth of fast-growing plants (Walther 2004), but also alter species size and composition (Sommer & Lengfellner 2008, Woodward et al. 2010). Changes in temperature will affect autotrophs and heterotrophs differently, primarily because heterotrophic metabolism are more sensitivity towards changed temperature (Brown et al. 2004). Consequently, herbivore-plant interactions can be strengthened (O'Connor 2009) and trophic mismatches in spring blooms can be induced (Woodward et al. 2010). Further, changes in the metabolic balance can turn shallow-water sediment systems net heterotrophic (Hancke & Glud 2004, Yvon-Durocher et al. 2010) with direct consequences on nutrient cycling (Engelsen et al. 2008). In seagrass meadows warming is even predicted to have a direct negative effect on *Zostera* plants (Reusch et al. 2005), potentially resulting in ecosystem shifts (Connell & Russell 2010). Overall, warming is predicted to synergistically interact with other stressors, primarily due to the extra input of energy into the system, thus strengthening the effect of the other stressor (Crain et al. 2008).

Elevated CO_2 concentrations. Increased concentrations of CO_2 will in the marine environment reduce the pH via a process known as 'ocean acidification' (Doney et al. 2009, Doney et al. 2012). Acidification may impact biofilm community composition by shifting from a bacterial- towards an algal dominance, thus affecting productivity and nutrient cycling (Rost et al. 2008). In general, acidification (and thus an increased availability to CO_2) seems to stimulate benthic microalgal and *Zostera* growth, probably due to carbon limitation (Kroeker et al. 2010, Hicks et al. 2011). However, a lowered pH can potentially induce sublethal negative effects on herbivores and predators (Hurd et al. 2011). Thus, the effects of ocean acidification on herbivore-plant interactions are likely to depend more on the specific responses of different species involved, compared to global warming. If herbivores are resistant to ocean acidification their presence can weaken effects of ocean acidification (Anthony et al. 2011). If, however, herbivores are negatively impacted, ocean acidification can stimulate plant growth via the combined effect of increased bottom-up stimulation and reduced top-down control.

Nutrient enrichment. Coastal eutrophication is the consequence of nutrient enrichment (Cloern 2001). Increased concentrations of nutrients and the consequence of eutrophication have large effects on shallow-water coastal ecosystems, mainly trough the formation of floating macroalgal mats that suffocates ecosystems such as eelgrass meadows and rocky shores (Cloern 2001). In shallow areas and estuaries there will even be a shift in primary production from the bottom to the pelagic zone (McGlathery et al. 2007), which in turn will affect the metabolic balance, i.e. turning the sediment systems more heterotrophic (Borum & SandJensen 1996). Still, nutrient enrichment and consequently the presence of floating macroalgal mats have only small effects on microphytobenthos in unvegetated sediment communities. Probably because microphytobenthos can withstand periods of darkness and anoxia (Kamp et al. 2011). However, for other groups of organism such as fish, sediment-associated fauna, macroalgae and eelgrass, floating algal mats can have devastating effects (Isaksson & Pihl 1992, Everett 1994, Norkko & Bonsdorff 1996).

Toxicants. The effects of toxins found in the marine environment can be very dramatic or hardly noticeable (Heugens et al. 2001, Chapman 2004, Turley et al. 2005, Porsbring et al. 2010, Johansson et al. 2012). There are, however, some common observed effects in shallow-water sediment systems, particular on microphytobenthos and meiofauna. For example, toxicants can often induce food-web mediated indirect effects through the 'removal' of one trophic level in the system (Fleeger et al. 2003, Chapman 2004). In shallow-water sediment systems microphytobenthos seems to be fairly tolerant to toxins, at least when compared to heterotrophic bacteria and meiofauna (Carman & Todaro 1996, Sundbäck et al. 2007, Sundbäck et al. 2010). The toxins used were Polycyclic Aromatic Hydrocarbons (PAH), which usually originate from oil spill and combustion in boat engines, and Copper Pyrithione (CuPT), which is an antifouling toxin, used in boatpaints. Both toxins have been studied with ecotoxicological approaches before (such as EC50, multiple concentration levels etc.) and are able to generate both direct and indirect effects in shallow-water sediment systems (Carman & Todaro 1996, Maraldo & Dahllöf 2004, Alsterberg et al. 2007, Larson et al. 2007).

AIM OF THIS THESIS

To date, there are a large number of stressor-related studies in terrestrial and aquatic environments. A large number of these studies, in the marine environment, are on species level (Crain et al. 2008), but the number of studies under ecologically more relevant conditions (i.e. several trophic levels included simultaneously) has started to increase during the last decade (Christensen et al. 2006, Dueri et al. 2009, Fitch & Crowe 2011). Even though single-species experiments are highly valuable for the understanding of ecological systems, there is still a growing need of stressor experiment under ecological more relevant conditions. Further, stressors seldom operate individually; ecosystems are rather exposed to a multiple array of different stressors simultaneously. With the exception of **paper I**, dealing only with one stressor, increased seawater temperature, all papers presented in this thesis involves multiple stressors on several trophic levels simultaneously. The results from this thesis will try to clarify the combined effects of multiple stressors and also generate basic knowledge about the biology and ecology of shallow-water sediment systems. Specific aim for each paper were:

Paper I: How will the autotrophic and heterotrophic compartments in unvegetated sediment systems respond to increased seawater temperature of 4 °C? Will net heterotrophy be induced and thus consequently change the nutrient cycling?

Paper II: Will an increased seawater temperature of 4 degrees Celsius exacerbate the direct consequences of nutrient enrichment in shallow-water sediment systems? Also, will there be non-additive effects (synergistic or antagonistic) on the nitrogen cycling?

Paper III: Are effects of the toxicant cupper pyrithione (CuPT) strengthened, i.e. synergistic, on the structure and function of SWS when the seawater temperature is increased with 4 °C?

Paper IV: Are effects of the toxicant pyrene dependent on nutrient status? And will pyrene induce sublethal effects on the meiofauna that affects their grazing rate on microphytobenthos?

Paper V: Will the effects of warming and acidification on benthic microalgae be regulated by mesograzers, because respiration by the mesograzers is more temperature-dependent than plant photosynthesis? Secondly, will the indirect effects of warming and acidification on benthic primary producers be at least as strong as the direct effects?

METHODS

Both functional (primary and bacterial production, community respiration, meiofaunal grazing, denitrification, nitrogen mineralization and sediment-water fluxes of oxygen, nutrients and alkalinity) and structural (biomass and composition of microphytobenthos and meiofauna) variables were studied. Many of these variables describes metabolic rates of shallow-water sediment systems as well as uptake and removal of nitrogen and are thus considered key ecosystem variables (e.g. Sundbäck et al. 2007). Also, performing experiments with realistic changes in the environment and using undisturbed sediment communities combined with methods from both ecology and biogeochemistry will make it easier to assess possible interactions between trophic levels as well as detecting both direct and indirect effects of environmental alterations. Nitrogen was of special interest in this thesis since it is usually considered to be limiting in marine environments (e.g. Howarth 1988, Antia et al. 1991) and also occurs in many forms and oxidation states caused by a variety of different transformation processes (Carpenter & Capone 1983). Individual and combined stressor effects were studied on intact sediment cores, which were placed in an outdoor flow-through facility with focus on the community response. When stressors were studied in the laboratory, homogenized sediment was used and focus was rather on mechanistic processes such as grazing by meiofauna on microphytobenthos.

SEDIMENT COLLECTION

Sediment to be used for experiments was collected at three different locations and also with different sampling techniques on the west coast of Sweden. In **paper I**, **II** and **III** the sediment was collected using an Olausson box corer (30 cm x 30 cm) at a depth of ca 2 meters. Cylinders of black Acrylonitrile Butadiene Styrene plastics (height = 25 cm, i.d. = 25 cm) were used to sample one core from each box-core sample and later also used for sediment-water incubations. The use of intact, not sieved or homogenized sediments, add to the ecological relevance of our mesocosm experiment in the outdoor flow-through system since we have all indigenous organisms as well as trophic levels, which also can allow for indirect food-web mediated effects. In **paper IV** the top 3 mm of surface sediment was scraped off using a plastic plate and later sieved, homogenized and placed on top of cleaned beach sand in 350 ml crystallization dishes. Sand was used as a foundation for the surface sediment but also to avoid the build up of too much organic matter. In the last paper, sediment was collected from a *Zostera marina* meadow in the Gullmar Fjord and mixed with cleaned beach sand and placed in 30L semi-transparent buckets. On top of the sand–sediment mix, a layer of surface sediment (<0.5 cm), also collected from the *Zostera* meadow, were placed to initiate a diatom biofilm.

EXPERIMENTAL SYSTEMS

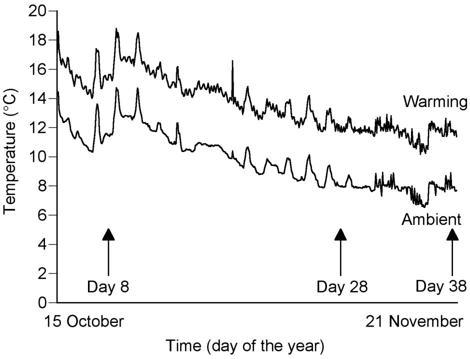

Figure 1. Temperature manipulation on incoming seawater (Figure from **paper II**).

In order to study the effects of changes in water temperature, alone and in combination with other stressors on intact sediment cores I constructed a flow-through system inside a green-house to allow close to natural light and temperature conditions, as well as protection from precipitation and birds (Fig. 1). The water was supplied directly from the Gullmar Fjord and was first entering two isolated and covered plastic containers of 1000L each. Water was bubbled to ensure no stratification and also filtered through cartridge filters before supplying water to each individual cylinder with a flow of 20 L h^{-1} (Fig 2). Experimental manipulation of temperature was performed using an immersion heater that was mounted in one of two larger containers. Two sensors inside the green house was placed in two cylinders or buckets (heated or ambient) and connected to a computerized united that controlled the immersion heater. A surveillance system of the temperature manipulation was installed and connected to my mobile phone. This system enabled me to monitor the heating of incoming water but also to make sure that all electrical components functioned correctly. Incoming water was monitored by measurements of temperature, pH, nutrients and oxygen to exclude possible side effects of rapid heating of the seawater. The temperature system was used in all papers, except for **paper IV**. However, besides heating incoming seawater, other manipulations were also performed.

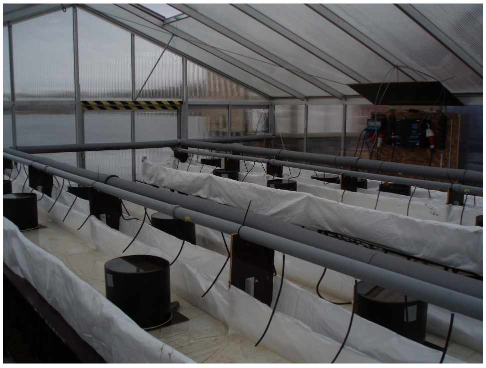

Figure 2. The flow-through system used in papers **I**, **II**, **III** and **V**.

In **paper II**, heated seawater was combined with nutrient addition. Nutrients were added by applying an in-situ enrichment technique using fertilizer pellets (Worm et al. 2000), with a total load of ~ 80 mmol N m^{-2} day^{-1} and ~ 20 mmol P m^{-2} day^{-1}. The pellets were placed in mesh bags at the water inlet of each cylinder, where inorganic nutrients where released to the surrounding water. In **paper III** heated seawater was instead combined with a toxicant. I used cupper pyrithione (CuPT) to simulate a toxicant exposure, with a low and natural occurring concentration (Petersen et al. 2004). For this I used a slurry technique to ensure that the toxicant came in rapid contact with the sediment, and was not dissipated in the water column (Sundbäck et al. 2007). The flow-through system was further developed in **paper V** by installing and connecting additional tanks (80 L) where the incoming seawater could be bubbled with enriched air or CO_2 using a Beckman Coulter pHi 460 data logger to manipulate seawater pH. **Paper VI** was rather a mechanistic grazing study, where I constructed a mini flow-through system using peristaltic pumps, placed inside a thermo-constant room. Pyrene (a PAH) was used as the toxicant and was mixed with the surface sediment slurry before placed in the glass vials. Nutrients were added as a spike to one of the two water-supply containers, corresponding to concentrations of ~ 34 mmol N m^{-2} day^{-1} and ~ 3.4 mmol P m^{-2} day^{-1}

RESPONSE VARIABLES

Structural variables. In all papers chlorophyll *a* (Chl *a*) concentration from the top 3 mm of sediment surface was used as a proxy for microphytobenthos biomass. Chl *a* was extracted with acetone over night and then measured spectrophotometrically (UV-2401PC, SHIMADZU) using the methods and equations by (Lorenzen 1967), which corrects for degradation products of chl *a*. In **paper IV**, living cells were also counted and measured (cell volume) in a Bürker counting chamber using epifluorescense microscopy at 500× magnification to complement Chl *a* data. Epifluorescense microscopy was used to look at the relative abundance and size group of species and cells were identified to the nearest taxon level possible. If floating algal mats were present (**paper I**, **III** and **V**) they were collected, wet and dry weights were measured as well as the carbon and nitrogen content using an Element Analyzer EA1108. *Zostera* leaves, roots and rhizomes were cleaned, dried, weighed and pooled to total *Zostera marina* biomass.

Meiofaunal biomass was assessed through staining sediment samples with formalin containing Bengal rose in room temperature for 24 hours. The sediment was then sieved through 500-, 200-, 100- and 40-um sieves and after each sieving step, all meiofauna were counted and allocated to major taxonomic groups and converted to ash-free dry weight according to (Widbom 1984). Meiofauna was further converted to carbon using a conversion factor of 0.43 (Båmstedt 1986). Macrofauna was collected by sieving the sediment of each cylinder/bucked through a 1-mm sieve and sorted, counted, dried and weighed.

Sediment variables included, loss on ignition (LOI), which is a measurement of organic content, as well as porosity to characterize the sediment as either sandy or silty.

Functional variables. Sediment-water fluxes of oxygen and total alkalinity in light and dark were used to measure net and gross primary production and community respiration (**paper I** – **III**). During all flux incubations the water flow was turned off and cylinders were sealed with transparent lids with an Teflon coated stirring bar attached to the inside of the lid enabling mixing while doing the incubations. Also, sediment-water fluxes were always measured before sediment variables were sampled. Concentration of oxygen was determined using the Winkler technique. For practical reasons I assumed that CR in light equaled CR in dark. Even though CR in light probably is higher than in the dark (Kühl et al. 1996), which probably means that the GPP values were somewhat underestimated, but this fact should not influence the interpretation of the results. Trophic state has been found to be a good proxy for the nutrient balance as well as net flux of nutrients between the sediment and overlaying water (Engelsen et al. 2008, Eyre et al. 2011). Thus, the trophic state was further determined by calculating net 24-h oxygen fluxes and if the net oxygen flux were negative, the system was considered to be net heterotrophic. Total alkalinity was measured using potentiometric titration and Gran evaluation, following the increase of H^+ after the second equivalence point. Sediment water fluxes of inorganic nutrients (ammonium, nitrate + nitrite, DIP and silicate) in light and dark were, as with oxygen, determined by start–stop incubations, assuming a linear increase/decrease of concentrations. Nutrients were analyzed using an automatic analyzer (Smart Chem; Westco Scientific Instruments) according standard colorimetric procedures (Strickland & Parsons 1972).

Primary production in **paper V** was measured by incubating sediment slurries in scintillation vials with ^{14}C-sodium bicarbonate under natural light and temperature conditions. Sediment bacterial production in **paper I** and **III** was measured with ^{3}H-leucin according to (Ask et al. 2009). To enable a good dpm reading (disintegrations per minute – a measure of radioactivity intensity) all scintillation vials were ultrasonicated (Branson 2510) for 10 min and then counted in a liquid scintillation analyzer (TRI-CARB 2900TR, software: Quanta Smart V 1.1).

In **paper II** I was also particular interested in the cycling of nitrogen since this experiment investigated the combined effects of nutrient enrichment and warming, and the effects on nitrogen cycling during nutrient enrichment is not completely understood (McGlathery et al. 2007). Thus I measured potential denitrification and nitrogen mineralization as a complement to fluxes of inorganic nutrients. Potential denitrification was measured with $^{15}NO_3$ on preincubated sediments (with helium removing the oxygen) in darkness and calculated according to (Thamdrup & Dalsgaard 2002). Nitrogen mineralization (ammonification) was measured in two ways (1) through closed sediment incubation i.e. changes in pore-water ammonium concentration and (2) calculated from measured sediment-water nitrogen fluxes and denitrification.

Meiofaunal grazing. In order to investigate potential sublethal effects of PAH and nutrient enrichment, meiofaunal species were allowed to graze on pre-labeled ^{14}C microphytobenthos pellets (**paper IV**). Microphytobenthos was cultivated with f/2 medium in cultivations flasks (Nunc®). The grazing pressure was assessed through DPM data for each major meiofaunal taxon when corrected for biomass (Nilsson et al. 1993). With this approach only meiofauna was exposed to experimental treatments and not microphytobenthos, which was done in a previous study (Alsterberg et al. 2007). All samples were counted in a liquid scintillation analyzer (TRI-CARB 2900TR, software: Quanta Smart V 1.1).

STATISTICS USED

In all papers I used an orthogonal design and differences between treatments were analyzed using model I (fixed factors) and model 3 (mixed factors) analysis of variance (ANOVA), permutational multivariate analysis of variance (PERMANOVA) and structural equation modeling (SEM). The combined use of ANOVA and PERMANOVA allowed me to test treatment effects on individual variables as well as the integrated community response, which showed a more general response of the community to experimental treatments. Further, by including SEM analysis, net effects analyzed with ANOVA could be allocated into direct and indirect effects. Analyzing indirect effects is of great importance considering the fact that they are just as important in structuring aquatic communities as direct effects (Wootton 2002, Harley 2011, Wernberg et al. 2012). In **paper I** and **IV** time were treated as fixed factors, thus I had a specific interest in the chosen days. In **paper II** time was treated as a random factor because I was more interested in a general temporal variation. I did not use repeated measurements, so after each sampling occasion the sampled mesocosms was discarded. All data were checked for normality and homogeneity of variance using box, residual and Q-Q plots. ANOVA were run in the R-environment (Development Core Team 2010).

Permutational multivariate analysis of variance. To analyze the integrated community responses to experimental treatments I used PERMANOVA in **papers I – VI**, which is a multivariate approach of ANOVA (Anderson 2001, McArdle & Anderson 2001). PERMANOVA partitions multivariate variation according to individual factors in balanced ANOVA designs. Thus, PERMANOVA tests simultaneous responses of one or more variables to one or more factors in an ANOVA design on the basis of a distance measure. The main difference, compared to ANOVA, is that PERMANOVA calculates *P*-values using permutations (Anderson 2001). Permutations are a mathematical, random, rearrangement of the observations to each treatment groups under each variable, which results in a number of recalculations of the F statistics. Hence, repeated permutations create distributions of F-values that can be compared with the F-values from the original data set, instead of using tabulated values. In all analyzes I used the Euclidean distances and raw data was never transformed but standardized through normalization. A total of 9999 unrestricted permutations of raw data were used in all tests. To detect differences in within-group dispersion between treatment groups, data was also analyzed with permdist, based on the sample distance to the group centroid. In **paper III** and **IV** principal coordinate analysis (PCO) was calculated and the replicate locations were plotted in a two-dimensional plot explaining the main part of the variation. Analyzes were run using the programs PERMANOVA v.1.6, PERMDIST and PCO3 (Anderson 2001).

Structural equation modeling. In **paper V** the observed net effects of warming and acidification on microphytobenthos was allocated into direct and indirect effects using structural equation modeling (SEM). The major advantage with SEM is that it incorporates casual processes, making it possible to distinguish between direct and indirect effects, as well as graphical and mathematical representations of the model(s) (Grace et al. 2012). Structural equation models, in a simplified explanation, consist of several structural equations (representing a univariate equation showing that x has a causal effect on y) that are modeled with multivariate relationships. Assessment of model fit and parameter estimation is performed through comparison between actual covariance matrices and estimated covariance matrices of the best model fit using maximum likelihood and Chi-squares as goodness of fit. A low *p*-value means that the model is significantly different from the observed data, hence *p*-values above 0.3 is usually accepted as probable (e.g. Arhonditsis et al. 2007). I further used a multigroup analysis, which is an extension to the use of structural equation and greatly enhances the applicability for experimental data (Grace 2003). A multigroup analysis permits determination of the degree to which two subsets of the data fit a common model and can be used to further investigate what parameters are the same or different across groups as well as comparisons between group means and intercepts (Grace & Jutila 1999). Hence, in **paper V** I used the multigroup SEM analysis to identify direct and indirect effects of warming and acidification, but also to test if the relationships between microphytobenthos, sediment fauna, filamentous macroalgae and *Zostera* differed between seagrass meadows having mesograzers present or absent. All SE models were run in AMOS (version 20).

THE RESPONSE OF SHALLOW-WATER SEDIMENT SYSTEMS TO ENVIRONMENTAL ALTERATIONS

The response of shallow-water sediment systems depend of the type of stressor being studied and in most cases there were very few interactive effects between stressors. However, there seemed to be a general pattern of response of processes and organisms being studied: heterotrophs, such as bacteria, meio- and macrofauna were more sensitive to all types of stressors, while autotrophs, appeared less sensitive. The apparent lack of individual and combined effects of stressors, particularly on microphytobenthos could however have been due to several direct and indirect effects counteracting each other. Regardless, the function of shallow-water sediment systems as a nutrient filter between the sediment and overlaying water, as well as sustaining net autotrophy was maintained due to the presence and activity of microphytobenthos.

CHANGES IN TROPHIC STATE AND NUTRIENT CYCLING WERE BUFFERED BY THE ACTIVITY OF MICROPHYTOBENTHOS

Changes in seawater temperature affected heterotrophic activity by increasing the respiration, bacterial production and faunal biomass. Heterotrophic activity is predicted to increase during warming (Brown et al. 2004) and this also means that the trophic state of ecosystems can be pushed from net autotrophic to net heterotrophic (Brown et al. 2004, Hancke & Glud 2004, Yvon-Durocher et al. 2010). Further, even small changes in the trophic state can affect the overall dynamics of nutrients in shallow-water sediment systems (Engelsen et al. 2008). Thus, warming can change the functions of SWS as a nutrient filter. However, the trophic state of SWS also varies with season in cold-temperate areas, and in general turns autotrophic during spring and heterotrophic during autumn (Sundbäck et al. 2000). Two studies, one in spring and one in autumn, were therefore performed in order to detect if warming affected the trophic state and consequently the nutrient cycling.

During spring, warming increased community respiration and likewise heterotrophic mineralization in the sediment. Dark and light fluxes of alkalinity indicated that warming stimulated anoxygenic mineralization, which is fueled with other electron acceptors than oxygen. Warming consequently challenged the net autotrophic state by decreasing the oxygenated zone in the surface sediment. But the sediment never turned net heterotrophic. Rather, warming strengthened the autotrophy even more, probably due to a high activity and biomass of microphytobenthos. Since the activity of microphytobenthos increased with warming, the filter function was likewise strengthened, particularly through an increased demand of inorganic nutrients by diatoms and bacteria, thus cutting of the supply of pore-water nutrients to the water column (Sundbäck & McGlathery 2005). During autumn, the combined and individual effects of warming and nutrient enrichment also stimulated community respiration and in this case actually induced net heterotrophy – but not during the entire experimental period. The trophic state was therefore, with a few exceptions, maintained net autotrophic during autumn. Probably an increased temperature initially increased the mineralization of the organic matter (strengthening heterotrophy), but later the mineralization was instead reduced through a shortage of labile organic matter (reducing heterotrophy). Since microphytobenthos was present and active, the trophic state therefore, once again was turning net autotrophic.

The presence of a well-developed diatom mat sustained net autotrophy in the shallow-water sediment systems. Since microbial mats usually are dominated by species with short generation times, adaptation to changes can be quick and hence hinder or buffer changes in environmental conditions (Admiraal et al. 1999, Witt et al. 2011). Previous experiments (Alsterberg et al. 2007, Larson et al. 2007, Montserrat et al. 2008) have already shown that microphytobenthos can quickly recover after perturbations. Thus, considering the importance of the autotrophic system in terms of its buffering capacity (de Wit et al. 2001), the presence of microphytobenthos can be suggested to contribute to the overall buffering of shallow-water sediment systems to environmental alterations.

WARMING AND SIMULTANEOUS NUTRIENT ENRICHMENT INCREASED THE INTERNAL LOADING OF NUTRIENTS TO THE OVERLAYING WATER – BUT NOT ALWAYS…

Warming combined with nutrient enrichment synergistically increased nitrogen mineralization and denitrification, which consequently resulted in an increased efflux of ammonium and denitrification. However, nitrogen mineralization and nitrification probably became temporally separated due to a faster response of nitrogen mineralization during warming, thus channeling ammonium to the water column rather than fueling nitrification. The stimulated denitrification, fueled by nitrates in the water column, contributed to counteract the nutrient enrichment by transforming nitrates to nitrogenous gas. However, denitrification in shallow sediments is seldom high enough to counteract nutrient enrichment (McGlathery et al. 2007), and this was also the case in this experiment. Thus, the combined effects of warming and nutrient enrichment have the potential to strengthen coastal eutrophication through the production of redundant amounts of ammonium in the sediment. However, the production of ammonium (through mineralization) and the actual loss of ammonium (sediment-water fluxes, adsorption to particles and bacterial uptake) were compared with the calculated nitrogen demand of microphytobenthos. These comparisons showed that the efflux of ammonium would have been much higher without an uptake by microphytobenthos. At larger water depths in the photic zone, however, microphytobenthos become less active due to lower light availability (Sundbäck et al. 2004), and hence the combined effects of warming and nutrient enrichment can be expected be stronger along with increasing heterotrophy of the sediment. In the very shallow-water, however, microphytobenthos will probably continue to sustain the filter-function.

WARMING MAY REMOVE THE TOXICANT EFFECT

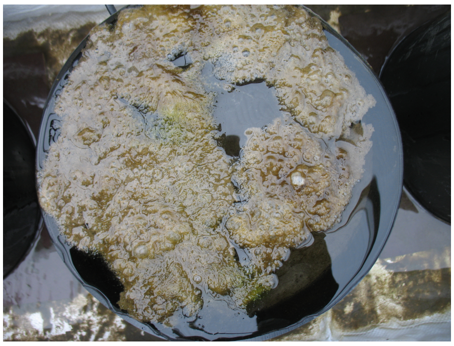

Figure 3. Floating algal mat (**paper III**).

An accelerated effect of warming was the flaking of sediment, which actually resulted in a formation of floating algal mats (Fig. 3). Flaking of diatom biofilms in shallow sediment areas is a fairly common phenomenon and usually occurs due to air bubbles trapped inside the microalgal mat (Admiraal 1984, Sutherland et al. 1998). The floating algal mats consisted not only of benthic, but also of pelagic and epiphytic microalgal species, which probably came from outside the flow-through system. Interestingly, however, was that the floating algal mats were negatively affected by a toxicant (CuPT), whereas microphytobenthos was not. Also, when warming was combined with the toxicant, antagonistic effects were observed. Hence, warming removed the toxicant effect. Tolerant species probably dominated in the sediment, whereas less tolerant species dominated in the floating algal mat, which suggests that warming accelerated pollution-induced community tolerance of the microphytobenthos by selecting for CuPT tolerant species (Admiraal et al. 1999, Blanck 2002). Further, the floating algal mat can be regarded as detached periphyton living in a planktonic environment, thus the habitat of seemingly similar communities can decide the mode of response to environmental alterations. The antagonism induced by warming can only be speculated about, but most likely there was an increased turnover due to warming, leading to a dilution effect and a lower bioavailability of the toxicant to individual cells (Heugens et al. 2001). Actually, since most temperature effects on microphytobenthos were rather modest, flaking might have been the most obvious direct temperature effect on microphytobenthos. Flaking of surface sediments also show that the effects of warming can induce a translocation of primary production from benthos to the pelagic zone, which actually resembles the same shift of primary producers observed in eutrophied shallow bays (Sundbäck & Snoeijs 1991, Cloern 2001, McGlathery et al. 2007).

TOXICANTS CAN INDUCE FOOD-WEB MEDIATED EFFECTS ON MICROPHYTOBENTHOS VIA MEIOFAUNA

The biomass of microphytobenthos increased during the exposure of a toxicant (pyrene). However, the increased biomass of microphytobenthos was interpreted as an indirect effect of the toxicant, caused by a sublethal effect on the meiofaunal grazing pressure. Difference in sensitivity to stressors both within and between trophic levels can result in food web mediated effects (Petchey et al. 1999, Wootton 2002, Fleeger et al. 2003). In shallow sediments, both meiofauna and macrofauna are known to graze (Blanchard 1991, Nilsson et al. 1993), and sometimes even exert a top-down control on microphytobenthos (Coull 1999). Thus, it was shown that toxicants can induce food-web mediated effects through sublethal effects on meiofaunal grazing pressure and also that increased concentrations of inorganic nutrients can exacerbate the toxicant effects. Copepods are usually the most sensitive meiofaunal taxa to toxicants (Carman et al. 2000), but in the experiment with pyrene and nutrient enrichment it was actually the temporary meiofauna, i.e. juvenile polychaetes that experienced a reduced grazing rate when exposed to pyrene. These results emphasize temporary meiofauna as important grazers on microphytobenthos, and also that microphytobenthos is an important food resource for higher trophic levels in shallow-water sediment systems. An increased grazing pressure by temporary meiofauna could therefore also explain the absence of warming effects on microphytobenthos biomass during spring, since the biomass of polychaete worms significantly increased by warming. Thus the direct positive effects by warming on microphytobenthos would have been masked by the increased grazing (indirect) by temporarily meiofauna.

HETEROTROPHS APPEAR MORE SENSITIVE THAN AUTOTROPHS

In general, it seems that autotrophs, primarily microphytobenthos, are less affected by environmental alternations when compared to heterotrophs, such as bacteria and meiofauna.

The effects of environmental alterations on microphytobenthos biomass and composition were in general quite week. The environmental alterations included in thesis all had a positive effect on microphytobenthos biomass, including positive effects of toxicants, which is quite in contrast to what would have been expected (Heugens et al. 2001). However, many of these effects were probably indirect effects (see discussions in previous paragraphs) rather than direct effects, with the exception of nutrient enrichment, which probably directly stimulated the growth of microphytobenthos. Low net effects of environmental alterations are quite in contrast to observations from phytoplankton communities (Sommer & Lengfellner 2008, Lewandowska & Sommer 2010, Sommer & Lewandowska 2011). However, similar responses in the benthic and pelagic zones are perhaps not to be expected because of large physical, as well as biological differences between the two habitats. Macroalgae and *Zostera* benefited from acidification, probably due to CO_2 limitation (Kroeker et al. 2010). For *Zostera* this effect disappeared when increased CO_2 was combined with warming, whereas acidification and warming synergistically increased the biomass of macroalgae. Since temperature dependence of photosynthesis is weaker than that of respiration (e.g. Brown et al. 2004, Yvon-Durocher et al.

2010), especially for aquatic ecosystems (Yvon-Durocher et al. 2012), it would have been natural to find more pure temperature effects for heterotrophic variables. Also, previous experiments exposing intact sediment to single and combined stressors have suggested that the heterotrophic compartments are more sensitive than the autotrophic compartments (Larson et al. 2007, Sundbäck et al. 2007).

Among heterotrophic compartments, warming stimulated bacterial production, meiofaunal biomass and community respiration. Sediment bacteria, in general, have a higher Q_{10} than benthic diatoms (Hancke and Glud 2004), and an increased bacterial production can be a direct effect of warming (Sander & Kalff 1993). However, the stimulation of bacteria could also partly be a bottom-up effect, since bacteria are known to use dissolved organic matter (DOM) produced by microphytobenthos (Goto et al. 1999, Evrard et al. 2008). Considering that temperature, together with organic matter content, is one of the main controlling factors of bacterial production (Sander & Kalff 1993), it was surprising to find that warming also decreased bacterial production. This result was the complete opposite of what was expected, but it could have been caused by a temperature-induced indirect effect mediated by food-web interactions (Kordas et al. 2011). Thus, a strengthened grazing pressure could have counteracted the potential stimulatory effect of warming on bacterial production (Nydahl et al. 2013). Apart from directly measuring bacterial production, it was apparent from the increased sediment-water nitrogen fluxes in darkness (primarily NO_3^-), as well as from denitrification and nitrogen mineralization that the microbial heterotrophic activity was affected by warming as well as warming combined with nutrient enrichment.

An increased meiofaunal biomass due to warming was mainly caused by the growth of temporary meiofauna (in our experiment mainly polychaete larvae). Typical meiofauna (e.g., harpacticoid copepods and nematodes) were, however, not affected by temperature, which also accords with observations by Baulch et al. (2005). On the basis of these results, short-term periods of warming, even when water temperatures are naturally high, are not expected to lead to any changes in the composition of the meiofauna community. This could be due to the fact that meiofauna within the littoral zone already are adapted to natural diurnal and annual fluctuations in water temperature. On the other hand, in situ, higher seawater temperatures can directly increase the recruitment of temporary meiofauna. But the increase in biomass may also be a consequence of increased food availability, larval settling or biomass production (Costil & Daguzan 1995, Feuchtmayr et al. 2007). Warming also increased the biomass of sediment-associated fauna such as *Corophium* sp. and *Hydrobia* sp., whereas acidification did not seem to have any effect on either sediment-associated fauna or mesograzers such as *Gammarus locusta* and *Littorina littorea*. As previously mentioned, an increased activity and biomass of fauna is expected during warming (O'Connor 2009, Kordas et al. 2011). However, the response of meiofauna to toxicants seems more difficult to predict. Exposing meiofauna to toxicants induced lethal, sublethal and non-lethal effects. Sublethal effects were observed as reduced grazing pressure on microphytobenthos (*see previous* *paragraph*). However, sublethal effects can eventually be lethal, thus the time of toxicant exposure does matter for the detection of lethal or sublethal effects (Fleeger et al. 2003). Further, CuPT did not affect meiofauna, not even when CuPT was combined with warming. However, previous studies have shown that CuPT can decrease meiofaunal biomass (Alsterberg et al. 2007). On the other hand, pyrene affected both the biomass and grazing pressure of meiofauna. Also, the mode of response by pyrene was dependent on the nutrient status of the sediment, mainly by strengthening the negative effects on meiofaunal grazing. This could be explained by a higher metabolic turnover rate with more efficient transport of the toxicant (Koelmans et al. 2001, Hjorth et al. 2008). One additional plausible explanation is that toxicant effects are easier to detect in a "richer system" because of a higher response potential (Larson et al. 2007). To conclude, the sensitivity of meiofauna to toxicants has been found to vary with taxonomic group and species, as well as with the toxicant and its concentration. Also, the generation time of meiofauna lies within days or months (Kennedy & Jacoby 1999), making it possible for the meiofauna community to respond quickly to environmental differences such as toxicants and to adapt and recover.

DIRECT AND INDIRECT EFFECTS OF CLIMATE CHANGE CAN COUNTERACT EACH OTHER

The direct positive effects of warming combined with ocean acidification on microphytobenthos in a seagrass ecosystem were counteracted by food-web mediated indirect effects. Actually, the strength of indirect effects even resulted in a net zero effect of warming and acidification on microphytobenthos. Warming is predicted to strengthen herbivore–plant interactions and consequently induce food-web mediated indirect effects (Brown et al. 2004, O'Connor 2009). Thus, in this study warming increased faunal activity, such as grazing, as well as the biomass of both consumers and algae, and hence strengthening the top-down control on microphytobenthos. No negative effects of ocean acidification on grazers were observed and macroalgae were stimulated by the availability of extra carbon with the exception of microphytobenthos, the only autotrophic component unaffected by acidification alone. But when acidification was combined with warming, microphytobenthos biomass was stimulated, and in general, acidification can have major effects on autotrophs when combined with warming (Connell & Russell 2010). This is due to temperature-induced changes in autotrophic metabolism, which increases the need of CO_2 for the build up of biomass (Walther 2004, Connell & Russell 2010). In this study the mesograzers (*Gammarus*, *Littorina*, *Rissoa*) grazed on filamentous macroalgae (affecting light availability and competition of nutrients) as well as on sediment-associated fauna (*Corophium*, *Hydrobia*, *Nereis*; grazing on microphytobenthos).

Thus, depending on the complexity of the system, i.e. the number of trophic levels, the effects of warming and ocean acidification were either abundant or completely absent. Using structural equation modeling (SEM), the strength of direct and indirect effects by warming and acidification was evaluated between groups with or without mesograzer. Results from the analysis showed that several stronger direct effects of warming and acidification actually were counteracted by indirect effects mediated by trophic interactions, but only in the group with no consumers present. Hence, the consumers acted as buffers towards environmental alterations, by removing the direct and indirect effects of warming and acidification on microphytobenthos. This also highlights the fact that indirect effects of climate change may often exceed direct effects in terms of their relative importance (Wootton 2002, Harley 2011, Wernberg et al. 2012). Thus, for understanding effects of climate change from a more multi-trophic and multi-factor perspective, we need to manipulate factors separately and jointly, and apply statistical analyses that can discriminate between direct and indirect effects.

CONCLUSIONS

Results presented in this thesis highlight the complexity behind effects of environmental alterations and emphasize the importance of understanding the consequences of climate change combined with abiotic stressors in a multi-trophic and multiple-stressor perspective. Global warming may, like eutrophication, contribute to a shift in the type of primary producers (i.e. from benthic to pelagic) with the formation of floating microalgal mats with similar ecological implications as green-algal mats. However, changes in trophic status are less likely, even though small changes in trophic status can have strong effects on nutrient cycling. The effects of toxicants on aquatic organisms under a warmer climate do not need to be amplified. Thus, different photoautotrophic communities can respond very differently to stressors, even within the same ecosystem, and the habitat of seemingly similar communities can decide the mode of response. Still, stressor effects will depend on how many trophic levels that is present in the system and the sensitivity or direction of effects will also vary with trophic- and species level. Hence, unexpected indirect effects can be expected.

Throughout this thesis I have also, several times, stressed that microphytobenthos seems to be particularly resistant to single and multiple environmental alterations. Although microphytobenthos are no group of super organisms, I do believe that they feature a number of characteristics that protects them for environmental alterations and hence contributes to the overall buffering of shallow-water sediment systems. First of all, microphytobenthos exist with a high compositional diversity; hence a moderate stressor cannot cause any substantial change in the composition. Secondly, microphytobenthos usually consist of well-developed and fully dense communities capsuled in a coating of extracellular polysaccharides substances (EPS). Living at high densities often lowers the risk of mortality in physical harsh environments because the presence of neighbors directly or indirectly buffers environmental extremes (Hay 1981, Bertness & Leonard 1997). Also, the EPS protects the microphytobenthos community by stabilizing the sediment environment (Underwood & Paterson 2003, de Brouwer et al. 2005). Thirdly, microphytobenthos can survive in both dark and anoxic conditions by respiring nitrate and quickly starting to produce oxygen again when environmental conditions improve (Kamp et al. 2011, Larson & Sundbäck 2012). Finally, many species among microphytobenthos have the ability to move up and down within the sediment, especially the pennate diatoms, thus if environmental conditions change these species will simply escape and later return when conditions have improved.

Therefore, compared to other ecosystems such as the pelagic, shallow-water sediment systems are probably more protected and less sensitive to environmental alterations, mainly because of the presence and activity of microphytobenthos.

FUTURE OUTLOOK

The results presented in this thesis, and other papers dealing with stressor experiments, are two-fold; firstly, they show the actual response of shallow-water sediment systems when exposed to environmental alterations; secondly, they give further basic knowledge about the biology and ecology of these ecosystems. Thus, performing experiments with realistic changes in the environment and using undisturbed sediment communities will generate a broader perspective in terms of ecosystem function and the effects of interactive stressors. Such studies will also emphasize the vitality and resilience of valued ecosystems, which in the end will be highly valuable for scientific and management considerations.

In order to extend the knowledge of environmental alterations I believe that controlled manipulative experiments need to be complemented with field data. Although this is not an easy task, it could for example be possible to work with natural gradients of disturbance such as salinity, nutrients, trophic state and temperature. Also, the use of several levels within one disturbance could help in predicting the actual consequences of environmental alterations and show if the effects can be extrapolated over a wider scale of variation. As mentioned in **paper V**, taking both the direct and indirect effects into consideration with various statistical and experimental methods will also be very valuable and most likely change our current knowledge about the consequences of environmental alterations in aquatic and terrestrial ecosystems.

ACKNOWLEDGMENTS – TACK

Jag skulle först och främst vilja tacka min handledare Kristina Sundbäck. Kristina – din dörr har alltid varit öppen för mig och oavsett hur stressad du har varit så har du ALLTID tagit dig tid för att hjälpa mig, jag är dig evigt tacksam för detta. När vi har genomfört experiment så har du alltid varit med och grävt i sedimenten och också erbjudit dig att städa båten – fast bara för skojs skull! På konferenser har du försökt göra mig till en mer social människa genom att släpa mig runt bland okända människor för att käckt spontanprata. En övning som jag fann extremt svår och obehaglig men nu i efterhand mycket utvecklande…
Trots dina många lektioner i engelsk grammatik så finns det inte mycket hopp för att *is* och *are* kommer hamna på rätt ställe i en mening författat av mig. Det slog mig också att vi aldrig har tjafsat eller inte kommit överens. Ja, kanske förutom en gång, när jag hade varit uppe hela natten och tagit prover för att försöka få experimentsystemet att fungera och du vid frukostbordet, kl 06:30, börjar ihärdigt tjata om någon (för stunden) helt oviktig detalj. Jag ber om ursäkt om jag snäste till. Slutligen vill jag tala om att du är den bästa handledare en doktorand kan önska sig! Jag hoppas att vi kan fortsätta utforska sediment tillsammans…

Jag vill också ägna extra mycket tack till Stefan Hulth, Johan Eklöf och Per Nilsson. Stefan, du har introducerat mig till sedimentens biogeokemiska värld, och genom ett gott samarbete och många, många manuskriptmöten så har vi, tillsammans med Kristina, två väldigt bra artiklar tillsammans. Johan, jag halkade in på ett bananskal i ditt projekt (eftersom du ville åt mitt värmesystem, jag vet). Det blev ett väldigt lyckat och roligt samarbete och något som jag aldrig kommer att ångra! Per, som min biträdande handledare har du alltid hjälpt mig, med allt ifrån att läsa manuskript till att svara på statistikfrågor.

Sedan så vill jag ju så klart tacka alla gamla och nya kollegor på BIOENV. Framförallt Malin och Karro som gjorde alla konferensresor och undervisning mycket roligare (särskilt i Portland med flera besök på en viss restaurang). Anna N och Sonja som var mina första rumskamrater, Josefin och Sussie som blev mina nya rumskamrater. Ni delade min passion för mat och producerande av data (eller?). Erik N för alla långa och kalla fältdagar på Kristineberg. Anders som lärarkollega, Fabian som min hotellvärd och som alltid menar att sediment är tråkigt (jag vet att du älskar sediment egentligen Fabian). Lars G som inspirationskälla, Erik S, också som inspriationskälla samt för att du startade running club (något jag verkligen saknar här nere i tropikerna). Jon för ett gott samarbete i Zostera projektet, Monica för alla roliga diskussioner i lunchrummet (jag kommer aldrig använda dubbdäck!), Per Å för alla cykeldiskussioner, Jenny och Johanna för alla uppmuntrande ord när man är på riktigt dåligt humör (jag försöker att inte vara ironisk, eller?), Mikael för trevligt sällskap under tre långa veckor i Danmark och så alla andra som jag träffat under mina år som doktorand, Fredrik, Pia, Henrik, Lars, Maria, Lisa, Angela, Stefan, Anna, Johan…

Tack mamma och pappa för att ni alltid har trott på mig och stöttat mig under mina studier. Tack också Isabel, David, Ago, Johannes, Cia & Anders, Martha och alla andra i familjerna Alsterberg/Ljungberg/Magyar. Csaba du förtjänar ett speciellt tack eftersom du konstruerade temperatursystemet som har använts i flera lyckade experiment!

Till sist, Eszter!!!! Du är min eviga kärlek! Utan dig, ditt stöd och förståelse så hade jag aldrig, aldrig, lyckats med denna avhandling. Tillsammans har vi vårat lilla gos Emi. Ni är mitt allt och när jag är tillsammans med er så är allting annat oväsentligt…

REFERENCES

Adams SM (2005) Assessing cause and effect of multiple stressors on marine systems. Marine Pollution Bulletin 51:649-657

Admiraal W (1984) The ecology of estuarine sediment-inhabiting diatoms, p. 269–314. In Round FE and Chapman DJ [eds.], Progress in phycological research. Biopress.

Admiraal W, Blanck H, Buckert-De Jong M, Guasch H, Ivorra N, Lehmann V, Nyström BAH, Paulsson M, Sabater S (1999) Short-term toxicity of zinc to microbenthic algae and bacteria in a metal polluted stream. Water Research 33:1989-1996

Alsterberg C, Hulth S, Sundbäck K (2011) Response of a shallow-water sediment system to warming. Limnology and Oceanography 56:2147-2160

Alsterberg C, Sundbäck K, Larson F (2007) Direct and indirect effects of an anti-fouling biocide on benthic microalgae and meiofauna. Journal of Experimental Marine Biololgy and Ecology 351:56-72

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Australian Journal of Ecology 26:32-46

Anthony KRN, Maynard JA, Diaz-Pulido G, Mumby PJ, Marshall PA, Cao L, Hoegh-Guldberg O (2011) Ocean acidification and warming will lower coral reef resilience. Global Change Biology 17:1798-1808

Antia NJ, Harrison PJ, Oliveira L (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1-89

Arhonditsis GB, Paerl HW, Valdes-Weaver LM, Stow CA, Steinberg LJ, Reckhow KH (2007) Application of Bayesian structural equation modeling for examining phytoplankton dynamics in the Neuse River Estuary (North Carolina, USA). Estuarine Coastal and Shelf Science 72:63-80

Ask J, Karlsson J, Persson L, Ask P, Byström P, Jansson M (2009) Terrestrial organic matter and light penetration: Effects on bacterial and primary production in lakes. Limnology and Oceanography 54:2034-2040

Baden S, Boström C, Tobiasson S, Arponen H, Moksnes PO (2010) Relative importance of trophic interactions and nutrient enrichment in seagrass ecosystems: A broad-scale field experiment in the Baltic-Skagerrak area. Limnology and Oceanography 55:1435-1448

Baden S, Emanuelsson A, Pihl L, Svensson C-J, Åberg P (2012) Shift in seagrass food web structure over decades is linked to overfishing. Marine Ecology Progress Series 451:61-73

Baulch HM, Schindler DW, Turner MA, Findlay DL, Paterson MJ, Vinebrooke RD (2005) Effects of warming on benthic communities in a boreal lake: Implications of climate change. Limnology and Oceanography 50:1377-1392

Bertness MD, Leonard GH (1997) The role of positive interactions in communities: Lessons from intertidal habitats. Ecology 78:1976-1989

Blanchard GF (1991) Measurment of meiofauna grazing rates on microphytobenthos - is primary production a limiting factor? Journal of Experimental Marine Biololgy and Ecology 147:37-46

Blanck H (2002) A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. Human and Ecological Risk Assessment 8:1003-1034

Borum J, SandJensen K (1996) Is total primary production in shallow coastal marine waters stimulated by nitrogen loading? Oikos 76:406-410

Breitburg DL, Sanders JG, Gilmour CC, Hatfield CA, Osman RW, Riedel GF, Seitzinger SB, Sellner KG (1999) Variability in responses to nutrients and trace elements, and transmission of stressor effects through an estuarine food web. Limnology and Oceanography 44:837-863

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771-1789

Burkepile DE, Hay ME (2006) Herbivore vs. nutrient control of marine primary producers: Context-dependent effects. Ecology 87:3128-3139

Båmstedt U (1986) Chemical composition and energy content, p. 1–58. In Corner EDS, O'Hara SCM [eds.], The biological chemistry of marine copepodes. Clarendon Press.

Carman KR, Bianchi TS, Kloep F (2000) Influence of grazing and nitrogen on benthic algal blooms in diesel fuel-contaminated saltmarsh sediments. Environmental Science & Technology 34:107-111

Carman KR, Todaro MA (1996) Influence of polycyclic aromatic hydrocarbons on the meiobenthic-copepod community of a Louisiana salt marsh. Journal of Experimental Marine Biololgy and Ecology 198:37-54

Carpenter EJ, Capone DG [eds.], (1983), p. 1–900. Nitrogen in the marine environment. Academic press.

Chapman PM (2004) Indirect effects of contaminants. Marine Pollution Bulletin 48:411-412

Christensen MR, Graham MD, Vinebrooke RD, Findlay DL, Paterson MJ, Turner MA (2006) Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Global Change Biology 12:2316-2322

Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series 210:223-253

Colijn F, Dejonge VN (1984) Primary production of microphytobenthos in the Ems-Dollar estuary. Marine Ecology Progress Series 14:185-196

Connell SD, Russell BD (2010) The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. Proceedings of the Royal Society B-Biological Sciences 277:1409-1415

Cook PLM, Van Oevelen D, Soetaert K, Middelburg JJ (2009) Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. IV. Inverse model analysis and synthesis. Marine Ecology Progress Series 394:35-48

Costil K, Daguzan J (1995) Effect of temperature on reproduction in *Planorbarius corneus* (L) and *Planorbis planorbis* (L) throughout the life span. Malacologia 36:79-89

Coull BC (1999) Role of meiofauna in estuarine soft-bottom habitats. Australian Journal of Ecology 24:327-343

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters 11:1304-1315

Dalsgaard T (2003) Benthic primary production and nutrient cycling in sediments with benthic microalgae and transient accumulation of macroalgae. Limnology and Oceanography 48:2138-2150

de Brouwer JFC, Wolfstein K, Ruddy GK, Jones TER, Stal LJ (2005) Biogenic stabilization of intertidal sediments: The importance of extracellular polymeric substances produced by benthic diatoms. Microbial Ecology 49:501-512

de Jonge VN, de Boer WF, de Jong DJ, Brauer VS (2012) Long-term mean annual microphytobenthos chlorophyll a variation correlates with air temperature. Marine Ecology Progress Series 468:43-56

de Wit R, Stal LJ, Lomstein BA, Herbert RA, van Gemerden H, Viaroli P, Cecherelli VU, Rodriguez-Valera F, Bartoli M, Giordani G, Azzoni R, Schaub B, Welsh DT, Donnelly A, Cifuentes A, Anton J, Finster K, Nielsen LB, Pedersen AGU, Neubauer AT, Colangelo MA, Heijs SK (2001) ROBUST: The ROle of BUffering capacities in STabilising coastal lagoon ecosystems. Continental Shelf Research 21:2021-2041

Defeo O, McLachlan A, Schoeman DS, Schlacher TA, Dugan J, Jones A, Lastra M, Scapini F (2009) Threats to sandy beach ecosystems: A review. Estuarine Coastal and Shelf Science 81:1-12

Development Core Team (2010) R: A language and environment for statistical computing. Vienna Austria

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009), Ocean Acidification: The Other CO_2 Problem, p. 169-192. In: Carlson CA, Giovannoni SJ [eds.], Annual Review of Marine Science

Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, Polovina J, Rabalais NN, Sydeman WJ, Talley LD (2012), p. 11–37. Climate Change Impacts on Marine Ecosystems. In: Carlson CA, Giovannoni SJ [eds.], Annual Review of Marine Science

Dueri S, Dahllöf I, Hjorth M, Marinov D, Zaldivar JM (2009) Modeling the combined effect of nutrients and pyrene on the plankton population: Validation using mesocosm experiment data and scenario analysis. Ecological Modelling 220:2060-2067

Eklöf JS, Alsterberg C, Havenhand JN, Sundbäck K, Wood HL, Gamfeldt L (2012) Experimental climate change weakens the insurance effect of biodiversity. Ecology Letters 15:864-872

Engelsen A, Hulth S, Pihl L, Sundbäck K (2008) Benthic trophic status and nutrient fluxes in shallow-water sediments. Estuarine Coastal and Shelf Science 78:783-795

Everett RA (1994) Macroalgae in marine soft-sediment communities – effects on benthic faunal assemblages. Journal of Experimental Marine Biology and Ecology 175:253-274

Evrard V, Cook PLM, Veuger B, Huettel M, Middelburg JJ (2008) Tracing carbon and nitrogen incorporation and pathways in the microbial community of a photic subtidal sand. Aquatic Microbial Ecology 53:257-269

Eyre BD, Ferguson AJP, Webb A, Maher D, Oakes JM (2011) Denitrification, N-fixation and nitrogen and phosphorus fluxes in different benthic habitats and their contribution to the nitrogen and phosphorus budgets of a shallow oligotrophic sub-tropical coastal system (southern Moreton Bay, Australia). Biogeochemistry 102:111-133

Feuchtmayr H, McKee D, Harvey IF, Atkinson D, Moss B (2007) Response of macroinvertebrates to warming, nutrient addition and predation in large-scale mesocosm tanks. Hydrobiologia 584:425-432

Fitch JE, Crowe TP (2011) Combined effects of temperature, inorganic nutrients and organic matter on ecosystem processes in intertidal sediments. Journal of Experimental Marine Biology and Ecology 400:257-263

Fleeger JW, Carman KR, Nisbet RM (2003) Indirect effects of contaminants in aquatic ecosystems. Science of the Total Environment 317:207-233

Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnology and Oceanography 44:864-877

Froelich PN, Klinkhammer GP, Bender ML, Luedtke NA, Heath GR, Cullen D, Dauphin P, Hammond D, Hartman B, Maynard V (1979) Early oxidation of organic-matter in pelagic sediments of the eastern equatorial atlantic – suboxic diagenesis. Geochimica Et Cosmochimica Acta 43:1075-1090

Goto N, Kawamura T, Mitamura O, Terai H (1999) Importance of extracellular organic carbon production in the total primary production by tidal-flat diatoms in comparison to phytoplankton. Marine Ecology Progress Series 190:289-295

Grace JB (2003) Comparing groups using structural equations*,* p 281-296. In Pugesek BH, von Tomer A [eds.], Structural equation modeling: applications in ecological and evolutionary biology. Cambridge University Press

Grace JB, Jutila H (1999) The relationship between species density and community biomass in grazed and ungrazed coastal meadows. Oikos 85:398-408

Grace JB, Schoolmaster Jr DR, Guntenspergen GL, Little AM, Mitchell BR, Miller KM, Schweiger EW (2012) Guidelines for a graph-theoretic implementation of structural equation modeling. Ecosphere 3

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. Science 319:948-952

Hancke K, Glud RN (2004) Temperature effects on respiration and photosynthesis in three diatom-dominated benthic communities. Aquatic Microbial Ecology 37:265-281

Harley CDG (2011) Climate Change, Keystone Predation, and Biodiversity Loss. Science 334:1124-1127

Hay ME (1981) The functional-morphology of turf-forming seaweeds – persistence in stressful marine habitats. Ecology 62:739-750

Heugens EHW, Hendriks AJ, Dekker T, van Straalen NM, Admiraal W (2001) A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Critical Reviews in Toxicology 31:247-284

Hicks N, Bulling MT, Solan M, Raffaelli DG, White PCL, Paterson DM (2011) Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system. BCM Ecology 11

Hjorth M, Forbes VE, Dahlloef I (2008) Plankton stress responses from PAH exposure and nutrient enrichment. Marine Ecology Progress Series 363:121-130

Howarth RW (1988) Nutrient limitation of net primary production in marine ecosystems. Annual Review of Ecology and Systematics 19:89-110

Hurd CL, Cornwall CE, Currie K, Hepburn CD, McGraw CM, Hunter KA, Boyd PW (2011) Metabolically induced pH fluctuations by some coastal calcifiers exceed projected 22nd century ocean acidification: a mechanism for differential susceptibility? Global Change Biology 17:3254-3262

IPCC (2007) Intergovernmental panel on climate change. 2007: Synthesis report, p. 45–47. In RK Pachauri and A Risinger [eds.], Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change. IPCC.

Isaksson I, Pihl L (1992) Structural-changes in benthic macrovegetation and associated epibenthic faunal communities. Netherlands Journal of Sea Research 30:131-140

Jaschinski S, Brepohl DC, Sommer U (2011) Seasonal variation in carbon sources of mesograzers and small predators in an eelgrass community: stable isotope and fatty acid analyses. Marine Ecology Progress Series 431:69-82

Johansson P, Eriksson KM, Axelsson L, Blanck H (2012) Effects of Seven Antifouling Compounds on Photosynthesis and Inorganic Carbon Use in Sugar Kelp *Saccharina latissima* (Linnaeus). Archives of Environmental Contamination and Toxicology 63:365-377

Kamp A, de Beer D, Nitsch JL, Lavik G, Stief P (2011) Diatoms respire nitrate to survive dark and anoxic conditions. Proceedings of the National Academy of Sciences of the United States of America 108:5649-5654

Kennedy AD, Jacoby CA (1999) Biological indicators of marine environmental health: Meiofauna - A neglected benthic component? Environmental Monitoring and Assessment 54:47-68

Koelmans AA, Van der Heijde A, Knijff LM, Aalderink RH (2001) Integrated modelling of eutrophication and organic contaminant fate & effects in aquatic ecosystems. A review. Water Research 35:3517-3536

Kordas RL, Harley CDG, O'Connor MI (2011) Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. Journal of Experimental Marine Biology and Ecology 400:218-226

Kristensen E, Penha-Lopes G, Delefosse M, Valdemarsen T, Quintana CO, Banta GT (2012) What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Marine Ecology Progress Series 446:285-302

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters 13:1419-1434

Kühl M, Glud RN, Ploug H, Ramsing NB (1996) Microenvironmental control of photosynthesis and photosynthesis-coupled respiration in an epilithic cyanobacterial biofilm. Journal of Phycology 32:799-812

Larson F, Petersen DG, Dahllöf I, Sundbäck K (2007) Combined effects of an antifouling biocide and nutrient status on a shallow-water microbenthic community. Aquatic Microbial Ecology 48:277-294

Larson F, Sundbäck K (2012) Recovery of microphytobenthos and benthic functions after sediment deposition. Marine Ecology Progress Series 446:31-44

Lee KS, Dunton KH (2000) Diurnal changes in pore water sulfide concentrations in the seagrass Thalassia testudinum beds: the effects of seagrasses on sulfide dynamics. Journal of Experimental Marine Biololgy and Ecology 255:201-214

Lewandowska A, Sommer U (2010) Climate change and the spring bloom: a mesocosm study on the influence of light and temperature on phytoplankton and mesozooplankton. Marine Ecology Progress Series 405:101-111

Lorenzen CJ (1967) Determination of chlorophyll and pheo-pigments – spectrophotometeric equations Limnology and Oceanography 12:343-346

Maraldo K, Dahllöf I (2004) Indirect estimation of degradation time for zinc pyrithione and copper pyrithione in seawater. Marine Pollution Bulletin 48:894-901

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology 82:290-297

McGlathery KJ, Sundbäck K, Anderson IC (2007) Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. Marine Ecology Progress Series 348:1-18

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMJ, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: An in situ C-13-labeling study. Limnology and Oceanography 45:1224-1234

Moksnes P-O, Gullström M, Tryman K, Baden S (2008) Trophic cascades in a temperate seagrass community. Oikos 117:763-777

Montserrat F, Van Colen C, Degraer S, Ysebaert T, Herman PMJ (2008) Benthic community-mediated sediment dynamics. Marine Ecology Progress Series 372:43-59

Nascimento FJA, Näslund J, Elmgren R (2012) Meiofauna enhances organic matter mineralization in soft sediment ecosystems. Limnology and Oceanography 57:338-346

Nilsson P, Sundbäck K, Jönsson B (1993) Effects of the brown shrimp *Crangon crangon* (L) on endobenthic macrofauna, meiofauna and meiofaunal grazing rates. Netherlands Journal of Sea Research 31:95-106

Norkko A, Bonsdorff E (1996) Population responses of coastal zoobenthos to stress induced by drifting algal mats. Marine Ecology Progress Series 140:141-151

Nydahl A, Panigrahi S, Wikner J (2013) Increased microbial activity in a warmer and wetter climate enhance the risk of coastal hypoxia. FEMS Microbiology Ecology, *in press*

O'Connor MI (2009) Warming strengthens an herbivore-plant interaction. Ecology 90:388-398

Pedersen O, Borum J, Duarte CM, Fortes MD (1998) Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*. Marine Ecology Progress Series 169:283-288

Petchey OL, McPhearson PT, Casey TM, Morin PJ (1999) Environmental warming alters food-web structure and ecosystem function. Nature 402:69-72

Petersen DG, Dahllöf I, Nielsen LP (2004) Effects of zinc pyrithione and copper pyrithione on microbial community function and structure in sediments. Environmental Toxicology and Chemistry 23:921-928

Porsbring T, Backhaus T, Johansson P, Kuylenstierna M, Blanck H (2010) Mixture toxicity from photosystem II inhibitors on microalgal community succession is predictable by concentration addition. Environmental Toxicology and Chemistry 29:2806-2813

Reusch TBH, Ehlers A, Hammerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences of the United States of America 102:2826-2831

Rost B, Zondervan I, Wolf-Gladrow D (2008) Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. Marine Ecology Progress Series 373:227-237

Sander BC, Kalff J (1993) Factors controlling bacterial production in marine and fresh-water sediments. Microbial Ecology 26:79-99

Sommer U, Lengfellner K (2008) Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. Global Change Biology 14:1199-1208

Sommer U, Lewandowska A (2011) Climate change and the phytoplankton spring bloom: warming and overwintering zooplankton have similar effects on phytoplankton. Global Change Biology 17:154-162

Strickland JD, Parsons TR (1972) A practical handbook of seawater analysis, 2nd ed. Bulletin 167. Fisheries Research Board of Canada

Sundbäck K, Alsterberg C, Larson F (2010) Effects of multiple stressors on marine shallow-water sediments: Response of microalgae and meiofauna to nutrient-toxicant exposure. Journal of Experimental Marine Biolology and Ecology 388:39-50

Sundbäck K, Linares F, Larson F, Wulff A, Engelsen A (2004) Benthic nitrogen fluxes along a depth gradient in a microtidal fjord: The role of denitrification and microphytobenthos. Limnology and Oceanography 49:1095-1107

Sundbäck K, McGlathery KJ (2005) Interactions between benthic macroalgal and microalgal mats. Coastal and Estuarine studies 60:7-29

Sundbäck K, Miles A (2000) Balance between denitrification and microalgal incorporation of nitrogen in microtidal sediments, NE Kattegat. Aquatic Microbial Ecology 22:291-300

Sundbäck K, Miles A, Göransson E (2000) Nitrogen fluxes, denitrification and the role of microphytobenthos in microtidal shallow-water sediments: an annual study. Marine Ecology-Progress Series 200:59-76

Sundbäck K, Petersen DG, Dahllöf I, Larson F (2007) Combined nutrient-toxicant effects on a shallow-water marine sediment system: sensitivity and resilience of ecosystem functions. Marine Ecology-Progress Series 330:13-30

Sundbäck K, Snoeijs P (1991) Effects of nutrient enrichment on microalgal community composition in a coastal shallow-water sediment system – an experimental study. Botanica Marina 34:341-358

Sutherland TF, Amos CL, Grant J (1998) The effect of buoyant biofilms on the erodibility of sublittoral sediments of a temperate microtidal estuary. Limnology and Oceanography 43:225-235

Thamdrup B, Dalsgaard T (2002) Production of N-2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Applied and Environmental Microbiology 68:1312-1318

Turley PA, Fenn RJ, Ritter JC, Callow ME (2005) Pyrithiones as antifoulants: Environmental fate and loss of toxicity. Biofouling 21:31-40

Underwood GJC, Kromkamp J (1999) Primary production by phytoplankton and microphytobenthos in estuaries, p 93-153. In Nedwell DB, Raffaelli DG [eds.] Estuaries Vol 29. Advances in Ecological Research.

Underwood GJC, Paterson DM (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. Advances in Botanical Research 40:183–240

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104:451-457

Walther GR (2004) Plants in a warmer world. Perspectives in Plant Ecology Evolution and Systematics 6:169-185

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck KL, Jr., Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106:12377-12381

Weerman EJ, Herman PMJ, van de Koppel J (2011) Macrobenthos abundance and distribution on a spatially patterned intertidal flat. Marine Ecology Progress Series 440:95-103

Wernberg T, Smale DA, Thomsen MS (2012) A decade of climate change experiments on marine organisms: procedures, patterns and problems. Global Change Biology 18:1491-1498

Widbom B (1984) Determination of average individual dry weights in different sieve fractions of marine meiofauna. Marine Biology 84:101-108

Witt V, Wild C, Anthony KRN, Diaz-Pulido G, Uthicke S (2011) Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef. Environmental Microbiology 13:2976-2989

Woodward G, Perkins DM, Brown LE (2010) Climate change and freshwater ecosystems: impacts across multiple levels of organization. Royal Society Philosophical Transactions Biological Sciences 365:2093-2106

Wootton JT (2002) Indirect effects in complex ecosystems: recent progress and future challenges. Journal of Sea Research 48:157-172

Worm B, Reusch TBH, Lotze HK (2000) In situ nutrient enrichment: Methods for marine benthic ecology. International Review of Hydrobiology 85:359-375

Yvon-Durocher G, Caffrey JM, Cescatti A, Dossena M, del Giorgio P, Gasol JM, Montoya JM, Pumpanen J, Staehr PA, Trimmer M, Woodward G, Allen AP (2012) Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature 487:472-476

Yvon-Durocher G, Jones JI, Trimmer M, Woodward G, Montoya JM (2010) Warming alters the metabolic balance of ecosystems. Philosophical Transactions of the Royal Society B-Biological Sciences 365:2117-2126