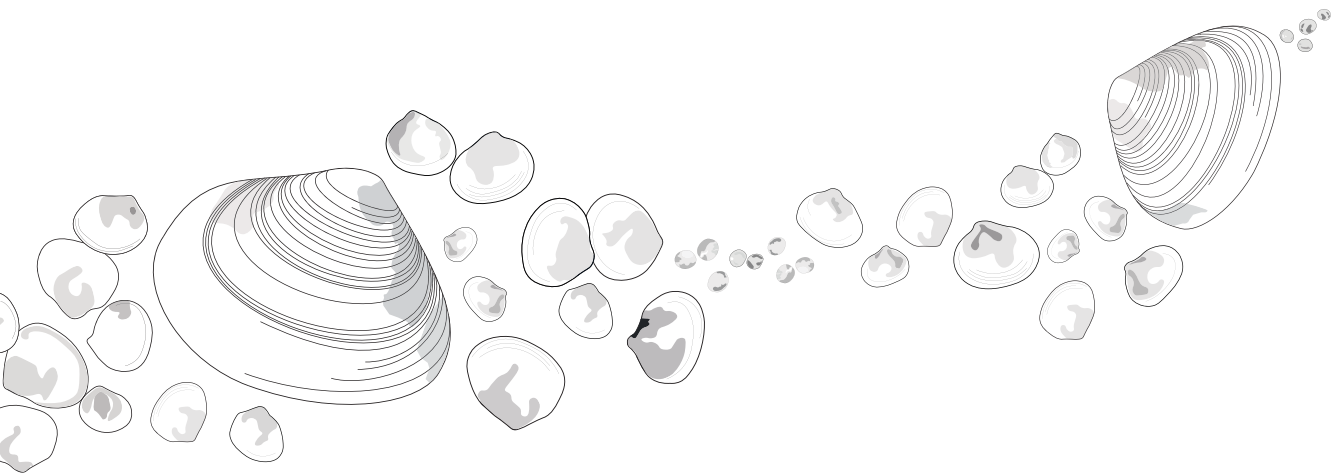


**OCEAN ACIDIFICATION IN THE BALTIC SEA —
IMPLICATIONS FOR THE BIVALVE *MACOMA BALTHICA***

Anna Jansson



Ocean acidification in the Baltic Sea –
Implications for the bivalve *Macoma balthica*

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Abstract

The Baltic Sea is one of the most human-impacted sea areas in the world and its ecosystems are exposed to a variety of stressors of anthropogenic origin. Large changes in the environmental conditions, species and communities of the Baltic Sea are predicted to occur due to global climate change, but the extent and magnitude of the future changes are challenging to estimate due to the multiple stressors simultaneously impacting the system. As an additional threat, future ocean acidification will play a role in modifying the environmental conditions, and these CO₂-induced changes are predicted to be fast in the Baltic Sea. This is especially of concern for the species-poor, but functionally essential benthic communities where key species such as bivalve *Macoma balthica* live at the limits of their tolerance range, and are already regularly disturbed by environmental stressors such as hypoxia. Currently, only very limited knowledge about the effects of future ocean acidification exists for this species.

The overall aim of my thesis was to develop an understanding of the effects of CO₂ increase on the vulnerability of Baltic Sea key species, and how this is related to other effects of climate change, e.g. an increase in bottom-water hypoxia. Specifically, I investigated how different life stages of the infaunal bivalve *M. balthica* could be affected by future ocean acidification. Survival, growth, behaviour and physiological responses were assessed in a combination of laboratory and mesocosm experiments by exposing different life stages of *M. balthica* to different pH levels over different time periods depending on the life stage in question. While some life stage-based differences in vulnerability and survival were found, the results indicate that reduced pH has a negative effect on all life stages. In larval *M. balthica*, even a slight pH decrease was found to cause significant negative changes during that delicate life stage, both by slowing growth and by decreasing survival. Other observed impacts included delayed settling of the post-larvae and increasing energetic demand of adult bivalves.

The results suggest consistent negative effects at all life stages with potential major implications for the resilience of *M. Balthica* populations, which are currently under threat from a range of anthropogenic stressors such as increasing hypoxia. The kind of experimental studies conducted in this thesis are useful for pinpointing mechanisms, but they are always simplifications of reality, however, and are usually conducted over time scales that are short in relation to the time scales over which ocean acidification is affecting populations, communities and ecosystems. To fully understand and to be able to estimate how the complex ecosystems are about to change in the future, incorporating more of the biotic interactions, impacting stressors and relevant environmental conditions are needed for increasing the level of realism in the experiments.

Keywords: ocean acidification, life stage, bivalve, hypoxia, climate change, pH, mesocosm, natural fluctuation

Sammanfattning (Swedish abstract)

Östersjön är ett av de mest påverkade havsområdena i världen och dess ekosystem är utsatta för en mängd olika typer av mänsklig påverkan. Stora förändringar i Östersjöns miljöfaktorer, artsammansättning och samhällsstruktur förväntas ske på grund av den globala klimatförändringen, men omfattningen av de kommande förändringarna är svår att uppskatta på grund av att flera stressorer samtidigt påverkar systemet. CO₂-inducerad havsförurning utgör ett nytt hot mot marina system och kommer att förändra miljöförhållandena i havet. Dessa förändringar förväntas vara snabba i Östersjön. Havsförurningen är potentiellt problematisk speciellt för Östersjöns artfattiga men funktionellt viktiga bottenfaunasamhällen, där nyckelarter så som Östersjömusslan *Macoma balthica* lever på gränsen av sin salthaltstolerans och där arterna redan nu regelbundet är utsatta för t.ex. syrebrist i bottenvattnet. Hittills har det dock endast funnits begränsad kunskap om havsförurningens potentiella effekter på denna art.

Ändamålet med min avhandling var att utöka förståelsen av CO₂-ökningens inverkan på sårbarheten hos denna nyckelart i Östersjön, och hur detta är kopplat till andra effekter av klimatförändringen såsom ökningen av havsbottnarnas syrebrist. Mera specifikt undersökte jag hur olika livsstadier av Östersjömusslan, *Macoma balthica*, påverkas av havsförurningen. Överlevnad, tillväxt, beteende och fysiologiska responser studerades i en kombination av akvarieexperiment och mesokosmstudier genom att utsätta olika livsstadier av *M. balthica* för olika pH-nivåer under varierande tidsperioder beroende på livsstadiet i fråga. Trots att känsligheten och överlevnaden varierade något mellan de olika livsstadierna, visar resultaten att minskande pH har negativa effekter på alla livsstadier. Hos larver av *M. balthica*, förorsakade även en liten pH-minskning relativt stora negativa förändringar såsom lägre tillväxthastighet och ökad mortalitet under detta känsliga livsstadium. Andra effekter av lägre pH på *M. balthica* var bl.a. försenad bottenfällning av larver och ökande energiförbrukning hos fullvuxna individer.

Mina resultat påvisar konsekvent negativa effekter av havsförurningen hos alla livsstadier, vilket kan ha allvarliga konsekvenser för de *M. balthica*

populationer som redan nu är utsatta för flera antropogena stressorer. Den typ av experimentella studier som har utförts i den här avhandlingen är värdefulla för att påvisa specifika processer och mekanismer som ligger bakom havsförsurningseffekterna. Experimenten representerar ändå alltid en förenklad version av verkligheten och de utförs under korta tidsperioder, som inte motsvarar de verkliga tidskalor över vilka havsförsurningen påverkar populationer, samhällen och ekosystem. För att bättre kunna förstå och förutsäga hur komplexa ekosystem kommer att förändras i framtiden, är det viktigt att experimenten görs mera realistiska genom att inkludera biotiska interaktioner, multipla stressorer samt naturligt varierande miljöfaktorer.

Nyckelord: havsförsurning, livscykel, östersjömussla, syrebrist, klimatförändring, pH, mesokosmstudie, naturlig fluktuerig

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List of original papers

This thesis is based on the following articles, which are referred to in the text by their Roman numerals (**paper I-IV**). The published papers have been reprinted with the kind permission of the copyright holders.

- I. Jansson A, Norkko J, Norkko A (2013) Effects of reduced pH on *Macoma balthica* larvae from a system with naturally fluctuating pH-dynamics. PLoS ONE 8(6): e68198. doi:10.1371/journal.pone.0068198
- II. Jansson A, Norkko J, Dupont S, Norkko A (2015) Growth and survival in a changing environment: Combined effects of moderate hypoxia and low pH on juvenile bivalve *Macoma balthica*. Journal of Sea Research 102: 41–47
- III. Jansson A, Lischka S, Boxhammer T, Schulz K, Norkko J (2016) Survival and settling of larval *Macoma balthica* in a large-scale mesocosm experiment at different $f\text{CO}_2$ levels. Biogeosciences 13: 3377–3385
- IV. Jansson A, Turja R, Lehtonen K. Long-term behavioral and physiological effects of decreased pH levels in the Baltic Sea *Macoma balthica* (manuscript)

1. Introduction

The Baltic Sea is one of the most human-impacted sea areas in the world. It is exposed to increasing amounts of external stress from various anthropogenic sources, and is strongly impacted by intense eutrophication and oxygen deficiency. Future global climate change is likely to have a strong impact on the Baltic Sea physico-chemical features, species (composition) and functioning. The extent and magnitude of the future changes are, however, challenging to estimate due to the variety of stressors simultaneously impacting the system with differing intensities. As an additional stressor, future ocean acidification will play a role in modifying the environmental conditions and potentially adding to the stress the species and communities experience. This is especially of concern for the species-poor, but functionally crucial benthic communities where key species such as bivalve *Macoma balthica* (L.) are already threatened e.g. by widespread hypoxia. *M. balthica* is a functionally important species in the Baltic Sea, but the effects of ocean acidification on this species have not been investigated in the northern Baltic Sea. Therefore the aim of this thesis is to study how reduced pH impacts the survival, growth, and physiology of *M. balthica* and to discuss the subsequent implications for the Baltic Sea ecosystem.

1.1 The Baltic Sea

The Baltic Sea is a shallow (mean depth 54 m), geologically young, strongly stratified brackish water body, that has a special topography consisting of several basins, and is connected to the North Sea only by narrow straights allowing a minimal water exchange with the fully marine North Atlantic waters (Leppäranta and Myrberg 2009). It is characterized by substantial riverine input and steep geographical and seasonal gradients in parameters such as temperature, salinity, alkalinity, pH and nutrient concentrations (Thomas and Schneider 1999, Leppäranta and Myrberg 2009, Beldowski et al. 2010). For example, the salinity in the Baltic Sea is in part extremely low, and ranges from limnic waters to nearly fully marine. It is at its lowest in the northern and north-eastern bays such as the Gulfs of Bothnia and Finland (ca. 1-5) and increases towards the Baltic Sea Proper and the Danish Straits in the southwest with the salinity of ca. 25 (Leppäranta and Myrberg 2009). The low salinity also means that alkalinity, the buffering capacity against pH changes, of the Baltic Sea is low, with important implications for the pH dynamics of the system (Hjalmarsson et al. 2008).

The Baltic Sea is surrounded by 14 countries, and a human population of ca. 85 million in its catchment area makes it highly susceptible to human-induced stressors (BACC II author team 2015). The Baltic Sea has suffered from severe eutrophication since the 1960s, with a large increase in the production of organic matter and the subsequent increased oxygen demand during decomposition of the produced biomass (Cederwall and Elmgren 1980, Andersen et al. 2016). Hence, the Baltic Sea is exposed to large-scale hypoxia, the extent of which varies seasonally, with large areas of the sea floor being permanently oxygen depleted both in the deep areas of the open sea and in coastal areas (Karlson et al. 2002, Conley et al. 2009, Carstensen et al. 2014). With an estimated area of 80 000 km², the central Baltic Sea represents one of the largest ecosystems in the world affected by hypoxia and anoxia (Diaz and Rosenberg 2008, Carstensen et al. 2014).

The macrobenthic fauna of the Baltic Sea is of marine, brackish and limnic origin (Remane 1934, Segerstråle 1957), and due to the short postglacial history of this sea area of ca. 8000 years, the biodiversity of the Baltic Sea is relatively low compared to fully marine systems as only a few species have yet fully adapted to the brackish conditions (Segerstråle 1957, Bonsdorff 2006). Hence salinity tolerance is the strongest driver of the species distribution ranges, with the number of species increasing towards the more saline southern Baltic Sea (Rumohr et al. 1996, Bonsdorff and Pearson 1999, Bonsdorff 2006). Due to the low species number, important ecosystem functions are often supported by only one or few key species, making them crucially important for the well-being of the local ecosystem (Villnäs et al. 2012). Living in a stressful environment such as in an oligohaline environment or at the edge of the distribution range, however, causes energetic constraints (e.g. osmotic stress) that can be seen as slow growth rates, thin and weak shells and small size like in the case of many Baltic Sea bivalves (Kautsky 1982, Tedengren and Kautsky 1986, Westerborg et al. 2002). Severe disturbances such as large-scale hypoxia, often co-occurring with reduced pH conditions, are strongly impacting the benthic communities both in the open sea areas and in the coastal zones with increasing frequency and extent (Conley et al. 2011). These conditions severely reduce macrofaunal biodiversity (Villnäs and Norkko 2011, Villnäs et al. 2012), and are predicted to increase in the Baltic Sea over the coming decades (Meier et al. 2011), leading to even higher risk of degraded benthic communities that are both structurally and functionally impaired.

A key species of the benthic system is the infaunal bivalve *M. balthica*. It is abundant throughout the Baltic Sea and is a key component of the local

community where it often dominates the soft-bottom ecosystem from a few meters depth to deep bottoms (Segerstråle 1960, Elmgren et al. 1986, Bonsdorff et al. 1995, Bonsdorff 2006). It is an important prey organism, and through its bioturbation, it oxygenates the sediment and facilitates the recycling of organic matter, contributing to the overall health and functioning of the benthic ecosystem (Norkko et al. 2013). In the species-poor northern Baltic Sea, no other species in the current community can fully replace its important functions (Villnäs et al. 2012) and hence it is of importance to understand the effects of different ecosystem disturbances on this species.

1.2 Climate change

The atmospheric layer of the Earth is constituted of ca. 0.04 % of carbon dioxide gas (CO_2), equaling to a partial pressure $p\text{CO}_2$ of ca. 400 ppm. Since the industrial period (1760's), atmospheric CO_2 has increased by ca. 100 ppm, as a consequence of anthropogenic CO_2 emissions (Etheridge et al. 1996). Despite the current agreements to control and reduce global CO_2 emissions, different models project an increase of CO_2 concentrations by at least 200 ppm by the year 2100, reaching 500 to even 1000 ppm (IPCC 2007). The large production of CO_2 has the potential to increase Earth's mean surface temperatures between 2°C to 4.5°C from 1990 to 2100 (IPCC 2007). Ca. 40 % of the emitted atmospheric CO_2 has been absorbed in the oceans during the past 200 years due to the $p\text{CO}_2$ difference in the ocean surface and atmosphere. This removal of CO_2 from the atmosphere helps moderate climate change (Sabine et al. 2004), however, the reaction of excess CO_2 in the seawater has severe effects on marine ecosystems in the form of future ocean acidification, the ecological and economic consequences of which are difficult to predict (see chapter 1.3.).

In the Baltic Sea area, like elsewhere in the world, warming climate and changing patterns in precipitation, winds and ocean currents are likely consequences of the global climate change. A record warming of the Baltic Sea has already occurred since the 1860s, especially during the recent decades (MacKenzie and Schiedek 2007, Belkin 2009). Since the last 30 years, a warming of surface waters is evident in all seasons (up to 1°C per decade in all areas), and the probability of extremely warm surface waters in winter and summer has increased since the 1990s by two- to fourfold (Lehmann et al. 2011, BACC II author team 2015). The future temperature increase is estimated to be large for the Baltic Sea area during this century, causing warming of the sea surface by $2\text{-}5^\circ\text{C}$ in different parts of the Baltic Sea (BACC II author team 2015,

Holopainen et al. 2016). In the northern sea areas, the recent decrease in the extent and duration of ice cover also has a strong influence on the trends in seawater temperature (BACC II author team 2015). A significant decrease has been found in the yearly maximum sea ice extent; during the past 100 years the decrease has been ca. 3400 km² per decade (BACC author team 2008, Vihma and Haapala 2009). The extent and/or duration of the ice-cover is estimated to strongly decline in the future due to warming temperatures (BACC II author team 2015). A salinity decrease of ca. 1.75 at the bottom water of the Bothnian Sea and Gulf of Finland is expected by the end of this century due to an increase in precipitation (of 12-14 %) and the subsequent increase in fresh water run-off from the catchment area (Meier et al. 2012, Holopainen et al. 2016). The increased riverine run-off also brings along an increasing nutrient load, impacting the nutrient levels in the Baltic Sea (Leppäranta and Myrberg 2009). Also, hypoxic conditions are predicted to increase due to the combination of rising temperatures and accelerated eutrophication in the Baltic Sea (Karlson et al. 2002, Conley et al. 2001, Kabel et al. 2012, Carstensen et al. 2014). These changes in environmental conditions modify populations and communities by impacting species distribution ranges, abundance and dominance relations, food-web structures and behavioural and physiological functions of species. Through these effects, the structure and functioning of whole ecosystems can be altered (Thomas et al. 2004, Doney et al. 2012).

1.3 Ocean acidification

Anthropogenic CO₂ emissions are causing severe changes in the ocean seawater chemistry and the global inorganic carbon balance of the oceans, as well as in their physico-chemical conditions (Caldeira and Wickett 2003, Feely et al. 2004). This other consequence of rising atmospheric CO₂ is termed ocean acidification (OA). It includes alterations in the inorganic carbon chemistry of the seawater due to the reactive nature of CO₂ in the water. As CO₂ dissolves in water it forms a weak acid, carbonic acid (H₂CO₃), which further dissociates into bicarbonate (HCO₃⁻) and carbonate ions (CO₃²⁻) losing hydrogen (H⁺) ions during the reaction, which causes the pH of the water to decrease: $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CO}_3^{2-} + 2\text{H}^+$. Due to the direction of the reaction of the inorganic carbon components in their natural state, the carbonate ion concentration declines while hydrogen ion concentration increases when excessive CO₂ is added to the system. This process also decreases the saturation states of aragonite (Ω_{arag}) and calcite (Ω_{cal}), the two mineral forms of calcium carbonate that most calcifying

organisms use to form their skeletal structures. The IPCC 2007 report predicts a decrease of surface ocean pH from the current global average of 8.1 to 7.7 by the end of the century. This corresponds to a ca. 50 % decrease in carbonate ion concentration (Feely et al. 2004, Orr et al. 2005), with likely significant consequences for the structure and function of many different ecosystems globally.

Certain areas are considered more vulnerable to future ocean acidification than others. The cold higher latitudes, such as the Arctic Ocean, are predicted to experience stronger and faster ocean acidification because the ocean absorbs more carbon dioxide in cold temperatures. In addition, undersaturation of aragonite already regularly occurs, and is estimated to spread to the whole surface of the Arctic Ocean by the year 2100 (Orr et al. 2005, Steinacher et al. 2009). Future ocean acidification is predicted to be severe also in the Baltic Sea due to the naturally low buffering capacity of the system caused by low alkalinity, with a drop of up to 0.5 pH units estimated for the surface waters during this century (Hjalmarsson et al. 2008, Omstedt et al. 2012). However, the natural pH fluctuations in the Baltic Sea are already large, with high pH fluctuations occurring seasonally, diurnally and spatially. Strong seasonal pH fluctuations occur mainly due to changes in primary production (Thomas and Schneider 1999, Schneider et al. 2003) or associated with seasonal hypoxia (Melzner et al. 2012). In the shallow coastal areas of the northern Baltic Sea, the CO₂ concentration in the water column frequently exhibits substantial diurnal variation resulting in pH changes of more than one pH unit per day, especially during peaks of high photosynthetic activity during phytoplankton blooms, or in productive habitats such as seagrass meadows and macroalgal belts (Jansson pers. obs., Almen et al. 2014). Future ocean acidification is nevertheless likely to have an impact by further increasing the pH fluctuations and making the occasionally experienced extreme pH levels even more pronounced (Thomas and Schneider 1999, Melzner et al. 2012). It is important to note that the modelling of the level of future ocean acidification has generally been made for open sea areas, but for the diverse, productive, variable coastal areas it is challenging to produce reliable estimates of the level of future acidification, both due to the naturally highly fluctuating conditions and the multiple parameters that impact these areas (Duarte et al. 2013, Waldbusser and Salisbury 2014).

Ocean acidification will rarely act alone, but in combination with other environmental factors. A range of studies has focused on the interactive effects of reduced pH in combination with other effects of climate change (e.g. temperature rise; Byrne 2011, Byrne 2013, Todgham and Stillman 2013), yet relatively little

is still known about the interactive effects of reduced pH with other environmental stressors such as low oxygen and pollutants. Due to the nature of the Baltic Sea, hypoxia being one of the most striking features of the local ecosystems, the combined effects of hypoxia and reduced pH are of great interest as the two stressors are strongly interconnected near the sea floor (Brewer and Peltzer 2009, Wesslander et al. 2011, Melzner et al. 2012). They are also known to have significant effects on the benthic communities and are increasing in magnitude and extent in the Baltic Sea as the climate change proceeds (Conley et al. 2011, Carstensen et al. 2014)

1.4 Biological effects of ocean acidification

The future ocean acidification is of growing concern, and it is estimated to impact a large range of marine species, communities and processes. Key biological processes such as growth, calcification, development, metabolism, physiological balance and reproduction play an important role in organisms' capacity to maintain viable populations. These processes are likely to be affected by future ocean acidification (Kroeker et al. 2010, 2013, Gazeau et al. 2013, Parker et al. 2013), and there is a growing interest to study them in a range of organism groups, some of the most studied including calcifying species such as corals and phytoplankton. Different organismal groups exhibit different responses to reduced pH conditions, however, and the responses have been found to differ even between closely related species (Kurihara 2008, Dupont and Thorndyke 2009, Kroeker et al. 2010), for example, due to the different local conditions where the organisms occur (Waldbusser and Salisbury 2014). Extrapolating from one species to another can therefore be challenging. In addition, in order to gain a realistic picture of impacts on a specific species or population, studies on different life history stages of the species are required to reveal the true impacts.

It is widely accepted that delicate early life history processes of marine invertebrates such as larval survival, development and subsequent settling are highly sensitive to CO₂-induced acidification (Green et al. 2004, Kurihara 2008, Byrne 2012). For example, a range of impacts from no effects to delayed and/or abnormal development, slower growth, reduced calcification to higher mortality has been found for bivalve early life stages when different species have been exposed to reduced pH, whereas adult life stages are rarely impacted as directly (Harvey et al. 2013, Kroeker et al. 2013, Waldbusser and Salisbury 2014). The impacts of reduced pH on adult life stages may manifest in the next generations

only. As the adult individuals are required to consume a larger amount of energy for the maintenance of physiological balance in the reduced pH conditions, less energy can be allocated to reproduction and the viability of embryos (Parker et al. 2012, Calosi et al. 2016).

The alteration of the carbonate system associated with ocean acidification has consequences on several marine species that form hard calcified structures by making the building of calcium carbonate structures (calcification) more energetically costly. The energy cost arises from the increased need for acid-base regulation to maintain the optimal conditions at the calcification site, and from the cost to prevent or compensate for dissolution, and thus calcification is one of the biological processes most sensitive to ocean acidification (Fabry et al. 2008, Waldbusser et al. 2011, Barton et al. 2012, Waldbusser et al. 2015). As a result calcifying organisms like bivalves, corals and calcareous plankton will potentially be highly affected by reduced pH. A decrease in the calcification has been found in response to reduced pH level and carbonate saturation state in a large range of marine species (Doney et al. 2009, Ries et al. 2009, Gazeau et al. 2013).

There is relatively little available information on the response of the Baltic Sea species and communities to reduced pH levels (Havenhand 2012). The impact of reduced pH on Baltic Sea macrofauna has been studied on certain key benthic components in the southern Baltic Sea in the Kiel Fjord, as well as in the northern Baltic Sea (this thesis). In the Kiel fjord the bivalve *Mytilus edulis* has been found to maintain shell and somatic growth rates and high recruitment rate at high $p\text{CO}_2$ conditions (1000-1400 ppm) that naturally occur in the area. Some general mechanisms that are behind the species responses to increasing $p\text{CO}_2$ have been identified. The reason behind the ability of the southern Baltic *M. edulis* and barnacle *Amphibalanus improvisus* (Pansch et al. 2012) to maintain the physiological functions in high level of CO_2 -induced acidification is suggested to be the high food availability in the area (Thomsen et al. 2010). It enables the maintenance of high energy levels despite the extra energy expenditure required to counteract the negative effects of increasing $p\text{CO}_2$. As many of the key processes (above) become energetically costly to the organisms in conditions where they have to allocate increasing amounts of energy to maintain internal pH balance or calcification rate (Melzner et al. 2011), the availability of adequate energy reserves in the form of food may determine the tolerance to ocean acidification in many species.

The implications and the full extent of future ocean acidification is difficult to estimate since the environmental conditions that the species experience vary,

ocean acidification is likely not to act alone, responses vary from one species and life stage to another and experiments investigating the effects of future ocean acidification have been of highly different types; the majority of relatively short-term consisting of single species and one to two stressors (Boyd 2013, Waldbusser and Salisbury 2014, Cornwall and Hurd 2015, Riebesell and Gattuso 2015). Problems involved in up-scaling from species- and population level studies to ecosystems are many, nevertheless, this is a top priority in order to benefit from the already existing wide information of the susceptibility of organisms to reduced pH for predicting the actual impacts of future ocean acidification on communities and ecosystems (Riebesell and Gattuso 2015).

1.5 The role of early life stages for population dynamics

Most benthic marine invertebrates have indirect, complex life cycles (Thorson 1950) composed of different life stages (e.g. larvae, juvenile, adult). Every life stage differs in morphology, function and ecological requirements, and the impact that different environmental conditions may have on the species depends on the response of the life stage in question. For example, a high proportion of the benthic species develop via a planktotrophic larval stage that feed on phytoplankton in the water column, spend a relatively long time in the plankton and have the potential to disperse over some, yet limited, distances (Pechenik 1999). Early developmental stages are also presumed to be the most vulnerable part of a species life cycle, particularly when it comes to environmental stressors (Thorson 1950, Spicer and Gaston 1999), and are therefore important to study to gain a realistic estimate of a species (and communities) response to changing environmental conditions. The population dynamics of a species is largely dependent on successful settlement and recruitment of the post-larvae into adult populations, and on dispersal of larval and post-larval stages (Bonsdorff et al. 1995, Hunt and Scheibling 1997, Pedersen et al. 2008, Pineda et al. 2009, Valanko et al. 2010).

Based on estimations of gamete production, larval densities and abundance of post-larvae and adults in the field, the natural mortality of the larvae is extremely high (Rumrill 1990, Pedersen et al. 2008). During the time in the plankton, marine larvae are at great risk from various sources of natural mortality including insufficient or low quality food supply, predation, unfavourable environmental conditions and abnormal development (Thorson 1950, Rumrill 1990). Loss ratios between larval and post-larval life stages in invertebrates including bivalves are estimated to range from 85 to 98% (Pedersen et al. 2008). Thus, a reduction in

growth or developmental rates, which is often observed as a response to reduced pH conditions, makes the slowly growing larvae face a risk of increased mortality due to the longer time they are required to spend in the plankton. Juvenile mortality is known to be high in a majority of taxa with multiple life stages. The mortality of the early juvenile stages exceeded 90 % in 70 % of the compared studies that investigated juvenile mortality rates in bivalves, gastropods, barnacles, ascidians, bryozoans and echinoderms, and up to 98 % mortality was found in > 50 % of the species (Gosselin and Qian 1997). Pedersen et al. (2008) estimated the mortality of post-larval invertebrates to range from 71 to 85%.

The main study organism of this thesis, the infaunal bivalve *M. balthica*, spawns at water temperatures > 7°C and subsequent gonadal re-development continues into autumn or early winter (Caddy 1967). The planktonic life stage lasts ca. 6 weeks and ends when a sufficient size and developmental stage for metamorphosis has been reached (Caddy 1969), commonly at the size of 250-300 µm in the Baltic Sea (Ankar 1980, Elmgren et al. 1986, Olafsson 1989). During the pelagic larval phase, abundances >12 000 larvae/m³ have been measured in the Baltic Sea with a settling population of around 30 000/m², at peak settling even up to 300 000/m² (Elmgren et al. 1986, Bonsdorff et al. 1995). The high mortality from larval stage to adult stage of up to 97.8–99.5%, as reported by Pedersen et al. (2008), would translate to ca. 1000 individuals surviving to adulthood from a batch of 300 000 settlers (Bonsdorff et al. 1995).

M. balthica is experiencing different variable conditions throughout its life cycle. During the planktonic larval phase, it is exposed to large pelagic diel pH fluctuations (Jansson pers. obs., Almén et al. 2014). The peak settling in the northern parts of the Baltic Sea typically occurs in July, coinciding with high organic flux to the seafloor during the productive summer period (Ankar 1980, Bonsdorff et al. 1995). Settling individuals are thus exposed to a strongly reduced environment at the sediment surface, often involving low pH and low oxygen levels, especially at the benthic boundary layer where strong gradients exist (Gundersen and Jørgensen 1990).

1.6 Aims of this thesis

Ecosystems such as the Baltic Sea, where the communities are already exposed to large variability regarding a range of environmental parameters, are often expected to be less impacted by future changes, as the organisms are considered adapted to living in such variable conditions (Hofmann et al. 2010, Hendriks et

al. 2015). In the Baltic Sea, however, many species are occurring at the limit of their tolerance range regarding several environmental parameters (Segerstråle 1957, Bonsdorff 2006). Evidence exists that a change in an environmental parameter can increase the sensitivity of an organism to changes in other conditions (Pörtner 2008). Thus, an additional intensity in a stressor might have a strong impact on the survival of the species.

When the susceptibility of a species is assessed in a multi-stressor, changing ocean, different aspects of its development have to be considered (e.g. Byrne 2012). In *M. balthica* there are some critical steps in the process of becoming an established part of the population (adult). First of all, successful fertilization and the development of the embryos to the larval stage have to occur (this step not included in this thesis). Second, the tolerance and performance of the delicate early life stages including the survival and development of the larvae has to be maintained, together with the ability of the newly-hatched larvae to calcify their shells (**paper I**). Third, the successful gaining of sufficient mass and the building of shell structure for the subsequent settling to the sea-floor (**paper III**), followed by fast growth of the juvenile stages in the sedimentary system (**paper II**) has to take place in order for the bivalves to recruit to the adult population. Continued adult survival and adequate energy allocation (**paper IV**) is required for successful development of gametes, and a disturbance of this life stage might lead to potential transgenerational effects. Developmental or other failure, regardless of the life stage, will cause a decrease in the number of individuals proceeding to the next stage, with negative impacts on the maintenance of the population dynamics and structure (Hofmann et al. 2010).

The overall aim of this thesis was to develop an understanding of the effects of future CO₂ increase on the vulnerability of the Baltic Sea key species *M. balthica*, and how this is related to other effects of climate change, e.g. an increase in bottom-water hypoxia. Specifically, I investigated how different life stages of *M. balthica* are affected by decreasing pH. Survival, growth, behaviour and physiological responses were assessed in a combination of laboratory and mesocosm experiments.

The main questions explored by this thesis were:

1. The response of early larvae of *M. balthica* to increasing $p\text{CO}_2$.
2. The response of pelagic larvae and settling individuals of *M. balthica* to increasing $p\text{CO}_2$ in their natural surroundings.
3. Effects of multiple stressors on the early life stage of *M. balthica*: newly-settled juveniles in low pH and hypoxic conditions.
4. The impact of increasing $p\text{CO}_2$ on physiology and behaviour of adult *M. balthica*.

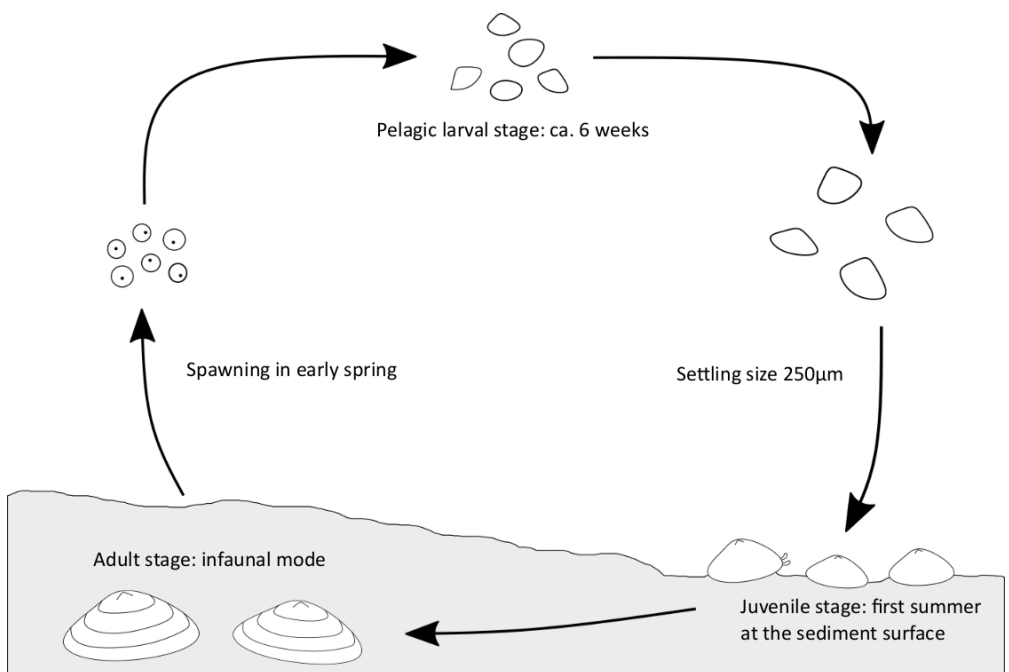


Fig 1. Life stages of *M. balthica*.

2. Materials and methods

2.1 The study system

The field measurements and the experimental work were conducted at the Tvärminne Zoological Station, Hanko, in the Southwest Finnish archipelago. The natural pH variability in the Baltic Sea is already large, and the highest seasonal pH differences are found in the northern areas of the Baltic Sea, such as the Gulf of Finland and the Bothnian Sea (Thomas and Schneider 1999). While these observations describe the highly fluctuating conditions of the Baltic Sea, they are traditionally based on monitoring data measured in the open sea areas, and do not give precise enough information on the conditions in the shallow coastal habitats. Moreover, current monitoring data is usually collected in the near-bottom water and not at the actual seafloor, most likely underestimating conditions encountered by the benthic communities. During peaks of high photosynthetic activity during phytoplankton blooms, the CO₂ concentration in the study area exhibits substantial diurnal variation resulting in high pH values of up to 8.6 during daytime and low pH values such as 7.5 due to respiration at night. Also, vertical variation in pH is large with a steep decrease with depth from ca. 8 in the surface to < 7.5 in the sea-floor. In addition, oxygen and therefore pH become reduced in the benthic boundary layer, so that in situ pH conditions relevant for the infaunal benthic organisms may be very low (Gundersen and Jørgensen 1990).

2.1.1 Quantification of natural pH fluctuations and local environmental conditions in the field

For better understanding of the local conditions in the micro-habitats where the macrofaunal communities of interest in this thesis reside (in the vicinity of Tvärminne Zoological Station), we conducted a field survey to quantify the fluctuating environmental conditions. The local pH conditions were characterized on diurnal to seasonal time scales at different types of key habitats; at one deep (ca. 35 m) soft-bottom site and four sheltered shallow (ca. 3.5 m each) habitats with varying sediment type and vegetation. The shallow sites consisted of a densely vegetated soft-sediment site where vegetation consisted mainly of *Myriophyllum* sp. and *Ceratophyllum* sp., an unvegetated sandy site, an unvegetated muddy site and a rocky bottom site with a dense cover of brown algae *Fucus vesiculosus*. All sites were visited on four different sampling periods

during April-September, at every sampling period on three different days with two day intervals, four times on each day (every six hours). A water sample was taken at the surface, and as close as possible to the bottom without disturbing the sediment, and temperature, pH, alkalinity, dissolved inorganic carbon, oxygen and salinity were determined from all water samples. Salinity (ca. 5) and oxygen (>10 mg/l) remained stable during the measurements, and only pH results are presented here. Also the faunal community was determined at each site by taking sediment and vegetation samples.

2.2 General experimental setup

The sensitivity of different *M. balthica* life stages to increased $p\text{CO}_2$ was tested in laboratory experiments (**papers I, II, IV**) and in mesocosms (**paper III**) by exposing the individuals to a range of pH levels over different time periods depending on the life stage in question. Given the large natural variability in the Baltic Sea pH, also a relatively low pH-level (7.1-7.2) outside of present natural variability was included in the treatments, in addition to pH 7.4, 7.7 and current average surface pH of ca. 8. **In papers I, II, and IV**, pH was manipulated in header tanks using a computerised control system (AquaMedic pH computer) attached to a CO_2 -gas bottle that released a suitable amount of pure gaseous CO_2 into the tank in order to reach and maintain a desired pH-level. From the header tanks the water flowed to the experimental containers. The appropriate parameters for determining the components of the carbonate system were measured regularly during the experiments (Dickson et al. 2007).

2.2.1 Early life stages in aquaria (papers I-II)

pH manipulation experiments on larval and newly settled juvenile (ca. 0.3 and 0.5 mm shell length) stages were conducted in bottles (3 l Erlenmeyer flasks) that received inflowing sea water from the header tanks where the mixing of water to each pH (and O_2 , hypoxic conditions induced using nitrogen gas, **paper II**) level was carried out. The organisms were fed regularly during the experiments with a mixture of algal species (5-10 μm) in high concentrations. Survival and growth parameters of the early life stages of bivalves were studied by studying mortality and measuring growth and changes in the shell structure using calcein staining. The experiments were run for 21 and 28 days in **paper I and II**, respectively.

2.2.2 Adult bivalves (paper IV)

pH manipulation experiments on adult bivalves were conducted in laboratory conditions. During 3 months, adult individuals were exposed to 4 pH-levels in tanks, where pH was manipulated as above. The organisms were kept in local sediment and were fed regularly during the experiment. Survival, condition, energetic status and species-specific activity of the bivalves were studied by measuring oxygen consumption, burrowing and cellular energy allocation (CEA; de Coen and Jansen 1997, Verslycke et al. 2004).

2.2.3 Mesocosms (paper III)

The survival, growth and subsequent settlement of early-stage *M. balthica* were studied while exposed to different levels of future $p\text{CO}_2$ in a mesocosm setting using 55m³ mesocosms encompassing the entire pelagic community. Bivalve larvae were collected from each mesocosm weekly and samples of settling juveniles were collected from settlement traps at the bottom of each mesocosm every second day. The 6 mesocosms comprised a large pH range, from 7.3 to the current pH of ca. 8, and included the whole planktonic community present in early summer when the experiment was initiated. In addition to the bivalve larvae development, the succession of the planktonic community was studied in different pH-levels, including the development of a phytoplankton bloom and changes in zooplankton and bacterial production (for a review of the studied parameters, see Paul et al. 2015). pH was manipulated at the start of the experiment to each target level by adding a suitable amount of CO₂ rich seawater to each mesocosm, after which the development of the pH dynamics was depending on the community response.

Table 1. Methods for data collection in the laboratory and mesocosm experiments.

Variable	Method	Paper I	Paper II	Paper III	Paper IV
Experimental Duration		21 days	29 days	45 days	87 days
Survival	Counts of living / dead individuals	x	x	x	x
Growth/size	Length measurements	x	x	x	
Settling	Settlement traps			x	
Burrowing	Burrowing tests				x
Oxygen consumption	Respiration measurements				x
Activity	Counts of moving		x		
Energy budget and allocation	CEA-measurements				x
Condition	Length/weight-ratios				x

2.3 Data analysis

Generalized linear mixed models (GLMM) (**papers I, II**), two-way ANOVAs (**papers II, IV**), chi-square tests (**papers I, III, IV**) and linear regression models (**paper III**) were used to analyse for differences on the investigated variables. The analyses were performed with the statistical software R (version 3.0.2; R Core Team 2013) and with SPSS 23.

GLMMs were used to study the relationship between larval survival, pH treatment and time (**paper I**), as well as the effects of time, pH and oxygen on the survival of juvenile bivalves (**paper II**). The impact of pH and oxygen level on the growth of juvenile *M. balthica* were analysed with ANOVA, and the differences in the activity of the individuals were assessed with two-way ANOVA (**paper II**). In **paper IV**, the influence of pH treatment and duration of the exposure on physiological and behavioural parameters (oxygen consumption, condition index and the different components of the energy budget) was tested

with a two-way ANOVA. Chi-square tests were used to analyse the size of the larvae in the different pH-treatments (**paper I**), the differences in post-larval settling between the mesocosms (**paper III**) and the burrowing performance of adult bivalves in different pH treatments (**paper IV**). For these analyses, the investigated parameters were divided into suitable classes (size, time). In the mesocosms, the sizes of both the larvae in the water column and the post-larvae in the settling traps in the different treatments were compared by linear regression models (**paper III**).

3. Results and discussion

3.1 Main findings of the thesis

In this thesis I show that reduced pH impacts different life stages of the bivalve *M. balthica* negatively (Fig. 2), based on findings both from aquarium experiments and in their natural surroundings in mesocosms. I observed impacts such as reduced growth (**paper I**), reduced survival (**papers I, IV**) and delayed settling (**paper III**) in the early life stages, whereas in adult *M. balthica*, significant reductions in the energy budgets as well as an increased energetic demand were found in reduced pH conditions (**paper IV**). Newly-settled *M. balthica* that were exposed to a combination of stressors reduced pH and low oxygen exhibited higher growth and survival rates when exposed to low oxygen conditions, whereas reduced pH alone had no impact (**paper III**).

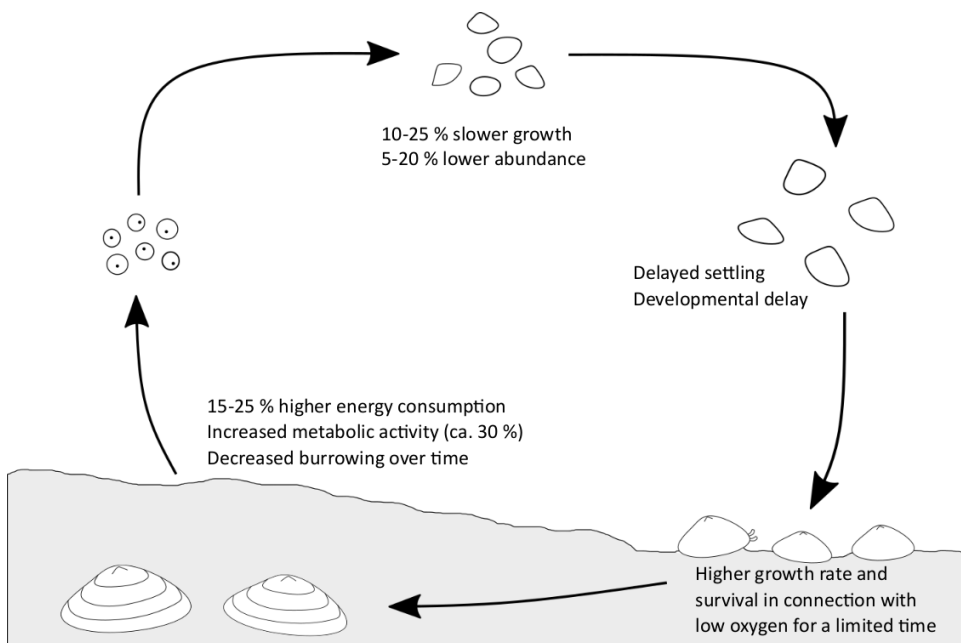


Fig 2. The main findings of the thesis. Observed impacts on different *M. balthica* life stages under different reduced pH conditions.

3.2 Natural pH conditions in the area

Different temporal pH patterns were found among the investigated sites (Fig. 3). In the surface waters, the pH variation was never larger than 0.2-0.3 pH units, with very slightly larger variations in April and in June when the primary production was the highest. Almen et al. (2014) have shown that along a larger depth range of ca. 30 m, a larger variation of > 0.5 pH units also occurs diurnally in the water column during the productive summer season. Measurements conducted in the seafloor of the deeper area (35 m) revealed a clear seasonal pH change in the bottom water with a decrease from 8.0 in June to 7.4 in July, whereafter it remained low. In both vegetated habitats, large pH variations (> 0.7 pH units) were regularly found in the bottom waters even diurnally; in the *F. vesiculosus* habitat in April, June and in September and in the vegetated habitat in July and September. Also in the muddy site, pH variations of over 0.5 units were measured diurnally. The large pH variability observed in some of the key habitats for the coastal invertebrate communities clearly highlights the inclusion of this characteristic in the improved design of future experiments testing the effects of reduced pH on coastal organisms.

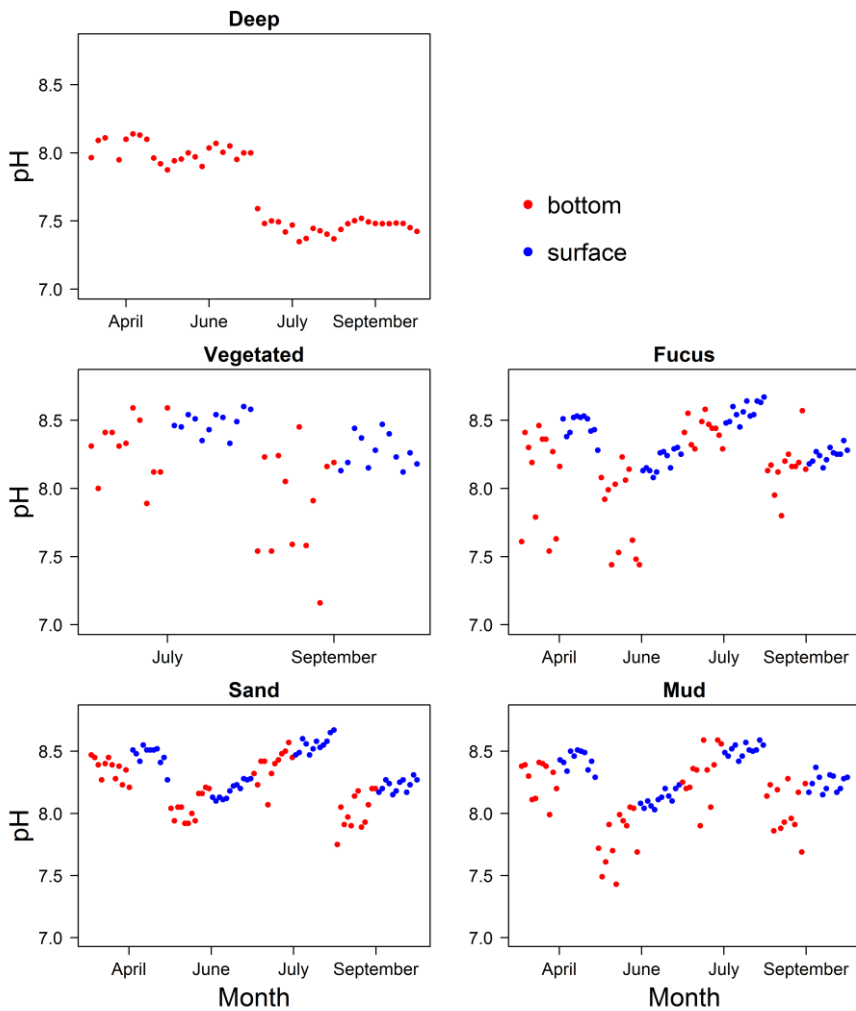


Fig 3. Natural pH fluctuations in key habitats in the area during the 5 most productive months of the year. In the vegetated habitat, measurements were conducted in July and September only.

3.3 The impact of reduced pH on different life stages of *M. balthica*

3.3.1 Newly-spawned larvae

During the exposure to different future pH levels, even a slight pH reduction was found to cause significant negative effects on the newly hatched larvae both by slowing growth and by decreasing survival (**paper I**). The newly hatched larvae of *M. balthica* grew 10-25 % slower in the reduced pH treatments 7.2, 7.4 and 7.7 than in the pH 8. During the experiment, 22% of the larvae grown in pH 8 reached the size of 250 μm that is considered indicative of imminent settling in our system, whereas in the reduced pH treatments the size of 250 μm was reached by 8%, 13% and 14% in 7.7, 7.2 and 7.4 pH, respectively. Significant pH-induced effects were also found on the abundance of *M. balthica* larvae over time (**paper I**). High mortality was measured in all treatments, which is natural for early life stages of invertebrates (Thorson 1950, Bonsdorff et al. 1995, Pedersen et al. 2008). Yet, at each sampling occasion during the 21 day experiment, the abundance of larvae was 15-20 % lower in all the reduced pH treatments than in the 8 pH treatment during the first days of the experiment, and ca. 5 % lower later on during the experiment (Fig. 4). During the planktonic phase, *M. balthica* larvae may encounter large pH fluctuations including very low pH values. Strong natural short-term pH fluctuations that occur in the Baltic Sea on a daily basis expose the bivalves to low pH levels already during early life stages. We detected a 70–85% drop in larval abundance after 8–10 days exposure to pH 7.2, but a similar sharp decrease in abundance in treatments 7.4 and 7.7 occurred later, after 10–12 days of exposure. This indicates that strong pH fluctuations might cause an immediate effect, i.e. sudden drops may be detrimental. To answer this question, however, there is a need to develop different experimental designs. Future ocean acidification will potentially exacerbate this by making the amplitude and frequency of the fluctuations even higher (Thomas and Schneider 1999, Melzner et al. 2012).

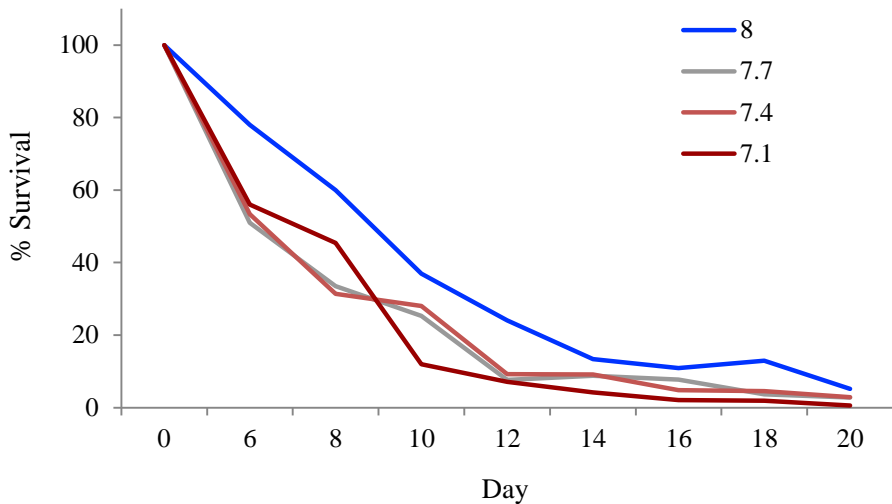


Fig 4. Larval abundance in the different pH treatments during the 21 day experiment (**paper I**)

3.3.2 Settling larvae and post-settlement juveniles

Similar responses to reduced pH as observed for the newly hatched larvae were found in a mesocosm experiment; in a system that better resembles the natural surroundings of the bivalves by incorporating several biotic interactions, trophic levels and potentially a larger diversity of food sources (**paper III**). When looking at the survival, growth and subsequent settlement of *M. balthica* larvae while being exposed to a range of future $p\text{CO}_2$ levels, we found that both the required size for settlement and the time to settlement increased along the increasing $p\text{CO}_2$ gradient, suggesting a developmental delay. An earlier settlement was found in the 319-469 $p\text{CO}_2$ mesocosms with 54-74 % of the individuals settling during the first 13 days, whereas in the 857 and 1072 $p\text{CO}_2$ mesocosms a smaller settling event occurred on days 13–15, and in the highest $p\text{CO}_2$ mesocosm (1347 μatm) the settling peaked on day 17 (Fig. 5).

Already during the planktonic larval stage, *M. balthica* start to incorporate the calcium carbonate shell and successful settling is related to their capacity to achieve high enough specific gravity to settle on the seafloor. In addition, the initial growth rate after settlement is of crucial importance for survival. Therefore the settling bivalves are likely to be vulnerable to future ocean acidification

conditions during this critical point of the life cycle, with subsequent implications for recruitment and recovery of disturbed habitats, as observed also in this study where the development seems to have been delayed in the high $p\text{CO}_2$ treatments. After 10 days of exposure to different $p\text{CO}_2$ levels, the average size of the larvae in the water column in the mesocosms increased significantly along the increasing $p\text{CO}_2$ gradient, with ca. 10 % larger larvae present in the highest $p\text{CO}_2$ mesocosms ($>1000 \mu\text{atm}$) than in the lowest $p\text{CO}_2$ mesocosms (ca. $320 \mu\text{atm}$) (**paper III**). Thus despite being relatively large in the high $p\text{CO}_2$, a large part of the bivalves remained in the upper water column without initiating settlement, whereas in the low $p\text{CO}_2$ the bivalves settled as expected, with only the small larvae remaining in the water column. Similar patterns have been found in other studies that have found the settling of post-larvae of a range of benthic organisms to be impacted by the changes in the water chemistry created by $p\text{CO}_2$ increase (Green et al. 2004, Cigliano et al. 2010, Clements and Hunt 2014). For example in polychaetes, gastropods and bivalves the larval recruitment was reduced in response to a $p\text{CO}_2$ increase from current to high levels (from ca. 900 ppm to up to 5000 ppm) at an underwater vent system (Cigliano et al. 2010).

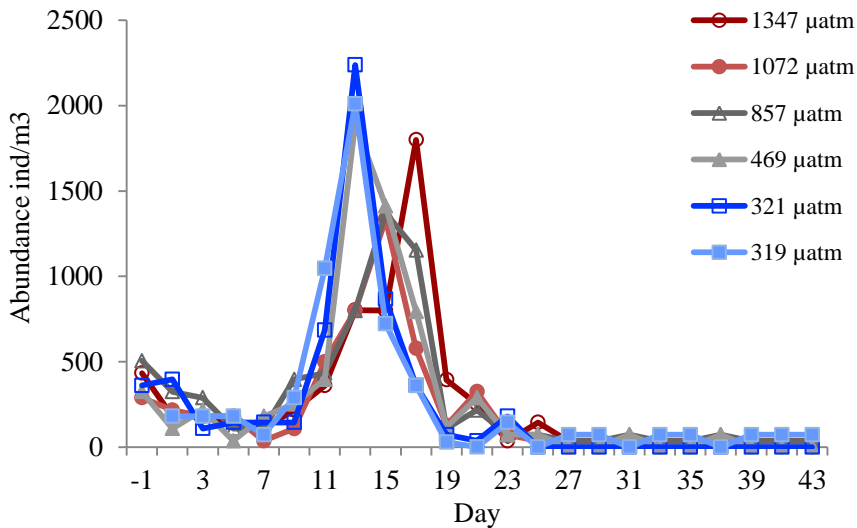


Fig 5. The abundance of settled individuals per cubic metre water mass enclosed in the different mesocosms over the course of the experiment (**paper III**).

3.3.3 Juveniles

The newly-settled juvenile *M. balthica* were exposed to conditions regularly occurring in their natural habitats; a combination of low oxygen concentration and reduced pH level. The pH-induced effects on the growth, survival and activity were negligible, whereas unexpected effects in combination with low oxygen conditions were found. The juveniles exhibited 2–2.4 times higher growth (Fig. 6) and 10–20 % higher survival rates at both treatments with moderately low oxygen conditions (3 mg/l) compared to high oxygen conditions, also in combination with pH 7.4. Reduced pH alone had no effect on the survival of juvenile *M. balthica* (**paper II**).

Benthic stages of *M. balthica* regularly experience low oxygen and low pH in tandem, both in the benthic boundary layer and even more so within sediments. *M. balthica* larval settlement occurs in late summer in Northern Europe, and coincides with high organic flux to the seabed. Settling individuals are thus exposed to a strongly reduced environment at the sediment surface, often involving low pH and low oxygen levels, especially in the benthic boundary layer where gradients in oxygen are strong and where oxygen deficiency may occur close to the sediment surface even in normoxic areas (Gundersen and Jørgensen 1990). They might thus have mechanisms to cope with these conditions for a certain time as is known for hypoxia alone (Theede 1973, Diaz and Rosenberg 1995, Burnett 1997, Morley et al. 2007). However, reduced pH seemed to exacerbate the coping mechanisms even further. Yet, it is well known that prolonged or frequently repeated hypoxic conditions severely degrade several aspects of benthic communities such as species richness, abundance and biomass (Modig and Ólafsson 1998, Norkko and Bonsdorff 1996, Villnäs et al. 2012). This shows the need to carefully consider all relevant parameters such as adequate experimental duration and potentially pre-exposing conditions, thus take the niche of the organism into account when designing an experiment.

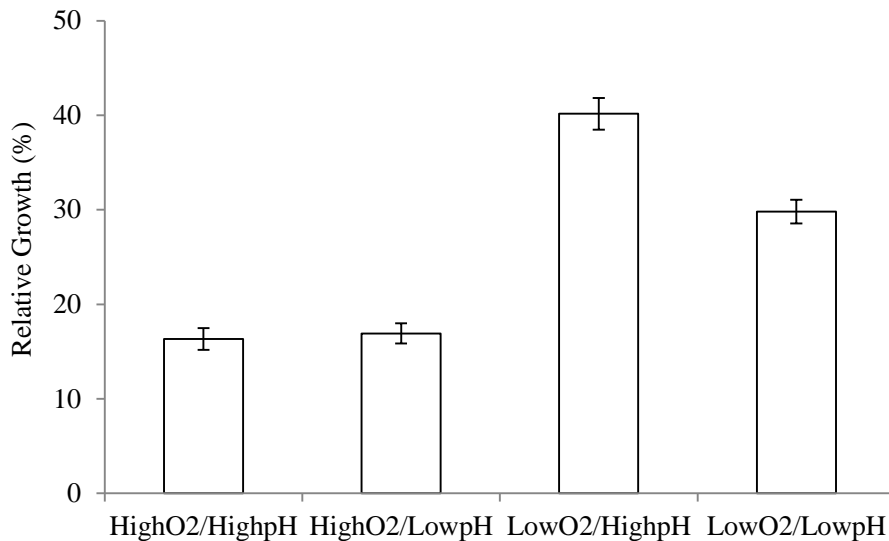


Fig 6. Relative growth in different treatments at the end of the 29 day-experiment (mean \pm SD; **paper II**).

3.3.4 Adults

In order to examine the effects of reduced pH on the adult individuals of *M. balthica*, the energy allocation measurements of the bivalves were studied together with other physiological and behavioural parameters under different pH conditions. The bivalves were adversely affected when exposed to a range of reduced pH from 7.1 to 7.7 for three months. Significantly increased energetic demand and reductions in the cellular energy allocation (CEA-index) were found in the treatments with reduced pH. After three months of exposure, the energy consumption was 15-25 % higher in the reduced pH treatments than in the pH 8 (Fig. 7), and correspondingly, the CEA was higher in the pH 8 than in the reduced pH treatments (on average 28 %). In addition, oxygen consumption (indicating metabolic activity), was found to be ca. 30 % higher in all the reduced pH treatments compared to pH 8 after two months of exposure, whereafter it largely decreased in all reduced pH treatments (**paper IV**).

As is becoming more and more the consensus within the ocean acidification field, the impacts of reduced pH are largely occurring through altered energy allocation requirements, with more energy needed for the maintenance processes of physiological balance (Wood et al. 2008, Thomsen and Melzner 2010, Stumpff

et al. 2011, Pan et al. 2015). Thus, a sufficient energy level and allocation is considered a key to withstanding the stress caused by reduced pH conditions. For example, the increased energy demands of higher metabolic rate can be compensated for in our system with abundant food during the productive season. If food availability (quality or quantity) decreases or is naturally lower such as in the winter, impacts of pH decrease could be larger.

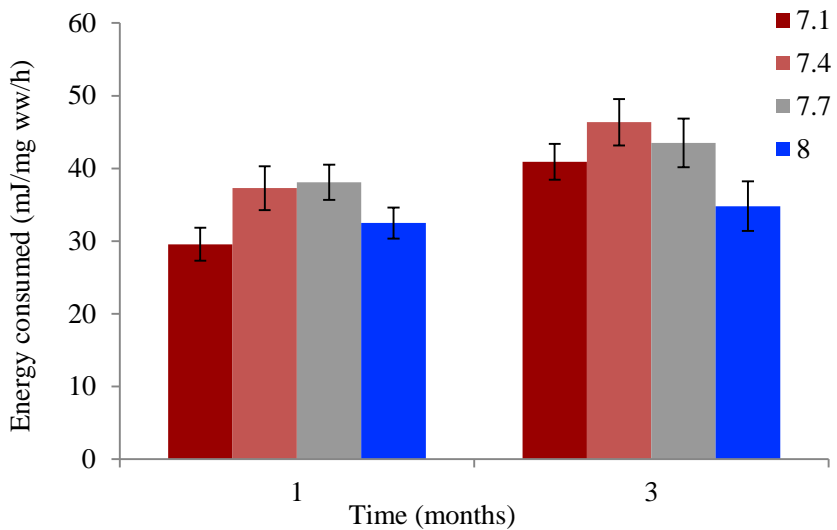


Fig 7. Energy consumption (mJ/mg ww/h) in the different pH treatments during the three month experiment (mean \pm SE, n=10; **paper IV**).

4. Implications and future research directions

4.1 The implications; future impacts on this *M. balthica* population

In this thesis, I found negative impacts of reduced pH on different life stages of *M. balthica*, despite the fact that the species could be assumed to be well-adapted to living in the highly variable conditions of the Baltic Sea. Increased larval mortality of even just 5 % as found in reduced pH conditions in **paper I** has the potential to translate into large impacts on adult population of *M. balthica*, as the natural larval mortality is already extremely high (Bonsdorff et al. 1995). Also, the slow growth and delayed development of larval stages in reduced pH conditions found in **papers I and III** are of great concern, as slowly growing larvae face a risk of increased daily mortality and higher predation risk in response to the longer time they are required to spend in the plankton (Rumrill 1990), leading to delayed and decreased abundance of settling individuals. Thus, even a slight change in their survivorship and performance may have long-lasting consequences as the population dynamics of a bivalve species is largely dependent on successful settlement and recruitment of the post-larvae (Pedersen et al 2008). Even if the juvenile and adult *M. balthica* were not found to be at imminent risk of declining due to direct effects of reduced pH (**papers II and IV**), even subtle changes in organisms' energy budgets, as found in **paper IV**, can affect the future resilience of the population. As more energy is required in reduced pH conditions for processes such as maintenance of the basal metabolism and regulating internal pH balance, or for behaviour such as burrowing, less energy remains available for other functions such as re-building of gonads for the next reproductive period. Thus, a disturbance of this life stage might lead to potential transgenerational effects (Parker et al. 2012).

M. balthica occurs in the Baltic Sea in an environment where, due to the severe eutrophication, the food supply to the benthic macrofauna is high during the highly productive spring and summer (substantially decreases for autumn and winter) (Tallberg and Heiskanen 1998), thus the organisms are possibly able to resist the negative effects of reduced pH better than in an environment with low food supply. If the quality or quantity of this major food supply to the benthic system changes due to global change (e.g. Hare et al. 2007, Rossoll et al. 2012, Schulz et al. 2013), it would likely have consequences to the energy supply to the organisms and to their capability of maintaining the required energy level. Also, due to the sporadic food supply, the responses of the bivalves might be highly

seasonal. These kinds of issues further add to the difficulties involved in up-scaling from species- and population level studies to community and ecosystem level.

In the Baltic Sea, *M. balthica* populations are in many areas severely disturbed by regularly occurring anoxic and hypoxic conditions. In these areas where *M. balthica* has decreased substantially, the functioning of the sedimentary system has been found to be disturbed (Norkko et al. 2013, Norkko et al. 2015). The loss of key species in sediment communities can potentially alter sediment biogeochemical processes and also lead to the reduction of structural complexity and hence habitat for associated organisms. The decrease in degradation of organic matter in sediments due to the reduction of benthic key players can directly affect the accumulation of organic carbon and cause the possible emergence of anoxic regions, with a rapid increase in the carbon flux to the sediments (Blackford and Gilbert 2007). Future survival of key species, such as *M. balthica* is essential for maintaining important sediment ecosystem functions, such as sediment cycling of oxygen and nutrients (Villnäs et al. 2012, van Colen et al. 2012) and secondary biomass production (Timmermann et al. 2012). The maintenance of such functions is of uttermost importance in disturbance-prone areas like the Baltic Sea. In the Baltic Sea, the pH conditions that are predicted for the future oceans, in combination with other stressors (e.g. low oxygen) are already regularly occurring. The system might thus work as an analogue for studying future ocean acidification scenarios.

4.2 Life in a changing ocean

Estimating and modelling the effects of ocean acidification and climate change on pelagic and benthic communities is exceedingly difficult. Ocean acidification rarely acts alone, but simultaneously in different combinations with other global change stressors. It exerts different impacts on different species, their life stages and species-specific processes with varying intensity and direction, potentially having consequences for biotic interactions. Many coastal systems are also highly variable in their natural state (Hofmann et al. 2011, Duarte et al. 2013, Waldbusser and Salisbury 2014), and with future climate change and ocean acidification, the variations in the natural conditions are expected to be magnified, and the timing of the occurrences is shifting (Doney et al. 2012). Other factors, which make the systems somewhat unpredictable for modelling are the uncertainties of how environmental parameters will change in the future. For example, predictions on temperature increase are considered relatively robust,

whereas there is a higher uncertainty regarding changes in precipitation and circulation patterns (Drinkwater et al. 2010). No ecosystem can, however, be expected to go through global climate change without some alterations.

In a changing world, the success of organisms is defined by their abilities to react by modifying their physiological and behavioural responses to match the changing conditions. Organisms usually respond to changes in their abiotic and biotic environment by altering behaviour, physiology or state. If the new conditions remain tolerable, acclimatization by plastic modifications can occur, which potentially, over a longer time period, leads to adaptation, i.e. the adjustment to environmental change between generations occurring through natural selection of individuals tolerant to new conditions (e.g. Somero et al. 2010, Sunday et al. 2014). On the other hand, if the conditions are unfavourable for acclimatization, migration (by individuals or populations), shifts in phenology or death and local extinction are possible outcomes for the organism. To determine the state of the future oceans, we need to know how species will respond to the changes, and how this in turn will impact the interactions of species within a community. By investigating to which extent species can respond to novel (additional) changes in their environment, we might be able to better predict which ones can become adapted in the future, and which ones will decline (Sunday et al. 2014, Calosi et al. 2016).

In environments that experience large spatial and temporal variability of different conditions, such as in the highly fluctuating Baltic Sea, the organisms are assumed being adapted to living in changing conditions. A wide range of mechanisms for coping with these types of stressful environments have indeed been evolved. The modifications of organisms to increase their survival in adverse conditions include e.g. changes in metabolic strategies (Burnett 1997, Pörtner et al. 2004, Morley et al. 2007). Low pH conditions regularly occur in the benthic realm, and e.g. calcifying organisms have evolved a number of mechanisms to increase their calcification capacity (Waldbusser et al. 2011, Hendriks et al. 2015). Regulating the conditions in the calcifying environment in order to alter the internal pH of the calcifying compartment is a necessary modification in response to decreasing and/or fluctuating pH level. Examples of such mechanisms are buffering of the intra- or extracellular fluids to avoid decreasing pH at calcification sites (Gutowska et al. 2010, Stumpp et al. 2012). Some species such as scleractinian corals actively manipulate the pH of the calcifying compartment by ion pumps, shifting the equilibrium composition of the fluid so that the effects of reduced carbonate saturation in seawater can be counteracted (e.g. Cohen et al. 2009). These modifications work as a buffer

against decreasing pH and/or oxygen conditions, but at an energetic cost (Pörtner et al. 2004, Stump et al. 2012), and other environmental factors such as food availability or the simultaneous pressure from other stressors play a role in determining the success of the coping mechanism. For example Thomsen et al. (2010) have showed that abundant bivalve communities flourish even in highly stressed systems when food supply is high.

In the Baltic Sea, it has been estimated that due to warming, new species requiring warmer waters might gain importance (Holopainen et al. 2016). The decreasing ice-cover can have implications for the spring plankton dynamics, leading to reduced quantity of food for zooplankton, and potentially changing the food quality and quantity for higher trophic levels (Leppäranta and Myrberg 2009). Also due to the warming temperatures, the timing of the temperature-induced spawning of invertebrate larvae in species such as *M. balthica* might be shifting, whereas the onset of annual spring primary production, the main food source for the developing organisms, might not shift. This would have consequences on the food quantity available for the developing larvae, and to the higher trophic levels through trophic cascade (e.g. Doney et al. 2012). Thus, the changing phenologies might have implications such as reduced food availability for organisms, whose maintenance of development and growth is highly dependent on the sufficient energy level, especially when exposed to changing environmental conditions such as reduced pH (**paper IV**, Pan et al. 2015). In the 45-day mesocosm study (**paper III**), no such change in phytoplankton community composition was observed (Bermúdez et al. 2016, Paul et al. 2015). However, the experiment was conducted in summer and the 45-day duration of the experiment is not likely to reflect future OA over decades; over real timescales it is almost certain that lower pH will have an effect on the phytoplankton communities, in spring as well as in summer.

pH-induced shifts in communities can already be seen in areas such as underwater volcanic systems, where changes in community composition such as reduction of bivalve species replaced with non-calcifying algal species occur along a gradient of pH exposure (Hall-Spencer et al. 2008). OA has also the potential to change habitat complexity (Fabricius et al. 2014), reduce biodiversity (Christen et al, 2013, Sunday et al. 2016) and cause ecosystem shifts via mechanisms such as altered competitive interactions and simplification of ecosystems (Connell et al. 2013, Kroeker et al. 2013). Thus the disturbances of current biological interactions have the potential to lead to changes in community composition and structure and loss of functionality, also in species-poor systems such as the Baltic Sea where not many substituting species exist.

4.3 Challenges of the ocean acidification research

The aquarium experiments have received critique for being unrealistic as they are mostly static, rarely include natural fluctuations of multiple parameters (Gobler and Talmage 2013), and are unable to include enough relevant parameters while mostly focusing on single-species and single stressors, out of natural context (Cornwall and Hurd 2015, Riebesell and Gattuso 2015, McElhany 2016). In addition, they are, usually by logistical necessity, very short-term in relation to the timescales over which ocean acidification occurs and induces changes in community structure and function, or in physiological adaptations (Sunday et al. 2014). Small-scale experiments do not, naturally, offer a realistic picture of the long-term and large-scale responses of patterns and processes occurring and impacting the communities and ecosystems. Contrary to the patterns observed in the field, small-scale experiments can, however, reveal mechanisms behind species responses to stressors (Dupont and Pörtner 2013).

For designing realistic experiments, the inclusion of locally important parameters and levels of stressors is essential. Most systems experience some degree of fluctuation of the abiotic conditions (Hofmann et al. 2010). The Baltic Sea is an example of a highly fluctuating system with diurnally, seasonally and also spatially large pH fluctuations (Fig. 3). Consequently, the selected treatments should be chosen based on the local conditions, including low pH levels in systems that experience a large range of pH fluctuations, preferably including the natural variability of the system, the impact of which is still poorly understood. The communities are also rarely exposed to single stressors, but face the challenge of coping with multiple stressors which impact with different intensity and magnitude, and exert different impacts on different components of the community. Even within a single species, the response to stressors may be context dependent, changing with different physical environmental conditions. This further complicates the scaling up of the existing knowledge to a broader level (Riebesell and Gattuso 2015).

Most empirical evidence of the effects of OA still comes from relatively short-term experiments and little is known about organisms' responses to long-term exposure to declining pH, and even less of their capacity to adapt (Riebesell and Gattuso 2015). Yet, for adult life stages, longer experimental durations are often needed to reveal effects of reduced pH (**paper IV**). Long-term exposure of adults may also have indirect impacts such as carry-over effects on future larval performance (Parker et al. 2012). Adaptation potential is not yet widely studied in the field of ocean acidification, however, if we are to identify which species, populations and assemblages are likely to persist through global change, and

which ones are more likely to go extinct, the investigation of populations' and species' ability to respond to environmental changes with plastic and adaptive mechanisms has an absolute priority (Calosi et al. 2016). Some studies show that in certain phytoplankton groups adaptation can take place relatively fast, after only a few hundred generations (about 6 to 12 months), and can potentially compensate for the negative effects of reduced pH (Lohbeck et al. 2012). A high capacity for adaptation has also been found in other taxa (Sunday et al. 2011, Kelly et al. 2013), but it still remains open if adaptation can occur fast enough to compensate for the negative impacts of decreased pH to a degree that allows the future maintenance of populations and communities (Sunday et al. 2014).

4.4 Future research priorities

For acquiring a more realistic view of the impact of a stressor on e.g. biological interactions, experiments should be conducted on whole communities, or at least a large amount of species and/or functions would need to be included in the experimental setting (Riebesell and Gattuso 2015). In addition to field studies conducted along natural gradients of the stressor or at sites of differing conditions, mesocosms can contribute to obtaining a more complete picture, although they also have constraints such as limited replication and exclusion of relevant parameters (predatory interactions, natural currents; **paper III**). Other approaches would be to conduct experiments (laboratory, or field if possible) on intact communities; benthic communities, for example, could be studied in incubation chambers, the main aim of this approach being to study multi-species assemblages, which would include the majority of the relevant functional groups. Thus, a diversity of approaches is needed to understand the impacts of ocean acidification at the level of communities and ecosystems (Riebesell and Gattuso 2015). Conducting all these types of experiments is practically impossible due to logistic or financial reasons, thus approaches allowing better comparisons of studies have been developed.

New types of attempts including research frameworks and modelling developments have been suggested to identify commonalities and unifying mechanisms and concepts, ultimately enabling a better comparison between studies. In this way the detailed, species-level data could be better integrated and used in ecosystem-level applications (Dupont and Pörtner 2013, Queiros et al. 2014, Griffen et al. 2016). Energy allocation could function as one such unifying mechanism, as all the responses of the species and communities to reduced pH ultimately depend on the changes in the energy budget and allocation (**paper**

IV). By focusing on key species (Boyd 2013), or species responsible for a major function in the community, this type of approach could reveal results beyond mere future species distributions or abundances (Queiros et al. 2014), which itself is already more than simple experiments can account for. The low-diverse Baltic Sea and its benthic communities would make a good study system for such, as the species and their functions are relatively few compared to the oceanic counterparts.

4.5 Management and mitigation of OA

To respond to the urgent demand for acquiring as reliable and realistic view as possible of the changing world, certain factors need to be accounted for when appropriate management is to be considered. Generally, more targeted research is still needed; the identification of the type of knowledge necessary to answer the remaining pressing questions is required. Climate change will impact the world's ecosystems on long time-scales. In the meantime the ecosystems are changing; some species will become adapted whereas others disappear (re-locate, become extinct), so communities undergo continuous, gradual change, and new ecosystems might emerge as a result. Thus, when planning for management practices, the assumption of a "steady-state ecosystem" cannot be applied (Jutterström et al. 2014). Before appropriate management practices can be implemented, a good estimate of the local conditions and of the future development has to be acquired, and management strategies should remain step ahead. Measures suggested as localized mitigation possibilities such as "artificial ocean alkalisation" (Ilyina et al. 2013), conserving and restoring ecosystems such as salt marshes, mangroves and sea grass meadows to capture CO₂ ("blue carbon"-method), as well as reducing stressors such as nutrient load and overfishing in order to improve the overall state of the ecosystem are ways to locally reduce CO₂ levels and increase the systems' resilience towards changes. Local (small-scale) strategies are, however, only ways to protect the systems from more damage ("buying time"), while the cause of the problem is global atmospheric CO₂ emissions, and a reduction of emissions is the level where the problem should be solved.

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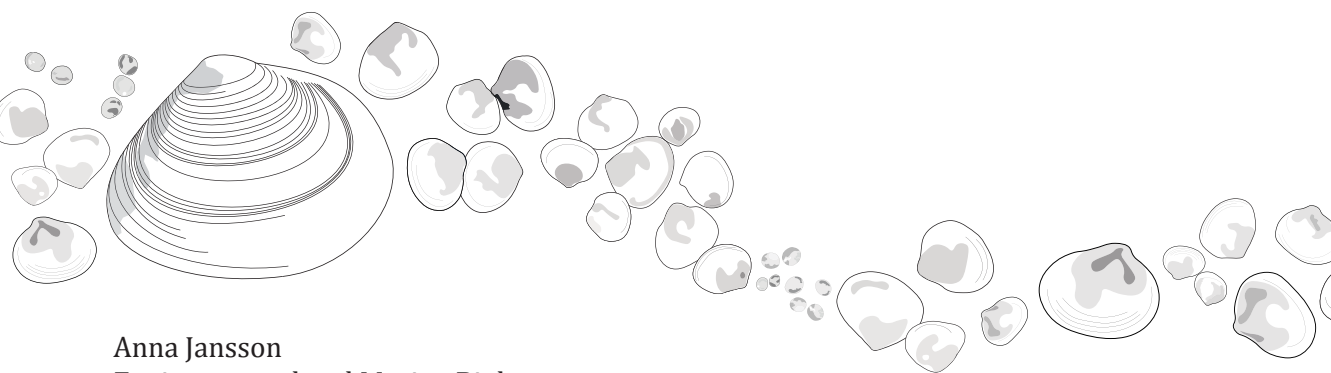
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