Copepods in a changing sea: Ocean acidification, long-term changes and short-term variability

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To Evelyn

Abstract

The rapid increase in atmospheric carbon dioxide is causing global warming but also changes in the ocean chemistry. When carbon dioxide dissolves in seawater it changes the carbonate chemistry towards lower levels of carbonate minerals and lowers pH, i.e., the water becomes less basic, a process called ocean acidification. This can have detrimental effects on many marine organisms. In eutrophic areas, such as the Baltic Sea, acidification is also caused by decaying organic matter as bacterial remineralisation releases carbon dioxide. Naturally, there are fluctuations in temperature, pH, and oxygen both with time and depth. In this thesis, I show that copepod zooplankton experience large daily fluctuations in temperature, oxygen and pH in a coastal pelagic area due to variability in physicochemical variables with time and depth and through their daily migrations in the water column. Further, I analysed monitoring data, and looked at the wintertime changes in pH, temperature and salinity in the Gulf of Finland during the past 36 years and found that all variables have changed. Sea temperature has increased and pH and salinity decreased.

Copepods are a vital part of the food chain as food for fish, and even small changes in their abundance could have far-reaching effects on the ecosystem. I studied the responses of two calanoid copepods, *Eurytemora affinis* and *Acartia* sp. to increased carbon dioxide and lowered pH in laboratory-based experiments using water and copepods from large mesocosms deployed in a coastal pelagic area in south-western Finland. *E. affinis* offspring production remained unaffected by carbon dioxide, whereas both food quantity in terms of phytoplankton availability and quality, as well as essential fatty acids of the copepod mothers, seemed to be important in regulating offspring production. Adult body size of *Acartia* sp. was reduced in high fCO_2, corresponding values projected for the end of the century, which is alarming considering the negative effect of warming on copepod body size. An egg-transplant experiment with *Acartia* sp. revealed maternal effects, however, the maternal effects could only compensate for negative effects of intermediate levels of fCO_2.

Based on the results in this thesis I conclude that although copepods are adapted to low pH on shorter time scales, there is a risk of negative effects of ocean acidification. Further, I discuss the secondary causes of acidification, and the importance of monitoring and management to reduce the negative effects on ecosystems.

Key words: ocean acidification, climate change, Baltic Sea, copepod, pH, carbon dioxide, mesocosm

Sammanfattning

Koldioxidhalten i atmosfären ökar snabbt vilket leder till global uppvärmning. Haven tar upp stora delar av både värmen och koldioxiden. Då koldioxiden löses i havsvattnet sjunker pH, dvs. vattnet blir mindre basiskt och halten karbonatjoner minskar. Den här processen kallas havsförsurning. Havsförsurningen framskrider snabbt och påverkar många vattenlevande organismer och utgör, tillsammans med uppvärmningen, ett hot mot ekosystemen. I övergödda områden bidrar också andra mekanismer till försurningen. Då organiskt material från tex algblomningar bryts ner av bakterier, avges koldioxid. Temperatur, syre och pH varierar naturligt både över tid och med djupet i havet. I den här avhandlingen beskriver jag de variationer i olika fysikalisk-kemiska variabler som hoppkräftor upplever i sin naturliga miljö nära kusten och visar på att de genom sin dygnsvandring mellan yta och botten upplever stora förändringar. Jag har också använt långtidsdata och undersökt hur temperatur, pH och salthalt förändrats i Finska viken under de senaste 36 vintrarna. Resultaten visar att havet blivit varmare emedan pH och salthalten minskat.

Hoppkräftor är en oersättlig del av födokedjan i haven, eftersom de utgör föda för fisk. Redan små förändringar i hoppkräftornas förekomst kan ha allvarliga effekter för ekosystemet. Jag har undersökt hur två arter av hoppkräftor, *Eurytemora affinis* och *Acartia* sp., påverkas av förhöjd koldioxidhalt och sänkt pH i experiment. Jag deltog i ett internationellt samarbete där havsförsurning simulerades i stora säckar i havet (mesokosmer) i ett kustnära område i sydvästra Finland. Produktionen av avkomma hos *E. affinis* påverkades inte till följd av försurningen, medan både mängden föda (växtplankton biomassa) och födokvaliteten (essentiella fettsyror och artsammansättning) påverkade produktionen av avkomma. Resultaten visar att vuxna individer av *Acartia* sp. hade mindre kroppsstorlek i hög koldioxid, vilket är alarmerande eftersom kroppsstorlek hos hoppkräftor även minskar med ökad temperatur. Ägg från *Acartia* sp. kläcktes i högre utsträckning i samma vatten som honorna vistats i än i kontrollvatten, men endast om modern inkuberats i vatten med medelhöga koldioxidnivåer. Resultatet tyder på att maternella effekter inte kunde kompensera för de negativa effekterna av havsförsurning motsvarande koldioxidhalten i havet år 2100.

Baserat på resultaten i den här avhandlingen drar jag slutsatsen att det finns en risk för att havsförsurningen har negativa effekter på hoppkräftorna, trots att de i sin naturliga miljö utsätts för stora variationer i pH. Vidare diskuterar jag de andra orsakerna till havsförsurningen och åtgärder som kunde vidtas för att begränsa den lokala belastningen på ekosystemen.

Nyckelord: havsförsurning, klimatförändring, Östersjön, hoppkräfta, koldioxid, pH, mesokosm

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List of original publications

I. **Almén**, **A.-K.**, Glippa, O., Pettersson, H., Alenius, P., Engström-Öst, J. (2017) Changes in wintertime pH and hydrography of the Gulf of Finland (Baltic Sea) with focus on depth layers. *Environmental Monitoring and Assessment*, 189: 147.

II. **Almén**, **A.-K**., Vehmaa, A., Brutemark, A., Engström-Öst, J. (2014) Coping with climate change? Copepods experience drastic variations in their physicochemical environment on a diurnal basis. *Journal of Experimental Marine Biology and Ecology*, 460: 120–128.

III. **Almén**, **A.-K**., Vehmaa, A., Brutemark, A., Bach, L., Lischka, S., Stuhr, A., Furuhagen, S., Paul, A., Bermúdez, J.R., Riebesell, U., Engström-Öst, J. (2016) Negligible effects of ocean acidification on *Eurytemora affinis* (Copepoda) offspring production. *Biogeosciences*, 13: 1037–1048.

IV. Vehmaa, A., **Almén**, **A.-K.**, Brutemark, A., Paul, A., Riebesell, U., Furuhagen, S., Engström-Öst, J. (2016) Ocean acidification challenges copepod phenotypic plasticity. *Biogeosciences*, 13: 6171–6182.

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Contributions[1]

I. Planned the study: JEÖ, OG, **A-KA**, HP, PA. Performed the analyses: OG, **A-KA**. Wrote the manuscript: **A-KA**, OG, JE-Ö, HP, PA.

II. Conceived and designed the experiment: **A-KA**, AB, JEÖ. Performed the study: **A-KA**, JE-Ö, AB, AV. Analysed the samples: **A-KA**. Statistical analyses: **A-KA**, AV. Wrote the manuscript: **A-KA**, JE-Ö, AB, AV.

III. Conceived and designed the experiment: JE-Ö, AB, **A-KA**, AV. Performed the experiment: **A-KA**, AV, JE-Ö, AB. Analysed the samples: **A-KA**, AV, SL, AS, JRB, LB, SF. Wrote the manuscript: **A-KA**, AV, JE-Ö, AB with help from the co-authors. Project coordinator: UR.

IV. Conceived and designed the experiment: AV. Performed the experiment: AV, **A-KA**, JE-Ö, AB. Analysed the samples and performed the statistical analyses: AV. Analysed TPC and C:N: AP. Analysed ORAC: SF. Shared the responsibility of writing the manuscript: AV and **A-KA,** with help from the co-authors. Project coordinator: UR.

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1 Introduction

1.1 Climate change and ocean acidification

Anthropogenic emissions have dramatically increased the levels of carbon dioxide (CO_2) in the atmosphere since pre-industrial time (IPCC, 2013, Le Quéré, 2015). The oceans and enclosed seas moderate climate change by absorbing additional heat and CO_2 (Gattuso et al., 2015). This comes with a great cost. When carbon dioxide dissolves in seawater, it changes the carbonate chemistry and lowers the pH (Caldeira and Wickett, 2003, Doney et al., 2009). The process is termed “ocean acidification” (OA), although surface waters remain alkaline. The simplified chemical reaction (Fig. 1) can be explained as follows: CO_2 reacts with water, forming carbonic acid that rapidly dissociates into bicarbonate and hydrogen ions. Carbonate reacts with hydrogen ions and form more bicarbonate. The concentration of hydrogen, bicarbonate and dissolved CO_2 is increasing, whereas the concentration of carbonate ions decreases. So far, the atmospheric pCO$_2$ has increased from 280 to 400 µatm and average ocean pH has decreased by 0.1 units (Le Quéré, 2015). The conditions in the oceans have already changed more than during the glacial-interglacial cycles (Pelejero et al., 2010). The rate of change is 100 times faster than during the last 50 million years (Caldeira and Wickett, 2003, IPCC, 2013), and too fast for weathering to have a significant impact on the saturation state of carbonate minerals. In today's oceans, the carbonate mineral saturation horizon is shoaling (Doney et al. 2009) and some areas, such as the Baltic Sea surface waters, will be under-saturated within a few decades (Orr, 2011). Under-saturation of calcium carbonate minerals, such as calcite and aragonite, has detrimental effects on especially corals, coccolithophores, pelagic molluscs such as pteropods, and shell-building organisms (Fabry et al., 2008, Doney et al., 2009, Kroeker et al., 2013).

Many physiological processes and intracellular enzymes are pH sensitive. CO_2 diffuses through the body, reacts with internal body fluids, causing H^+ to increase inside the body and pH to drop. The regulation of internal pH increases the energy demand and respiration and can reduce the scope for growth (Pörtner et al., 2005, Pedersen et al., 2014a). Increased pCO$_2$ and lowered pH can induce effects on many different traits, such as survival, reproduction, metabolism, development (Fabry et al., 2008, Kroeker et al., 2013) and body size (Fitzer et al., 2012). Behavioural changes, such as reduced activity and impaired escape ability have been reported for molluscs, crustaceans and fish (Briffa et al., 2012, Jutfelt et al., 2013).

Copepods are the most abundant multicellular animals on Earth (Schminke, 2007), with approximately 11,500 species (Humes, 1994). They constitute an essential part of the food web as food for fish (Cushing, 1990). Copepods also control the phytoplankton bloom by grazing and thereby affect the cycling and vertical flux of nutrients (Banse, 1995, Wassmann et al., 1998). Copepods show variable sensitivity to OA, with effects on lowered egg production and hatching (Thor and Dupont, 2015) and changes in metabolic processes and respiration (Cripps et al., 2016, Pedersen et al., 2014a), whereas many studies show high phenotypic buffering of adult copepods (Kurihara and Ishimatsu, 2008, Weydmann et al., 2012, McConville et al., 2013, Vehmaa et al., 2013, Zervoudaki et al., 2014). In contrast, eggs and early life stages seem to be more vulnerable (Fitzer et al., 2012, Cripps et al., 2014, Thor et al., 2016). There is also a risk of indirect effects of OA through changed food quantity and quality for the copepods. The timing and composition, as well as nutritional value in terms of increased carbon: nitrogen (C:N) ratio (Schoo et al., 2013) or essential fatty acids (Rossoll et al., 2012) of primary producers can be affected.

When considering potential impact on marine biota it is, however, important to consider the synergistic effects of both natural and anthropogenic contributions to OA, as well as changes on different time scales. Other processes that generate dissolved inorganic carbon and CO_2, such as mineralisation of primary production and dissolved organic matter (DOM), also add to the acidification (Hoikkala et al., 2015, Mostofa et al., 2016). The rate and degree of acidification vary with latitude, water temperature, strength of the different processes involved and distance from the coast (Mostofa et al., 2016).

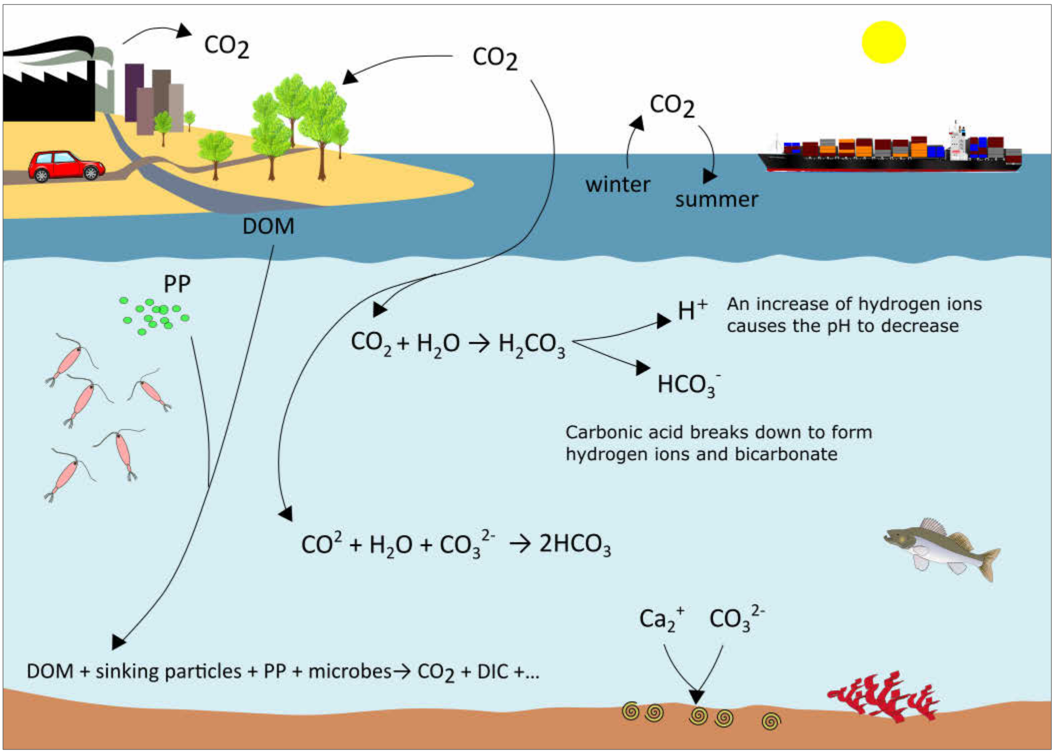

Fig. 1. The simplified explanation of processes involved in ocean acidification. Carbon dioxide (CO_2) dissolves in the water and reacts to form carbonic acid (H_2CO_3), which dissociates into bicarbonate (HCO_3^-) and hydrogen ions (H^+). Excess CO_2 reacts with water and carbonate leading to decreased levels of carbonate minerals. In coastal pelagic areas, primary production (PP) and dissolved organic matter (DOM) from runoff are remineralized by microbes, which also causes acidification.

The consequences of climate change are already seen in many areas of the world. OA affects many different parts of the food web and ecosystem processes. Any significant change could have severe consequences for the ocean and the people depending on it. Many ecosystem services are threatened, such as fisheries, coastal protection and recreation (Gattuso et al., 2015). Since climate change and OA are affecting all parts of the world, it is, opposite to many other problems, a common concern. Despite this, the awareness of OA is still limited. The projected increase in pCO_2 is 1000 µatm and 0.3-0.4 unit pH decrease by the end of the century (IPCC, 2013). Still UNFCCC (United Nations Framework Convention on Climate Change) does not mention OA. The Paris Agreement on limiting global warming to 2 ºC came into force in November 2016, however, recent calculations show the goal to be impossible to meet with current national action plans (http://ec.europa.eu/clima/policies/international/negotiations/paris_en). Even if the 2 ºC change would be met, it is still not enough to avoid ocean acidification (Gattuso et al., 2015).

1.2 Natural fluctuations and coastal areas

In coastal pelagic areas, there is a large variability in the carbonate chemistry (Cai et al., 2011, Sunda and Cai, 2012, Kelly and Hofmann, 2013, Wallace et al., 2014). There are natural fluctuations in pH on inter-annual, seasonal, monthly, daily time scales, and sometimes even shorter scales (Johnson et al., 2013, Waldbusser and Salisbury, 2014, Mostafa et al., 2016). For the open ocean, the atmospheric CO_2 is the main cause of acidification, whereas the biogeochemistry affecting the coastal system is more complex and many processes combined drive the changes (Duarte et al., 2013). pH is largely affected by phytoplankton photosynthesis and respiration, hypoxia due to increased stratification and warming (Wallace et al., 2014) and increased runoff of freshwater and organic matter (Gustafsson et al., 2015). Eutrophication causes large CO_2 uptake, through algal blooms, and there is a high correlation between dissolved oxygen (O_2) and acidification in coastal waters (Cai et al., 2011, Sunda and Cai, 2012, Melzner et al., 2013, Wallace et al., 2014). The blooms take up CO_2 and cause rapid increase in O_2 and pH in the surrounding water. When organic matter is respired by bacteria, uptake of O_2 by the bacteria, causes oxygen depletion and acidification close to the bottom (Sunda and Cai, 2012, Wallace et al., 2014). This aspect of coastal acidification has been largely overlooked until recently. CO_2 in deep water is dependent on these processes and on the water renewal. Upwelling can bring the anoxic bottom water, high in CO_2 derived from respiration, up to shallow depths (Feely et al., 2008, Melzner et al., 2013). In addition, high CO_2 in deeper water layers can decrease the buffering capacity and thereby enhance the effect of CO_2 from the atmosphere (Cai et al., 2011, Sunda and Cai, 2012). To a minor extent, downfall of acid sulphur and nitrogen compounds (HNO_3 and H_2SO_4) adds to the acidification of surface waters, especially in coastal areas in the Northern hemisphere (Doney et al., 2007).

In the open ocean surface, pH varies 0.1 unit inter-annually (Doney et al., 2009), whereas the variability in coastal pH can be 0.3-0.5 units; hence, some areas may already experience a change even larger than the predicted average decrease for the end of the century (Johnson et al., 2013, Kelly and Hofmann, 2013, Melzner et al., 2013, Wallace et al., 2014). Consequently, some organisms already experience high CO_2 in their natural environment, as shown for coral reef fish (Shaw et al., 2013). However, the response of the organisms is dependent on exposure time and magnitude of CO_2. Organisms inhabiting these areas experience a more fluctuating environment and are adapted to larger variations than those of the open ocean. They may therefore be less sensitive to increased CO_2 and lowered pH (Rossoll et al., 2012), and have more effective mechanisms to deal with stress. The natural variability in combination with the gradual increase of CO_2 will alter the exposure times to high CO_2 through increased frequency and amplitude of variation, as well as prolonged duration of extreme events (Waldbusser and Salisbury, 2014).

In addition to the natural temporal variations, all environmental variables in the sea vary with depth. Some organisms undertake diel vertical migrations (DVM, Hays, 2003). For Arctic copepods, vertical migration behaviour has been correlated with sensitivity to OA, so that species or life stages migrating deep, i.e., experiencing larger amplitude in pH, were more tolerant to lowered pH (Lewis et al., 2013). Copepods in the Baltic Sea perform diel vertical migration, residing at greater depths during daytime and close to the surface during night (Ackefors, 1969, Burris, 1980, Holliland et al., 2012). This behaviour adds to the range of change in physicochemical environment that the organisms experience.

1.3 The vulnerable Baltic Sea

The Baltic Sea is especially sensitive to ocean acidification due to the low buffering capacity of brackish water (Hjalmarsson et al., 2008, Schulz and Riebesell, 2013). The projected change in pH for the Baltic Sea is 0.2-0.4 units by the end of the century (BACC II Author Team, 2015). The pH change is, however, quite difficult to predict for the Baltic Sea. There are regional differences in alkalinity that modulate the CO_2 uptake and pH (Gustafsson et al., 2014). The buffering capacity, or the Revelle factor, is affected by the ratio of dissolved inorganic carbon (DIC) to total alkalinity (A_T). A_T is dependent on both external (riverine) and internal (sediment) sources and is related to salinity (Gustafsson et al., 2014, 2015).

The Baltic Sea has a long history of eutrophication (Ahtiainen et al., 2014). High nutrient levels cause high primary production in spring, with high remineralisation and CO_2 production in winter, which amplifies the seasonal pH cycle (Omstedt et al., 2010). In the Baltic Sea, A_T is basically unaffected by formation, precipitation or dissolution of calcium carbonate minerals (Gustafsson et al., 2014). However, there is a difference between the basins. The lowest A_T is found in the Bothnian Bay and Bothnian Sea, whereas the highest is found in the south–eastern Baltic (Baltic proper and Gulf of Riga), and the Gulf of Finland (GoF) has a somewhat intermediate A_T (Beldowski et al., 2010, Gustafsson et al., 2014). Despite this, the low productive Gulf of Bothnia is considered a source of CO_2 to the atmosphere due to high river input of carbon, whereas the Baltic proper, the Gulf of Riga, and the Gulf of Finland are sinks because of their high productivity (Kulinski et al., 2014).

Increased nutrient loads can increase, to some extent, the A_T through increased particulate organic matter (POC) and processes in anoxic areas (Gustafsson et al., 2015). Recently Müller and co-authors (2016) calculated that up to 50% of the atmospheric induced acidification has been compensated for by increased alkalinity in the Baltic proper, whereas in the Bothnian Sea, the alkalinity has totally compensated (atmospheric) OA during the last two decades. Despite this, increased alkalinity will not inhibit OA in the Baltic Sea (Omstedt et al., 2014).

Calculations of the carbon budget show contrasting results of the direction of net CO_2 flow and that the direction can vary inter-annually (Hoikkala et al., 2015). In addition to the inter-annual variability, there is a large seasonal variability in the carbon dioxide system and the Baltic Sea can therefore act both as sink and source of CO_2 (Wesslander et al., 2010, Omstedt et al., 2010, 2014). During summer, $p\mathrm{CO}_2$ is low in the surface waters due to uptake by primary producers. During autumn mixing, CO_2-rich water is brought up to the surface and CO_2 is released to the atmosphere between November and March. The wintertime $p\mathrm{CO}_2$ in surface water can reach ~500 µatm (Wesslander et al., 2010, Omstedt, 2014) and be much higher in anoxic deep water (Beldowski et al., 2010, Melzner et al., 2013). In winter, sea ice coverage dampens the air-sea flux and mixing of the water mass (Soomere et al., 2009). In addition to the seasonal changes, there are daily natural fluctuations due to ecosystem metabolism. During the day, pH rises high in the euphotic zone due to phytoplankton photosynthesis, and at night respiration uses oxygen, and CO_2 is released. Although many internal and external processes affect the Baltic Sea carbon budget and pH, modelling indicates that the direction and magnitude of future pH changes in the Baltic Sea will be controlled by atmospheric CO_2 (Omstedt et al., 2014).

In response to warming, organisms, and especially fish, can move northwards, but that does not help them avoid acidification, in fact, the decline in pH is more rapid in cold water and higher latitudes (Pelejero et al., 2010). Climate change and eutrophication have already caused changes in the hydrography and plankton communities in the Baltic Sea. This causes shifts in the food web to phytoplankton of low food quality and smaller zooplankton leading to decreased energy availability for predators (Suikkanen et al., 2013). Since copepods are the most important food for herring, sprat and fish larvae, changes in zooplankton abundance or behaviour can affect the abundance and behaviour of fish. In the GoF, herring growth was likely reduced due to dominance change from *Pseudocalanus acuspes* to *E. affinis* in the 1980s (Rönkkönen et al., 2004). In the late 1990s, a climate-induced regime shift took place in the North Sea and Baltic Sea due to a change in the North Atlantic Oscillation Index (NAO) from negative to positive (Alheit et al., 2005). Dominance changed from the copepod *Pseudocalanus acuspes* to *Acartia* spp. in surface waters, probably due to increased water temperature, shown to have changed the vertical migration pattern of sprat (*Sprattus sprattus*) (Voss et al., 2007). This shows that even if the change in copepod abundance, behaviour or species composition due to environmental change would be very subtle, the cascading effect throughout the food web can be strong.

2 Stress and adaptation

Marine organisms are exposed to a variety of potentially stressful environments due to changes in temperature, acidification, harmful algal blooms, pollution, eutrophication and hypoxia, to mention a few (Lesser, 2006, Dam, 2013). In a stressful environment, physiological compensatory mechanisms can produce reactive oxygen species (ROS) in the body (Monaghan et al., 2009). ROS are involved in normal cellular signalling, but if more ROS are formed than can be combated by the antioxidants, oxidative stress and potential damage to biological molecules such as proteins, lipids and DNA can occur (Lesser, 2006, Monaghan et al., 2009, Gorokhova et al., 2013). Oxidative stress in terms of increased damage or reduced levels of antioxidants can affect investment in different life-history traits, such as growth (Smith et al., 2016). The organism's ability to quench/combat these ROS is measured in the form of oxidative radical absorbance capacity (ORAC). ORAC has been shown to react more strongly to decreased pH in combination with increased temperature, than to pH alone (Vehmaa et al., 2013, Brutemark et al., 2015). Antioxidant capacity is not necessarily dependent on dietary antioxidants, but nutritional conditions influence the level of oxidative stress (Monaghan et al., 2009).

Considering the rate of the current change in ocean pH, organisms need to be able to adapt rapidly. Organisms can respond phenotypically or evolutionary (shifting genetic composition of a population) to the changing environment. Animals can change their distribution (Doney et al., 2009), behaviour, or their phenology (Richardson, 2008) and allocate energy towards important life functions (Fitzer et al., 2012). In maintaining the acid base balance, organisms may reallocate energy from growth or reproduction, if not excess resources (phytoplankton food for zooplankton) are available (Reusch, 2014).

In general, marine species, populations, and especially plankton should have large genetic diversity due to large population sizes, which could enhance their adaptive potential. On the other hand, many species live close to their temperature tolerance limits already now (Reusch, 2014), which may enhance their vulnerability. Transgenerational plasticity means that the parent(s), (i.e. the mother or father or both), can affect the traits of the offspring or enhance the tolerance of the offspring. These effects can alleviate the negative effect of stressors, such as OA (Vehmaa et al., 2012, Dupont et al., 2013, Cripps et al., 2014, Thor and Dupont, 2015). Phenotypic buffering, i.e. the ability to maintain different life functions in a changed environment (Reusch, 2014), can then give organisms more time for genetic adaptation (Kelly and Hofmann, 2013).

3 Aims of the thesis

The aim of my thesis is to increase the understanding of ocean acidification and changes in terms of hydrography, and of the ecology of zooplankton living in the sea. I use long-term monitoring data to broaden the knowledge of the characteristics and changes of the hydrography and pH in the Gulf of Finland, as there are, to the best of my knowledge, only a few studies describing changes for this area, as opposed to the western Baltic Sea and the Baltic proper. I also aim to describe the variability and the vertical profiles of food availability for copepods, hydrography and pH in a coastal pelagic area, as this issue has largely been overlooked previously. Moreover, I also studied direct and indirect effects of OA on copepod zooplankton. More specifically the aims of the four papers are:

I) to analyse the changes in wintertime salinity, temperature and pH in the Gulf of Finland during the past 36 years,

II) to study the chemical and physical characteristics of the copepods' habitat in a coastal area and to describe the environment they experience on a daily basis during their vertical migration during summer,

III) to study the offspring production of *Eurytemora affinis* in response to ocean acidification, food quantity, food quality as well as fatty acid content of females and eggs,

IV) to study the direct and indirect effects of ocean acidification (i.e., via food quantity and food quality) on the copepod *Acartia* sp. egg production (EPR), egg hatching success (EH) and female body size (measured as prosome length, PL).

4 Methods

4.1 Study organisms

In the studies presented in this thesis, I have worked with two species of calanoid copepods. The most common copepods in the study area (Viitasalo, 1992) in the Baltic Sea, *Acartia* spp. and *Eurytemora affinis* (Fig. 2) were used in the experiments. *Acartia* spp. includes mainly *Acartia bifilosa*, but also *Acartia tonsa* that occasionally occurs in the study area during late summer (Katajisto et al., 1998). *Acartia* spp. is a free spawner and broadcasts the eggs into the seawater, whereas *Eurytemora affinis* is an egg-bearing copepod, holding the eggs in a clutch until nauplii hatch. Both species have a life cycle of six nauplius stages and five copepodite (young) stages and do not grow after reaching maturity (Mauchline, 1998).

E. affinis (Poppe, 1880), previously called *E. affinis hirundoides* or *E. hirundoides*, is abundant throughout the Baltic Sea and in the Bothnian Bay (Kankaala, 1987). E. *affinis* has a global distribution and is found in both marine, brackish and freshwater. It is euryhaline, i.e., tolerant to a wide range of salinities (0.2-12‰) (Lee, 1999). In the Baltic Sea, the species prefers salinities below 6.5 and temperatures below 15°C (Diekmann et al., 2012). It produces subitaneous eggs in summer and diapause eggs in the autumn (Katajisto et al., 1998).

Acartia bifilosa occurs in both open sea and coastal areas (Hernroth and Ackefors, 1979). Both species can recruit from overwintering populations and by hatching from the sediment (Katajisto et al., 1998). The copepods constitute the main part of the diet for juvenile cod (Hinrichsen et al., 2002) and perch, cyprinids and herring in the study area (Flinkman et al., 1998). In the Gulf of Finland especially *E. affinis* is an important prey for clupeids, the three-spined stickleback (Peltonen et al., 2004) and mysid shrimp (Viherluoto, 2001).

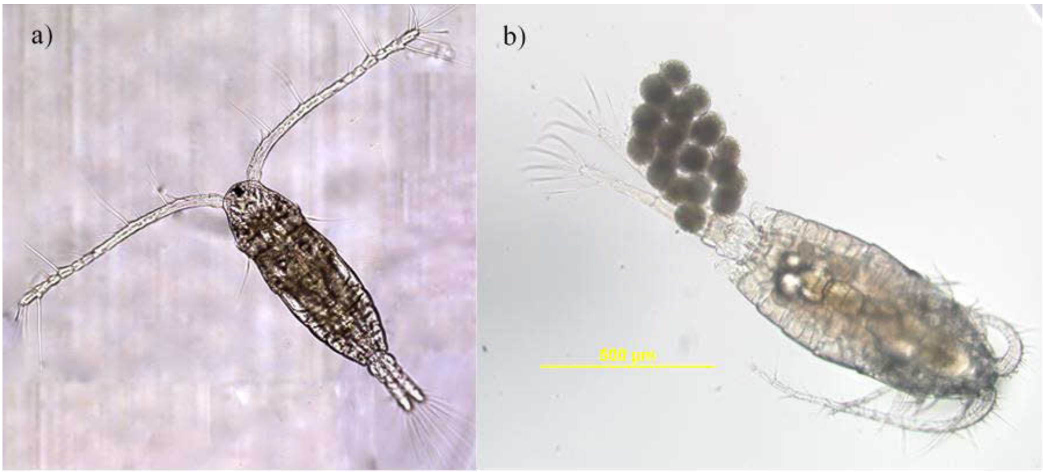

Fig. 2. a) The calanoid copepod *Acartia bifilosa* (Photo: Anu Vehmaa) and b) *Eurytemora affinis* with eggs (Photo: Olivier Glippa).

4.2 Methods applied in the individual studies

In **paper I**, long-term winter monitoring data from four stations in the Gulf of Finland were used (Fig. 3). The Finnish Environment Institute provided the data. I used the longest winter data sets available for pH, sea temperature and salinity from monitoring cruises by R/V *Aranda* for the stations LL3A, LL7 and LL12, and coastal monitoring data from the station Suomenlahti Haapasaari Kyvy-11 (hereafter referred to as Kyvy-11). To reduce the seasonal variability, winter months December-February were used. The data were analysed with linear mixed effects models (LMM) using four different depths (Surface; 5-10 m, Deep layers; 40-50 m, 50-60 m, and 60-70 m depending on station).

The studies (**paper II - IV**) were conducted at Storfjärden, close to Tvärminne Zoological Station (University of Helsinki) on the south–western coast of Finland, Baltic Sea (59º51'20"N, 23º15'42"E) (Fig. 3). In **paper II**, copepods were sampled in June, July and August with a closing net from depth intervals, between surface and bottom, every sixth hours, starting at noon. Adult copepods of the species *Acartia* spp. and *E. affinis*, as well as copepodites were counted using a Bogorov-trough and weighted mean depth (WMD) was calculated for the different copepod groups. Light was measured from the same depths with a LI-COR LI-1000 light meter. Water samples were taken at the same time, from the same location, from surface to 30 m depth with 2.5 m depth intervals for measurement of pH and Chl *a*. Oxygen was measured with an YSI meter and temperature and salinity measured with an FSI NXIC CTD-ADC probe from the surface to the bottom.

The studies for **paper III and IV** were conducted during a large mesocosm set-up arranged by GEOMAR, Helmholtz Centre for Ocean Research, (Riebesell et al., 2013) within the framework of the SOPRAN project (Paul et al., 2015). Ocean acidification was simulated in 55 m^3, 17 m deep bags (Fig. 4) deployed at Storfjärden, and by treating the mesocosms with a range of CO_2, by adding CO_2 enriched seawater. Weekly lab based incubations were conducted with seawater and copepods sampled from the mesocosms.

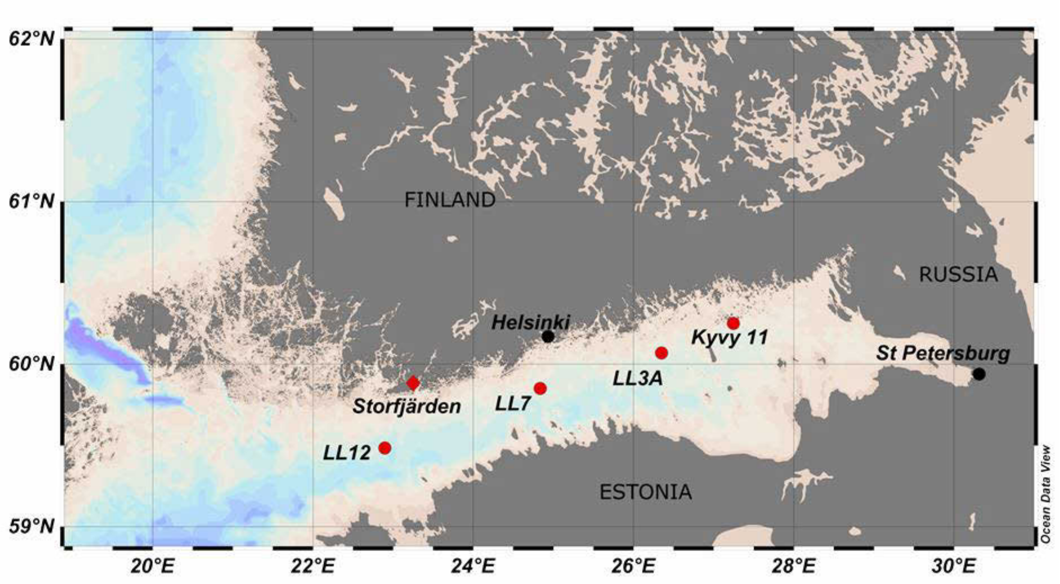

Fig. 3. Sampling stations in the Gulf of Finland. Data from LL stations and Kyvy-11 were used in paper I and field sampling for paper II-IV was done at Storfjärden.

The two units pCO_2 and fCO_2 are both used in this thesis and in references as analogues. The fugacity fCO_2 is the partial pressure corrected for non-ideal behaviour of the gas. The difference between the two is dependent on temperature, but only 3-4 ‰ (Zeebe and Wolf-Gladrow, 2001).

In **paper III,** copepod offspring (nauplii) production and fatty acid content of females and eggs of the copepod *E. affinis* were studied in response to pH, Chl *a* concentration, dinoflagellate and diatom biomass, C:N (<55 µm) and incubation temperature. In addition, ORAC was measured. We sampled copepods and water from the mesocosms on four different occasions (*t*3, *t*10, *t*17, *t*24, *t*0 being the day with first addition of CO_2). Zooplankton was sampled with a plankton net (300 µm, 17 cm) from 17 m depth to the surface and transferred to containers with water from the respective mesocosm. Unfiltered water samples were taken with depth integrated water samplers into air-tight 1.2 L glass bottles. Zooplankton and water were transferred to a light and temperature controlled room at Tvärminne Zoological Station. Female *E. affinis* were picked and incubated in the pre-filled bottles. The incubation lasted four days and the water was exchanged once daily and nauplii filtered out and preserved in acid Lugol's solution. At the end of the four-day incubation, females were frozen in -80°C for fatty acid measurements. At the beginning of each weekly incubation, gravid female *E. affinis* were picked from the mesocosms for analyses of ORAC. Samples for phytoplankton counting, Chl *a* and Carbon (C) and Nitrogen (N) concentrations were collected and analysed by other researchers (Paul et al., 2015), whose data were later shared. For details on the methods for C and N, see **paper IV**, section 2.4.

In **paper IV**, I studied *Acartia* sp. egg production (EPR), egg hatching success (EH), female body size (measured as prosome length, PL), as well as ORAC in response to $f\mathrm{CO}_2$ and food quantity as total particulate carbon (TPC) and quality (C:N). Sampling of water and mesozooplankton followed the same procedure as explained for **paper III**. Twenty adult *Acartia* sp. (17 females and 3 males) were incubated in pre-filled glass bottles with respective mesocosm water (24.3 ± 2.3 h, average ± SD). Copepods and eggs were gently filtered out by pouring the water through a plankton sieve. The copepods were counted and preserved in RNA*later* (Sigma). Prosome length of the preserved female copepods was measured.

An egg transplant experiment was performed to study if transgenerational effects can alleviate possible negative effects of OA. From each treatment, the collected eggs were divided into two 50 mL petri dishes; one dish filled with respective mesocosm water, and the other filled with Baltic water. Twice a day egg hatching was followed by counting the number of remaining eggs on the dish through the lid. *Acartia* sp. nauplius stages were determined, the number of nauplii and remaining copepod eggs counted and the development index (DI) calculated (Knuckey et al., 2005). On *t*3, *t*10, *t*17 and *t*31, adult female *Acartia*. sp were picked for ORAC analyses. For details on analyses of C:N and TPC, please see **paper IV** section 2.4.

4.3 Statistical analyses

A short summary of the statistical methods used is given for the different studies. In **paper I**, linear mixed effects models (LMM) were applied to test if pH, salinity and sea temperature have changed with time for station and depths as well as to look at differences between stations.

In **paper II**, a weighted mean depth[2] (WMD) was calculated. For each time point, WMD was calculated for the different groups of copepods (*E. affinis* males, *E. affinis* females, *E. affinis* ovigerous females, *Acartia* spp. males, *Acartia* spp. females and copepodites). The difference between the daytime (12:00) WMD and nighttime (24:00) WMD was tested with an LMM to verify if copepods perform diel vertical migration. Differences between months were tested with Kruskal-Wallis rank sum test.

In **paper III**[3], an LMM with nested design was applied. Day nested within week, nested within mesocosm was used as random intercept as nauplii production of the same groups of individuals was measured four times per week and weekly incubation were dependent on each other (sampled from the same mesocosms each week). LMM was also applied to test if pH has a direct effect on the fatty acid content of adult female copepods and on their ORAC concentration.

In **paper IV,** the effect of acidification, food quantity and food quality on *Acartia* sp. egg production (EPR), prosome length (PL), antioxidant capacity (ORAC) and nauplii development index (DI) were tested with LMM. Due to the binomial nature of the data, a generalized linear mixed model (GLMM) was applied to test the effect of $f\mathrm{CO}_2$, TPC (<55 µm) and C:N (<55 µm) on egg hatching success (EH).

For the egg transplant experiment, the ratio of Mesocosm EH (or DI) / Baltic EH (or DI) was calculated to separate the effect of hatching environment from maternal environment. A ratio >1 indicates that eggs hatch or develop better in maternal conditions (Mesocosm water), whereas a ratio <1 indicates that eggs hatch or develop better in Baltic Sea water. The effect of maternal environment ($f\mathrm{CO}_2$, TPC (<55 µm) and C:N (<55 µm)) on the ratio was tested with LMM, where the ratio of Mesocosm EH / Baltic EH and Mesocosm DI / Baltic DI were used as response variables; $f\mathrm{CO}_2$, TPC (<55 µm) and C:N (<55 µm) as fixed explanatory variables; and repeated measure of the mesocosms over time as a random factor. Spearman rank correlation tests were used to test if maternal ORAC correlates with egg hatching success. All the statistical analyses were performed using software R 2.15 or R 23.0.2 (R Core Team, 2013), and the significance level was 0.05.

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[2] Correction: In **paper II** formula on p. 123, section 2.3, should be: $\mathrm{WMD} = \Sigma(n_i d_i) / \Sigma n_i$

[3] In **paper III**, in Fig. 2c, should be: Dinoflagellates µg C L^{-1}

5 Results and discussion

5.1 Changes in wintertime pH, salinity and sea temperature

As part of this thesis, I analysed wintertime monitoring data from the last 36 years in the Gulf of Finland (GoF) (**paper I**). I found long-term changes in the hydrography and pH (summarized in Table 1). During 1979-2015, pH decreased significantly both in the surface (0.14) and the deep layers (0.3) in the western GoF. The decrease in pH was twice as large for the deep layers as for the surface. This finding is in line with the increased difference between summer and winter pH since 1990 in the GoF, reported by the Finnish Environment Institute (SYKE, 2016). It is most likely due to increased stratification and stagnation of the deep water in the GoF during the last two decades and high remineralisation of organic material in the deep water during winter (Omstedt et al., 2014, SYKE, 2016). Whole year data from SYKE (2016) show an obvious reduction in pH at 70 m depth 1990-2010 at station LL7. The report does unfortunately not state whether the authors base their conclusions on statistical analyses and if so, how analyses were applied, but it is indicated that the decrease in pH is not only observed during winter months.

In many coastal areas, OA derived from anthropogenic CO_2 is not the main cause of detected pH changes. Enhanced primary production and respiration are instead more important in driving the changes (Havenhand, 2012, Duarte et al., 2013). This is probably true for the GoF, too. Despite decreasing nutrient conditions, summer cyanobacteria blooms have not decreased in the GoF, instead they have increased (Suikkanen et al., 2007, 2013). It seems that pH in the GoF is regulated by internal processes regulating oxygen and pCO_2 levels, rather than by atmospheric CO_2. In the Danish straits and the southern North Sea, pH peaked around mid-1980s, but then declined rapidly by 0.3 and 0.4 units, respectively, which could not be due to OA alone (Duarte et al., 2013). According to Carstensen and co-authors (2011), nutrient levels decreased drastically from late 1980s onwards, but primary production remained high in these areas.

Table 1. Wintertime changes in the Gulf of Finland since 1979 for the four sampling stations (west to east). A statistically significant decrease is indicated by a downward arrow and an increase by an upward arrow, no significant change is indicated by a hyphen (**paper I**).

	LL12	LL7	LL3A	Kyvy-11	
pH	↓	↓	-	-	surface
	↓	↓	-	-	deep
Temp.	-	↑	-	-	surface
	-	↑	↑	-	deep
Salinity	↓	↓	-	↓	surface
	-	-	-	↓	deep

A change in pH was not seen for the easternmost part of GoF (Kyvy-11); however, pH was lowest in this area. This can partly be explained by a large freshwater input by the Neva River and dissolved organic matter (DOM) in the river water affecting pH and $p\mathrm{CO}_2$ (Kulinski et al., 2014, Ylöstalo et al., 2016). DOM dissolves into inorganic carbon, which reacts with CO_2 and lowers pH. According to a review by Hoikkala and co-authors (2015), dissolved organic carbon concentrations are 50-60% higher in the Neva bay than in the western GoF, most likely due to discharge from the Neva River. They reported a clear increase in total organic carbon (TOC) between 1975 and 2011 in the coastal area of the Bothnian Bay, the Quark and the eastern GoF, as opposed to the open sea area, probably due to elevated TOC import from rivers (Hoikkala et al., 2015). Production and mineralisation of TOC affect the seasonal changes in surface CO_2 and pH (Gustafsson et al., 2015). Recently, Gustafsson et al. (2015) showed that the CO_2 flux in coastal waters of the Baltic Sea is highly dependent on river loads of carbon, A_T, nutrients and freshwater input. In addition to high nutrient loads leading to high production and consequently oxygen consumption, it leads to increased internal alkalinity. The internal alkalinity (through processes in anoxic sediments) enhances the ability of the water to bind CO_2. Despite this fact, the exact impact of the river on the Baltic Sea carbon cycle remains unclear (Ylöstalo et al., 2016). The internal alkalinity has a strong effect on air water CO_2 flux, but the processes behind the internal production are still unclear (Gustafsson et al., 2015). How large part of the acidification that has been compensated for by increased alkalinity in the GoF during the past two decades, has not been verified due to scarcity in data (Müller et al., 2016).

The air temperature has increased in the Baltic Sea area since the 1980s (Lehmann et al., 2011) and consequently also the sea temperature in the middle of the GoF (shown here in **paper I**). However, no changes in sea temperature were found for the easternmost and westernmost stations, probably due to high inter annual variability making it difficult to detect the changes. During 1979-2015, a positive North Atlantic Oscillation Index (NAO) was dominating. A positive NAO leads to stronger and more persistent westerly winds, which influences the circulation in the Baltic Sea and leads to warmer and rainier winters with warmer seawater and less sea ice (BACC II Author Team 2015). In GoF, the ice coverage (Lehmann et al., 2011), thickness and length of the ice season have decreased during the last century (Merkouriadi and Leppäranta, 2014).

Baltic Sea sediment core samples dating 1000 years back have shown a high correlation between warming and the spreading of anoxic areas. The total organic carbon was higher in warmer years, which indicates the importance of temperature in regulating for example cyanobacteria blooms through increased sea surface temperature in summer (Kabel et al., 2012). Despite actions taken to reduce nutrient loads into the Baltic Sea, they may not have been enough to avoid the spreading of dead bottoms. Feedback mechanisms, through release of phosphate from hypoxic sediments, allow for sustained cyanobacteria blooms (Funkey et al., 2014) leading to remained high CO_2 production in the deep water of the Baltic Sea.

At the easternmost station, close to the Neva River, I found a decline in salinity both in the surface and in the deep layers. The prevailing atmospheric forcing and runoff can cause fluctuations in temperature and salinity on shorter time scales as salinity is affected by river discharge, currents and water exchange with the Baltic proper. The Neva River is very important for regulating the salinity, as it stands for almost 70% of the freshwater input into the GoF (Launiainen and Koljonen, 1982). Although the data for the easternmost station have two larger gaps, the decreased salinity is in accordance with the increased freshwater input during the last decade. The question remains whether salinity fluctuates on time scales longer than 40 years, in this area. For example, in Tvärminne, the annual average salinity at 10 m depth has been fluctuating greatly during the 1900's; it was below 5.5 in the 1920's, increased thereafter, reaching 6.8, and was high in the 1970's, and then declined to below 6 during a long stagnant period without salt water intrusions (Westerbom, 2006).

5.2 The physicochemical environment

In **paper II**, I studied the natural variability and change with depth of several physicochemical variables as well as the vertical migration behaviour of two common copepods at Tvärminne, Storfjärden during summer. The physicochemical variables changed both with time of the day and with depth. The largest diurnal variation was observed in August with 0.1 unit change in pH at 2.5 and at 30 m. A sharp change (0.5 unit) with depth was observed between 17.5 and 20 m. There was a ~10°C difference in temperature and 0.8-1.0 difference in salinity between surface and bottom. I found that both species, *E. affinis* and *Acartia* sp., performed vertical migration (average amplitude 5-22.5 m), with a more pronounced migration for adults than for copepodites. The copepods faced highly variable conditions of up to 0.5-unit change in pH and 5°C change in temperature. My results showed that *E. affinis* females carrying eggs migrated deeper than non-ovigerous females did, probably because of the prominent egg clutch that makes them more susceptible to predators. However, this behaviour also exposes them to lower oxygen saturation levels, lower pH, higher salinity and colder water. The changes in pH and temperature with time (and/or depth) are larger than the average projected change for the end of the century (BACC Author Team II, 2015) and the changes in this sampling area can be even larger than reported for this study, as upwelling of cold and more saline water takes place (Tvärminne monitoring data, unpublished data).

As shown by Lewis et al. (2013), copepods experiencing great change in their environment during migration are more tolerant towards lowered pH. The natural variability and changes that copepods experience in their habitat may partly explain their robustness towards OA, shown in short-term experiments (Vehmaa et al., 2012, 2013). However, the exposure times to low pH are relatively short now, but as pH decreases also at more shallow depths, copepods are exposed to possible unfavourable conditions for longer times. In addition, the amplitude of these variables will increase, especially during summer months (Schulz and Riebesell, 2013).

The results of this study show how highly variable local conditions can be in terms of pH, salinity, oxygen, temperature and food availability. As the local environmental conditions and consequent local adaptation of species to these conditions may be of great importance, I suggest that local background conditions should be considered in experimental design and interpretation of results. In addition, this study shows the importance of ensuring the sampling depth to be deep enough during the day in summer not to risk excluding a large part of the zooplankton community.

5.3 Mesocosms

Mesocosms (Fig. 4) provide a great opportunity to study natural plankton communities, where two or more trophic levels can be included. Many scientists from a variety of disciplines can collaborate, frequent sampling and measurements can be performed, and data can be shared. Due to the large size of the mesocosms, replication is difficult. However, this can be overcome by repeated sampling considered in the statistical analyses.

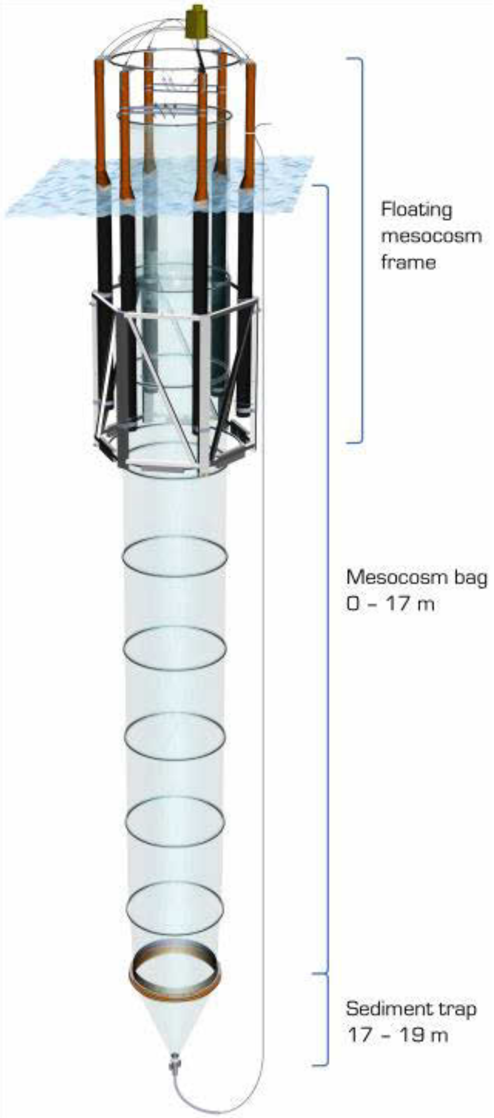

Fig. 4. Sampling a mesocosm unit (left) and a schematic drawing of the mesocosm (right) (Source: GEOMAR, Paul et al., 2016).

In the current mesocosm study, $f\mathrm{CO}_2$ was let to fluctuate naturally (except an addition of CO_2 on day 15), which causes the $f\mathrm{CO}_2$ to decrease towards the end and treatments to have less difference between them. Due to this and to the fact that all processes in the mesocosms are affected by the natural temperature, the different time points are not directly comparable to each other. There may also be a difference in, for example, zooplankton abundance from start. This can cause a significant deviation in the community composition at the end of the experiment.

During the mesocosm studies presented here, zooplankton were frequently sampled and counted and no significant differences in the main mesozooplankton species were detected (Lischka et al., 2015). Other weaknesses of the mesocosms are wall effects (growth of periphyton) and unnatural mixing and turbulence causing deviation from the natural conditions outside the mesocosms (Riebesell et al., 2010). Discrete sampling may not be optimal, if the water column inside the mesocosms is not well mixed. However, an integrated water sampler that takes equal amounts of water at different depths from bottom to surface was used to sample the mesocosms. Considering sampling of mesozooplankton, there is a risk of bias in abundance if the net tows are done during different times of the day, as a result of the light conditions and the consequent vertical migration (Riebesell et al., 2010).

5.4 Copepod offspring production, fatty acids, antioxidant capacity and body length

During a mesocosm study at Tvärminne Storfjärden, lab based incubations were performed to study how the two calanoid copepods, *E. affinis* (**paper III**) and *Acartia* sp. (**paper IV**), respond to ocean acidification and to changes in food quantity and food quality. For *E. affinis*, there was no statistically significant difference in nauplii production between CO_2 treatments, whereas Chl *a*, dinoflagellate biomass, and incubation temperature stimulated nauplii production. The nauplii production was unaffected by C:N (<55 µm) ratio, whereas negatively affected by diatom biomass. PUFA content of the females correlated with PUFA in their eggs and stimulated nauplii production. Egg lipid composition is shown to affect hatching success; in *Calanus finmarchicus* hatching can increase with PUFA content in females (Koski et al., 2012). However, PUFA was not affected by pH in my results. The results indicate that food availability was a more important factor for *E. affinis* than the effects of high $f\mathrm{CO}_2$/lowered pH. This is also supported by analyses of seston and copepod fatty acid content during the same mesocosm campaign, showing that PUFA of copepods declined towards the end, following a decrease of PUFA in the seston (Bermúdez et al., 2016). PUFA of seston decreased due to changed community composition and nutrient limitation in terms of silica and phosphorous. The positive effect of dinoflagellate biomass was probably due to their high nutritional value in terms of PUFA. The change in phytoplankton community composition was not due to $f\mathrm{CO}_2$, except for the less abundant Chlorophyta (Paul et al., 2015, Bermúdez et al., 2016).

When studying stress responses in animals one should preferably measure several of the four components mentioned; free radical production, antioxidant defence, oxidative damage and repair mechanisms (Monaghan et al., 2009). In my work, I only have the measure of antioxidant capacity (ORAC) which makes it more difficult to draw definite conclusions on possible stress effects. However, it gives an indication of changes in the animals' environment. Female ORAC was unaffected by pH and did not affect the nauplii production, which indicates that *E. affinis* did probably not face acute pH stress.

For *Acartia* sp., on the other hand (**paper IV**), neither food quantity (TPC <55 µm), food quality (C:N <55 µm), nor ocean acidification ($f\mathrm{CO}_2$) had a statistically significant effect on egg production. I have summarized the responses of the two species to OA in Figure 5. In contrast to what I expected, higher food quantity (TPC <55 µm) correlated negatively with egg hatching success, adult female size and ORAC, whereas C:N ratio did not correlate with any of the measured variables. This could, at least partly, be explained by a sharp decline in Chl *a* concentrations and in the phytoplankton community size structure after Day 17 (Paul et al., 2015). Picophytoplankton (<2 µm), which dominated in the mesocosms towards the end of the experiment, may have been too small to be consumed by *Acartia* sp. (Rollwagen Bollens and Penry, 2003). Even if the copepods seem able to ingest some picophytoplankton despite their small size (Motwani and Gorokhova, 2013), I suggest that decreasing PUFA in the seston in combination with decreasing cell size could partly explain this result, as the copepods would have needed to feed large quantities of picocyanobacteria to satisfy their need of PUFA.

In high CO_2, the somatic growth can be reduced due to higher energy requirement to maintain homeostasis, i.e., reduced scope for growth (Pedersen et al., 2014a). *Acartia* sp. body size (prosome length) was affected by $f\mathrm{CO}_2$, but seemed driven by the highest $f\mathrm{CO}_2$ concentration. The effect on body size is species specific and dependent on other factors than simply OA (Hildebrandt, 2014, Garzke et al., 2016). For example, there was no effect on body size in *Pseudocalanus acuspes* after a 3-month-long incubation in high $f\mathrm{CO}_2$ during a similar mesocosm campaign (as in papers III and IV) in Gullmarsfjord, Sweden (Almén et al., unpublished data).

Acartia sp. egg hatching (**paper IV**) remained >90% in both controls and varied less than in the CO_2-treated mesocosms. However, no statistically significant effect of $f\mathrm{CO}_2$ on hatching was found, whereas TPC affected hatching negatively. The egg transplant experiment revealed no effect of the maternal food quantity (TPC) nor the food quality (C:N) on the ratio of egg hatching and development between Baltic and $f\mathrm{CO}_2$ treatment conditions. The $f\mathrm{CO}_2$ in the maternal environment influenced the ratio of EH and DI between mesocosms and Baltic conditions. Egg hatching was higher in the Baltic water than in the maternal environment, when the mothers were reared in high $f\mathrm{CO}_2$. When the maternal environment was low in $f\mathrm{CO}_2$, eggs hatched better in that same environment. This indicates a possible threshold above which transgenerational effects may not be able to alleviate the negative effects of $f\mathrm{CO}_2$.

The maternal effects were weaker towards the latter end of the experiment, which could be due to 1) an overall low egg production after *t*17 causing difficulties to detect differences, 2) weakening of the mothers or general senescence 3) an upwelling event evening out differences between the $f\mathrm{CO_2}$ mesocosms and the Baltic conditions, 4) long-term stress and food limitation.

Only TPC (<55 µm) had a significant, negative effect on ORAC. ORAC and egg hatching success correlated positively (**Fig 3. in paper IV**) and was stronger for eggs developing in the mesocosm water than for eggs developing in the Baltic water. This indicates a possible allocation of ORAC to the eggs to protect the embryo from oxidative stress. Moreover, the ORAC levels seemed to be upregulated in the beginning of the study. The change in pH right after the first additions of CO_2 to the mesocosms could probably trigger the antioxidant defence (**paper IV**). The low ORAC at *t*31 indicates either an adaptation to the high $f\mathrm{CO_2}$ concentration or exhaustion of the antioxidant resources (**Fig 1. in paper IV**).

In general, indirect effects through food quantity and food quality seem to be more important than the direct effects of $f\mathrm{CO_2}$ (**paper III and IV**). Meunier et al. (2016) found no direct effect of $f\mathrm{CO_2}$ on *A. tonsa* development and respiration, but both microzooplankton and *A. tonsa* were negatively affected by higher C: nutrient ratio of algae and had higher respiration in high $p\mathrm{CO_2}$. Changed food quality in terms of fatty acids of phytoplankton monocultures has been reported (Rossoll et al., 2012, Torstensson et al., 2013). Consequently, the quality of phytoplankton affected development of *A. tonsa* (when using only one species of algae (Rossoll et al., 2012), whereas dinoflagellate *Heterocapsa* sp. did not show changes in stoichiometry, when grown in high $p\mathrm{CO_2}$, and no effect on copepods was detected (Isari et al., 2016). In addition to species-specific differences of algae in sensitivity as well as ability to compensate for different nutrients for higher trophic levels, protozoan herbivores may dampen the negative effects by trophic upgrading (Meunier et al., 2016). In addition to phytoplankton, microzooplankton constitute an important part of the diet for copepods.

In the current studies, it is unclear which species of algae or microzooplankton the copepods preyed upon. Indirect effects of $f\mathrm{CO_2}$ on copepods via phytoplankton in the mesocosms remain unclear, because the positive correlation between $f\mathrm{CO_2}$ and Chl *a* (mainly picocyanobacteria) was only discernible in the mesocosms after finishing the experiments (*t*25 for *E. affinis*). In this thesis, I did not study the development of *E. affinis* due to logistic restrictions during the mesocosms campaign. The abundance of *E. affinis* inside the mesocosm enclosures did, however, not differ significantly due to $f\mathrm{CO_2}$ (Lischka et al., 2015).

Although the studies of *E. affinis* and *Acartia* sp. are not completely comparable in terms of the measured variables, the data suggest that *E. affinis* is the more robust species of these two. *E. affinis* exists in many parts of the world and is known to invade both brackish and freshwater systems (Torke, 2001). The species seems to be tolerant towards a wide range of environmental conditions. The reproductive strategies of the two species differ, which probably could affect their sensitivity. Carrying the brood in a clutch until they hatch may protect the embryos and gives the female an opportunity to seek more favourable environmental conditions for the developing brood.

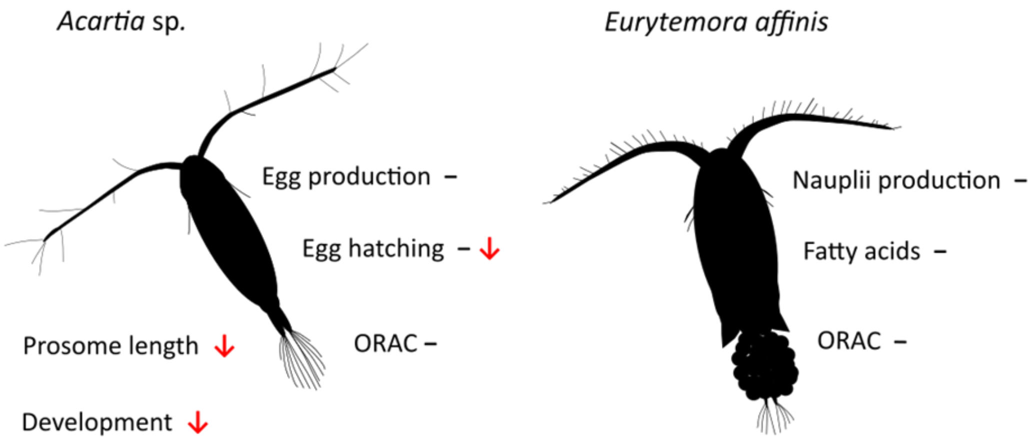

Fig. 5. The effects of high $f\text{CO}_2$/lowered pH on different traits of *Acartia* sp. and *E. affinis*. A downward arrow indicates a decrease and a hyphen indicates no effect. Negative effects of OA on *Acartia* sp. egg hatching were likely, as the result was very close to statistical significance (p = 0.052). There were negative effects on hatching observed in the transfer experiment at high $f\text{CO}_2$ levels.

The behaviour between the two species does also differ. Although *A. bifilosa* is exposed to natural variability in pH environment due to daily fluctuations, and stays at greater depths during the day (low pH in deep water), it does not reside as deep down as *E. affinis*. The fact that both species were able to maintain offspring production and hatching in all mesocosms, but that development was slower for *Acartia* sp. in high $f\text{CO}_2$, indicates the very important aspect of considering also other traits than simply the production of offspring. For example, Pedersen and co-authors (2014b) found the first feeding stage of nauplii to be more sensitive than later nauplius stages and slowed development has been reported for several other species (Weydmann et al., 2012, Pedersen et al., 2014a).

Even though we ought to extrapolate study results, the strong salinity gradient throughout the Baltic Sea makes it more difficult to make projections conclusive for all basins based on studies made in only one part of the sea. Salinity affects for example the alkalinity of the seawater and the osmoregulatory capacity of the organisms; hence, the base abiotic conditions need to be considered. Different populations of organisms within the same species may show a difference in physiological responses and sensitivity to OA, as shown for two populations of isopods from the North Sea and the Baltic Sea (Wood et al., 2016) and for an Arctic and a boreal population of the copepod *Pseudocalanus acuspes* (Thor and Oliva, 2015). Food availability in combination with lowered pH increased metabolism by 195% in the Arctic copepod *Calanus glacialis* CII-CIII, whereas CV was unaffected, probably because of their life strategy of hibernation and metabolic suppression at that stage (Thor et al., 2016). Thus, strong stage specific differences exist.

Although coastal plankton systems may be more resilient to changes in pH and temperature, coastal pelagic areas are at the same time prone to other forms of stress due to eutrophication, pollution, run off and over exploitation (Kelly and Hofmann, 2013, Melzner et al., 2013). In general, copepods seem robust when tested for direct effects of lowered pH (Kurihara and Ishimatsu, 2008, Mayor et al., 2012, Weydmann et al., 2012, McConville et al., 2013, Hildebrandt, 2014, Pedersen et al., 2014b), but there are still risks of unseen indirect effects. The natural variability can enhance or mitigate the vulnerability (Kelly and Hofmann, 2013, Pelejero et al., 2010). If the copepods are already living close to their physiological limits, further change can decrease their fitness and survival.

There are risks of indirect effects through primary producers on the grazers in terms of changed cell sizes, community composition and abundance, and fatty acid content. The observed long-term changes in temperature, pH and salinity (**paper I**) may affect the timing of copepod hatching in early spring and the potential match or mismatch between the copepods and their phytoplankton food. Warming causes decreased body size of adult copepods and other large zooplankton, as well as increased respiration (Daufresne et al., 2009). Decreased body size due to warming and OA is alarming, as it affects the transfer of energy to higher trophic levels. The proportion of large zooplankton declines with salinity in the Baltic Sea (Vuorinen et al., 1998), and this means that changes in the community composition (due to temperature and/or salinity) towards smaller zooplankton (e.g., small cladocerans and rotifers instead of large copepods) could decrease the food availability for fish, but also influence the vertical flux of nutrients.

The local environment and variability, especially in coastal areas, expose the copepods to many other stressors than simply OA. The background environment and its changes and variation, will certainly affect the organisms' responses. Moreover, the abundance of one species affects the abundance of other species, which leads to changes in community composition, size fractions, and changes in trophic interactions. Species and genetic diversity, which in turn affect the functional diversity and redundancy, can be impaired. If key species or groups, such as the Baltic copepods, herring, sprat or cod (Havenhand, 2012), are negatively affected, the whole ecosystem may have difficulties maintaining its functions and productivity, and may even collapse. The sea temperature has increased (**paper I**) and is projected to increase during the coming centuries for the Baltic Sea, facilitating cyanobacteria blooms and hypoxia (Kabel et al., 2012). OA, warming, hypoxia and toxic cyanobacterial blooms pose a combined threat to copepods.

5.5 Future research directions

Despite a long record of monitoring the Baltic Sea, there are only a few published papers on the long-term changes based on the data from the eastern Baltic Sea. Although there has been sampling going on for many decades, the continuity and accuracy have not met scientific standards at all times. The accuracy for standard pH measuring methods is 0.02-0.1, which is not good enough for high-standard monitoring purposes. This is the reason why high-quality pH monitoring data is lacking from the Baltic Sea. In addition, there are some gaps in the data, which makes statistical analyses difficult. OA is, for example, currently not included in the monitoring programs of HELCOM (Helsinki Commission) and there is only a brief mention of OA in the report "Climate change in the Baltic Sea area" (HELCOM, 2013). The awareness of OA would greatly increase, if OA were included in the HELCOM process. *Acartia* sp. showed higher sensitivity to OA in combination with warming (Vehmaa et al., 2012, 2013) and therefore warming should be considered in combination with OA. In a multi-stress experiment, the zooplankton community was also more affected by warming than by OA, although OA modified the effects of temperature (Garzke et al., 2016). In highly eutrophicated areas, there is also a need for experiments that combine the effects of hypoxia and OA (Melzner et al., 2013), as well as experiments including fluctuating control conditions corresponding to *in situ* conditions experienced by the organisms (Reum et al., 2014, Hendriks et al., 2015). These kinds of experiments are, however, both expensive and time consuming to perform. Another aspect, not yet studied in the Baltic Sea, is the zooplankton species stage specific sensitivity and the sensitivity at different times of the year.

6 Conclusions

6.1 Key findings

In this thesis, I studied the long-term changes in hydrography and pH of the Gulf of Finland (**paper I**), as well as the local variability of hydrography and the physicochemical variables at the entrance to the gulf. Results show that climate-related changes are detectable also in the GoF with decreased pH and salinity and higher sea temperature. The impact of the prevailing climate forcing and the input from Neva River seem to have a large effect on the hydrography and pH of the GoF. There is a natural variability in pH and hydrography on different time scales, and organisms may be adapted to large variations. In **paper II** vertical profiles of pH, salinity, oxygen and Chl *a* showed large amplitudes between bottom and surface and at different times of the day. Both species of copepods, *E. affinis* and *Acartia* spp., perform diel vertical migration in summer and experience changes in pH and temperature exceeding the projected average end of century values for this area. Moreover, laboratory-based incubations using copepods and water from large *in situ* mesocosms showed *E. affinis* egg production to be affected by both food quantity and food quality, but not directly by $f\mathrm{CO}_2$ (**paper III**), whereas *Acartia* sp. was negatively affected by TPC and showed reduced body size in high $f\mathrm{CO}_2$ (**paper IV**). On the other hand, maternal/transgenerational effects may alleviate the adverse effects of OA, as shown here in **paper IV**; however, the maternal effects seemed weak and could only protect the embryo under intermediate levels of $f\mathrm{CO}_2$.

There are still parameters yet not tested for these species; we lack, for example, knowledge on how OA affects respiration of *E. affinis* and *A. bifilosa*. Even though *E. affinis* showed robustness towards the levels of $f\mathrm{CO}_2$ used in the presented studies here, there are still many possible physiological processes not studied that could be affected by high CO_2. It is difficult to draw definite conclusions from experiments using only one generation of animals.

For copepods, the species and stage-specific responses seem to be large and, hence, definite conclusions about future risks are difficult to make for these organisms as a group. Due to warming, phytoplankton cell sizes are decreasing and species community composition changes will also occur (Sommer and Lengfellner, 2008, Daufresne et al., 2009). The results of this thesis also show the importance of food quality and food quantity and that copepods are very dependent on how the primary producers respond to climate change.

6.2 A few thoughts on management

In addition to decreasing pH, warming, rising sea surface, extreme weather events, floods and erosion pose a great threat to ecosystems and to millions of people living close to the waterline. With OA, the coral reefs will decline and erode, leaving large areas unprotected from storms. In addition, fisheries and shellfish will decline (Gattuso et al., 2015). In the Baltic Sea, our coastline and the life in the water will be very different within 50 years from now, but still there are only few actions taken to ensure a viable future sea.

Drastically reducing CO_2 emissions globally is the only way to manage the occurring climate change; however, even if CO_2 emissions stopped today, the ocean will still be affected for thousands of years in terms of changed carbonate chemistry (Pelejero et al., 2010). Nevertheless, with restricted emissions the high threat on many ecosystems will be less severe (Gattuso et al., 2015). Although the changing climate and acidifying oceans are a global concern, the perception of threats to the ocean, and how we value the ocean vary between different countries and cultures. In addition, the perception of climate change and possible ways to manage it differs between natural and social scientists, with the latter more often focusing on governance and policy decisions (Boonstra et al., 2015). The effects on ecosystems can, however, be delayed or reduced by protecting sensitive areas and ecosystems on local scales, by establishing marine protected areas and no-take-zones and reduce other stressors, such as pollution and eutrophication. We as humans can adapt our behaviour by relocating or changing activities, such as fishing and land use (Strong et al., 2014, Gattuso et al., 2015).

For the Baltic Sea, one management option is to reduce nutrients and pollutants. In the Baltic Sea region, Ecosystem based management (EBM) has been successful when mitigating for example organic contaminants (Blenckner et al., 2016). The EBM includes regulation of resource use and other human activities. There are only a few studies available on the effects of OA on Baltic Sea organisms and ecosystems, but this approach is probably one of the most effective ways in reducing the so-called secondary stressors and thereby alleviating the negative effects or by increasing ecosystem resilience through maintained diversity and abundance. As suggested in a report for the Finnish organisation SITRA (Bäck et al., 2016), there is a need for improved local measuring accuracy and in the Arctic area extended and well-coordinated monitoring with shared responsibilities and resources amongst countries.

Through improved communication and collaboration, better projections of the possible effects of climate change can be made (Bäck et al., 2016). Although much of the effort in the Baltic Sea region has been made on mitigating eutrophication and hypoxia, without including aspects of OA, the process will be beneficial in mitigating the latter. Due to large differences in local adaptation and responses, as well as differences in the local stressors, it is motivated to start working on a more local scale if we wish to alleviate future effects. This can be done by gathering data on local variability, identify stressors, build models and involve local decision makers and inhabitants to take actions against climate change, pollution, etc., especially in areas where the local environmental pressure is strong. We cannot stop OA from proceeding but by alleviating other stressors, we can buy time or reduce the extremes in terms of high CO_2, low O_2 and low pH.

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“Där vandrade hans hav förbi i våg på våg, fräsande och överdådigt,
lugnt och våldsamt. Pappan slätade ut alla sina funderingar,
han bara levde helt och hållet, från svansspetsen ända ut i öronen”

Tove Jansson, ur Pappan och havet

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