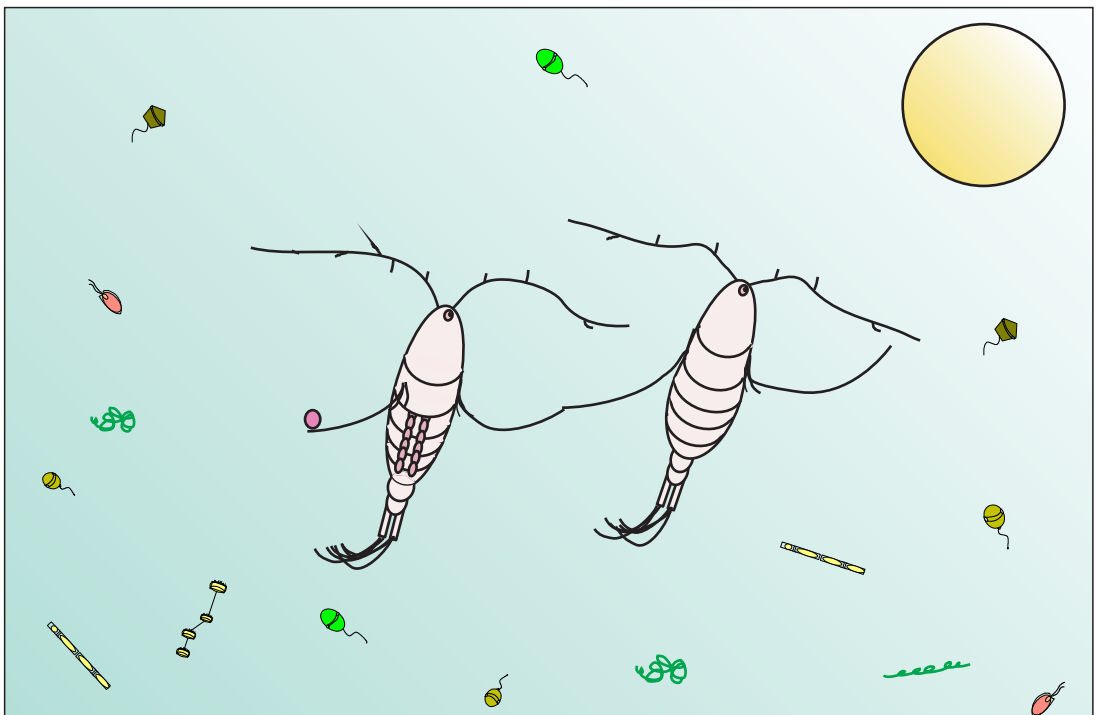**Åbo Akademi
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Anu Vehmaa

# Climate driven changes in temperature, pH and food quality

Effects on copepod reproduction

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-Effects on copepod reproduction

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II   Vehmaa A, Brutemark A, Engström-Öst J. Maternal effects may act as an adaptation mechanism for copepods facing pH and temperature changes. Conditionally accepted manuscript

III  Vehmaa A, Kremp A, Tamminen T, Hogfors H, Spilling K, Engström-Öst J (2012) Copepod reproductive success in spring-bloom communities with modified diatom and dinoflagellate dominance. *ICES Journal of Marine Science* 69(3):351-357 doi:10.1093/icesjms/fsr138

IV   Vehmaa A, Larsson P, Vidoudez C, Pohnert G, Reinikainen M, Engström-Öst J (2011) How will increased dinoflagellate:diatom ratios affect copepod egg production? – A case study from the Baltic Sea. *Journal of Experimental Marine Biology and Ecology* 401:134-140 doi:10.1016/j.jembe.2011.01.020

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## CONTRIBUTIONS

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| Satistical analysis | AV, HH | AV | AV | AV |
| Responsible for manuscript preparation | AV, HH | AV | AV | AV |

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## ABSTRACT

Increased emissions of greenhouse gases into the atmosphere are causing an anthropogenic climate change. The resulting global warming challenges the ability of organisms to adapt to the new temperature conditions. However, warming is not the only major threat. In marine environments, dissolution of carbon dioxide from the atmosphere causes a decrease in surface water pH, the so called ocean acidification. The temperature and acidification effects can interact, and create even larger problems for the marine flora and fauna than either of the effects would cause alone.

I have used Baltic calanoid copepods (crustacean zooplankton) as my research object and studied their growth and stress responses using climate predictions projected for the next century. I have studied both direct temperature and pH effects on copepods, and indirect effects via their food: the changing phytoplankton spring bloom composition and toxic cyanobacterium. The main aims of my thesis were: 1) to find out how warming and acidification combined with a toxic cyanobacterium affect copepod reproductive success (egg production, egg viability, egg hatching success, offspring development) and oxidative balance (antioxidant capacity, oxidative damage), and 2) to reveal the possible food quality effects of spring phytoplankton bloom composition dominated by diatoms or dinoflagellates on reproducing copepods (egg production, egg hatching, RNA:DNA ratio). The two copepod genera used, *Acartia* sp. and *Eurytemora affinis* are the dominating mesozooplankton taxa (0.2 – 2 mm) in my study area the Gulf of Finland.

The 20°C temperature seems to be within the tolerance limits of *Acartia* spp., because copepods can adapt to the temperature phenotypically by adjusting their body size. Copepods are also able to tolerate a pH decrease of 0.4 from present values, but the combination of warm water and decreased pH causes problems for them. In my studies, the copepod oxidative balance was negatively influenced by the interaction of these two environmental factors, and egg and nauplii production were lower at 20°C and lower pH, than at 20°C and ambient pH. However, presence of toxic cyanobacterium *Nodularia spumigena* improved the copepod oxidative balance and helped to resist the environmental stress, in question. In addition, adaptive maternal effects seem to be an important adaptation mechanism in a changing environment, but it depends on the condition of the female copepod and her diet how much she can invest in her offspring.

I did not find systematic food quality difference between diatoms and dinoflagellates. There are both good and bad diatom and dinoflagellate species. Instead, the dominating species in the phytoplankton bloom composition has a central role in determining the food quality, although copepods aim at obtaining as a balanced diet as possible by foraging on several species. If the dominating species is of poor quality it can cause stress when ingested, or lead to non-optimal foraging if rejected.

My thesis demonstrates that climate change induced water temperature and pH changes can cause problems to Baltic Sea copepod communities. However, their resilience depends substantially on their diet, and therefore the response of phytoplankton to the environmental changes. As copepods are an important link in pelagic food webs, their future success can have far reaching consequences, for example on fish stocks.

# SAMMANFATTNING (in Swedish)

Ökade utsläpp av växthusgaser i atmosfären leder till klimatförändring förorsakad av människan. Den globala uppvärmningen medför utmaningar för organismer att anpassa sig till nya förhållanden. Varmare klimat utgör inte det enda hotet. Koldioxid från atmosfären löses i haven och orsakar en försurning av ytvattnen. Temperaturförändringen och sänkt pH kan samverka och förorsaka ännu större problem för marin flora och fauna än vad faktorerna ensamma orsakar.

Som försöksorganism har jag använt hoppkräftor (Calanoida) från Östersjön och undersökt tillväxt och stressresponser på klimatförändringen motsvarande prognosen för det kommande seklet. Jag har undersökt både direkta effekter av temperatur och pH på hoppkräftor, samt indirekta effekter via födan: ändrad artsammansättning av växtplankton i vårblomningen och giftiga cyanobakterier. Den huvudsakliga målsättningen för min avhandling var 1) att ta reda på hur varmare klimat och havsförsurning kombinerat med giftiga cyanobakterier påverkar reproduktionsframgången hos hoppkräftor (äggproduktion, äggöverlevnad, äggkläckning, utveckling av avkomman) och oxidativ balans (antioxidantkapacitet, oxidativ skada) och 2) att observera eventuella effekter av födans kvalitet i vårblomningar dominerade av kiselalger eller dinoflagellater på reproducerande hoppkräftor (äggproduktion, äggkläckning, RNA:DNA förhållandet). De två hoppkräftsgenus som använts, *Acartia* sp. och *Eurytemora affinis*, är dominerande mesodjurplanktontaxa (0.2 – 2 mm) i undersökningsområdet i Finska viken.

Temperaturen 20°C verkar vara inom toleransgränsen för *Acartia* sp., eftersom hoppkräftor kan anpassa sig till temperatur fenotypiskt genom ändrad kroppsstorlek. Hoppkräftor tål också sänkning av pH med 0.4 enheter från nuvarande, medan kombinationen av varmt vatten och sänkt pH förorsakar problem för dem. I mina undersökningar påverkades hoppkräftornas oxidativa balans negativt av samverkan mellan två omgivningsfaktorer och ägg- och naupliiproduktionen var lägre vid 20°C och lågt pH än vid 20°C och oförändrat pH. Däremot förbättrades hoppkräftornas oxidativa balans och motståndet mot stress förorsakat av närvaron av den giftiga cyanobakterien, *Nodularia spumigena*. Adaptiva maternella effekter föreföll också vara en viktig anpassningsmekanism i en förändrad omgivning, men honans kondition och kvaliteten på tillgänglig föda påverkar hur mycket hon kan investera i avkomman.

Det framkom inga systematiska skillnader i födokvalitet mellan kiselager och dinoflagellater. Kiselalger och dinoflagellater kan delas upp i arter av både bättre och sämre kvalitet. Däremot har den dominerande arten i växtplanktonblomningen en central roll för födokvaliteten, fastän hoppkräftorna försöker uppnå en så balanserad diet som möjligt genom att beta flera olika arter. Om den dominerande arten är av dålig kvalitet kan intag av den medföra stress, eller om den aktivt väljs bort leda till icke-optimalt födointag.

Min avhandling visar att varmare temperatur och sänkt pH som en följd av klimatförändringen kan orsaka problem för hoppkräftorna i Östersjön. Deras motståndskraft beror långt på dieten och därför på växtplanktonens respons på förändrade miljöförhållanden. Eftersom hoppkräftor utgör en viktig del i den pelagiska födoväven kan deras framgång ha långtgående konsekvenser för till exempel fiskbestånd.

## TIIVISTELMÄ (in Finnish)

Ihmistoiminnasta aiheutuva kasvihuonekaasupäästöjen lisääntyminen ilmakehässä aiheuttaa ilmastonmuutoksen. Seurauksena on eliöiden sopeutumiskyvyn haastava ilmaston lämpeneminen. Lämpötilan nousu ei kuitenkaan ole ainoa ilmastonmuutoksen aiheuttama uhka. Hiilidioksidin liukeneminen ilmakehästä veteen laskee merten pintakerrosten pH:ta, eli aiheuttaa merten happamoitumista. Lämpeneminen ja happamoituminen voivat yhdessä aiheuttaa merieliöille vielä suuremman haasteen kuin kumpikaan vaikutus yksin.

Tutkimuskohteena olen käyttänyt hankajalkaista eläinplanktonia (lahko: Calanoida). Olen tarkastellut niiden kasvu- ja stressivastetta ensi vuosisadalle ennustetuissa Itämeren oloissa. Olen tutkinut sekä lämpötilan ja pH:n suoria vaikutuksia että kevään ja kesän ravintotilanteessa tapahtuvien muutosten aiheuttamia epäsuoria vaikutuksia hankajalkaisiin. Tämän väitöskirjatyön päätavoitteet olivat: 1) saada selville kuinka lämpötilan nousu ja pH:n lasku yhdistettynä myrkylliseen syanobakteeriin (sinilevä) vaikuttavat hankajalkaisäyriäisten lisääntymismenestykseen (munantuotanto, munien elinkykyisyys, kuoriutumismenestys ja jälkeläisten kehitys) ja oksidatiiviseen tasapainoon (antioksidantit ja oksidatiivinen stressi), sekä 2) selvittää piilevien tai panssarisiimalevien hallitseman keväisen kasviplanktonkukinnan mahdolliset ravinnonlaatuvaikutukset hankajalkaisten lisääntymiseen (munantuotanto, kuoriutumismenestys, RNA:DNA suhde). Tutkimuksissa käytetyt kaksi hankajalkaisäyriäissukua, *Acartia* sp. ja *Eurytemora affinis* ovat vallitsevia mesoeläinplankton-taksoneita (0.2 – 2 mm) tutkimusalueella Suomenlahdella.

*Acartia* sp. hankajalkaiset näyttävät kestävän 20 °C lämpötilan, sillä ne pystyvät sopeutumaan eri lämpötiloihin muuttamalla kokoaan. Hankajalkaiset sietävät myös 0.4 laskun pH-asteikolla nykyisestä, mutta lämpimämpi vesi yhdistettynä matalampaan pH-tasoon aiheutti niille ongelmia ja heikensi oksidatiivista tasapainoa. Lisäksi, munantuotanto ja nauplius-toukkien tuotanto oli matalampi 20 °C lämpötilassa ja alennetussa pH:ssa kuin 20 °C ja normaali pH-tasolla. Myrkyllinen syanobakteeri *Nodularia spumigena* paransi hankajalkaisten oksidatiivista tasapainoa ja auttoi kestämään ympäristön aiheuttamaa stressiä. Myös maternaaliefektit (äidin vaikutus jälkeläisen kehitykseen) ovat tärkeä sopeuttamismenetelmä muuttuvassa ympäristössä, mutta kyky panostaa jälkeläisiin riippuu naaraan kunnosta ja ravinnosta.

En löytänyt systemaattisia ravinnon laatueroja piilevien ja panssarisiimalevien välillä. On olemassa sekä hyviä että huonoja piilevä- ja panssarisiimalevälajeja. Sen sijaan kasviplanktonkukinnan vallitsevalla lajilla on ratkaiseva rooli ravinnon laadussa, vaikka hankajalkaiset yrittävätkin hankkia mahdollisimman monipuolisen ruokavalion syömällä useita kasviplanktonlajeja. Jos kukinnan vallitsevalla lajilla on huono ravinnonlaatu, se voi aiheuttaa syöjälleen stressiä tai johtaa epäoptimaaliseen ravinnonhankintaan, jos sitä vältellään.

Väitöskirjatyöni osoittaa, että ilmastonmuutoksesta johtuva veden lämpötilan ja pH:n muuttuminen voivat aiheuttaa ongelmia Itämeren hankajalkaisyhteisöille. Hankajalkaisäyriäisten sietokyky riippuu kuitenkin olennaisesti niiden ruokavaliosta, ja siksi ympäristön muutoksen vaikutuksesta kasviplanktoniin. Koska hankajalkaiset ovat tärkeä osa ulapan ravintoverkkoa, niiden tulevaisuuden menestyksellä voi olla kauaskantoisia seurauksia, mm. kalakantoihin.

# 1. INTRODUCTION

## 1.1. Why study copepods?

Zooplankton is an essential group in pelagic ecosystems, because of its wide distribution, and functional and morphological diversity. As a main utiliser of primary production, i.e., phytoplankton, herbivorous/omnivorous zooplankton itself provides nutrition for animals in the range from carnivorous zooplankton and fish larvae to the largest animals on earth, baleen whales. Copepods typically dominate the zooplankton biomass, and they are estimated to be the most plentiful multi-cellular animals on earth (Humes 1994). Unique survival, feeding, and reproduction features have evolved in copepods making them very successful among zooplankton in the dangerous and meagre three-dimensional environment that they live in (Kiørboe 2011).

In the famous article by Costanza et al. (1997) the value of ecosystem services that marine and freshwater ecosystems provide globally is estimated to be 22.65 trillion USD per annum. Although not directly consumed by humans, copepods contribute significantly to many of these services by being an indispensable part of functioning pelagic ecosystems (Bron et al. 2011), and therefore ultimately benefiting also humans (Millenium Assessment 2005). Top down control of primary production, i.e., grazing of phytoplankton, is a regulating service that can prevent nuisance algal blooms, improve water transparency, and thus increase recreational value of the waters. Copepods are engaged in nutrient cycling and vertical nutrient flux in the water column by performing diel vertical migration, and creating marine snow, as faecal pellets, dead eggs and carcasses that feeds the aphotic zone (Noji 1991, Frangoulis et al. 2005). Another supporting service, and simultaneously the most distinct one is copepod's role as fish food. They nourish early life-history stages of many economically important fish species (Beaugrand et al. 2003, Bi et al. 2011) as well as some adult fishes, such as sprat and herring (Rudstam et al. 1992,

Flinkman et al. 1998).

The Baltic Sea is a relatively young sea; permanent ice receded in 11.000-10.000 cal yr BP, and it has undergone many different phases after that, both saline and fresh (von Storch & Omstedt 2008). As the world's largest brackish water basin its nature is unique. Young geological age and brackish water, which excludes many marine and freshwater species, are reasons for low species number in the Baltic (HELCOM 2009). That is why the Baltic Sea is especially vulnerable and susceptible to pressures such as pollution, habitat destruction and overfishing (HELCOM 2010). There are in total ~1200 zooplankton species in the Baltic Sea with the open Baltic hosting 86% of them and estuaries 14% (Ojaveer et al. 2010). Between ten and twenty out of 40 mesozooplankton (0.2-2 mm) species that regularly inhabit the Baltic are dominating taxa. The two dominating zooplankton species in the Gulf of Finland are calanoid copepods *Acartia bifilosa* and *Eurytemora affinis* (Ojaveer et al. 2010). *Acartia tonsa* can be present when water temperature becomes warm enough at the end of summer (Katajisto et al. 1998), and *Temora longicornis*, *Limnocalanus macrurus* and cyclopoid copepods are sometimes found from the zooplankton samples (Koski et al. 1999). *Bosmina* spp. cladocerans can constitute a major proportion of zooplankton biomass in late summer and early autumn (Koski et al. 1999). The dominating zooplankton taxa have a major role in functioning of the whole pelagic food webs, because of the low species diversity. Therefore, studying those leverage species can also promote important knowledge of the possible cascading effects in the ecosystem.

## 1.2. Global climate change and Baltic Sea

Global climate change does not impact all parts of the world equally. Some areas and seasons will be affected more than others. The warming trend for the whole globe was 0.05°C in the decade between 1861 and 2000, whereas

for the Baltic Sea basin it was 0.03°C higher (HELCOM 2007). The warming is continuing with accelerated speed; the projected global temperature increase for the next century is 0.5-3.5°C, and for the Baltic basin 3-5°C depending on the emission scenario (HELCOM 2007, IPCC 2007). Rising temperatures will lengthen the growing season and shorten the ice cover period, as well as decrease the ice extent in the Baltic (HELCOM 2007). Mild winters will also result in increased precipitation, which will affect nutrient run-off and salinity (Graham et al. 2008). Higher temperatures strengthen spring and summer water stratification by preventing convective mixing, whereas decreasing salinity counteracts the warming by increasing the mixing temperature and thus also mixing probability (Hordoir & Meier 2012).

The surface ocean experiences gradual and global carbon dioxide enrichment due to the continuous rise of atmospheric $CO_2$. The net flux of $CO_2$ into the surface ocean causes a change in seawater carbonate chemistry (Riebesell 2004). Acidification, the increase of protons ($H^+$) in water, shifts the pH-dependent equilibrium between $CO_2$, bicarbonate ions and carbonate ions: dissolved $CO_2$ concentration increases, concentrations of bicarbonate ions and total dissolved inorganic carbon (DIC) increases slightly, and carbonate ion concentration and pH decreases. Since pre-industrial times, oceans have absorbed approximately 50% of the $CO_2$ produced by burning fossil fuels and cement production, which have decreased the surface ocean pH values by 0.1 units (The Royal Society 2005). If global emissions of $CO_2$ from human activities continue to rise as current trends show, the average pH of the ocean could fall by 0.5 units by the year 2100 (Caldeira & Wickett 2003, The Royal Society 2005).

When environmental conditions change, fate of the species in the area is dependent on several aspects. First, phenotypic plasticity, i.e., the acclimatisation potential, will be challenged. Physiologically, organisms need to compensate disturbances in their body functions, or cope with resulting oxidative stress. If the challenge is too strong, the consequence can be an acute death, or slow disappearance after losing the ability to grow and reproduce due to high maintenance costs. Further, if populations can move, local populations can be replaced by congener species, or even con-specific, nearby populations that can better adapt to the new conditions. Finally, climate change is a continuing process, which does not stop after the first set of changes. Population size and genetic variability, as well as generation time will have an effect on the species ability to 'keep up with' the changing environment (Somero 2010). Species with short generation times might be able to respond to the changes through adaptive evolution (Lohbeck et al. 2012).

## 1.2.1. It is getting hot in here

Increasing temperatures affect zooplankton growth and reproduction directly. The immediate response can be negative or positive, depending on the optimal temperature of the species in question. Temperature affects physiological processes, such as heart function, membrane fluidity, protein synthesis and stability, and mitochondrial respiration (Somero 2002). Species that already live close to their thermal limits are the most vulnerable to increasing temperatures (Harley et al. 2006). For a species living in an area with seasonal variability, the first way to adapt to changing temperature is to alter its phenology so that the most vulnerable stages of the life-cycle are in synchrony with the physical environment (Beaugrand 2009). A few degrees change in temperature can possibly be combated, for example, by a few weeks earlier start of the reproductive season.

Copepod growth and fecundity are universally positively related to temperature (Hirst & Bunker 2003). Organisms in the northern Baltic Sea experience large seasonal temperature changes, from 0°C below the ice up to 20°C and above in summer (Suikkanen et al. 2007). Both *A. bifilosa* and *E. affinis* are eurythermal species, whereas *E. affinis* is more sensitive to temperature changes (stenotherm) (Viitasalo et al. 1994 and references therein). However, it

is possible that the two copepod species reach their thermal limits during warm summers. Higher temperature could force copepods to change their dial vertical migration patterns and stay in deeper and cooler water columns. The migrations patterns are determined by a complex matrix of environmental conditions such as predator avoidance and food availability, therefore search for optimal temperature might cause trade-offs between these individual needs (Vuorinen 1987, Holliland et al. 2012). Increasing temperature decreases the carbon content of reproducing females, which probably is caused by accelerated metabolism and increased food demand (Koski & Kuosa 1999). Thus temperature correlates positively with foraging and metabolism, and negatively with body size. Another *Acartia*-species inhabiting the Baltic Sea, *A. tonsa*, has an even wider temperature tolerance (González 1974). It is able to produce >10 eggs d-1 at temperatures between 10 and 30°C, and its egg production peaks at 22.9°C (Holste & Peck 2006). The high reproductive success over a wide temperature range is thought to be one of the reasons why *A. tonsa* is a cosmopolitan with a wide distribution within productive, estuarine habitats.

Long-term data analyses in the Baltic Sea have shown that temperature has a greater influence on the surface communities of smaller zooplankton than on the neritic and submergent populations of larger species (*Pseudocalanus* and possibly *Temora*), which are affected more by salinity changes (Möllmann et al. 2000, Dippner et al. 2008). Further, rotifers, cladocerans and smaller copepods may benefit from elevated surface water temperatures both during cold and warm seasons, due to increased wintertime survival, faster population build-up in spring, and higher growth and reproduction success in summer (Möllmann et al. 2000, 2002, Dippner et al. 2001, Hansson et al. 2010). After harsh winters, the reproduction of the copepods *Acartia* spp. and *T. longicornis* begins later and is slower (Dippner et al. 2000, Möllmann et al. 2000). Furthermore, a significant correlation between high winter North Atlantic Oscillation index (NAO, mild winters) and spring and early summer biomasses of *E. affinis*, *Acartia* spp. and *T. longicornis* has been found in coastal areas (Hansson et al. 2010). As parthenogenetic reproducers, rotifers multiply their numbers rapidly in optimal conditions. The abundance of *Synchaeta*-rotifers is observed to be especially high after mild winters (Dippner et al. 2000). Cladocerans also benefit from elevated spring temperatures and warm summers (Möllmann et al. 2002). It seems that while mild winters are becoming more common in temperate regions, the role of small mesozooplankton species is increasing and their biomasses are growing. This is true at least in springtime when environmental conditions are more relevant than top-down control by predators (Dippner et al. 2000).

## 1.2.2. Acidifying sea

As with all abiotic variables, the effects of elevated $CO_2$ concentrations on animal physiology depends on the organism's structure, e.g., is the circulatory system open or closed, and are the blood pigments intra- or extracellular (Pörtner 2008). In water-breathing crustaceans, the $CO_2$ concentration in the haemolymph (extracellular compartment) can increase when the excretion across the gills is endangered due to changes in the seawater carbon chemistry (Whiteley 2011). Decreased pH in the haemolymph can be tolerated on a short-term basis, but it can affect oxygen supply and delivery to tissues on a longer perspective (Whiteley 2011). Maintenance of the acid-base equilibrium of the body fluids is also important, because imbalance might result in dysfunctional key metabolic processes, such as protein synthesis, metabolism, and cell volume control (Whiteley 2011).

Mechanisms behind acid-base balance control are closely associated with ion-regulation; therefore strong ion- and osmoregulator species are presumably less vulnerable to ocean acidification (Whiteley 2011). Species inhabiting shallow coastal and estuarine areas, influenced by fluctuating salinity, temperature, oxygen and pH conditions, are at least in theory, the least

likely to suffer from ocean acidification. In my study area, both seasonal (Viitasalo et al. 1995, Brutemark et al. 2011) and rapid upwelling induced changes happen in the four parameters (Lips et al. 2009).

One prominent physiological response of ocean acidification is the effect on the calcification process of marine organisms. Considering all organisms in the surface oceans the impacts are thought to be most severe for the calcifying species, such as molluscs, crustaceans, echinoderms, corals, large calcareous algae, foraminiferans and certain phytoplankton (The Royal Society 2005). Calcium carbonate, $CaCO_3$, that builds up the shells of marine organisms are dependent on the presence of bicarbonate and carbonate forms of DIC in the seawater. If the surrounding water is not saturated with carbonate ions, calcium carbonate will dissolve back into the water. From the copepod's point of view, calcification is of a lesser concern, because $CaCO_3$ in the crustacean exoskeletons is a more stable form of calcite, different from aragonite in molluscs and echinoderms (Whiteley 2011). The atmospheric $CO_2$ concentration predicted for the year 2300 (~2000 ppm) and its corresponding pH decrease in the surface waters (0.77 units) (Caldeira & Wickett 2003) does not seem to affect the survival rate of *Acartia* copepods (Kurihara et al. 2004, Kurihara & Ishimatsu 2008). However, laboratory experiments with marine copepods have shown that responses to increased $CO_2$ concentrations can vary between different life stages. Concentrations (5000 and 10000 ppm) which did not affect the survival rates of adult female *Acartia* copepods (*A. erythraeae* and *A. steueri*) had a negative effect on reproduction and larval development (Kurihara et al. 2004). The study also indicated that the tolerance capacity to increasing $CO_2$ levels differs between copepod species, depending on the variation of abiotic factors in their natural habitat (Kurihara et al. 2004). Watanabe et al. (2006) confirmed this by discovering that deep-living copepods were more tolerant to high $CO_2$ levels than shallow-living copepods. This is true especially in the subarctic and transitional regions where the natural $CO_2$-concentrations are higher, due to higher primary production and thus higher decomposition compared to that in subtropical regions. Mayor et al. (2007) carried out experiments with the calanoid copepod *Calanus finmarchicus*, the keystone species in the North Sea and the North Atlantic Ocean, which overwinters in depths between 500 and 1500 m. Viability of *C. finmarchicus* eggs, produced and incubated in the acidified seawater (8000 ppm, pH 6.95), was significantly impaired. It was concluded that the threshold of $CO_2$, which generates a reduction in copepod egg hatching success lies between 5000 and 8000 ppm (Mayor et al. 2007, cf. Kurihara et al. 2004).

## 1.2.3. A high quality meal or not?

The effects of the increasing temperature in aquatic environments are versatile. Higher water temperature accelerates the metabolism of aquatic organisms, which usually means, until a certain point, faster growth and reproduction, but also a higher energy demand. Changes in water temperatures affect mixing conditions; winter water temperature above the temperature of maximum density prevents spring convective mixing. However, turbulent mixing caused by strong winds might compensate the weakened convective mixing (Dippner et al. 2008). Enhancing stratification will influence the surface water nutrient availability and thus the rate of primary production. In the Northeastern Atlantic, sea surface warming is expected to increase phytoplankton abundance in cooler regions and decrease it in warmer regions, due to differences in stratification and nutrient availability (Richardson & Schoeman 2004). Because of tight trophic coupling, the regional differences are extended through the food web to both herbivorous and carnivorous zooplankton.

The consequences of increasing water temperatures on zooplankton depend on changes in food availability, because food demand increases with temperature. Diatoms

are the phytoplankton group that dominate the spring bloom in the temperate zone. In parts of the Baltic Sea, however, cold-water dinoflagellates co-occur with diatoms or even exceed their biomass (e.g. Kononen & Niemi 1984). In recent decades, dinoflagellates have increased in proportion to diatoms (Wasmund & Uhlig 2003, Klais et al. 2011, Wasmund et al. 2011), but there are also signs of alternating oscillations between the two groups (Wasmund et al. 2011). The success of dinoflagellates over diatoms has been explained by a period of warm winters caused by a positive NAO phase and eutrophication associated changes in dissolved silica availability and decreased dissolved silica:nitrogen ratios. Fast-growing small diatoms are superior competitors in turbulent unstable conditions, whereas large and slow-growing, but motile dinoflagellates can outcompete diatoms if they get a head-start with a massive inoculum population (Klais et al. 2011). When the diatom bloom fails to emerge, the dinoflagellates compensate the loss and even surpass the customary diatom biomass (Wasmund & Uhlig 2003). Similar changes in the spring phytoplankton bloom composition have also been reported in other sea areas in the northern hemisphere. In the North Sea, the changes in phytoplankton community composition occurred synchronously with changes in the Central Baltic Sea (Alheit et al. 2005). Dinoflagellates became more abundant in the late 1980s and their abundance was positively correlated with NAO.

The utilisation of the diatom-dominated spring bloom is generally low and a significant proportion of it sinks ungrazed. It has been argued that the main grazers of the spring bloom, calanoid copepods could benefit from the change in the species composition, because some common spring bloom diatoms species diminish the reproductive success of copepods (Ianora et al. 2004). Diatom species are observed to impair copepod reproduction, either by affecting egg production directly, or egg hatching success indirectly (Ban et al. 1997, Pierson et al. 2005, Ask et al. 2006, Poulet et al. 2006, 2007), or by preventing the development

of juvenile stages (Poulet et al. 2007). This has been explained by the lack of essential nutrients, such as polyunsaturated fatty acids (PUFAs) that are necessary for copepod reproduction (Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996), or by inhibitory substances, such as polyunsaturated aldehydes (PUAs), which are released when cells are disrupted (Miralto et al. 1999, Pohnert 2000). However, there are also studies showing no effect of diatom bloom-derived aldehydes on copepods (Koski et al. 2008, Jónasdóttir et al. 2011). Alternative hypotheses of diatom-induced reproductive failure in copepods include other oxylipins than aldehydes that induce oxidative stress (Fontana et al. 2007), or nutritional deficiencies due to incomplete digestion (Dutz et al. 2008).

Besides the potential positive effects of higher temperatures on spring bloom dinoflagellate abundances, increasing temperature also has its downsides. Harmful algal blooms (HAB) caused by, amongst other taxa, toxic dinoflagellates and cyanobacteria might be favoured by the warming climate (Hallegraeff 2010). HABs causing paralytic shellfish poisoning or producing cyanobacterial toxin may disrupt food webs by accumulating toxins in zooplankton (Doucette et al. 2005, Karjalainen et al. 2007), but also by affecting behaviour and energy allocation of co-occurring organisms (Buskey & Hyatt 1995, Karjalainen et al. 2007). In many studies pelagic bacteria and other heterotrophs are more stimulated by increased temperature than primary producers are, due to differences in the temperature dependence of photosynthesis and respiration (Dippner et al. 2008). The higher activity of the heterotrophic communities increases nutrient recycling and mineralisation in surface waters and thus, decreases the sedimentation of organic matter. In the Baltic Sea and in several lakes around the world, cyanobacterial blooms are a frequent summer phenomenon, and a symptom of human-induced eutrophication. Cyanobacteria flourish in warm water, which gives them a competitive advantage, while temperatures are elevating and surface waters are becoming more stable (Paerl & Huisman 2008). Cyanobacteria are

low quality food for zooplankton, and they also use allelopathic mechanisms to outcompete co-occurring phytoplankton, such as cryptophytes (Suikkanen et al. 2005), which are high quality food for copepods (Brett & Müller-Navarra 1997). One prominent effect of a temperature increase on the phytoplankton community is a shrinking cell size (Sommer & Lengfellner 2008). The impacts of a changing phytoplankton composition can be extensive. A shift from large diatoms to pico- and nanosized phytoplankton for example impairs the transfer efficiency of energy from primary production to copepod and fish production, as well as the function of the biological carbon pump (Sommer & Lengfellner 2008). Diatoms are considered as important carbon vectors to the deep water, both as fast sinking cells and colonies and as components of copepod faecal pellets, whereas carbon of smaller phytoplankton is recycled in surface waters by protist respiration. A shift to smaller phytoplankton species can therefore impair the ocean's role as a carbon sink, and further accelerate climate change.

## 1.2.4. Non-linear and non-independent changes

One huge challenge that climate change sets for scientists is the possibility of abrupt changes with wide consequences. A prime example of non-linear change in temperature could be caused by a shut-down of thermohaline circulation in the North Atlantic Ocean (Harley et al. 2006). We have difficulties to foresee such huge changes because they are out of our experience limits. A little less dramatic example is a regime shift, which is a transition in an ecosystem from alternative stable state to another. Starting from the late 1980s, a positive winter time NAO index in the North Sea and the Baltic Sea caused an increase in sea surface temperature. Together with warm water inflow to halocline in summertime, the temperature rise lead to a regime shift in the pelagic ecosystem of the Baltic Sea that extended several trophic levels from phytoplankton to cod (Alheit et al. 2005).

In addition, interaction effects of several climate related variables could cause unexpected consequences. The combined effect can be larger (synergistic) or smaller (antagonistic) than expected result from the single effects. Further, the combination of several stressors can push an individual or a population beyond a critical threshold that would not be reached by any variable acting alone (Harley et al. 2006). For example, the interaction of several stressors, such as $CO_2$, salinity change, and hypoxia will affect the organism's performance, especially at extreme temperatures, which can lead to narrowed thermal window and biogeographical range (Pörtner & Farrell 2008). In addition, temperature-induced phenological changes can vary between functional groups and trophic levels, and cause a temporal mismatch between predators and prey (Cushing 1990, Edwards & Richardson 2004). Temperate pelagic environments are especially vulnerable to changes in phenology, since synchronised planktonic production feeds the higher trophic levels.

## 2. AIMS OF THE THESIS

The ongoing human-induced climate change is an incomprehensibly large phenomenon that stretches to every corner of our planet. Climate change related publications in the field of marine ecology have increased tremendously in recent decades (see literature surveys by Harley et al. 2006 and Gattuso & Hansson 2011). To add my part to the growing but still desperately needed knowledge, I have studied calanoid copepods, a key order in pelagic food webs. This thesis work touches upon direct and indirect climate driven aspects concerning copepod reproductive success: single and combined effects of acidification and warming, and the effects of changing food quality, i.e., toxic cyanobacteria and spring bloom composition (Fig. 1).

The objectives of the four chapters of my thesis are:

**Paper I**     To study how copepod oxidative balance (antioxidant capacity and oxidative stress) and reproductive success (egg production, egg viability and offspring development) are influenced by a multi-stress situation created by decreased pH, elevated temperature and presence of toxic cyanobacteria. The pH decrease and the temperature increase were selected according to a 2100 climate scenario.

**Paper II**     To study the effects of decreased pH and elevated temperature on copepod egg production, and to reveal the importance of adaptive maternal effects on egg hatching speed and success. The same pH and temperature treatments as in **Paper I** were used.

**Paper III**     To study if copepod vernal growth (egg production, egg hatching and RNA:DNA ratio) is higher when the spring bloom composition is dominated by dinoflagellates, instead of diatoms.

**Paper IV**     To study if copepod egg production is higher when copepods feed on *Scrippsiella hangoei* dinoflagellates rather than *Skeletonema marinoi* diatoms or a combination of the two, and to see if the food quality parameters polyunsaturated aldehydes, fatty acids, sterols, and minerals can explain the egg production results.
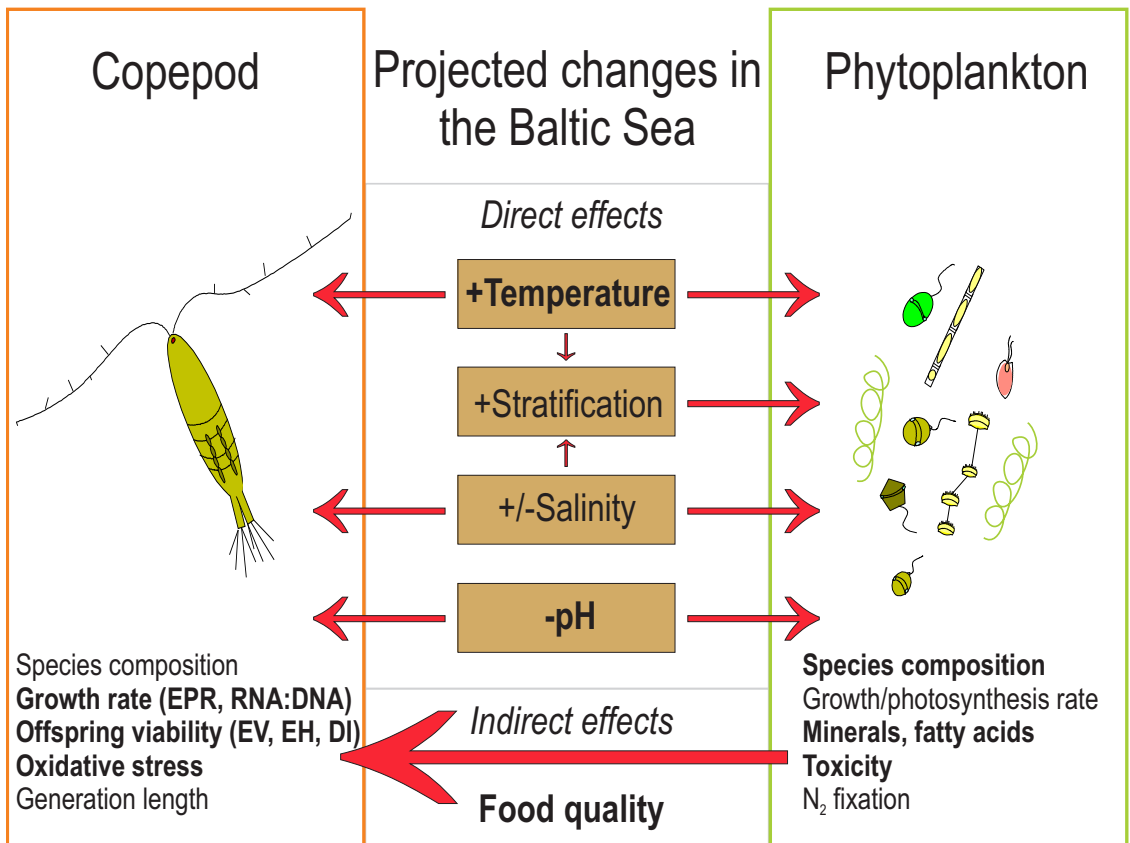


**Fig. 1** A schematic of the projected climate induced changes in the Baltic Sea and their direct effects on copepods, and their indirect effects via the food source, phytoplankton. The effects that I have studied in this thesis are emphasised in bold.
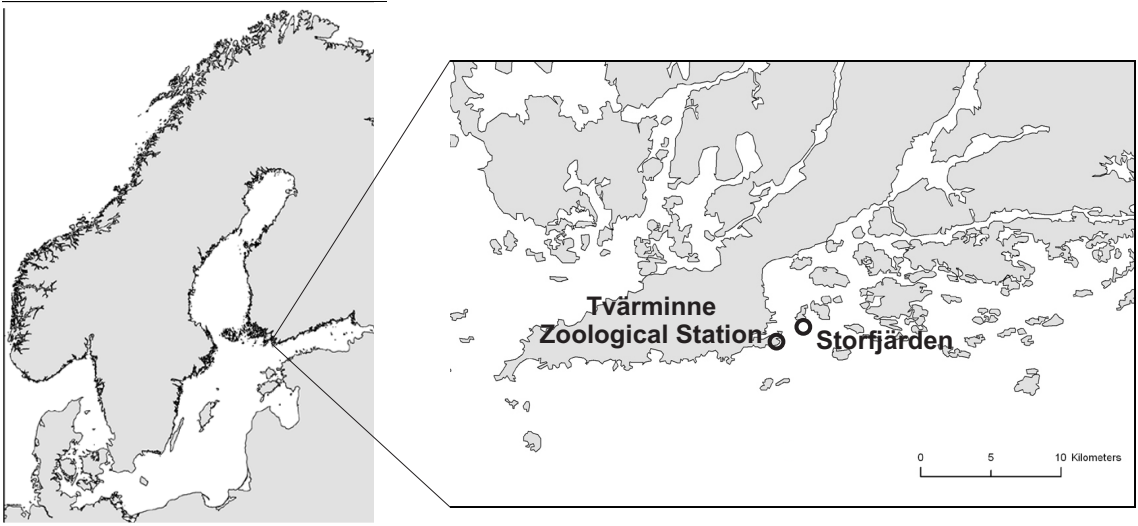
**Fig. 2** Map showing the sampling station Storfjärden and Tvärminne Zoological Station at the entrance of Gulf of Finland, Baltic Sea.

## 3. METHODS

All chapters of my thesis are based on experimental studies, which were performed at Tvärminne Zoological Station (University of Helsinki), SW coast of Finland, Baltic Sea. The animals, phytoplankton and water needed for the experiments were collected from Storfjärden (59°51′20′′N and 23°15′42′′E), a pelagic area near the field station (Fig. 2). In **Paper III**, the copepods were grown from hatched resting eggs, which were isolated from surface sediment collected from Storfjärden two months prior to the experiment. More information about the sampling station can be found from Hällfors et al. (1983).

A diverse set of methods was used for assessing reproduction and condition of the two dominating copepod species in the study area, *Acartia bifilosa* and *Eurytemora affinis* (Viitasalo 1992) (Table 1). Copepods were provided with live (**Papers I**, **II**, **III** & **IV**) or preserved (**Paper II**) cultured phytoplankton as food. The cyanobacterium *Nodularia spumigena* (AV1) (**Paper I**) was grown in Z8 medium (Kótai 1972). The chlorophyte *Brachiomonas submarina* (TV 15) (**Papers I** & **III**), the cryptophyte *Rhodomonas* spp. (TV 22, 07B6)

(**Papers II**, **III** & **IV**) and the dinoflagellates *Gymnodinium corollarium* (GC TV B4) (**Paper III**) and *Scrippsiella hangoei* (SHTV-5) (**Paper IV**) were grown as batch cultures in f/2 without silica medium (Guillard & Ryther 1962). The diatoms *Chaetoceros* cf. *wighamii* (**Paper III**), *Skeletonema marinoi* (**Papers III** & **IV**) and *Thalassiosira baltica* (**Paper III**), which were all originally isolated from the study area, were grown in f/2 medium. In **Paper II**, the copepods were additionally fed with Bioplankton (Liquid Life), a commercially available food solution containing *Isochrysis* sp., *Nannochloropsis* sp. and *Tetraselmis* sp. phytoplankton cells. The quality and the quantity of the food were estimated in multifaceted ways (Table 2).

The pH treatments (**Papers I** & **II**) were created by bubbling filtered seawater (Sartobran P 300 0.2 μm) with industrial $CO_2$-gas (AGA Finland) until the target level was reached using a TUNZE pH/$CO_2$-controller-set (7074/2). TUNZE pH-controller was also used for measuring pH in the experiment bottles (**Paper II**) after calibrating it with at least two buffers (pH 4.00, 7.00 or 10.00) in the operating temperature. In addition to the pH-measurements, dissolved inorganic carbon was measured according to the acidification and bubbling method of Salonen (1981) in **Paper I**.

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**Table 1.** Summary of methods used for assessing copepod reproduction, growth and condition.

| Measurement | Method | Paper in the thesis | Reference |
|---|---|---|---|
| Copepod reproductive success | Egg production (*Acartia* sp.) | **I, II, IV** | Runge & Roff 2000 |
| Copepod reproductive success | Egg production *Eurytemora affinis* | **III** | Runge & Roff 2000 |
| Copepod reproductive success | Egg hatching | **II, III** | Runge & Roff 2000 |
| Copepod reproductive success | Egg viability | **I** | Gorokhova 2010 |
| Offspring development | Development index | **I** | Knuckey et al. 2005 |
| Copepod protein synthesis | RNA : DNA | **III** | Gorokhova & Kyle 2002 |
| Copepod oxidative status | Oxygen radical absorption capacity, ORAC | **I** | Prior et al. 2003 |
| Copepod oxidative status | Lipid peroxidation assay, TBARS | **I** | **Paper I** |
| Feeding | Cell enumeration in grazing and in control bottles | **IV** | Frost 1972 |

**Table 2.** Summary of methods used for measuring food quality and quantity.

| Measurement | Method | Paper in the thesis | Reference |
|---|---|---|---|
| POC[1], PON[2] | Mass spectrometry | **I, II, III, IV** | Grasshoff 1976 |
| POP[3] | Dry combustion, acid hydrolysis | **IV** | Solórzano & Sharp 1980 |
| Cyanobacterium toxin nodularin | Enzyme-linked immunosorbent assay (ELISA) | **I** | Metcalf & Codd 2003, as in Engström-Öst et al. 2011 |
| PUFA | Direct transesterification, gas chromatography-mass spectrometry (GC-MS) analysis | **IV** | Lepage & Roy 1984, **Paper IV** |
| Sterols | Direct transesterification, GC-MS analysis | **IV** | Lepage & Roy 1984, **Paper IV** |
| PUA | GC-MS | **IV** | Wichard et al. 2005, **Paper IV** |
| Cell enumeration | Ütermöhl | **I, II, III, IV** | Ütermöhl 1958 |
| Cell enumeration | Haemocytometer, Bürker | **I, IV** | Guillard & Sieracki 2005 |

[1]Particulate organic carbon, [2]particulate organic nitrogen, [3]particulate organic phosphorus

# 4. RESULTS AND DISCUSSION

## 4.1. Direct effects of climate change on copepods

Sudden changes in the organism's environment challenges the phenotypic plasticity, i.e., the ability of an organism to respond to the change by altering its physiological state, appearance or behaviour (West-Eberhard 2003). Although climate change induced changes are not reckoned to be sudden, but rather slow alterations over decades and centuries, testing the organism's plasticity in short-term studies gives also valuable information about future ecosystem changes. If an organism can tolerate a rapid change and start acclimating or adapting to the new conditions within hours or days, a change of the same magnitude on a longer time-scale will not cause problems (Pörtner 2008). Moreover, time-scales are very much taxon-dependent. Changes over decades or centuries can occur in a part of a lifetime for long-lived organisms, but comprise hundreds or thousands of generations for short-lived organisms, such as copepods. Therefore, long-term adaptation prospects are also very much dependent on the generation time of the taxa, as well as population size and extent of the genetic variation in it, enabling adaptation via evolutionary pathways (Harley et al. 2006).

### 4.1.1. Temperature

The body size of an adult copepod is very plastic. Prosome length of *Acartia* copepods can vary >40% seasonally (Halsband & Hirche 2001) and >20% also in my study area (Viitasalo et al. 1995). The variations are mostly due to changing temperature and less to food availability. The temperature-size rule describes how temperature impacts on growth and development in ectotherms: individuals reared at colder temperatures grow slowly, but develop even slower, and therefore mature as larger adults than individuals reared at warmer temperatures (Atkinson 1994). Copepod

nauplii sizes do not appear to be temperature sensitive, whereas copepodite sizes from stages CIII and CIV onwards indicate a strong negative relationship (Foster et al. 2011). A meta-analysis on *A. tonsa* and *C. finmarchicus* showed that the temperature-size response is established when approximately 0.2 of the adult weight has been achieved (Foster et al. 2011). Further, egg clutch sizes are positively related to adult body size on *Acartia*-copepods (Landry 1978, Durbin et al. 1983). As I have used wild copepods that are caught from the sea shortly before the study, the origin of the animals affects the reproduction via temperature effect on body size, regardless of the acclimatisation to the experimental conditions prior to the actual measurements. Therefore it is difficult to do direct comparisons between the egg production results obtained from the different studies.

The temperature treatments 17°C and 20°C in **Papers I** and **II** had different effects on egg production of *Acartia* (Fig. 3). In August 2010, the temperature either did not have an effect on egg production, or the higher temperature was influencing egg production negatively, depending on the diet that the copepods were provided with (Fig. 3a & 3b, **Paper I**). In June 2011, the effect of higher temperature was negative (Fig. 3c, pilot study for **Paper II**), but in August the same year, it was clearly positive (Fig. 3d, **Paper II**). I did not measure prosome length from these copepods, but when looking at the temperature at Storfjärden at the time of the experiments (Fig. 3), it seems plausible that the varying temperature effects and slightly different egg production rates in the experiments are caused by ambient sea temperature. The water column was warmer in summer 2011 compared with 2010, and copepod populations had developed phenotypically to match those conditions.

Adaptive maternal (paternal or parental) effects are a type of phenotypic plasticity, and they are determined as a means of the mothers to adjust their offspring, according to signals that they receive from their surrounding environment (Lacey 1998). A maternal effect can be prezygotic, indicating that the condition and

the quality of gametes depend on the phenotype and the condition of the mother, or postzygotic-prenatal implying that the mother can still affect the fertilised and already developing eggs (Wade 1998), by reabsorption of the zygote or choosing the spawning site for example. Postzygotic-postnatal maternal effects (Wade 1998) on copepods are limited to predator avoidance by sac spawning species. Higher temperature induced an adaptive maternal effect in **Paper II**; the eggs were developing faster when they were produced in 20°C independent on the hatching temperature.

Harmful changes in the environment, such as suboptimal temperature, UV radiation, ozone and pollutants (exogenous sources), and the resulting physiological compensatory mechanisms (endogenous sources) can lead to increased reactive oxygen species (ROS) production (Monaghan et al. 2009, Metcalfe & Alonso-Alvarez 2010). To prevent ROS to cause further reactions and lead to oxidative damage (TBARS in **Paper I**), the antioxidant defence mechanism (ORAC in **Paper I**) is needed (Costantini 2008). When ROS production increases, the antioxidant defence system must keep up with the growth so that the homeostatic position remains (Fig. 4). If ROS
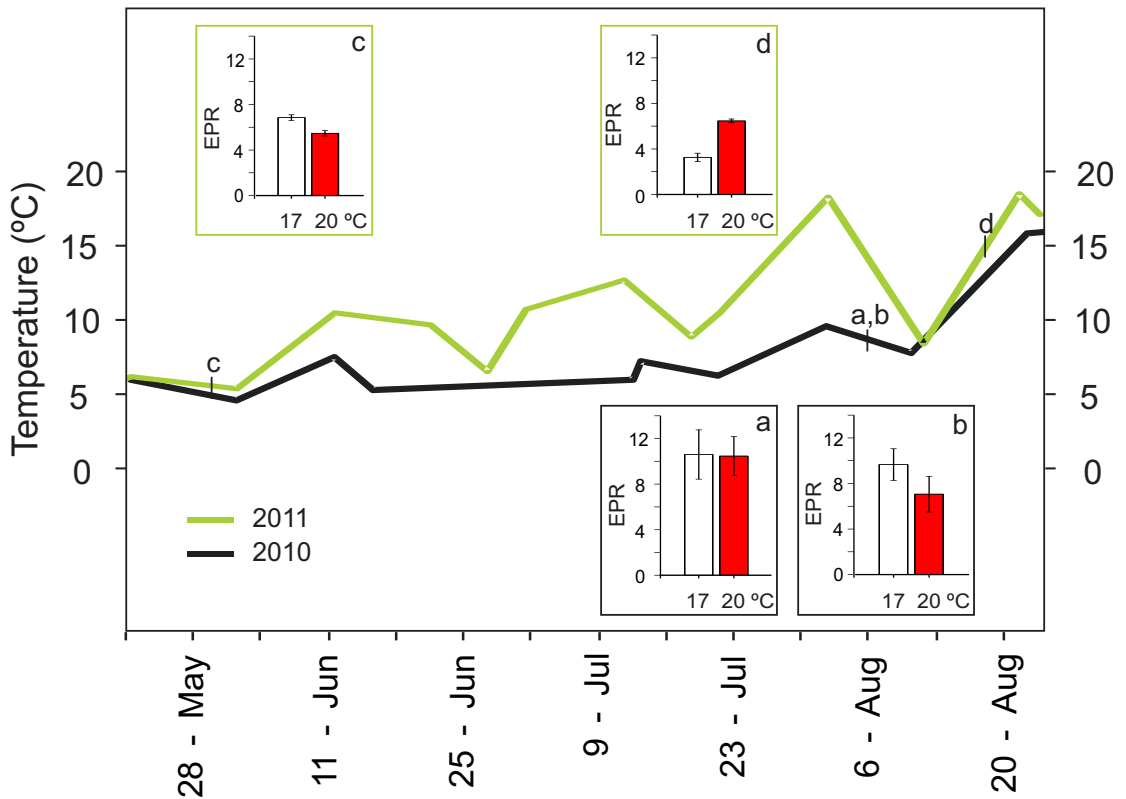


**Fig. 3** Average water temperature weighted with depth at Storfjärden monitoring station in summers 2010 and 2011 (data from Tvärminne ZS web page, VACCIA-project, http://maps.tvarminne.helsinki.fi/graphics/images/graphs/frontpage.html), and egg production rate (EPR, eggs copepod$^{-1}$ d$^{-1}$) at temperatures 17 °C and 20 °C. Letters a–d indicate days when the copepods were caught from Storfjärden for the egg production experiment. Copepods were provided with *Brachiomonas submarina* (1060 μg C L$^{-1}$) (a), *B. submarina* + *Nodularia spumigena* (970 + 100 μg C L$^{-1}$) (b), and *Rhodomonas* sp. + Bioplankton (270 + 120 μg C L$^{-1}$) (c) and (d) as food. EPR results (a) and (b) are from **Paper I**, (c) is from a pilot study made for **Paper II**, and (d) is from **Paper II**.

production cannot be combated, the resulting oxidative stress can damage key biological molecules, in particular proteins, lipids and DNA (Monaghan et al. 2009). Germline cells might be especially vulnerable to oxidative damage, which could have transgenerational effects on offspring viability (Metcalfe & Alonso-Alvarez 2010). I found evidence of these effects as the oxidative balance of the copepod mother correlated significantly with the egg viability and development (**Paper I**). Higher temperature increased antioxidant capacity and the oxidative damage, so that the resulting effect on the oxidative balance (ORAC:TBARS ratio) was negative (Fig. 4a & 4c). When the mother's oxidative state was balanced, they were able to invest more in their eggs and this led to better egg quality, indicated as egg viability and development. Further, even though egg production as such was unattached of oxidative balance, the production of viable eggs correlated positively with it (**Paper I**).

Projected future warming trend for the Baltic Sea exceeds the expected mean global warming by 0.9°C (von Storch & Omstedt 2008). In the northern parts of the Baltic basin main warming would occur during the winter months, whereas in southern parts summers would get warmer. For organisms inhabiting an area with large seasonal temperature changes, it is the extreme temperatures that have the largest effect on them. Because of their temperature-dependent body size, copepods are able to adjust their metabolism and energy demand according to the prevailing temperature. However, copepod reproductive output, and further, population sizes might be negatively mediated through the decreasing body size. In addition, the limits of the body size reaction norm (continuously variable plasticity) might be reached during summer heat waves. As many marine organisms already live close to their thermal tolerances (Somero 2002), increasing temperature can impact their performance and survival negatively (Harley et al. 2006).

## 4.1.2. pH

Although not as apparent as temperature-dependent effects, pH also influences phenotypes of marine organisms, especially calcifyers. Larval sand dollars, for example, develop narrower bodies in elevated $CO_2$ levels (Chan et al. 2011), and bryozoan colony composition can be modulated by acidification (Lombardi et al. 2011). Fitzer et al. (2012) observed that the body size of harpacticoid copepod *Tisbe battagliai* was decreased considerably when incubated in pH 7.82 and in pH 7.67 compared to the control (pH 8.06), but calanoid copepod body size has not been found to be affected by elevated $CO_2$ conditions even in studies lasting over generations (Kurihara & Ishimatsu 2008). Copepod egg production and hatching success have also shown substantial tolerance to moderate (0.3-0.7 decrease on pH scale) acidification (Kurihara et al. 2004, Mayor et al. 2012); however, nauplii mortality was increased when pH decreased 1.1 on pH scale (Kurihara et al. 2004). Egg production and hatching success have been found to reduce only at pH below 7.0 (Kurihara et al. 2004, Mayor et al. 2007). My results are in line with these studies; a pH decrease of 0.4 units according to the 2100 scenario did not affect egg production, egg viability, egg hatching success or early development of the offspring alone (**Papers I** & **II**). However, pH derived adaptive maternal effects were found (**Paper II**). Hatching success was suppressed when the eggs were incubated in different pH environment than what the mothers experience while laying the eggs, but the effect faded towards the end of the study. This might have been caused by improved copepod female condition and increased provisioning of the eggs. The result indicates that in a fluctuating environment such as the Baltic Sea, maternal effects can be an important adaptation mechanism, and that copepods are quite resistant to future changes in their environment, if they are able to invest in the cross-generational plasticity and adjust the offspring to the environment.

## 4.1.3. Interaction of pH and temperature

In recent years, it has become increasingly clearer that several climate change induced and other anthropogenic stressors should be combined in order to enable predictions of the fate of marine organisms (e.g. Harley et al. 2006, Whiteley 2011). An area that is already heavily influenced by anthropogenic stressors like eutrophication, fishing and pollution, can be less resilient to new challenges, such as global climate change (Richardson 2008). Further, climate change variables can act synergistically making the effects stronger than expected by simply summing up the single effects. Exposure to high $CO_2$ levels enhances the sensitivity of organisms to thermal extremes (Pörtner 2008). I found this interaction between acidification and warming in both of my studies (**Papers I** & **II**). In **Paper I**, the interaction between temperature (factor) and DIC (a continuous covariate) on the copepod antioxidant capacity (ORAC) means that the acidification effect is different in the two temperatures, and therefore, ORAC is different in the two temperatures (Enqvist 2005). At 17°C and in low pH, ORAC was up-regulated, whereas at 20°C the low pH resulted in lower ORAC (Fig. 4b & 4d, see also **Paper I** Fig. 2e). My results are in line Todgham & Hofman (2009), who found that the oxidative balance of sea urchin larvae is negatively affected by elevated $CO_2$. Exposure to moderately elevated or high $CO_2$ affects the organism's physiology also beyond calcification, and impairs oxidative balance by reducing expression of genes involved in defence against oxidative stress (e.g., antioxidants) (Todgham & Hofman 2009). In **Paper I**, copepod antioxidant capacity correlated positively with egg viability, viable egg production and offspring development. This implies that future copepods, inhabiting lower pH conditions and temperatures which are on the higher level of the species tolerance limits, might have fewer viable offspring. The negative effect on antioxidant capacity may also imply that longer incubations in those conditions lead to oxidative stress and further, to clear negative direct effects on the reproductive parameters.
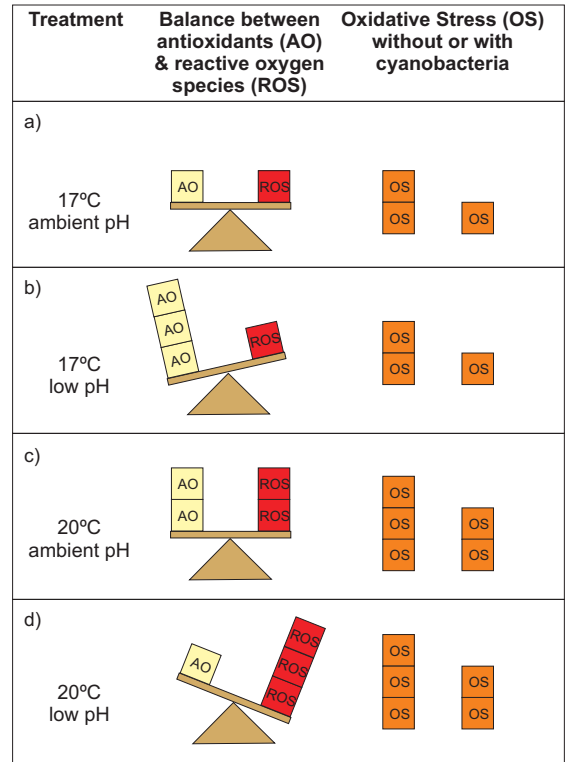


**Fig. 4** A schematic of the balance between antioxidants (AO, ORAC in **Paper I**) and reactive oxygen species (ROS) at 17°C or 20°C and in ambient or low pH, and the resulting oxidative stress (OS, TBARS in **Paper I**) with or without toxic cyanobacteria present. a) Ambient pH and temperature conditions do not induce antioxidant responses and oxidative stress levels are moderate. The risk (ROS) and the defence (AO) are balanced. b) A change in pH conditions triggers the antioxidant defence and antioxidants are up-regulated. c) Higher temperature increases the ROS production. The antioxidant defence is triggered, but oxidative stress levels increase anyhow. d) When pH is decreased at higher temperature, the antioxidant capacity decreases and ROS production exceeds it, which leads to an imbalance between the threat and the defence. Oxidative stress is decreased by the presence of cyanobacteria. Inspired by Monaghan et al. (2009).

This was indeed the case in **Paper II** where the incubations lasted longer than in **Paper I**. A lower pH treatment increased egg production during the first 2 days at 17°C, but decreased it after the third incubation day; whereas at 20°C the egg production was lower in low pH

treatment than in ambient pH treatment during the whole 5-day study. However, egg quality (egg hatching success) was not affected (**Paper II**).

## 4.2. Indirect effects via the food source

Ecological regime shifts occurred synchronously in the North Sea and the central Baltic Sea in the late 1980s (Alheit et al. 2005, Beaugrand 2009). The temperature driven change altered the mixing conditions, which led to changes in the spring phytoplankton bloom species composition (Alheit et al. 2005). The diatom biomass dropped dramatically, the dinoflagellate biomass increased steadily, and the total phytoplankton biomass decreased in the central Baltic Sea after the late 1980s. In the zooplankton community, the regime shift was observed as a lower total zooplankton biomass and altered species size spectrum. The dominating copepod taxa, *Acartia* spp. and *Temora longicornis* benefitted from the temperature change, whereas *Pseudocalanus* spp. populations declined. The positive response of especially *Acartia* spp. was explained by increased hatching of resting eggs, since hatching of nauplii from resting eggs is strongly temperature dependent (Dutz et al. 2004). The alternative hypothesis suggested that copepods can benefit from the change from diatoms to dinoflagellates, because some diatoms induce strong negative effects on copepod reproduction (Ianora et al. 2004). I did not find evidence for the latter hypothesis in my studies (**Papers III** & **IV**). Egg production of *A. bifilosa* was higher when fed with *Scrippsiella hangoei* dinoflagellates than *Skeletonema marinoi* diatoms, or a 1:1 mixture of the two species (**Paper IV**). However, egg production and egg hatching success of *Eurytemora affinis*, fed with a multi-species spring phytoplankton mixture, dominated by a diatom or a dinoflagellate revealed that the food quality effect is species-dependent instead of there being a systematic clear difference between diatoms and dinoflagellates (**Paper III**). Therefore, the generalisation that copepods will benefit from the climate warming driven decreasing diatom:dinoflagellate ratio in the spring bloom is too simplistic.

### 4.2.1. Role of dominating species in the bloom

The balanced diet hypothesis suggests that a diet consisting of a diverse set of prey organisms provides more complete nutrition and leads to higher consumer biomass (DeMott 1998). Benefits of multi-species diets on copepods have been observed in many studies (Bonnet & Carlotti 2001, Li et al. 2008). Both *A. bifilosa* and *E. affinis* are selective feeders; however, *Acartia* spp. are considered to be more selective, because they can switch between suspension feeding and ambush feeding types, than *E. affinis* that is a pure suspension feeder (Tiselius & Jonsson 1990, Kiørboe et al. 1996). Therefore, both species can select prey, for example, in a multi-species spring bloom. In **Paper III**, I provided *E. affinis* copepods with natural spring bloom community, which was dominated by the dinoflagellate *Biecheleria baltica*. The other treatment diets were created by combining a 1:1 natural spring bloom and a cultured common spring bloom species, either a diatom or a dinoflagellate. In addition to egg production and egg hatching success, I measured copepod RNA:DNA ratio as an estimate of short term growth (Saiz et al. 1998). The method was developed to assess copepod in situ growth, because the traditional egg production method has its drawbacks, in terms of tedious and laborious work that prevents intensive sampling within short spatial and temporal scales (Saiz et al. 1998). Holmborn et al. (2009) showed that the RNA:DNA ratio is not necessarily the best predictor for egg production of copepods provided with high food concentrations. Fortunately, I was able to confirm this statement; there were discrepancies between egg production and RNA:DNA ratios, which showed that the two parameters seem

to reveal different aspects of the copepod's condition when living in a bloom (**Paper III**). The *Gymnodinium corollarium* dinoflagellate dominated diet brought about the highest egg production, but induced only a moderately high RNA:DNA ratio (**Paper III**). A diet dominated by the *Thalassiosira baltica* diatom, on the other hand, resulted in low egg production, but high RNA:DNA ratio. When *Skeletonema marinoi* dominated the bloom, both the copepod egg production and the RNA:DNA ratio were low. The results indicate that the active protein synthesis (high RNA:DNA ratio) is used for something else than growth (reproduction), for example for coping with a stress-induced denaturation of proteins (Sanders 1993). According to the optimal foraging theory, copepods have two poor alternatives to choose from if the phytoplankton composition is dominated by a poor quality food species: 1) copepods might consume it, which then leads to low production and possible physiological costs, or 2) avoid eating it and search for better food (DeMott 1989) (Fig. 5). When the dominating species is of good quality, copepods can feed efficiently without consuming energy for search, and the resulting egg production is high and RNA:DNA ratio is moderate. Further, although there were no statistically significant differences in egg hatching success between the different food types, the lowest median hatching success on *T. baltica* and the highest median on

*G. corollarium*-dominated diet give additional support for the inter-connection between food quality and egg production / RNA:DNA ratio.

## 4.2.2. What is balanced diet?

Zooplankton must gain essential elements from their food, whereas some biochemicals can be chemically altered or synthesised from precursors (semi-essential) (Müller-Navarra 2008). The food quality of phytoplankton is high when the ratios of nutritional components are close to the grazer's own elemental ratios, e.g., C:N:P ratio (DeMott et al. 1998), which varies between life-stages and species (Andersen & Hessen 1991, Sterner & Hessen 1994). Imbalance between the stoichiometry of a producer and a consumer can lead to compensatory feeding and affect the strength of trophic cascades (Sterner & Hessen 1994, Siuda & Dam 2010), whereas excretion of excess nutrients by the grazers can alter the selective regime of the primary producers. As warming and acidification change elemental ratios as well as lipid content of primary producers, zooplankton dynamics can be affected too (Fu et al. 2007, Rossoll et al. 2012).

In all my studies, food concentration was high enough (200-1310 µg C L$^{-1}$) to prevent copepod food limitation (Koski et al. 1999, Holmborn et al. 2009). POC:PON ratios of the
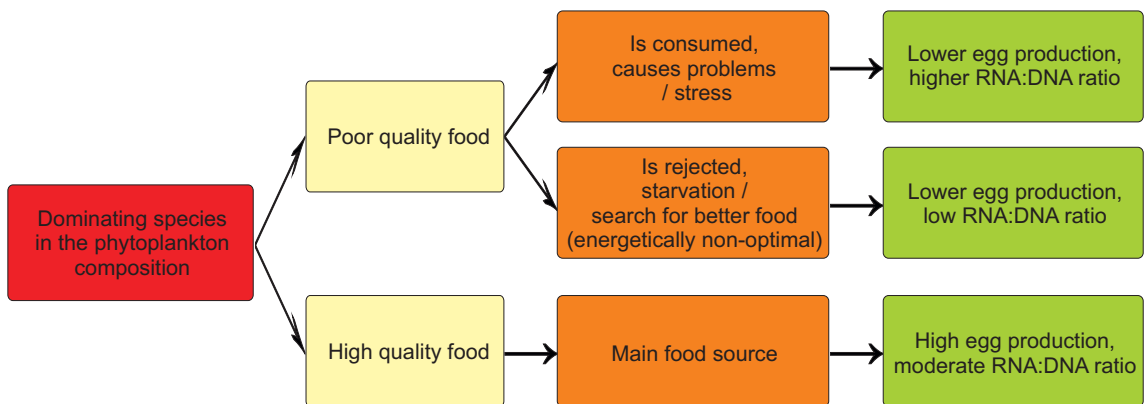


**Fig. 5** Hierarchical schematic graph concerning the hypothesis on quality of the dominating phytoplankton bloom species and its effects on copepod growth.

food offered in different treatments (**Papers III & IV**) were relatively low, and close to natural POC:PON ratios of *A. bifilosa* and *E. affinis* copepods in the area (Pertola et al. 2002), indicating that nitrogen-limitation probably did not cause the differences in reproduction. I did not measure copepod elemental ratios in my studies. However, by using published results from the study area (Pertola et al. 2002) and a gross growth efficiency of 30% (Straile 1997) to calculate a critical threshold for the food mineral ratio ($Q^{\star}_{c-e} = K_c/Q_{z-e}$, where $K_c$ is the gross growth efficiency for carbon and $Q_{z-e}$ is the elemental ratio of the consumer) (Urabe & Watanabe 1992), it can be estimated that copepods did not seem to be phosphorus limited either (**Paper IV**).

Polyunsaturated fatty acids (PUFAs), such as ω3 and ω6 fatty acids, and sterols are essential for copepods since they sustain membrane fluidity and serve as precursors for tissue hormones (Müller-Navarra 2008). PUFAs 22:6ω3 (DHA) and 20:5ω3 (EPA) are important for copepod reproduction, but have different purposes. High EPA concentration or high EPA:DHA ratio in phytoplankton is often a sign of diatom dominance (Peters et al. 2007), which supports high copepod egg production but low hatching success (e.g. Koski et al. 2011). DHA and DHA:EPA ratio, on the other hand, correlate positively with egg hatching success (Jónasdóttir et al. 2009). Sterols content of the diet might be of high importance for somatic growth of juveniles (Martin-Creuzburg et al. 2005). High EPA content of *Skeletonema marinoi* diatoms was not reflected as high egg production in my study (**Paper IV**). This might have been caused by depletion of fatty acids during grazing resulting in an inferior DHA:EPA ratio than in the *Scippsiella hangoei* dinoflagellates. In the feeding experiment, *A. bifilosa* copepods were trying to obtain as balanced diet as possible by feeding on both of the offered phytoplankton species, the diatom and the dinoflagellate. This suggests that multi-species diets have benefits for other aspects of copepod life than egg production.

### 4.2.3. Eat well, be well

Nutritional conditions can influence the oxidative stress that an organism experiences, as some antioxidants, e.g., vitamins and carotenoids must be gained from the food (Müller-Navarra 2008, Monaghan et al. 2009). Therefore, a balanced diet can help to cope with the stress created by climate change. In **Paper I**, copepod antioxidant capacity was better and oxidative damage lower when copepods were provided with a mixed diet of *Brachiomonas submarina* chlorophyte and *Nodularia spumigena* cyanobacterium, instead of *B. submarina* only. Since the cyanobacterium produces hepatotoxins that increase mortality and decrease reproduction in copepods (DeMott et al. 1991, Karjalainen et al. 2007), it was expected to be a stress factor in the experiment. The results showed that when *N. spumigena* was present, copepods were producing fewer eggs (**Paper I**, see also Fig. 3a & 3b) but with higher viability, giving no net effect on viable egg production. However, copepod eggs were developing faster in the presence of cyanobacteria (**Paper I**). Copepod grazing rates were not monitored in the study, so there is no certainty that the cyanobacterium was ingested, but the positive effects could be explained by complementary nutritional components (e.g., antioxidants) that the cyanobacterium added to the copepod diet, making it more balanced than the single chlorophyte diet. The complementarity hypothesis (DeMott 1998) would, therefore, explain the improved antioxidant capacity and resulting decrease in oxidative damage. Copepods are known to benefit from a multi-species diet, and in addition, the chlorophytes are not the best quality food for reproducing copepods (Brett & Müller-Navarra 1997). The allocation of antioxidants to the eggs could also have caused the positive maternal effect and resulted in a better offspring development. An alternative but highly controversial hypothesis suggests that low levels of harmful substances, such as cyanobacteria toxins, would cause a so-called hormesis effect (Costa et al. 2005); boost the antioxidant defence, and lead to the

other positive effects. The latter hypothesis is supported by the fact that the toxin (nodularin) concentration was actually explaining the copepod antioxidant capacity better than the *N. spumigena* biomass. However, the connection between the toxin and copepod offspring development could also be an artificial one, created by a correlation with a food quality variable, such as nitrogen, and the toxin production.

When studying the role of maternal effects on offspring development in changing pH and temperature conditions, I took extra care that the copepods received a diet as diverse as possible, consisting of four flagellate species (**Paper II**). The egg production increased during the experiment, which is an indication of high food quality (Jónasdóttir & Kiørboe 1996). Further, the negative effect of changing pH condition between the maternal environment and the hatching environment diminished during the study. This might have been caused by the improved food environment when the individuals were transferred from the sea to the laboratory, and thus by the improved copepod condition. As the experiment progressed, copepod females were able to invest more in their eggs, which made them more resistant to pH changes (**Paper II**).

## 5. CONCLUSIONS

In my thesis, I have studied both the direct and indirect climate change induced effects on copepod reproduction, oxidative status and RNA:DNA ratio. My results show that it is important to consider several variables in combination, instead of studying their effects separately. A twenty-degree temperature induced both positive and negative consequences for *Acartia* copepods in my studies (**Papers I & II**), but the temperature seems to be within the tolerance limits of the species. Copepods inhabiting the Baltic Sea with large seasonal temperature fluctuations can tolerate the changes by adjusting their body size in a plastic

manner. However, smaller adult copepods can produce fewer eggs than larger counterparts, which might affect the population structures. Further, increasing extreme temperatures might reach beyond their plasticity and force them to change their vertical migration patterns or geographical distributions.

Temperature effects are strengthened by acidification effects. I found out that regardless of the direction of the temperature effect on copepod antioxidant capacity (**Paper I**) or egg production (**Papers I & II**), the combined effect of elevated temperature and decreased pH is always negative. Higher temperature induces a stronger acidification effect or vice versa. Maternal effects seem to be an important adaptation mechanism in acidifying environment, but the food quality determines how much the females can invest in their eggs (**Paper II**).

There does not seem to be an apparent food quality difference between the taxa diatoms and dinoflagellates, according to my studies (**Papers III & IV**). Therefore, if climate warming increases dinoflagellate abundances in relation to diatoms, the copepod response will not necessarily be positive. Further, increasing toxic cyanobacteria blooms are not always a nuisance for marine organisms that they have co-occurred with for a long time. Low levels of cyanobacteria biomass seem to supplement the diet, or toxin may vitalize the body functions (**Paper I**)!

According to this thesis work, climate change induced water temperature and pH changes can cause problems to Baltic Sea copepod communities. However, their resilience depends substantially on their diet. A shift in phytoplankton community towards nano- and picosized species dominance, as well as increasing frequency of single species blooms could create a non-optimal food environment. In addition, other climate induced changes such as decreasing salinity in the northern parts of the Baltic Sea basin might benefit freshwater species and, for example, force *Acartia* species to alter their area of distribution and move southwards. Warming waters could further

decrease oxygen concentrations in deep water, and thus hamper spring population build-up of zooplankton species that depend on benthic resting stages.

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*On mulla asiat joskus osuneet kohdalleen.*
*Se saattoi vahinko olla,*
*eikä tapahdu uudelleen.*
*Suru joskus käy, ja ikävää riittää;*
*Kantaa laineet laivatkin.*
*Mistä mun pitäis ketäkin kiittää?*
*- Jossain on kai vastauskin.*
Paula Vesala

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Anu Vehmaa

# Climate driven changes in temperature, pH and food quality

## Effects on copepod reproduction

This thesis demonstrates that climate change induced water temperature and pH changes can cause problems to Baltic Sea copepod communities. In addition, copepod resilience depends substantially on their diet and, therefore, the response of phytoplankton to environmental changes.

The author graduated from Väinö Linna High School, Urjala in spring 2000. She received her Master of Science degree in hydrobiology and limnology from University of Jyväskylä in 2006. Since 2008, she has been working as a project scientist and a PhD student in ARONIA Coastal Zone Research Team, Åbo Akademi University and Novia University of Applied Sciences.