The Effect of Changing Environmental Conditions on the Reproduction Success of Copepod *Acartia bifilosa*

Anni Rein

Degree thesis for Bachelor of Natural Resources

Degree Programme in Sustainable Coastal Management

Raseborg 2016

BACHELOR'S THESIS

Author: Anni Rein
Degree Programme: Sustainable Coastal Management
Supervisors: Olivier Glippa, Jonna Engström-Öst, Maria Kihlström

Title: The Effect of Changing Environmental Conditions on the Reproduction Success of Copepod *Acartia bifilosa*

Date: May 4, 2016 Number of pages: 22 Appendices: Yes (1)

Summary

The objective of this thesis is to shed light on how climate-induced changes in the environmental conditions of the Baltic Sea, mainly pH and temperature, affect the reproduction success of copepods, specifically *Acartia bifilosa*. The possible changes in the egg production rate and hatching success of *A. bifilosa* due to climate change may affect the future plankton trophic dynamics in the Baltic Sea as it is the most abundant zooplankton species and a major food source for many species, for example Baltic herring.

The research was carried out by gathering quantitative data in a laboratory experiment in Tvärminne Zoological Station, in Hanko, southern Finland in August 2015. In the laboratory experiment, the copepods were sampled directly in the field and incubated in ambient pH and temperature conditions after which the egg production rate and hatching success were analyzed. The experiment was complemented with a description of long-term monitoring data of seawater pH collected since 1996 at Storfjärden, a pelagic area near Tvärminne Zoological Station and the sampling location for this experiment.

The results show that elevated temperature and pH had a positive effect on the egg production rate. The hatching success results indicate that elevated temperature may increase hatching rates at least when the temperature is within the optimum range for copepod. These results imply that copepods are used to adapting to changing conditions at least in the short term due to their behaviour of diurnal vertical migration. Further studies would be needed to determine if this adaptability enhances the copepods' ability to endure the effects of climate change also in the long term.

Language: English Key words: ocean acidification, climate change, copepod, *Acartia bifilosa*, reproduction, pH, temperature

---

Table of contents

1 Introduction

Carbon dioxide emissions are one of the major causes of anthropogenic (i.e. human-induced) climate change. In turn, ocean acidification is seen as one of the main effects of anthropogenic climate change (Pörtner et al. 2014, 415), together with temperature increase. Ocean acidification is defined as a change in ocean carbonate chemistry caused by rising atmospheric carbon dioxide (CO_2) that diffuses into the ocean surface waters and lowers the pH (Pörtner et al. 2014, 415).

Climate change, with its related effects, may directly affect the growth, survival and productivity of individual organisms through changes in the properties of the water such as temperature, salinity and chemical composition. The effects can also be detected indirectly through changing species interactions or seasonal succession, which may create a mismatch between predator and prey. In addition to oxygen concentration and pH, seawater temperature is the fundamental climate-driven factor affecting individual organisms directly. (HELCOM 2013, 42) The effects of ocean acidification on biota are largely poorly understood, thus more research is needed on the effects of decreasing pH on Baltic biota (HELCOM 2013, 54).

In the field of pelagic organisms, early studies of ocean acidification have focused on phytoplankton and calcifying invertebrates. Much less information has been gathered about the effects of ocean acidification on zooplankton (Riebesell & Tortell 2011, 109). Research on copepods is necessary since they are the most abundant zooplankton (Almén et al. 2014, 120). Copepods are integral to marine trophodynamics (Fitzer et al. 2012, 31), meaning that they transfer biomass from primary producers to higher trophic levels thus forming an important link in the plankton ecosystem and influencing biogeochemical cycles (Cripps et al. 2014, 3377). As copepods are a major food source for many species, they play a major role in the food web interactions. Changes in ocean acidity and temperatures can have a cascading effect in the food web if a key species is affected (Busch 2013, 829).

In this thesis, the effects of changing pH and temperature conditions on the reproduction success of copepod are studied. This may give an indication on how organisms respond to climate-induced changes in water ecosystems.

2 Research question

Since copepods form the bulk of the biomass in most pelagic zooplankton communities, evaluating the impacts of seawater temperature and pH changes on copepods is essential for projecting possible changes of marine ecosystems (Nybakken 2001 according to Kurihara & Ishimatsu 2008, 1087). Furthermore, as copepod *Acartia bifilosa* is one of the most abundant zooplankton species in the Baltic Sea, its reproductive success and possible changes in it due to climate change may affect the future plankton trophic dynamics in the Baltic Sea.

The research question is: **How does pH and temperature affect the reproduction success of copepod *Acartia bifilosa*?**

The research question will be examined with the help of the literature review as well as by describing the long-term pH data and analyzing the results of a laboratory experiment. In the experiment, a total of eight *A. bifilosa* sample populations were sampled from the field and incubated in ambient conditions to gain insight on the egg production and hatching rates in different pH and temperature conditions.

3 Changing environmental conditions: ocean acidification and temperature increase

The effects of climate change have been increasingly researched over the past two decades. The research has shown that the global ocean acts as a carbonate buffer by taking up about 25% or even one-third of the anthropogenic CO_2 emitted to the atmosphere, which leads to acidification of the marine environment (HELCOM 2013, 54; Sabine et al. 2004 according to Fabry et al. 2008, 414). Most of the accumulated energy stored in the climate system is stored in oceans, which causes ocean warming. Globally, the ocean warming is largest near the surface. (IPCC 2014, 4)

3.1 General trends in ocean pH and temperature

Over the last 250 years, the atmospheric concentration of CO_2 has increased by over 40% from 280 ppm (parts per million) to 394 ppm (NOAA 2014). In January 2016, the CO_2

levels were at 402.5 ppm (CO_2.earth 2016). Globally, increasing atmospheric concentrations of CO_2 are causing an increase in the mean sea surface acidity (Raven et al. 2005, according to Mayor et al. 2007, 91). The surface ocean pH has decreased on average by more than 0.1 units below the pre-industrial average of 8.17 (Pörtner et al. 2014, 418).

This decrease in ocean pH is likely to continue, since the atmospheric CO_2 levels are expected to more than double by 2050 compared to the pre-industrial times (SRES, IPCC 2000 according to Pörtner et al. 2014, 418). According to other projections, the atmospheric CO_2 levels will reach 788 ppm by 2100 resulting in a mean surface ocean pH of 7.8 (Orr et al. 2005 according to Kelly et al. 2011, 2544) and one worst-case scenario suggest CO_2 levels will reach 970 ppm, resulting even lower mean surface ocean pH levels (Houghton et al. 2001 according to Pedersen et al. 2014, 535). In their study, Caldeira and Wickett (2003, according to Raven et al. 2005, 10) have in turn predicted a decrease in pH of 0.5 units in the surface oceans by the year 2100. Regardless of the chosen projection of the future pH levels, the trend and its effects are clear: the effects of ocean acidification on the ocean systems will be detrimental and far-reaching (IPCC 2014, 13).

The effect on marine ecosystems may be even stronger because ocean acidification is occurring simultaneously with ocean warming (Caldeira & Wickett 2003, according to Mayor et al. 2012, 1). During the last four decades, oceans have warmed at an average rate of >0.1°C per decade in the upper 75 meters and the strongest warming trends have occurred at higher latitudes in the Northern hemisphere. The mean sea surface temperature in 2090 is estimated to be on average 2.7°C higher than in 1990. (Pörtner et al. 2014, 418) Ocean acidification is likely to have synergistic effects with an increase in the ocean temperature and other climate change related environmental changes (Pörtner et al. 2014, 418).

3.2 Ocean acidification in the Baltic Sea

The Baltic Sea is a semi-enclosed brackish water sea that drains into the North Sea via the Danish Straits (HELCOM 2013, 18). The low salinity and limited water exchange makes it particularly vulnerable to the effects of climate change. The distribution of total CO_2 is regulated by the alkalinity, which derives from the weathering of the limestone in the catchment area. The highest pH occurs during the productive period of spring and summer and reaches up to 8.5 and the lowest pH of around 7.9 occurs during autumn and winter.

(HELCOM 2013, 40) The seasonal fluctuation is very strong and the changes in pH can reach up to one pH unit for some months (Brutemark et al. 2011, 93). This seasonal pH variation is mostly driven by massive phytoplankton blooms but there are also other several factors contributing to the pH of the Baltic Sea, such as ice cover and wind-induced surface mixing, upwelling of deep waters, and precipitation of anthropogenic atmospheric sulphur and nitrogen oxides (Soomere et al. 2008, Hällfors et al. 2008, Doney et al. 2007 according to Brutemark et al. 2011, 93).

The increase of atmospheric CO_2 from the pre-industrial 280 ppm to over 400 ppm in 2015 has caused an increase in the mean partial pressure of CO_2 (pCO_2) in surface water (HELCOM 2013, 40). The pH of the Baltic Sea has decreased since 1993 and the largest changes have occurred in the Bothnian Sea and the southern Baltic Proper. (HELCOM 2013, 46) However, ocean acidification has not progressed at an alarming rate in the Baltic Sea (HELCOM 2013, 54). The increase in alkalinity over the past 60 years has weakened the effect of increased atmospheric CO_2 in the upper water layers during spring and summer. The increased alkalinity occurs due to both limestone dissolution and the eutrophication-related high biological production during which large quantities of CO_2 are consumed. This has, in turn, resulted in a calcium carbonate oversaturation of surface water during spring and summer. (HELCOM 2013, 40)

3.3 Seawater temperature in the Baltic Sea

In the Baltic Sea, the seasonal cycle of water temperature is affected by the salinity stratification. The temperature ranges from 3°C to 8°C below 100 meters depth and in surface waters the temperature can range up to 25°C. The Baltic Sea surface waters have been getting warmer in all seasons since 1985. Based on remote sensing data, there has been an estimated increase in the annual mean surface temperature of up to 1°C per decade from 1990 to 2008. One of the largest increases has occurred in the Gulf of Finland. (HELCOM 2013, 20)

The salinity of the Baltic Sea is already low due to its characteristics and may decline as a result of climate change induced higher precipitation, but currently there is no scientific consensus on how climate change will impact future river runoff and salinity stratification and thus water temperature cycles in the Baltic Sea (HELCOM 2013, 5).

According to HELCOM's (2013) scenarios, the largest temperature changes are projected to occur in the Bothnian Bay and Bothnian Sea during summer and in the Gulf of Finland in the spring. The expected summer sea-surface temperature will increase about 2°C in the southern parts of the Baltic and about 4°C in the northern parts. In all parts of the Baltic the surface water is projected to warm more than the deep water, which is likely to increase vertical stratification and stability across the seasonal thermocline. (HELCOM 2013, 34) Higher temperatures will accelerate many biological and biochemical processes and increase hypoxia (HELCOM 2013, 41).

4 Copepods

Copepods are part of the mesozooplankton (0.2–20 mm) and together with cladocerans among the major zooplankton groups in the Baltic Sea. In the northern part of the Baltic Sea, calanoid copepods such as *Acartia bifilosa* are the most important zooplankton species. *A. bifilosa* (figure 1) is a brackish water species and thus common in the Gulf of Finland. It is a major food source for many species, for example Baltic herring (*Clupea harengus membras*). (Luontoportti 2015)

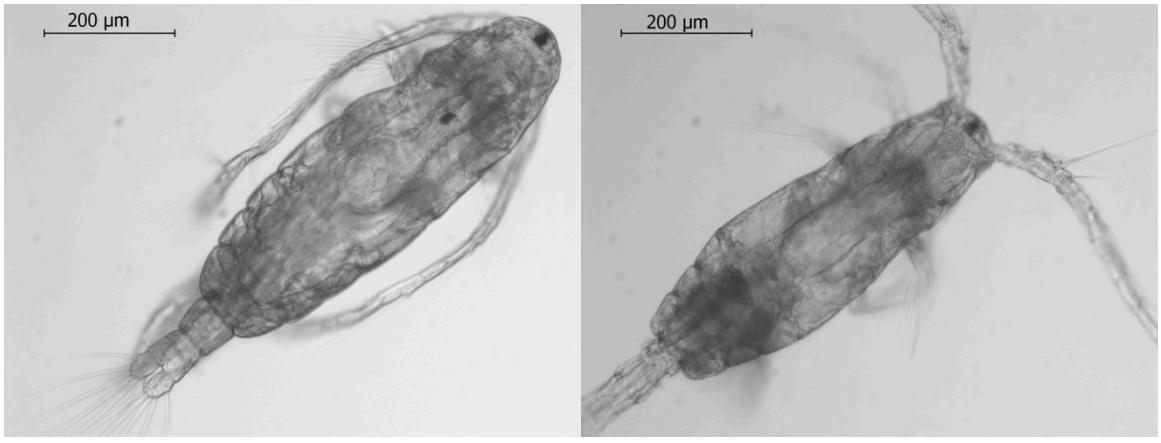

Figure 1. **Acartia bifilosa** ***female (left) and male (right)***

Copepods migrate vertically during the day, i.e., they ascend at night and descend at dawn in order to avoid their predators. Through this behavior, they experience widely varying conditions in their physicochemical environment on a diurnal basis. Almén et al. (2014) discovered that copepods experience a change in pH of more than 0.5 units and 5°C change in temperature during diurnal vertical migration. These variations in their physicochemical

environment are greater than the estimated future climate change scenarios. This may positively affect the copepods' ability to withstand the effects of climate change. (Almén et al. 2014, 120–124)

4.1 Effects of climate change on copepods

Climate change can have direct effects on zooplankton in the Baltic Sea. The effects of temperature on Baltic Sea ecosystems depend on sensitivities, limits and functional properties of organisms. Organisms have limited temperature ranges within which they live and function and they are sensitive to temperature extremes. Changes in temperature may affect e.g. diversity, development and reproductive success of marine species as well as the composition of communities in both pelagic and benthic systems. (Pörtner et al. 2014, 427-432)

The future salinity and temperature changes are likely to have a negative effect on marine copepod abundance because temperature and salinity are important factors for key mesozooplankton species such as copepods (HELCOM 2013, 44; 50).

Zooplankton dynamics are controlled by both hydroclimatic conditions and predation pressure, which, in turn, are affected by environmental conditions and fisheries. Food quality, indirectly affected by climate change, also affects the zooplankton growth rates and population dynamics. These factors make the evaluation of climate change effects on zooplankton more difficult. (HELCOM 2013, 44)

Despite the several factors, the combined effects of various climate change factors can be studied. For example, when studying the combined effects of ocean acidification, global warming and cyanobacteria on the calanoid copepod, Vehmaa et al. (2013) discovered that the ocean acidification together with the higher temperature increased the risk of oxidative stress in copepods. In turn, higher temperature caused a decrease in egg viability, nauplius development and oxidative status. (Vehmaa et al. 2013, 4552–4554)

4.2 Effects of ocean acidification on copepod reproduction success

Recent studies indicate that ocean acidification has negative effects for many calcifying organisms. It may also lead to changes in biodiversity, trophic interactions and other

ecosystem processes. (Royal Society 2005, Kleypas et al. 2006 according to Fabry et al. 2008, 414) Specifically, it has been shown that extremely low pH levels have adverse effects on the fertilization rates and early development in several marine fauna groups, such as copepods, sea urchins and molluscs (Kurihara et al. 2004, 724; Fabry et al. 2008, 422).

4.2.1 Effects on reproduction rate

Some of the previous studies investigating the response of copepods have discovered that the ocean acidification has no significant effect on reproduction parameters of copepod (e.g. egg production rate, egg viability) with the pH levels predicted for 2050 or 2100 (pH 7.6–7.8) (McConville et al. 2013; Vehmaa et al. 2013; Zervoudaki et al. 2014). Only when the atmospheric CO_2 and thus pH reaches extreme levels (at or below 7), has ocean acidification been noted to have clear potential to damage populations and the reproduction of copepods (Cripps et al. 2014; Kurihara et al. 2004; Pedersen et al. 2014). For example, in a study by Kurihara et al. (2004, 724–725), an extreme pH level of 6.82, equivalent of pCO_2 1.036 and 10 000 ppm higher than the control, was found to affect copepod's egg production rate adversely.

The reproductive output of copepods, in terms of egg production and development index, has also shown to increase as a response to short-term incubations in low pH. In a laboratory study, *A. bifilosa* responded to low pH (between 7.15 and 7.6) with an increased reproductive output, which suggests that copepods may have considerable capacity to adapt to future pH decline. (Engström-Öst et al. 2014, 178–180)

There might be other factors that have stronger effect on the reproduction rate of copepods than ocean acidification. For example, in a study where the combined effects of ocean acidification and temperature on the reproduction rate of copepods were studied, the lower pH level had no noticeable effect, whereas warming, nutrition and the duration of exposure to low pH levels were more significant for the reproduction rate of copepods (Zervoudaki et al. 2014, 79). In turn, Koski and Kuosa (1999, 1782) discovered that higher temperature and food availability have a direct, positive effect on the egg production.

4.2.2 Effects on hatching success

Elevated CO_2 levels have been found to affect the hatching success of copepods, but only with extreme CO_2 levels (Kurihara et al. 2004, 724). Another study by Mayor et al. (2007)

examined the effects of 8000 ppm carbon dioxide levels (pH 6.95) on the growth and reproduction of copepod *Calanus finmarchicus*, a keystone species in the Northern Atlantic. In the experiment, the simulated ocean acidification representative of the predicted atmospheric CO_2 worst-case scenario levels in 2300 did not affect egg production and biomass loss in adult female copepods. However, there were significant negative effects on egg hatching success and naupliar survival. This suggests that the earlier developmental stages of copepods may be more pH sensitive than adult copepods and that the reproduction of copepod is pH sensitive under extreme CO_2 levels. (Mayor et al. 2007, 91–95)

In turn, when using modestly acidified seawater (2000 ppm above ambient air), significant effects on hatching success rates have not been found (Kurihara et al. 2004, 724; Kurihara & Ishimatsu 2008, 1088).

5 Methods and procedures

The research was carried out by gathering quantitative data in a laboratory experiment in Tvärminne Zoological Station, in Hanko, southern Finland. The experiment was complemented with a description of long-term monitoring data of seawater pH collected since 1996 at Storfjärden, a pelagic area close to Tvärminne Zoological Station and the sampling location for this experiment. Simultaneously with the copepod samplings, a set of environmental parameters were measured to get information on the environmental conditions during the sampling and to be able to define what role, if any, the environmental conditions played when interpreting the results.

5.1 Research protocol

For researching the effect of pH on copepods, the preferred methodology usually includes using two or more subsequent generations (see for example Kurihara et al. 2008) so that the results are not affected by the drastic change between natural environment and laboratory conditions. The lifespan of one generation of copepods is usually three weeks (depending on food and temperature), thus using two subsequent generations in the

experiment would have taken 2–3 months. This method was rejected due to time constraints. Another way to improve validity of the results is to use copepods that have been acclimated for several days before incubations (see for example Mayor et al. 2007). This would also have been too time consuming for the circumstances of this study. These reasons led to the chosen methodology of laboratory experiment where the copepods were sampled directly in the field and incubated in ambient conditions. Many of the previous studies have used controlled pH conditions by aerating water with CO_2 (e.g. McConville et al. 2013; Pedersen et al. 2014; Vehmaa et al. 2013), but here the focus was on ambient conditions.

5.1.1 Sampling and analyses

The long-term monitoring data of seawater pH was obtained from Tvärminne Zoological Station and examined for the years 1996–2014. This time period was considered to be sufficient by the research team for giving indication of the pH trend at the sampling location.

The sampling was carried out in August 2015 at Storfjärden (59° 52' 56" N, 23° 15' 14" E), in the proximity of Tvärminne Zoological Station, which is situated in the western Gulf of Finland in the Baltic Sea (figure 2). The samples were taken during two weeks and during each week on four consecutive days (10.–14.8.2015 and 17.–21.8.2015). The time of sampling varied each day (table 1).

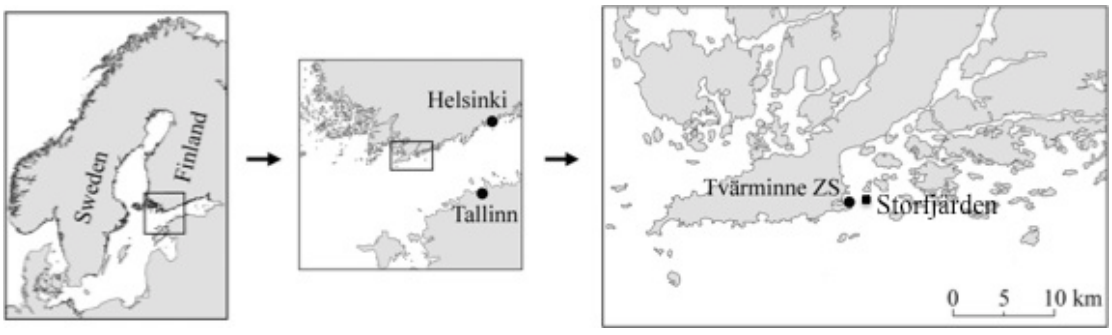

Figure 2. Location of Tvärminne Zoological Station and Storfjärden (original map by R. Rancken)

The copepods were sampled with a closing net (200 µm) to collect copepod population living at specific depths, except for the night samplings (3.1 and 3.2), which were done with a cod end (1 liter, 200 µm) since the sampling was done from 10 meters to the

surface. Sampling depth varied according to the time of day to match the diurnal vertical migration of copepods and the day length was studied beforehand to determine the photoperiod for the climate chambers (table 1). After each haul, the animals were immediately transferred to 32-liter cooling boxes filled with 10 liters of water from the sampling depth and kept in the cooling boxes in a climate chamber until they were sorted for incubations.

The environmental parameter measurements for temperature and oxygen were done with a CTD and YSI ProODO Optical Dissolved Oxygen Instrument. The water samples for pH, Chlorophyll a (Chl a) and dissolved inorganic carbon (DIC) analyses were collected with 2-liter Limnos. For pH analyses, seawater was sampled in 250 ml airtight glass bottles. Two replicates from each depth 25, 15 and 5 meters were taken and kept in cooling boxes on board. The pH meter (Jenway 3510 pH) was calibrated (pH 4.0, 7.0 and 10.0, room temperature) before every measurement and kept in pH 7.0 after calibration. The room-temperature (19.3 ± 0.80 °C, mean ± SD) samples were measured from the more alkaline to the more acidic ones.

Table 1. Sampling information and sunrise and sunset times

Sample	Date	Sampling time	Depth (m)	Sunrise	Sunset	Day length
1.1	10.8.2015	12:00	20-30	5:21	21:31	16:09:14
2.1	11.8.2015	18:00	10-20	5:24	21:28	16:04:03
3.1	13.8.2015	0:00	0-10	5:29	21:22	15:53:38
4.1	14.8.2015	6:00	15-25	5:31	21:19	15:48:23
1.2	17.8.2015	12:00	20-30	5:38	21:11	15:32:35
2.2	18.8.2015	18:00	10-20	5:41	21:08	15:27:17
3.2	20.8.2015	0:00	0-10	5:46	21:02	15:16:38
4.2	21.8.2015	6:00	15-25	5:48	20:59	15:11:19

(Timeanddate.com; location Espoo)

For Chl a analyses, the samples were taken from three different depths (25, 15 and 5 meters), three replicates from each depth. The samples were taken into 1000 ml clear plastic bottles and protected from the light right after sampling. 100 ml of the samples were filtered on a Millipore vacuum filtration device using GF/F Whatman 0.7µm (Ø 25mm). The filters were then placed in ethanol for 24 h in the dark, prior to analyzing them with a Varian Cary Eclipse Fluorescence Spectrophotometer.

For DIC analyses, three replicates of unfiltered water were taken from each of three depths (25, 15 and 5 meters). The samples were placed into 25 ml acid-washed, airtight glass bottles stored on ice and protected from light in a climate chamber at 3°C until the measurements the next day. The measurements were performed approximately 24 h later using the Uras 3G after subsampling from the room-tempered samples.

For female incubations and egg incubations, seawater (*in situ* depth and temperature) was filtered on site with Millipore Sterifil Aseptic Vacuum Filter (volume 250 ml) using GF/C Whatman 1.2µm (Ø47mm) filters. For egg incubations and rinsing, seawater (*in situ* depth and temperature) was filtered with 20 µm mesh at the site. All seawater was stored in airtight 1000 ml glass bottles and kept in cooling boxes until stored in a climate chamber (*in situ* temperature).

5.1.2 Experimental set-up

Female incubations: Originally, five replicates in each of the eight samples were planned (total 40), but the difficulties in obtaining the adequate amount of copepods for these samples resulted in fewer replicates. Thus, the amount of replicates was 1–5 per sample (sample 2.1 one replicate, sample 4.1 three replicates), depending on the amount of copepods available. In total, there were 34 replicates. Ten *Acartia bifilosa* females and two males were picked for each replicate to be incubated in 250 ml plastic beakers with false bottom chambers and kept in *in situ* seawater and temperature in climate chambers (photoperiod 16:08 h (L:D); T 11°C for samples 1, 2, and 4; T 15°C for samples 3) for 24 h. pH was measured before and after the incubations. After the incubations, the eggs were counted on a petri dish using a microscope to determine the egg production rates (eggs per copepod female per day).

Egg incubations: After the eggs were counted they were moved back to the 250 ml plastic beakers with 200 ml *in situ* water and placed in climate chamber according to their *in situ* temperature (see above). pH and dissolved oxygen concentration were measured before the incubations. The incubation period was 72 h after which the pH was measured and the eggs and nauplii were counted on a petri dish using a microscope to determine the hatching success (hatching rate = number of nauplii per total number of eggs in a sample). To facilitate the counting, the eggs and nauplii were fixed and colored with Lugolin.

5.2 Statistical analysis

For environmental parameters, the results are described in the following chapter using mean and standard deviation. The statistical analysis was done with the SPSS 21.0 software for egg production rate and hatching success results (H_0= pH and temperature have no effect on egg production and hatching success). All data were checked for normal distribution and homogeneity of variances to begin with. Egg production rates were analyzed against temperature using linear regression, and to reveal differences between depths and hours of day, a GLM (general linear model) was used in combination with Tukey HSD. The hatching success data did not have normal distribution; therefore, the data were analyzed using non-parametric tests, Mann-Whitney U test and Kruskal-Wallis one-way ANOVA.

6 Results

6.1 Seawater pH in Tvärminne

The description of the long-term pH data for years 1996–2014 shows that pH is higher in the spring/summer and lower in the winter with the maximum value of 9.13 (May 2005) and minimum of 7.00 (September 2003). The average pH is 8.01 ± 0.30. Despite the great seasonal variability, which occurs e.g., due to ice conditions in the winter, and elevated nutrition levels and warm periods in the summer, there is a slight declining trend in the seawater pH (figure 3).

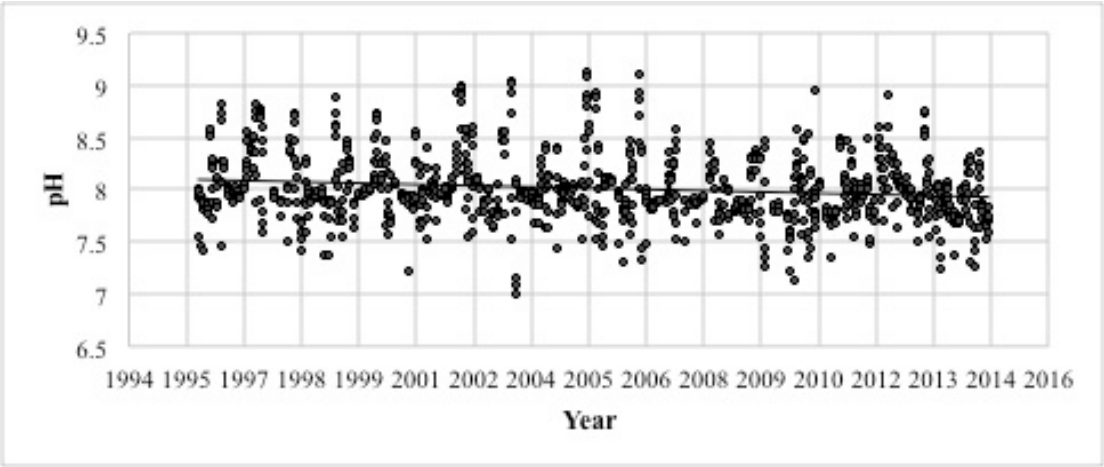

Figure 3. pH at Storfjärden (Tvärminne Zoological Station's long-term data series, years 1996–2014)

6.2 Environmental parameters

During the first sampling week, the mean seawater temperatures ranged from 10.18 to 16.10°C and during second week from 12.08 to 17.96°C (table 2) thus there was a noticeable increase during the second week. The salinity was quite stable between week 1 and 2, with values ranging from 5.80 to 6.03 psu (table 2). Dissolved inorganic carbon (DIC) values ranged between 17.63 and 22.73 mg/l during week 1 and between 20.21 and 22.25 mg/l during week 2 (table 2).

Table 2. Seawater temperature, salinity and dissolved inorganic carbon (DIC) at sampling depths

Sample	Date	Sampling time	Depth (m)	T°C (mean ± SD)	Salinity (psu) (mean ± SD)	DIC (mg/l) (mean ± SD)
1.1	10.8.2015	12:00	20-30	10.18 ± 0.67	10.18 ± 0.67	22.73 ± 0.16
2.1	11.8.2015	18:00	10-20	13.08 ± 0.74	13.08 ± 0.74	20.56 ± 0.15
3.1	13.8.2015	0:00	0-10	16.14 ± 2.27	16.14 ± 2.27	20.26 ± 0.27
4.1	14.8.2015	06:00	15-25	11.30 ± 0.89	11.30 ± 0.89	17.63 ± 0.26
1.2	17.8.2015	12:00	20-30	12.08 ± 0.28	12.08 ± 0.28	20.71 ± 0.19
2.2	18.8.2015	18:00	10-20	15.62 ± 1.47	15.62 ± 1.47	21.27 ± 0.17
3.2	20.8.2015	0:00	0-10	17.96 ± 0.47	17.96 ± 0.47	20.21 ± 0.30
4.2	21.8.2015	06:00	15-25	14.66 ± 1.04	14.66 ± 1.04	22.25 ± 0.13

The temperature and salinity profiles (figure 4) were obtained from the CTD data. In the surface, the temperature ranged between 16.73 and 19.88°C during week 1 (figure 4). The temperature declined with similar profiles towards the bottom, where it ranged between 9.05 and 10.1°C. Salinity ranged from 5.19 to 5.79 psu in the surface and from 6.00 to 6.06 psu in the bottom (figure 4; see appendix for temperature and salinity profiles for week 2).

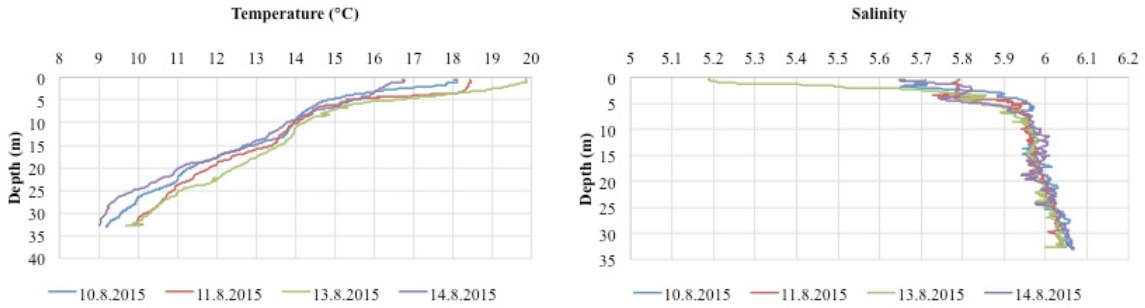

Figure 4. Temperature and salinity profiles for the samplings during week 1. For week 2, see appendix.

Oxygen levels were higher for the surface and intermediate copepod sampling depths during the second week of sampling (figure 5, see framed values). The oxygen levels ranged between 10.1 and 10.5 mg/l (week 1) and between 10.9 and 11.6 mg/l (week 2) in 5 meters, between 8.40 and 9.07 mg/l (week 1) and between 8.90 and 9.85 mg/l (week 2) in 15 meters, and between 5.71 and 6.25 mg/l (week 1) and between 6.69 and 7.79 mg/l (week 2) in 25 meters (figure 5).

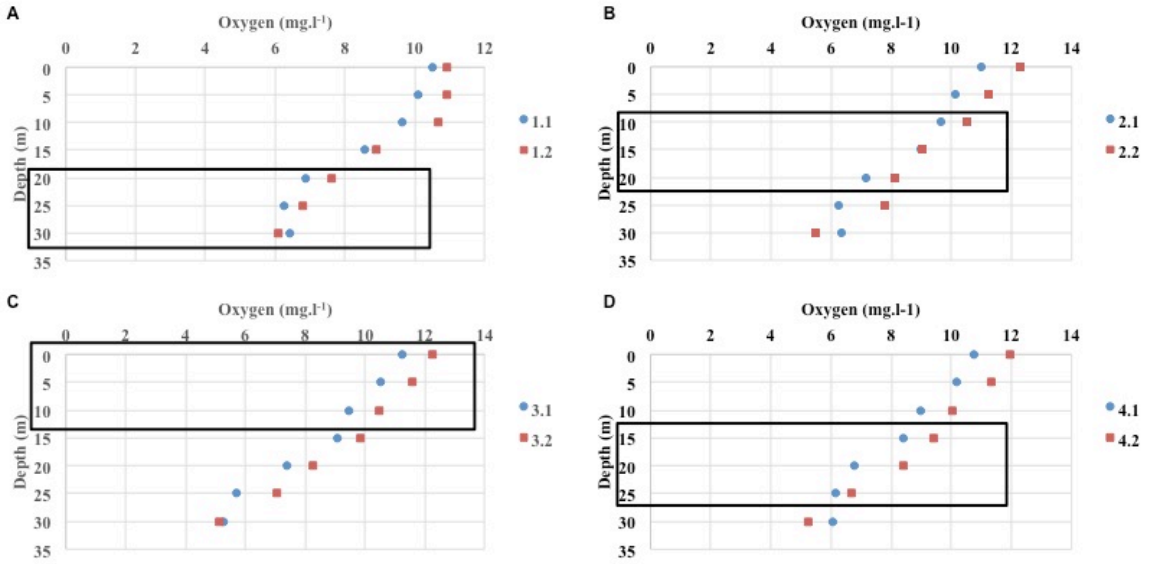

Figure 5. Oxygen levels for samplings. The framed values depict sampling depth for each copepod sampling. Week 1: full circle, week 2: square.

pH values decreased with depth (figure 6) with the values ranging between 7.96 and 8.06 (week 1) and between 8.36 and 8.52 (week 2) in 5 meters, between 7.70 and 7.84 (week 1) and between 7.84 and 8.40 (week 2) in 15 meters, and between 7.32 and 7.40 (week 1) and between 7.48 and 7.68 (week 2) in 25 meters. During week 1, pH varied less between the

surface and the bottom compared to week 2, because the pH values were higher during the second week.

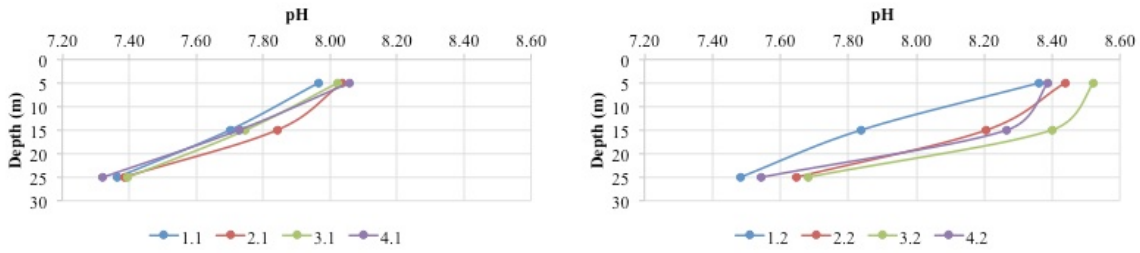

Figure 6. pH values at different sampling times and depths week 1 (left) and week 2 (right)

Chlorophyll a (Chl a) content (table 3) was highest on the surface and decreased with depth as a result of less sunlight. Values increased between week 1 and 2 from 2.3–4.6 µg/l to 5.0–8.5 µg/l in 5 meters, from 0.7–2.7 µg/l to 2.6–4.8 µg/l in 15 meters, and from 0.4–1.1 µg/l to 0.6–2.1 µg/l in 25 meters for week 1 and 2 respectively.

Table 3. Chlorophyll a content during different sampling times in various depths

Sample	Date	Sampling time	Depth (m)	Chl *a* ($\mu g.l^{-1}$) (mean ± SD)
1.1	10.8.2015	12:00	5	2.4 ± 0.7
			15	1.8 ± 1.5
			25	1.1 ± 0.4
2.1	11.8.2015	18:00	5	2.3 ± 1.3
			15	0.8 ± 0.1
			25	0.4 ± 0.0
3.1	13.8.2015	0:00	5	4.6 ± 1.4
			15	0.7 ± 0.3
			25	0.6± 0.2
4.1	14.8.2015	6:00	5	4.1 ± 2.5
			15	2.7 ± 2.4
			25	0.7 ± 0.1
1.2	17.8.2015	12:00	5	5.4 ± 1.0
			15	2.6 ± 0.1
			25	0.6 ± 0.1
2.2	18.8.2015	18:00	5	8.5 ± 1.7
			15	4.5 ± 0.5
			25	1.7 ± 0.2
3.2	20.8.2015	0:00	5	5.0 ± 1.2
			15	4.8 ± 1.0
			25	2.1 ± 0.10
4.2	21.8.2015	6:00	5	7.7 ± 0.2
			15	3.2 ± 0.1
			25	2.1 ± 0.1

6.3 Reproduction success

For the copepod female incubations, climate chambers were used. Samples 1, 2, and 4 were incubated in 11°C climate chamber, whereas 15°C was used for samples 3 to simulate the warmer surface water temperatures in both weeks. *In situ* water (for stable pH conditions) and temperatures were maintained during the female incubations. The average temperatures in the climate chambers were 10.6°C ± 0.94 and 15.3°C ± 0.99, which were close to the intended temperatures, 11°C and 15 °C, respectively.

The pH at the beginning of female incubations varied between 7.55 and 8.53. The pH at the beginning and end of both female and egg incubations (table 4) was below the long-term average pH of 8.01 (see 6.1) for all the samples except 3.1, 2.2 and 3.2. pH values for the beginning of egg incubations are not available for samples 1.1, 2.1 and 3.1 due to a measurement error.

Table 4. pH values at the start and end of female and egg incubations (*=1 replicate, **= 3 replicates)

		Female incubations		**Egg incubations**	
Sample	**T°C**	**pH start**	**pH end**	**pH start**	**pH end**
1.1	11	7.55 ± 0.02	7.72 ± 0.07	n/a	7.92 ± 0.03
2.1*	11	7.86	7.87	n/a	7.98
3.1	15	8.06 ± 0.04	8.02 ± 0.02	n/a	8.14 ± 0.06
4.1**	11	7.55 ± 0.00	7.82 ± 0.01	7.60 ± 0.04	7.90 ± 0.01
1.2	11	7.66 ± 0.04	7.75 ± 0.03	7.58 ± 0.03	7.92 ± 0.04
2.2	11	8.12 ± 0.04	8.06 ± 0.04	8.14 ± 0.03	8.06 ± 0.05
3.2	15	8.53 ± 0.04	8.26 ± 0.05	8.40 ± 0.06	8.18 ± 0.10
4.2	11	7.84 ± 0.04	7.96 ± 0.02	7.80 ± 0.02	8.00 ± 0.05

6.3.1 Egg production

The duration of female incubations was 25.7 ± 3.12 hours. The average egg production rate (EPR) per female varied between 1.9 and 10.6 (figure 7). The EPR was calculated using the amount of females found alive after the incubation period.

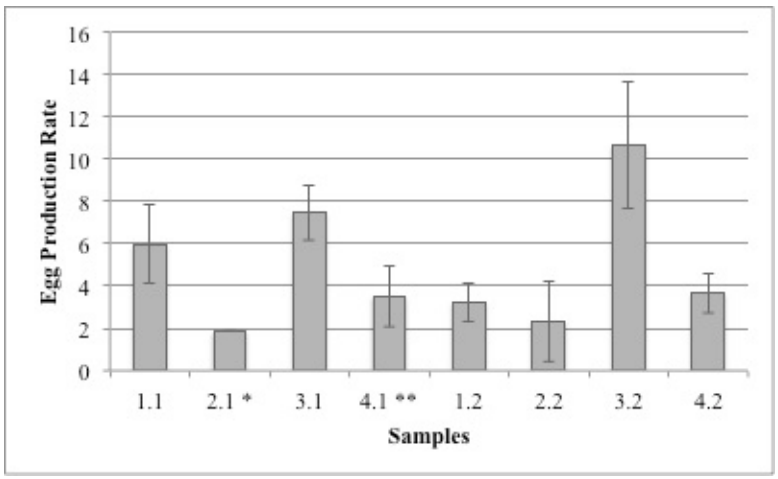

Figure 7. Egg production rate counted as egg production per day per female copepod found alive. N= 34 with each sample including 5 replicates, except * = 1 replicate, **= 3 replicates. Bars mean standard deviation.

There was a significant positive relationship between egg production rate and temperature (figure 8) (Linear regression, R = 0.76, df = 32, $p < 0.001$). There were no significant differences in egg production between weeks (General Linear Model, $F_{1,32} = 2.996$, $p = 0.095$), whereas time of day affected rates significantly ($F_{1,32} = 11.423$, $p < 0.001$). Pairwise comparisons showed differences in the EPR of *A. bifilosa* between midnight and all the other sampling times (Tukey HSD, $p < 0.001$), as well as between noon and evening. ($p = 0.022$). pH at experiment start had also a slight effect on the egg production rates ($F_{1,32} = 4.554$, $p < 0.042$) (figure 8). There was also a strong interaction between pH at the experiment start and temperature on egg production rates ($F_{1,32} = 36.471$, $p < 0.001$), indicating that the EPR increases with elevated pH and temperature.

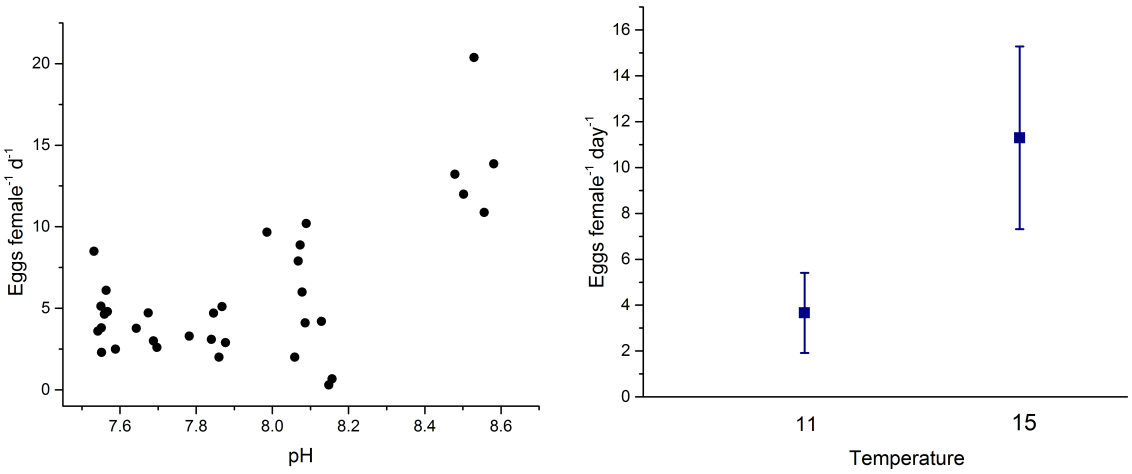

Figure 8. The relationship between egg production rate and a) pH at experiment start (left) and b) incubation temperature (right)

6.3.2 Hatching success

The duration of the egg incubations (n=34) was ~3 days, except for sample 2.1 the incubation time was ~4 days. There was great variation in the hatching rates between samples (figure 9) as for some samples hatching rate was close to or at zero and for samples 3.1 and 3.2 around 90%. There were no differences found in egg hatching success between weeks (Mann-Whitney U test, N = 33, p = 0.221), whereas egg hatching differed between sampling times significantly (Kruskal-Wallis one way-ANOVA, N = 33, p < 0.001).

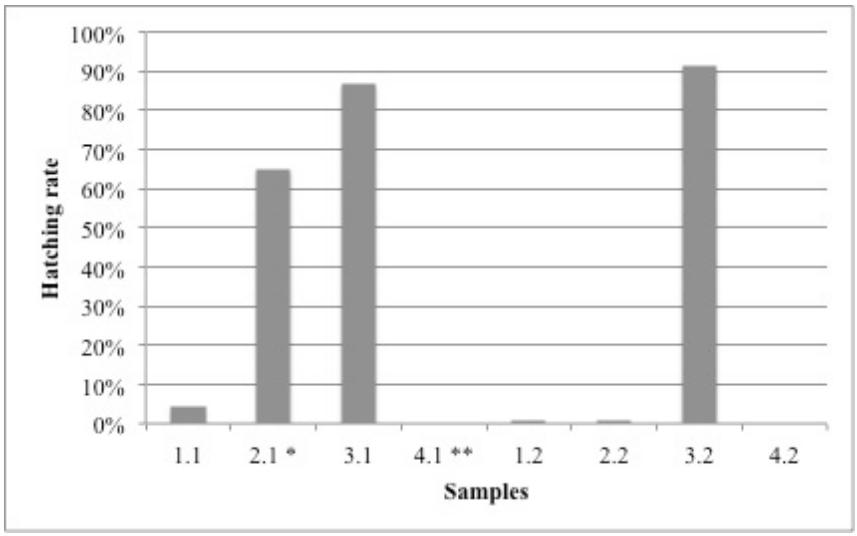

Figure 9. Hatching rates. N=34 with each sample including 5 replicates, except * = 1 replicate, **= 3 replicates.

7 Discussion

The objective of this thesis was to shed light on how pH and temperature changes in the Baltic Sea affect the reproduction success of copepods, specifically *Acartia bifilosa*. The focus of the experiment was on the ambient conditions experienced by copepods on diurnal basis in their natural environment. Increasing the understanding of copepods' resilience is important as the Baltic Sea surface waters have been getting warmer in all seasons during the past thirty years with an estimated increase in annual mean surface temperature of up to 1°C per decade (1990–2008). Also the pH of the Baltic Sea has decreased over the past 20 years, but increased alkalinity has weakened the pH decrease in the surface waters. (HELCOM 2013)

The main findings of this study were that egg production rate increased with elevated temperature and pH and that sampling time affected hatching success significantly. Thus, increasing pH and temperature conditions have a positive effect on the reproduction success of *A. bifilosa*. No conclusions can be made about the effect of pH in the hatching success but it seems that elevated temperature may increase hatching rates at least when the temperature is within the optimum range for copepod.

7.1 Environmental conditions

There was an increase in the seawater temperatures at all sampling depths during the second sampling week due to warm weather that was prevalent at the time. Oxygen levels were higher for surface and intermediate sampling depths during the second week. This is also a result of the warm weather, which likely caused the increase of cyanobacteria blooms and thus the increase in the photosynthesis in shallower depths. Both pH and Chl a values were higher during the second week, which is an indication of higher level of productivity caused by the warm temperature. In this study, the sampling conditions variations were 1.2 pH units (7.32–8.52) and 7.8 °C (10.18–17.96°C), which were higher than found by Almén et al. (2014) (change of 0.5 pH units and 5°C).

The purpose of including the long-term monitoring pH data was to demonstrate natural pH variations in the coastal areas of the Baltic Sea and to widen the discussion about the survival and adaptability of copepods. The long-term pH data from the same location as the copepods were sampled from reveals large seasonal variations and a slight declining trend in the pH values. This is not necessarily a proof of ocean acidification since pH is affected by several factors (Brutemark et al. 2011) and dissolved inorganic carbon measurements would be needed to in order to determine the effect of elevated CO_2. Instead, this shows how drastic seasonal pH variations copepods already endure, as the difference between the minimum and maximum value in the dataset was more than two pH units. In addition to strong seasonal pH and temperature variations, copepods experience widely varying pH and temperature conditions also through diurnal vertical migration, which may enhance their ability to endure the effects of climate change as these natural variations are greater than expected climate change induced pH and temperature changes (Almén et al. 2014). Great variations during diurnal vertical migration, which were also demonstrated during this study, show that the copepods are used to adapting to changing

conditions at least in the short term. It can be speculated if this enhances the copepods' ability to endure the effects of climate change also in the long term.

7.2 Egg production rate

There was a significant positive relationship between the egg production rate (EPR) and the incubation temperature, which is in line with the findings of Koski and Kuosa (1999). The positive relationship of the EPR and temperature may be due to the difference in the incubation conditions and the natural environment: The seawater temperatures were 1-2 degrees higher during the second week but as the incubation temperatures remained the same, the difference between the seawater temperature and the incubation temperature in the climate chamber increased especially for samples 1, 2, 4. Thus, the incubation conditions in the higher temperatures (samples 3) may have been more favorable for reproduction because 15°C could be closer to the optimal temperature for *A. bifilosa*. Also, the time of day affected the EPR significantly: the EPR was higher for midnight samples (3.1 and 3.2) compared to all the other sampling times and also higher during noon samplings compared to evening samplings. This could be due to the change in the ambient conditions as described above but conclusions are difficult to draw due to the relatively small amount of replicates per each sampling time.

The results suggest that the EPR increases with elevated pH. This is not in line with findings of Engström-Öst et al. (2014) who discovered that *A. bifilosa* responded to low pH with increased reproductive output. According to other previous studies, decreased ocean pH has been noted to decrease the reproduction rate of copepod only at extreme levels (Cripps et al. 2014; Kurihara et al. 2004; Pedersen et al. 2014), thus there is a positive relationship between low pH and low EPR. It must be noted that other factors, such as warming, nutrition, and the duration of exposure to the low pH levels (Zervoudaki et al. 2014), may have stronger effect on the reproduction rate of copepod than pH change alone.

7.3 Hatching success

The hatching success differed between sampling times significantly. This may be due to the different temperature conditions as samples 3.1 and 3.2 were incubated in warmer

conditions, which could be more favorable to copepod. Higher temperature (20°C) has also been noted to cause a decrease in egg viability and nauplii development (Vehmaa et al. 2013), but in this study, the incubation temperature for samples 3 was quite close to the ambient conditions and apparently within the range of optimal conditions for hatching.

Another factor affecting the hatching rate may be the variation in the incubation hours since the incubation time for sample 2.1 was ~4 days thus 40% longer than average incubation time, which may have increased the hatching rate. This is supported by the low hatching rate of sample 2.2. as the conditions for these two samples were otherwise similar. Hatching success (Kurihara et al. 2004; Mayor et al. 2007) has been found to be adversely affected by pH decrease but only at extreme CO_2 levels. With modest decreases (~2400 ppm vs. ambient air ~400ppm) in the seawater pH, hatching success rates have not been found to be significantly affected. (Kurihara et al. 2004, 724; Kurihara & Ishimatsu 2008, 1088) This is also supported by the results of this study.

7.4 Limitations of the study and suggestions for future research

Instead of using acclimatized populations or populations reared over subsequent generations in laboratory conditions, the methodology chosen for this thesis was a laboratory experiment with animals sampled from the field due to time constraints. The relatively small amount of samples (8) and replicates (34) should be noted before making far-reaching conclusions. The reproduction success of copepods is controlled by several other factors besides pH and temperature, such as predation pressure and food availability and quality (HELCOM 2013; Koski & Kuosa 1999), thus the combined effect of changing environmental conditions on the reproduction success is hard to predict.

The climate chamber temperatures were chosen before the start of the experiment according to the estimated *in situ* temperatures. Due to the increase in the temperature during the second week of the experiment, the difference between the ambient and climate chamber temperature increased. This unpredicted change in the natural conditions may have affected the egg incubations. One indication of this is the hatching success of sample 2.1: the same temperature but longer incubation time led to significantly higher hatching success compared to sample 2.2.

As the copepods were incubated in ambient conditions without controlling pH beyond using *in situ* seawater, the pH conditions were not stable during the incubations and the analysis takes only the pH at the beginning of females into account. For some samples, pH decreased in the course of incubations due to respiration by copepods whereas for other samples pH increased due to oxygen production in algal photosynthesis.

This study focused on impacts of ambient pH and temperature using copepod populations sampled from the field. Future research could shed light on long-term impacts of pH and temperature with controlled pH conditions and using copepods that have been reared for subsequent generations.

8 Acknowledgments

My sincere thanks to Tvärminne Zoological Station and to its personnel for supporting the research for this thesis during the experiment and for providing the long-term pH data. This thesis was financially supported by Societas pro Fauna et Flora Fennica for which I would like to express my gratitude. Many thanks to my thesis supervisors Olivier Glippa and Jonna Engström-Öst from Aronia Research and Development Institute, and Maria Kihlström from Novia University of Applied Sciences for their continuous support and collaboration.

Works cited

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 (2014)*, 120–128.

Brutemark, A., Engström-Öst, J., Vehmaa, A. (2011). Long-term monitoring data reveal pH dynamics, trends and variability in the western Gulf of Finland. *International Journal of Oceanography and Hydrobiology 40 (3),* 91–94.

Busch, D.S., Harvey C.J & McElhany P. (2013). Potential impacts of ocean acidification on the Puget Sound food web. *ICES Journal of Marine Science, 70 (4),* 823–833.

CO_2.Earth (2016). Monthly CO_2. www.co2.earth/monthly-co2 (retrieved 27.2.2016)

Cripps, G., Lindeque, P. & Flynn, K.J. (2014). Have we been underestimating the effects of ocean acidification in zooplankton? *Global Change Biology, 20 (11),* 3377–3385.

Engström-Öst, J., Holmborn, T., Brutemark, A., Hogfors, H., Vehmaa, A. & Gorokhova, E. (2014) The effects of short-term pH decrease on the reproductive output of the copepod *Acartia bifilosa* – a laboratory study. *Marine and Freshwater Behaviour and Physiology, 47 (3),* 173–183.

Fabry, V.J., Seibel, B.A., Feely, R.A. & Orr, J.C. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science 65:* 414–432.

Fitzer, S.C., Caldwell, G.S., Close, A.J., Clare, A.S., Upstill-Goddard, R.C. & Bentley, M.G. (2012). Ocean acidification induces multi-generational decline in copepod naupliar production with possible conflict for reproductive resource allocation. *Journal of Experimental Marine Biology and Ecology*, *2012 (418–419)*, 30–36.

HELCOM (2013). *Climate change in the Baltic Sea Area. HELCOM thematic assessment in 2013*. Baltic Sea Environment Proceedings No. 137. Helsinki: Helsinki Commission.

IPCC (2014). Summary for policymakers. In: *Climate Change 2014: Impacts, Adaptation, and Vulnerability*. *Part A: Global and Sectoral Aspects.* Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1–32.

Kelly, M.W., Padilla-Camino, J.L. & Hofmann, G.E. (2011). Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus. Global Change Biology 19 (8),* 2536–2546.

Koski, M. and Kuosa, H. (1999). The effect of temperature, food concentration and female size on the egg production of the planktonic copepod *Acartia bifilosa*. *Journal of Plankton Research* 21 (9), 1779–1789.

Kurihara, H., Shimode, S. & Shirayama, Y. (2004). Effects of raised CO_2 concentration on the egg production and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*). *Marine Pollution Bulletin, 49 (9–10),* 721–727.

Kurihara, H. & Ishimatsu, A. (2008). Effects of high CO_2 seawater on the copepod (*Acartia tsuensis*) through all life stages and subsequent generations. *Marine Pollution Bulletin, 56 (6)*, 1086–1090.

Luontoportti (2015). Hankajalkaiset.www.luontoportti.com/suomi/fi/itameri/hankajalkaiset (retrieved 19.2.2015)

Mayor, D.J., Matthews, C., Cook, K., Zuur, A.F. & Hay, S. (2007). CO_2-induced acidification affects hatching success in *Calanus finmarchicus*. *Marine Ecology Progress Series, 350,* 91– 97.

Mayor, D.J., Everett, N.R. & Cook, K.B. (2012). End of century ocean warming and acidification effects on reproductive success in a temperate marine copepod. *Journal of Plankton Research 34 (3)*, 258–262.

McConville, K., Halsband, C., Fileman, E.S., Somerfield, P.J., Findlay, H.S. & Spicer, J.J. (2013). Effects of elevated CO_2 on the reproduction of two calanoid copepods. *Marine Pollution Bulletin, 73 (2)*, 428–434.

NOAA (2014). *NOAA Ocean Acidification Program.* http://oceanacidification.noaa.gov/ (retrieved 16.11.2014)

Pedersen, S.A., Våge, V.T., Olsen, A.J., Hammer, K.M. & Altin, D. (2014). Effects of elevated carbon dioxide (CO_2) concentrations on early developmental stages of the marine copepod *Calanus finmarchicus* Gunnerus (Copepoda: Calanoidae). *Journal of Toxicology and Environmental Health, Part A*, *77 (9–11),* 535–549.

Pörtner H-O., Karl, D.M., Boyd, P.W., Cheung, W.W.L., Lluch-Cota, S.E., Nojiri, Y., Schmidt, D.N. & Zavialov, P.O. (2014). Ocean systems. In: *Climate Change 2014: Impacts, Adaptation, and Vulnerability*. *Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. 411– 484.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C., Watson, A. (2005). *Ocean acidification due to increasing atmospheric carbon dioxide.* The Royal Society policy document 12/05, Royal Society, London, UK. http://www.us-ocb.org/publications/Royal_Soc_OA.pdf (retrieved 8.1.2016)

Riebesell, U. & Tortell, P.D. (2011). Effects of ocean acidification on pelagic organisms and ecosystems. In: Gattuso, J-P. & Hansson, L. *Ocean acidification.* 99–121. New York: Oxford University Press. Thor**,** P. & Dupont, S. (2015). Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. *Global Change Biology 21 (2)*. (E-publication)

Timeanddate.com. (2015). Sunrise and sunset times in Espoo, August 2015. www.timeanddate.com/sun/finland/espoo?month=8&year=2015 (retrieved 9.12.2015)

Vehmaa, A., Hogfors, H., Gorokhova, E., Brutemark, A., Holmborn, T. & Engström-Öst, J. (2013). Projected marine climate change: effects on copepod oxidative status and reproduction. *Ecology and Evolution 3 (13)*, 4548–4557.

Zervoudaki, S., Frangoulis, C., Giannoudi, L. & Krasakopoulou, E. (2014). Effects of low pH and raised temperature on egg production, hatching and metabolic rates of a Mediterranean copepod species (*Acartia clausi*) under oligotrophic conditions. *Mediterranean Marine Science, 15 (1),* 74–83.

Appendix

Temperature and salinity profiles for the second sampling week (17.-21.8.2015).