Negligible effects of ocean acidification on *Eurytemora affinis* (Copepoda) offspring production

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Abstract. Ocean acidification is caused by increasing amounts of carbon dioxide dissolving in the oceans leading to lower seawater pH. We studied the effects of lowered pH on the calanoid copepod *Eurytemora affinis* during a mesocosm experiment conducted in a coastal area of the Baltic Sea. We measured copepod reproductive success as a function of pH, chlorophyll *a* concentration, diatom and dinoflagellate biomass, carbon to nitrogen (C : N) ratio of suspended particulate organic matter, as well as copepod fatty acid composition. The laboratory-based experiment was repeated four times during 4 consecutive weeks, with water and copepods sampled from pelagic mesocosms enriched with different CO$_2$ concentrations. In addition, oxygen radical absorbance capacity (ORAC) of animals from the mesocosms was measured weekly to test whether the copepod’s defence against oxidative stress was affected by pH. We found no effect of pH on offspring production. Phytoplankton biomass, as indicated by chlorophyll *a* concentration and dinoflagellate biomass, had a positive effect. The concentration of polyunsaturated fatty acids in the females was reflected in the eggs and had a positive effect on offspring production, whereas monounsaturated fatty acids of the females were reflected in their eggs but had no significant effect. ORAC was not affected by pH. From these experiments we conclude that *E. affinis* seems robust against direct exposure to ocean acidification on a physiological level, for the variables covered in the study. *E. affinis* may not have faced acute pH stress in the treatments as the species naturally face large pH fluctuations.

1 Introduction

The concentration of carbon dioxide (CO$_2$) in the atmosphere is rising at a ten times faster rate than during the past 55 million years. The oceans absorb CO$_2$ from the atmosphere leading to lower seawater pH and a reduction in carbonate concentration. Since pre-industrial times the ocean acidity has increased by 28 % (IPCC, 2013). The fast increase in CO$_2$ and change in seawater chemistry will have adverse effects on many marine species and ecosystems (Fabry et al., 2008; Kroeker et al., 2010). Due to lower buffering capacity of brackish water, the Baltic Sea is especially sensitive to elevated CO$_2$ (Havenhand, 2012). Modelling suggests a decrease of 0.26–0.40 pH units for the Baltic Sea by the year 2100 (BACC II, 2015). In addition, high CO$_2$ levels interact with other climate change related factors that may have
negative effects on marine organisms (Kroeker et al., 2013; Talmage and Gobler, 2012). Especially the coastal zones are under heavy pressure from anthropogenically driven ocean acidification due to eutrophication and oxygen minimum zones (Fabry et al., 2008; Melzner et al., 2013; Wallace et al., 2014).

Copepods are the most abundant zooplankton in the oceans. They constitute major parts of the diet of juvenile fish, and are hence an important part of the food web. Lowered pH may disturb the acid-base balance, thereby altering the reproduction, hatching, and development (Kurihara et al., 2004; Mayor et al., 2007; Weydmann et al., 2012). Besides the direct effects of acidification, rising CO$_2$ can adversely affect consumers and food webs due to changed nutritional value of prey (Rossoll et al., 2012). Polysaturated fatty acids (PUFA) are essential metabolites for copepods and need to be obtained from the diet. Certain PUFA have specific roles in central processes of copepod reproduction including egg production (20:5ω3 EPA), egg hatching (22:6ω3 DHA), and development (18:3ω3 and 18:5ω3) (Jónasdóttir et al., 2009). Important ω3 fatty acids decreased significantly in the diatom Thalassiosira pseudonana grown at high CO$_2$ with lower levels of PUFA with following decreased egg production in the copepod Acartia tonsa (Rossoll et al., 2012). Further, CO$_2$-related changes in the fatty acid composition and content of several primary producers have been reported (Bermúdez et al., 2016, and references therein). Furthermore, ocean-acidification-induced changes in phytoplankton species composition can have an indirect effect on food quantity and quality for heterotrophic consumers. Elevated CO$_2$ levels can increase C:N ratios of primary producers, which alter their nutritional value and can adversely affect the growth and reproduction of copepods (Schoo et al., 2013).

Ocean acidification can induce oxidative stress in marine organisms (Tomanek et al., 2011; Kaniewska et al., 2012). Hence, biochemical responses to low pH conditions, such as changed activity of antioxidants and enzymes may show higher sensitivity than, for example, survival and reproduction (Gorokhova et al., 2010; Zhang et al., 2012). An enhanced antioxidant defence in response to increased reactive oxygen species (ROS) concentration may occur at the expense of reduced investment in other metabolic processes, such as growth and reproduction. The defence capacity against oxidative stress can be assessed by measuring the capacity to quench ROS (see review by Monaghan et al., 2009).

E. affinis is a common copepod in the Baltic Sea and dominates the zooplankton community together with Acartia bifilosa in the study area during summer. E. affinis is an egg-bearing copepod that produces subitaneous eggs during summer and diapause eggs in autumn. The copepods recruit from small overwintering populations, and by hatching from the sediment (Katajisto et al., 1998). Previous studies on the effects of ocean acidification on A. bifilosa from the Baltic Sea have shown adverse effects in combination with warming (Vehmaa et al., 2012a, 2013). The increase in egg production with warmer temperature was lower when copepods were simultaneously exposed to warmer temperature and lowered pH (Vehmaa et al., 2012a).

The main objectives of this study were to examine effects of ocean acidification on reproductive success and antioxidant defence of the copepod E. affinis, as well as measuring the effects of food quality and quantity on offspring production. We studied how lowered pH, phytoplankton biomass (indicated as chlorophyll $a$), biomass of diatoms and dinoflagellates and the C:N ratio of particulate organic matter (POM) affect the offspring, i.e., nauplii production in E. affinis. In addition, we looked at the effect of pH on essential fatty acids of incubated egg-bearing females to reveal indirect effects via the food. We also tested whether the fatty acid levels of the females were reflected in their eggs under a range of $f$CO$_2$ values representative for the future ocean (IPCC, 2013).

2 Material and methods

2.1 Experimental set-up

The study was conducted using KOSMOS mesocosms (Riebesell et al., 2013) within the framework of the SOPRAN project (Paul et al., 2015). The mesocosms were located at Storfjärden, an offshore pelagic area in the vicinity of Tvärminne Zoological Station (University of Helsinki) Baltic Sea (59°51′20″N, 23°15′42″E) from the beginning of June until the middle of August, 2012. Storfjärden has a maximum depth of 34 m. The water is brackish with a mean salinity of 6. The area receives inflow of freshwater from the river Svartån, and periodical inflows of cold water from the open Baltic Sea with higher salinity (Niemi, 1976). Six mesocosms, consisting of 17 m deep bags made of thermoplastic urethane, each enclosing ∼55 m$^3$, were moored on site on June 12. The mesocosms were covered by a net (mesh size 3 mm) at the top and the bottom during filling and left open for 4 days before the net was removed and the top was pulled up 1.5 m above the water surface and closed at the bottom (see Riebesell et al., 2013 and Paul et al., 2015 for details on the experimental design) to enclose the natural plankton community. The water column was mixed at the beginning of the experiment in order to avoid a salinity stratification. Four of the mesocosms were stepwise manipulated with CO$_2$-enriched seawater, during 3 consecutive days. Two bags were untreated and used as controls. Due to outgassing, CO$_2$ was also added on day 15 of the experiment to the upper 7 m of the high CO$_2$ mesocosms to maintain the treatment levels. No nutrients were added. The average $f$CO$_2$ levels during the period of our incubation experiments ($t1 − t30$) were 346, 348, 494, 868, 1075, and 1333 μatm (Paul et al., 2015).
2.2 Sampling and incubations

Our copepod experiment was conducted during a 4-week period with weekly incubations. We sampled water and copepods from the mesocosms on days t3, t10, t17 and t24 (t0 being the day of first addition of CO2 into the bags). Zooplankton was sampled with a 300 µm net (Ø 17 cm) from 17 m depth to the surface from all mesocosms and transferred to containers pre-filled with 4 L of seawater from a depth of 9 m from the respective mesocosm. On the same day, unfiltered water samples were taken from each mesocosm with depth-integrated water samplers (IWS, HYDRO-BIOS, Kiel) which take equal amount of seawater from every depth (0–17 m). In order to minimize handling of the restricted water available, to keep food conditions as similar to in situ conditions as possible, and to avoid gas exchange, the water was directly transferred into airtight 1.2 L Duran bottles for incubations. Water samples and zooplankton were transported to a light- and temperature-controlled room at Tvärminne Zoological Station. Egg-bearing females of *E. affinis* (*n* = 10 per treatment) were incubated in the 1.2 L Duran glass bottles which contained mesocosm water. Temperature and pH were measured before adding the copepods to the bottles. Bottles were filled up and sealed without airspace, ensuring no air bubbles were present, to prevent CO2 outgassing. The bottles were slowly inverted after sealing and incubated in a 16:8 h light-dark cycle at in situ temperature, as an attempt to match the natural environment. A light source was installed above the incubation bottles, yielding 7 µmol m–2 s–1 (LI-COR LI-1000). All pH and temperature measurements were conducted with an Ecosense pH10 pH/temperature Pen directly from the bottles before closing and directly after opening (Table 1). The pen was calibrated with standard buffer solutions (Centipur, TitriCAD, 80 °C until analyses. For further details on sampling and storage samples were frozen in an ultra-freezer (−80 °C) until fatty acids were measured by gas chromatography as fatty acid methyl esters (FAMEs) following instructions in Klein Breteler et al. (1999). Fatty acids were separated into three groups that were used in the analyses; polyunsaturated (PUFA), monounsaturated (MUFA), and saturated fatty acids (SAFA) and were expressed as ng mg dry weight–1.

With each start of the weekly, sub-experiments, female *E. affinis* with egg sacs were picked from the mesocosms for analyses of oxygen radical absorbance capacity (ORAC). The animals (*n* = 30 ± 2) were carefully moved with tweezers onto a piece of plankton net gauze and stored in Eppendorf tubes in −80 °C until they were homogenised in 150 µL Tris-EDTA buffer containing 1% sarcosyl. The antioxidative capacity was assayed as ORAC according to Ou et al. (2001). As a source of peroxyl radicals, we used 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) (152.66 mM) and fluorescein was used as a fluorescent probe (106 mM). We used trolox (218 µM, Sigma-Aldrich) as a standard and the assay was performed on a 96-well microplate and to each well, 20 µL sample, 30 µL AAPH and 150 µL fluorescein were added. ORAC values were normalized to protein concentration and expressed as mg Trolox equivalents mg protein–1. Protein concentration was measured with NanoOrange® (Life Technologies).

Phytoplankton was sampled every second day, fixed with acidic Lugol’s iodine (2% final concentration) and counted with the inverted microscope method (Utermöhl, 1958). Samples for chlorophyll *a* (Chl *a*) measurements were collected onto GF/F filters and measured as described by Welschmeyer (1994). Samples for carbon (C) and nitrogen (N) concentrations were collected as for Chl *a* and stored in glass petri dishes at −20 °C until analyses. For further details on sampling and analyses, please refer to Paul et al. (2015).

2.3 Statistical analyses

2.3.1 Nauplii production

A linear mixed-effects model (LMM) was applied, as we did repeated measures of nauplii production of the same groups of individuals from the same mesocosms, to test if pH or food quantity and quality affected the nauplii production of *E. affinis*. Collinearity between all explanatory variables was checked (Pearson’s product-moment correlation). Chl *a* concentration and the abundance of filamen-
Table 1. $f CO_2$ values ($t1 - t30$), average weekly pH, temperature, and dissolved oxygen (DO) and saturation in incubation bottles.

<table>
<thead>
<tr>
<th>$fCO_2$ treatment (µatm)</th>
<th>Mesocosm</th>
<th>Week</th>
<th>pH</th>
<th>temp. (C$^\circ$)</th>
<th>DO mg L$^{-1}$</th>
<th>DO%</th>
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<td>7.62</td>
<td>15.14</td>
<td>9.72</td>
<td>99.7</td>
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</table>

tous cyanobacteria correlated. As these correlating variables explain partly the same thing, the variable that explained the variation in nauplii production the best (Chl a) was included in the model. In the model the average number of nauplii produced female$^{-1}$ day$^{-1}$ (log-transformed) for each treatment was set as response variable. Incubation pH (calculated as weekly mean values from daily measurements from incubation bottles), Chl a concentration, biomass of diatoms (Chaetoceros sp., Skeletonema marinoi and pennate diatoms, total µg C L$^{-1}$), C : N < 55 µm fraction of POM, biomass of mixotrophic dinoflagellates (Amylax triacantha, Dinophysis spp., Heterocapsa triqueta and Micraacanthodinum spp., size range $\sim$10–100 µm, total µg C L$^{-1}$) and incubation temperature were used as fixed effects (Table 2). We used only the most abundant diatoms as the other species had a very scarce and inconsistent abundance in the samples. The main groups of diatoms were present in all mesocosms. The smaller fraction of C : N < 55 µm was used instead of total fraction may have included large zooplankton such as copepods which could affect the results. The explanatory variables used included data of each mesocosm of the corresponding day of sampled water used for the incubations. When sampling days were missing, the average values (of total µg C L$^{-1}$ for diatoms and dinoflagellates, and mol : mol of C : N) for the previous and the next day were used. Day nested within week, nested within mesocosm, was used as random intercept as nauplii production of the same animals was measured four times per week and as weekly incubations were dependent on each other, and they were repeatedly sampled from the same mesocosms. The model simplifications were done manually in a backward stepwise manner by removing the non-significant effects and by using Akaike’s information criterion (AIC) to achieve the minimum adequate model for the data. We report $t$-statistics of the retained variables for the LMMs (Table 3).

### 2.3.2 Fatty acids

Linear mixed-effects models were applied to test if pH has a direct effect on the fatty acid content of female copepods. EPA, DHA, and their precursor 18:3ω3 autocorrelated strongly with each other, and with total PUFA (Pearson’s product-moment correlation); therefore we decided to use PUFA in the LMM. Separate models were made for each fatty acid group, which was set as a response variable, with pH as fixed effect and mesocosm as random effect. To test the effects of essential fatty acids on weekly nauplii production, we used separate LMMs, as PUFA and MUFA autocorrelated. In the models, PUFA, MUFA, and SAFA were used as fixed effects and mesocosm was tested as a random factor (Table 2).

To test whether female fatty acid content are reflected in the fatty acid content of eggs, each fatty acid group (PUFA, MUFA and SAFA) was tested separately in a LMM.
Table 2. Variables that were used in the full LMM models (numbers indicate separate models). Repeated measures were used as random effects in the models, as samples from the same enclosures are dependent on each other.

<table>
<thead>
<tr>
<th>LMM</th>
<th>Fixed effects</th>
<th>Definition</th>
<th>Response variable</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>The ocean acidification effect</td>
<td>Nauplii production</td>
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<tr>
<td></td>
<td>Chl a</td>
<td>The food quantity effect</td>
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<tr>
<td></td>
<td>Diatoms</td>
<td>The food quality effect</td>
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<td></td>
<td>C : N &lt; 55 µm</td>
<td>The food quality effect</td>
<td></td>
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<tr>
<td></td>
<td>Dinoflagellates</td>
<td>The food quality effect</td>
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<tr>
<td></td>
<td>Incubation temp.</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>Incubation pH</td>
<td>The ocean acidification effect</td>
<td>Fatty acids in females:</td>
</tr>
<tr>
<td>3</td>
<td>Incubation pH</td>
<td>The ocean acidification effect</td>
<td>Fatty acids in eggs:</td>
</tr>
<tr>
<td>4</td>
<td>Incubation pH</td>
<td>The ocean acidification effect</td>
<td>MUFA</td>
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<tr>
<td></td>
<td></td>
<td>Fatty acids in females:</td>
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<tr>
<td></td>
<td>PUFA</td>
<td>Relationship between female fatty acids and their eggs</td>
<td>MUFA</td>
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<tr>
<td>5</td>
<td>MUFA</td>
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<td>6</td>
<td>SAFA</td>
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<td>11</td>
<td>pH</td>
<td>ORAC</td>
<td></td>
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</table>

model, fatty acids of eggs was set as a response variable and female fatty acid content as fixed effect; mesocosm was used as a random factor. Not all females had egg sacs left at the end of weeks 3 and 4 and therefore not enough material (egg sacs) was obtained for all treatments. The variables of corresponding samples that were missing the egg data were therefore removed.

### 2.3.3 Antioxidative capacity

We tested whether there was an effect of pH on the copepods’ antioxidant capacity (ORAC) with a LMM. ORAC was set as response variable, pH (measured the same day from water samples taken for incubations) as fixed factor and mesocosm was set as random factor. In addition, to test for potential correlation between ORAC and nauplii production, a Pearson’s product-moment correlation was performed. In the ORAC data, values for mesocosms 5 (control) and 6 (868 µatm) were missing.

For all models, model validation was done by plotting the standardised residuals against the fitted values. All statistical analyses were performed with R 2.15.2 and the nlm-package (Pinheiro et al., 2012) was used for the LMM analyses (R Development Core Team, 2012).

### 3 Results

The oxygen saturation was continuously high (> 93.8%) in all incubations (Table 1). Temperature in the climate-controlled room followed the in situ temperature except during the fourth weekly incubation (t24-t28) when the room was not adjusted to the sudden in situ drop in temperature that occurred. Temperature in the treatment bottles increased from around 10°C in the first week to 15°C during the fourth week (Table 1). The pH remained stable in the bottles (SD < 0.08 within a week based on daily measurements, Table 1) and matched the in situ pH and CO₂ treatments.

Chl a concentration was relatively stable at ∼ 2 µg L⁻¹ in all mesocosms but then decreased to ∼ 1 µg L⁻¹ on t17. A significant positive effect of CO₂ on Chl a was observed after t17 (Paul et al., 2015). Dinoflagellates were on average 4.41 ± 1.39 µg CL⁻¹ (+SD) (range 0–7.32) and declined rapidly after t17. The C : N values included in our analyses (our sampling days) were on average 7.66 ± 0.42 (range 6.13–8.77). A more comprehensive description of C : N is found in Paul et al. (2015). The diatoms included in our analyses were on average 0.06 ± 0.10 (range 0–0.53 µg C L⁻¹). CO₂ treatment did neither effect dinoflagellates, C : N < 55 µm, nor diatoms.

Nauplii production in incubations was highest in water from M3, 1075 µatm (pH 7.6) with on average 12.6 ± 9.6 nauplii produced per female per day during the whole study period. For clarity and easier comparison between studies within this mesocosm project, average fCO₂ lev-
els (t1–t30) are included in Fig. 1 to describe the treatments. The effect of pH on nauplii production was not statistically significant. Particulate matter C : N (< 55 µm) had no impact on nauplii production. Chl a concentration, as an indicator of total food availability had a positive effect (LMM; \( t = 5.440, p < 0.001 \), Fig. 2a). Dinoflagellate biomass (\( t = 2.731, p = 0.008 \), Fig. 2c) stimulated nauplii production, whereas diatom biomass (LMM; \( t = -4.231, p < 0.001 \), Fig. 2b) had an adverse effect. There was a positive relationship between incubation temperature and nauplii production (\( t = 3.388, p < 0.004 \)) (Table 3).

The fatty acid contents (ng mg dry weight\(^{-1}\)) of the females were not affected by pH (LMM \( p > 0.5 \), see also Supplement). Female MUFA and PUFA content significantly affected the MUFA and PUFA content of the eggs (LMM MUFA; \( t = 2.922, p = 0.012 \), LMM PUFA; \( t = 2.864, p = 0.013 \)), whereas female SAFA did not (Fig. 3a–c, LMM; \( t = -1.497, p = 0.158 \)). Female PUFA concentration stimulated nauplii production (LMM; \( t = 3.989, p = 0.001 \)), whereas MUFA (LMM; \( t = 2.031, p = 0.058 \)), and SAFA content had no statistically significant effect (LMM; \( t = 0.644, p = 0.528 \), Fig. 4a–c, Table 3).

ORAC was not affected by pH (LMM; \( t = -0.057, p = 0.580 \)) and there was no correlation between female ORAC and nauplii production (\( \text{rho} = 0.297, p = 0.180 \)) (Fig. 5).

![Figure 1. Weekly nauplii production, as averages of 10 females per bottle, for all mesocosms (treatment target fCO2 in brackets, as averages of t1–t30). Time point 1 is the average weekly nauplii production t3–t7, 2 = t10–t14, 3 = t17–t21, and 4 = t24–t28.](image)

**Table 3.** \( T \)-statistics of the retained fixed effects in the LMM.

<table>
<thead>
<tr>
<th>LMM</th>
<th>Response variable*</th>
<th>Variable</th>
<th>Value ( \pm ) SE</th>
<th>df</th>
<th>( t )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nauplii production</td>
<td>Chl a</td>
<td>1.09 ± 0.20</td>
<td>69</td>
<td>5.440</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diatoms</td>
<td>-2.79 ± 0.66</td>
<td>69</td>
<td>-4.231</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dinoflagellates</td>
<td>0.14 ± 0.05</td>
<td>69</td>
<td>2.731</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incubation temp.</td>
<td>0.16 ± 0.05</td>
<td>17</td>
<td>3.388</td>
<td>0.004</td>
</tr>
<tr>
<td>2</td>
<td>Fatty acids in females:</td>
<td>Incubation pH</td>
<td>75.99 ± 112.80</td>
<td>16</td>
<td>0.673</td>
<td>0.510</td>
</tr>
<tr>
<td>3</td>
<td>Fatty acids in females:</td>
<td>Incubation pH</td>
<td>-7.70 ± 34.60</td>
<td>16</td>
<td>-0.223</td>
<td>0.827</td>
</tr>
<tr>
<td>4</td>
<td>Fatty acids in females:</td>
<td>Incubation pH</td>
<td>-135.27 ± 325.21</td>
<td>16</td>
<td>-0.416</td>
<td>0.683</td>
</tr>
<tr>
<td>5</td>
<td>Fatty acids in eggs:</td>
<td>PUFA</td>
<td>1.15 ± 0.40</td>
<td>13</td>
<td>2.864</td>
<td>0.013</td>
</tr>
<tr>
<td>6</td>
<td>Fatty acids in eggs:</td>
<td>MUFA</td>
<td>1.08 ± 0.37</td>
<td>13</td>
<td>2.922</td>
<td>0.012</td>
</tr>
<tr>
<td>7</td>
<td>Fatty acids in eggs:</td>
<td>SAFA</td>
<td>-2.51 ± 1.68</td>
<td>13</td>
<td>-1.497</td>
<td>0.158</td>
</tr>
<tr>
<td>8</td>
<td>Fatty acids in females:</td>
<td>MUFA</td>
<td>0.09 ± 0.02</td>
<td>17</td>
<td>3.989</td>
<td>0.001</td>
</tr>
<tr>
<td>9</td>
<td>Fatty acids in females:</td>
<td>MUFA</td>
<td>0.185 ± 0.09</td>
<td>17</td>
<td>2.031</td>
<td>0.058</td>
</tr>
<tr>
<td>10</td>
<td>Fatty acids in females:</td>
<td>SAFA</td>
<td>0.006 ± 0.01</td>
<td>17</td>
<td>0.644</td>
<td>0.528</td>
</tr>
<tr>
<td>11</td>
<td>ORAC</td>
<td>Incubation pH</td>
<td>-0.02 ± 0.04</td>
<td>15</td>
<td>0.057</td>
<td>0.580</td>
</tr>
</tbody>
</table>

*\( \log \)-transformed
This is also in line with findings of Niehoff et al. (2013), who found no effect of CO$_2$ on zooplankton community development or abundance of single taxa in a similar mesocosm study in Kongsfjorden, Svalbard.

The physicochemical conditions in the research area is naturally fluctuating, therefore the plankton community may be adapted to large variability in CO$_2$ concentration and pH. In addition, organisms such as copepods are exposed to daily variation in pH and there is evidence that species performing vertical migration may be more robust to changes in CO$_2$ (Lewis et al., 2013). *E. affinis* undertakes diel vertical migration and particularly ovigerous *E. affinis* females stay below 20 m depth and experience > 0.5 units change (7.51–8.1) in pH on a daily basis (Almén et al., 2014), in the area where the current study was conducted. Thus, this could partially explain why *E. affinis* reproduction did not respond to lowered pH. Cripps et al. (2014), on the other hand, found severely reduced nauplii survival for *Acartia tonsa* kept at a $p$CO$_2$ of 1000 µatm, while other life stages were less affected. There appears to be a large variation in CO$_2$ sensitivity between species, even for organisms from the same study area. During this KOSMOS study, Vehmaa et al. (2015) found a nega-
We found that nauplii production was positively affected by food availability (Chl $a$ concentration, Fig. 2a). Our results are in agreement with Zervoudaki et al. (2014) who neither found discernible effects of lowered pH, whereas both higher temperature and food concentration (Chl $a$) positively affected egg production in $A. clausi$ in a low nutrient Mediterranean system. According to fractionated Chl $a$ measurements during the mesocosm campaign (Paul et al., 2015) > 90% of the Chl $a$ consisted of nanophytoplankton (< 20 $\mu$m), which possibly constituted an important food source for the filter-feeding $E. affinis$ (Motwani and Gorokhova, 2013).

Although nauplii production of $E. affinis$ was negatively affected by diatoms, no effect of CO$_2$ on diatom abundance was found. The abundance of diatoms was high during the first days but then declined rapidly. Low hatching frequency has, however, previously been observed for $E. affinis$ during the diatom spring bloom in the same area (Ask et al., 2006). Some diatoms contain inhibitory compounds or lack essential nutrients that may be crucial for copepod reproduction (Lee et al., 1999). In the current study, diatoms consisted of Chaetoceros spp., Skeletonema marinoi and pennate diatoms. Vehmaa et al. (2012b) reported low egg production for $E. affinis$ on a $S. marinoi$ dominated diet in the study area. Skeletonema can produce potentially harmful aldehydes affecting copepod egg production (Ianora and Miralto, 2010). Significant negative correlation between Chaetoceros spp. and $E. affinis$ hatching frequency has also been reported (Ask et al., 2006). However, there could potentially be a non-causal relationship between low diatom abundance and high nauplii production. It is possible that the end of the diatom bloom and peak abundance coincided (Ask et al., 2006). Dinoflagellates are in some cases considered superior food source for copepods, as opposed to diatoms (Ianora et al., 2004; cf. Vehmaa et al., 2012b). In this study dinoflag-
ellates positively stimulated nauplii production. Dinoflagel-
lates probably contributed to nutritional quality as they are
high in essential fatty acids (Galloway and Winder, 2015).
We do not know to which extent the copepods fed on the dif-
ferent species; however, E. affinis is able to feed on both H.
triquetra and Dinophysis spp., although the latter has toxic
strains (Setälä et al., 2009).

We realize that some copepods and nauplii probably were
introduced with the unfiltered water to the incubation bot-
tles. We assume that it did not have a major effect on the
results as the copepod nauplii abundance did not vary be-
tween the mesocosms (Lischka et al., 2015), and only E. affi-
nis nauplii were counted. We observed a lot of epibionts (Vor-
ticella) attached to adult copepods during the third week in
the mesocosms. This was probably due to ageing (Jamieson
and Santer, 2003), or the lack of predators that would other-
wise have removed infested individuals which are more visi-
ble due to epibionts causing impaired escape ability (Souissi
et al., 2013). The age of the E. affinis adults incubated in our
experiments, was estimated to be 2–3 weeks to > 1 month.
The higher age structure of E. affinis occurring in the meso-
cosms, as well as the decreasing Chl a levels could partly ex-
plain the decreased nauplii production in the third and fourth
week of the experiment. Decreasing levels of PUFA in fe-
male towards the fourth week (Bermúdez et al., 2016), could
also have affected copepod nauplii production. In the current
study, the natural phytoplankton composition in the meso-
cosms did not change significantly due to CO2 (Bermúdez et
al., 2016; Annegret Stuhr, personal communication, 2015).
Rossoll et al. (2013) and Bermúdez et al. (2016) suggest
that a dampening of CO2 effects can be expected for coastal
communities adapted to strong natural fluctuations (cf. Wald-
busser and Salisbury, 2014), as also proposed here. Rossoll
et al. (2013) found no changes in phytoplankton community
composition and no direct effect of lowered pH or indirect
CO2 effect, via changed food quality on A. tonsa reproduc-
tion, exposed to similar treatment levels as in the present
study.

4.3 Antioxidative capacity and fatty acids

Our results suggest that the oxidative balance was maintained
in the copepods in all treatments regardless of pH, as we did
not observe any change in ORAC. As noted by Vehmaa et
al. (2013), ORAC is affected by lowered pH, rather in com-
brination with warmer temperatures, but not by moderately
lowered pH alone. An oxidative imbalance, favouring ROS
production can result in oxidative stress, as ROS can attack
biomolecules, such as lipids, proteins, and DNA (Monaghan
et al., 2009). Developmental stage (Fanjul-Moles and Gon-
sebatt, 2012), environmental condition (Luschak, 2011), as
well as feeding activity (Furuhaug et al., 2014) can affect
levels of oxidative stress, suggesting the importance of mea-
suring several biomarkers (Monaghan et al., 2009). We con-
clude that E. affinis did not face pronounced pH stress and
therefore seems fairly robust to future ocean acidification, at
least based on results in the present manuscript.

Analyses of fatty acid concentration in E. affinis females
from our incubations revealed that PUFA in females was
transferred to the eggs and stimulated nauplii production sig-
nificantly, whereas no significant effect of pH on FA con-
tent in females was revealed. Despite the fact that Rossoll et
al. (2012) found CO2-induced changes in fatty acid content
of phytoplankton in laboratory-based experiments, no CO2-
induced changes on phytoplankton or copepod fatty acid
composition were found during the current mesocosm study
(Bermúdez et al., 2016). The authors suggest that phospho-
rus limitation, being homogeneous in all mesocosms as nu-
trient addition was not practised, may have a stronger influ-
ence on community composition and their associated fatty
acid profile than CO2. Isari et al. (2015) found neither direct
effects on copepod vital rates, nor indirect effects, via phy-
toplankton fatty acid composition, in two copepods Acartia
granii and Oithona davisae. However, most PUFA showed
a positive correlation with pCO2 during part of a mesocosm
study in Svalbard, which the authors attribute to taxonomical
changes due to rising dinoflagellate abundances (Leu et al.,
2013). In the present study female MUFA were reflected in
their eggs, whereas SAFA were not, and none of them had
a significant effect on nauplii production. These fatty acids,
at least MUFA, are rather used for metabolism and storage
(McMeans et al., 2012).

5 Conclusions

From our results we conclude that E. affinis is not sensitive
to near-future levels of ocean acidification on a physiological
level for the variables measured in the study. Offspring pro-
duction was not affected after one generation. Food quality,
in terms of dinoflagellate biomass and higher PUFA stimu-
lated nauplii production, but we observed no difference in
fatty acid composition due to pH. We also did not observe an
effect of pH on ORAC. In the study area E. affinis is proba-
bly adapted to high pH variability due to diel vertical migra-
tion and may, therefore, not have faced pronounced pH stress
from the treatment levels used in this study. We found that the
effects of food quantity had an impact on nauplii production
of E. affinis. For the time we conducted the laboratory-based
experiments, we, however, did not observe an indirect CO2
effect via phytoplankton biomass. Chl a concentration corre-
lated positively with CO2, but only clearly discernible for
picophytoplankton from r25 onwards (Paul et al., 2015) and
we sampled no longer than r27. How the indirect effect of
CO2 (via the food) would affect the copepods on a longer
timescale remains unclear. Future studies should focus on
copepod adaptation in relation to coastal pH variability and
tolerance towards extreme events.
Author contributions. Anna-Karin Almén, Anu Vehmaa, Andreas Brutemark andonna Engström-Ost designed and conducted the laboratory experiment. Anna-Karin Almén counted the nauplii samples, Silke Lischka counted plankton from the mesocosms and Annegret Stuhr counted phytoplankton. Sara Furuhagen analysed ORAC, Allanah Paul analysed C:N samples, J. Rafael Bermúdez analysed fatty acids and Lennart Bach analysed Chl a. Anna-Karin Almén and Anu Vehmaa performed the statistical analyses and Anna-Karin Almén wrote the manuscript with contributions from all co-authors. Project coordinator: Ulf Riebesell.

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