

# 1 Ocean acidification challenges copepod phenotypic 2 plasticity

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## 22 **Abstract**

23 Ocean acidification is challenging phenotypic plasticity of individuals and populations.  
24 Calanoid copepods (zooplankton) are shown to be fairly plastic against altered pH conditions,  
25 and laboratory studies indicate that transgenerational effects are one mechanism behind this  
26 plasticity. We studied phenotypic plasticity of the copepod *Acartia* sp. in the course of a  
27 pelagic, large-volume mesocosm study that was conducted to investigate ecosystem and  
28 biogeochemical responses to ocean acidification. We measured copepod egg production rate,

1 egg hatching success, adult female size and adult female antioxidant capacity (ORAC) as a  
2 function of acidification ( $f\text{CO}_2 \sim 365\text{--}1231 \mu\text{atm}$ ), and as a function of quantity and quality of  
3 their diet. We used an egg transplant experiment to reveal if transgenerational effects can  
4 alleviate the possible negative effects of ocean acidification on offspring development. We  
5 found significant negative effects of ocean acidification on adult female size. In addition, we  
6 found signs of a possible threshold at high  $f\text{CO}_2$ , above which adaptive maternal effects  
7 cannot alleviate the negative effects of acidification on egg hatching and nauplii development.  
8 We did not find support for the hypothesis that insufficient food quantity (total particulate  
9 carbon  $< 55 \mu\text{m}$ ) or quality (C:N) weakens the transgenerational effects. However, females  
10 with high ORAC produced eggs with high hatching success. Overall, these results indicate  
11 that *Acartia* sp. could be affected by projected near future  $\text{CO}_2$  levels.

12 Keywords: *Acartia biflosa*, climate change, maternal effects, total particulate carbon, C:N,  
13 oxidative stress

14

## 15 **1 Introduction**

16 Increased concentrations of carbon dioxide ( $\text{CO}_2$ ) in the atmosphere is changing the carbon  
17 chemistry of the world's oceans.  $\text{CO}_2$  dissolves in seawater thereby decreasing ocean pH.  
18 Ocean acidification is increasing fast and pH is expected to decrease by a further 0.14–0.43  
19 pH units during the coming century (IPCC, 2007). Acidification can cause various problems  
20 to biochemical and physiological processes in aquatic organisms. In addition to affecting  
21 calcification of calcareous organisms, maintenance of acid-base equilibrium of body fluids  
22 may become more difficult and have consequences for example on protein synthesis,  
23 metabolism and volume control (Whiteley, 2011).

24 In a changing environment, populations can respond in three main ways: through plastic  
25 responses of individuals, through genetic changes across generations, or through escaping in  
26 space or time by modification of phenology. During rapid change, phenotypic plasticity, i.e.,  
27 the ability of an individual or a population to alter its physiological state, appearance or  
28 behaviour in response to the environment, is of major importance (West-Eberhard, 2003).  
29 Theory predicts that higher plasticity evolves in extreme environments and that spatial  
30 heterogeneity and dispersal select for higher plasticity (Chevin et al., 2013). One could  
31 therefore hypothesise that organisms inhabiting a variable environment, such as the study

1 area, could be fairly plastic in their response to ocean acidification because they have to cope  
2 with both seasonal and sudden changes in pH (Almén et al., 2014; Lewis et al., 2013).

3 Proteomic studies suggest that oxidative stress is a common co-stress of temperature and  
4 acidification (Tomanek, 2014). Increased production of reactive oxygen species (ROS) may  
5 result in increased antioxidant and/or repair costs, and furthermore in reduced investment in  
6 reproduction or other functions, such as immune defence. In addition, increased production of  
7 ROS may lead to accumulation of oxidative damage and furthermore to acceleration of  
8 senescence (Monaghan et al., 2009). There can also be a connection between maternal  
9 oxidative balance and offspring quality. In birds, for example, females allocate diverse  
10 antioxidants to the eggs that protect the embryo from oxidative stress. This maternal effect  
11 has a positive effect on offspring development and growth (Rubolini et al., 2006).

12 Copepods (zooplankton) are indispensable to the functioning of the whole pelagic ecosystem  
13 and contribute significantly to many ecosystem services (Bron et al., 2011). They provide, for  
14 example, food for early-life stages as well as some adult fishes of many economically  
15 important fish species (Steele, 1974; Cushing, 1990).

16 Previous results suggest that calanoid copepods have high buffering capacity against  
17 projected ocean acidification for the year 2100 and beyond (Kurihara and Ishimatsu, 2008;  
18 Weydmann et al., 2012; McConville et al., 2013; Vehmaa et al., 2013), meaning that they are  
19 able to survive, grow, develop and reproduce in lower pH (Reusch, 2014). However, there are  
20 also studies showing negative impacts on moderate CO<sub>2</sub> levels (Fitzer et al. 2012), whereas  
21 most of the negative impacts have been discovered for extreme, carbon storage scenarios  
22 (Kurihara et al., 2004; Mayor et al., 2007; Weydmann et al., 2012). Many studies have tested  
23 only one life-stage, adult females, and have therefore possibly underestimated the effects of  
24 ocean acidification on copepods (Cripps et al., 2014a). There are indications that  
25 transgenerational effects are one mechanism responsible for the high plasticity of copepod  
26 reproduction against altered pH conditions (Vehmaa et al., 2012). This maternal effect is most  
27 likely dependent on the condition of the mother and the availability of food and quality of her  
28 diet (Vehmaa et al., 2012; Pedersen et al., 2014a). Paternal effects can also influence  
29 offspring traits. Exposure of both parents to CO<sub>2</sub> leads to fewer adverse effects on egg  
30 production and hatching than exposure of only gravid copepod females (Cripps et al., 2014b).  
31 Thor and Dupont (2015) also highlight the importance of testing transgenerational effects.  
32 They found significantly lower copepod egg production after two generations when exposed

1 to 900 and 1500  $\mu\text{atm}$  compared to 400  $\mu\text{atm}$ , but transgenerational effects alleviated the  
2 negative  $\text{CO}_2$  response in 1500  $\mu\text{atm}$  (Thor and Dupont, 2015).

3 We tested direct and indirect effects of ocean acidification (i.e., via food quantity and quality)  
4 on the copepod *Acartia* sp. egg production (EPR), egg hatching success (EH), female body  
5 size (measured as prosome length (PL)), as well as antioxidant capacity (ORAC). The study  
6 was conducted in association with the KOSMOS (Kiel Off-Shore Mesocosms for Ocean  
7 Simulations) project in the Baltic Sea (Paul et al., 2015). The study was intended to cover the  
8 low productivity late spring and early summer period, i.e., the post-spring bloom period when  
9  $p\text{CO}_2$  concentrations are at the annual minimum. Over the annual cycle,  $p\text{CO}_2$  and pH vary  
10 substantially at the study site as a result of biological activity and mixing/upwelling of  $\text{CO}_2$ -  
11 enriched deep water (Niemi, 1975; Omstedt et al., 2014). There are also strong spatial  
12 gradients in seawater  $p\text{CO}_2$ /pH, most prominently between the surface layer and the  $\text{CO}_2$ -rich  
13 deeper waters (Almén et al., 2014). Thus, the copepods in the study area are likely to  
14 experience strong changes in seawater carbonate chemistry, both seasonally and during their  
15 diurnal migration. Total particulate carbon (TPC  $<55 \mu\text{m}$ ) was used as the measure of food  
16 quantity. Food quality was indicated by carbon to nitrogen ratio of the same size fraction of  
17 seston (C:N  $<55 \mu\text{m}$ ) (Elser and Hasset, 1994; Sterner and Hessen, 1994). In addition, in  
18 order to separate transgenerational plasticity (i.e., maternal and paternal effects) and the effect  
19 of environment on copepod egg hatching and development, we performed an egg-transplant  
20 experiment. Half of the produced eggs were allowed to develop in respective mesocosm water  
21 and the other half in water collected outside the mesocosm bags.

22 Due to the high buffering capacity of *Acartia* sp., we hypothesised that there are no  $f\text{CO}_2$   
23 related differences in egg production rate, egg hatching success and prosome length between  
24 the mesocosms. In addition, we hypothesised that copepod eggs hatch and develop better in  
25 the same environment in which they are produced, because transgenerational effects can  
26 alleviate the negative effects of environmental change. Our third hypothesis stated that low  
27 food quantity (TPC) and poor quality (high C:N) will weaken the maternal effect by  
28 deteriorating the condition of the mother. Finally, we tested if mothers with higher antioxidant  
29 capacity (ORAC) produce better quality offspring (EH) by calculating correlation coefficients  
30 between the two variables.

## 1 **2 Materials and Methods**

2 The study was performed in summer 2012 in the vicinity of Tvärminne Zoological Station on  
3 the south-western coast of Finland. Six large mesocosms were moored on site in the  
4 beginning of June. To enclose the natural plankton community, the mesocosms were left open  
5 with only 3 mm mesh size net covering the top and the bottom during filling. After four days,  
6 the net was removed and the top was pulled up 1.5 m above the water surface and closed at  
7 the bottom (Riebesell et al., 2013; Paul et al., 2015). pH was ~8 and  $f\text{CO}_2$  concentrations in  
8 the mesocosms prior to adjustment were  $237 \pm 9$   $\mu\text{atm}$  (average  $\pm$  std of daily measurements  
9 from all bags). Four mesocosms were manipulated with  $\text{CO}_2$  enriched seawater, during three  
10 consecutive days to reach  $f\text{CO}_2$  concentrations of 600-1650  $\mu\text{atm}$  (Paul et al., 2015). Two  
11 untreated mesocosms were used as controls. The water column was mixed in the beginning of  
12 the experiment to avoid salinity stratification. Due to outgassing,  $\text{CO}_2$  was also added on day  
13 15 to the upper 7 m of the high  $\text{CO}_2$  mesocosms to maintain the treatment levels. No nutrients  
14 were added.

### 15 **2.1 Sampling**

16 Sampling took place once a week during the first four weeks of the experiment, and once  
17 more at the end of the whole experiment (days 3, 10, 17, 24 and 45). Mesozooplankton were  
18 sampled from all mesocosms by taking two hauls with a 300  $\mu\text{m}$  net (17 cm diameter) from  
19 17 m depth. The samples were rinsed into containers with 4 l of seawater from respective  
20 mesocosm, taken from 9 m depth with a water sampler (Limnos, Hydrobios). On the same  
21 day, integrated water samples (0-17 m) were collected from all mesocosms and the Baltic Sea  
22 directly into 1.2 l Duran bottles that were closed without head space. Water samples were  
23 kept in cool bags and zooplankton samples were protected from light until transported to a  
24 temperature and light controlled room at Tvärminne Zoological Station within 4 h. The light:  
25 dark cycle in the room was 16:8 h and light intensity was 7  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (LI-COR LI-  
26 1000). Temperature followed the *in situ* temperature [ $9^\circ\text{C}$  (day 3),  $11^\circ\text{C}$  (day 10),  $15^\circ\text{C}$  (day  
27 17),  $16^\circ\text{C}$  (days 24 and 45)].

## 2.2 Measurements of egg production, egg hatching success and prosome length

Twenty adult *Acartia* sp. (17 females and 3 males) were picked with pipettes from each sample using stereo microscopes, and gently placed in pre-filled glass bottles with respective mesocosm water. The bottles were closed without head-space, to minimise CO<sub>2</sub>-outgassing during the incubation. pH in the bottles was measured before closing and right after opening them at the end of the incubation using an Ecosense pH10 pH/temperature pen (Table S1). The pen was calibrated with standard buffer solutions (Certipur, Titripac pH 4.00, 7.00, and 10.00) every second day. The bottles were incubated in temperature and light controlled room in conditions described above (Materials and Methods 2.1), and mixed three times a day and their place on the shelf was changed randomly. After the incubation ( $24.3 \pm 2.3$  h, average  $\pm$  std), the copepods and produced eggs were filtered using 250  $\mu$ m and 30  $\mu$ m sieves, respectively. The copepods were counted and their viability checked before preserving them in RNAlater (Sigma). RNAlater can affect size (Foley et al., 2010), and the effect depends on the number of segments in the animal, i.e., the more segments the larger effect. Shrinkage is ~15% for copepods (Prof. Elena Gorokhova, Stockholm University, pers. comm.). Prosome length of the preserved female copepods was measured using a stereo microscope (Leica MZ12) and ocular micrometer (total magnification 100  $\times$ ). As all the measured copepods were adult females, we assume the shrinkage to be in proportion similar for all individuals, which means that our results are quite conservative and comparable between mesocosms.

In the egg transplant experiment, the collected eggs were divided for hatching into two 50 ml petri dishes with different conditions; one dish was filled with respective mesocosm water and the other filled with Baltic water. pH of the water was measured as above before the incubations and right after the petri dishes were opened after the incubation (Table S1). The eggs were counted before the petri dishes were completely filled and sealed without head-space using Parafilm. Egg hatching was followed by counting the number of remaining eggs on the dish through the lid using a stereomicroscope twice a day. When the number of eggs had remained the same on two consecutive counting times, the dishes were opened and the water containing the remaining eggs and hatched nauplii was preserved with acid Lugol's solution. The hatching incubation time varied between 63.9 and 137.6 h, depending on incubation temperature. *Acartia* sp. nauplii stages were determined and the number of nauplii and remaining copepod eggs counted using a stereo microscope.

1 Some adults, copepodites, nauplii or eggs could have ended up in the incubation bottles or  
2 petri dishes with the unfiltered incubation water. The possible extra adults and their  
3 contribution to the egg production rate (EPR, eggs copepod<sup>-1</sup> d<sup>-1</sup>) were taken into account as  
4 EPR was calculated using the number of eggs and adult *Acartia* sp. females found in the  
5 incubation bottles after the 24 h incubation. When estimating the egg hatching success (EH,  
6 %), the total number of hatched *Acartia* sp. nauplii and remaining eggs at the end of the  
7 hatching incubation were compared with the number of eggs counted before the hatching  
8 incubation. If the total number exceeded the egg number prior to hatching, the most  
9 developed nauplii (>N4) were considered to be carry-over individuals, and were therefore not  
10 considered in the estimation of EH. For estimation of nauplii development rate, the  
11 development index (DI) was calculated (Knuckey et al., 2005) accordingly,

$$12 \quad DI = \frac{\sum_{i=0}^3 (N_i \times n_i)}{\sum_{i=0}^3 n_i} \quad (1)$$

13 where  $N_i$  is the assigned stage value (0 for eggs, 1 for N1, 2 for N2 and 3 for N3 and N4) and  
14  $n_i$  the number of individuals at that stage. We assume all the *Acartia* sp. adults and nauplii to  
15 be species *A. bifilosa*. However, because another *Acartia* species, *A. tonsa* occurs in the area  
16 in late summer too (Katajisto et al., 1998), we cannot be totally sure that we only had one  
17 species in the experiments.

### 18 **2.3 Antioxidant capacity**

19 For antioxidant capacity (ORAC) samples ~25 live female *Acartia* sp. were picked from  
20 every zooplankton sample onto a piece of plankton net in the temperature and light controlled  
21 room on days 3, 10, 17 and 31. The net containing the copepods was folded and stored in  
22 Eppendorf tubes at -80°C. The samples were homogenised in 150 µl Tris-EDTA buffer  
23 containing 1% sarcosyl. The antioxidative capacity was assayed as ORAC (Ou et al., 2001).  
24 As a source of peroxy radicals, 2, 2-azobis (2-amidinopropane) dihydrochloride (AAPH)  
25 (152.66 mM) was used and fluorescein was used as a fluorescent probe (106 nM). We used  
26 trolox (218 µM, Sigma-Aldrich) as a standard and the assay was performed on a 96-well  
27 microplate and to each well, 20 µL sample, 30 µL AAPH and 150 µL fluorescein were added.  
28 ORAC values were normalized to protein and expressed as mg Trolox eq. mg protein<sup>-1</sup>.  
29 Protein concentration was measured with NanoOrange<sup>®</sup> (Life Technologies).

## 1 **2.4 C:N and TPC**

2 Samples for TPC and C:N were collected onto GF/F filters (Whatman, nominal pore size 0.7  
3  $\mu\text{m}$ ) using gentle vacuum filtration ( $<200$  mbar) and then stored in glass petri dishes at  $-20^\circ\text{C}$ .  
4 GF/F filters and petri dishes were combusted at  $450^\circ\text{C}$  for 6 hours before use. Gauze pre-  
5 filters were used to separate the size fraction  $< 55 \mu\text{m}$ . Filters were not acidified to remove  
6 inorganic carbon, therefore total particulate carbon was used. C and N concentrations were  
7 determined on an elemental analyser (EuroEA) following Sharp (1974), coupled by a Conflo  
8 II to a Finnigan Delta<sup>Plus</sup> mass spectrometer and were used to calculate C:N ratios in mol:mol.  
9 For further details on sampling and analyses, please refer to Paul et al. (2015).

## 10 **2.5 Statistics**

11 The effect of acidification and food quantity and quality on *Acartia* sp. egg production (EPR),  
12 prosome length (PL), antioxidant capacity (ORAC) and nauplii development index (DI) was  
13 tested using linear mixed effect models (LMM) with restricted likelihood (REML)  
14 approximation from the nlme-package (Pinheiro et al., 2014), where EPR, PL or ORAC were  
15 used as response variables,  $f\text{CO}_2$ , TPC ( $<55 \mu\text{m}$ ) and C:N as fixed explanatory variables and  
16 repeated measure of the mesocosms over time as a random factor (Table 1). Due to the  
17 binomial nature of the data, the effect of  $f\text{CO}_2$ , TPC ( $<55 \mu\text{m}$ ) and C:N on egg hatching  
18 success (EH) was tested with generalized linear mixed model (GLMM) with Laplace  
19 likelihood approximation, binomial error structure and logit-link function from the lme4-  
20 package (Bates et al., 2014) (Table 1). The average of  $f\text{CO}_2$ , TPC ( $<55 \mu\text{m}$ ) and C:N  
21 measurements from each mesocosm within three days before the zooplankton sampling were  
22 used as explanatory variables for EPR, ORAC and EH, because 2–3 days are considered to be  
23 an appropriate acclimatisation period for *A. bifilosa* (Yoon et al., 1998; Koski and Kuosa,  
24 1999). For PL, the average of all  $f\text{CO}_2$ , TPC ( $<55 \mu\text{m}$ ) and C:N measurements from the start  
25 of the mesocosm experiment were used since PL reflects the environmental conditions of the  
26 whole lifespan of the animal. In addition, Day 3 was excluded in the LMM testing the PL  
27 (Table 1), since three days is too short period for detecting differences in copepod size.  
28 Egg–adult generation time for *A. bifilosa* at  $17^\circ\text{C}$  is approximately 16 days of which  $\sim 7.5$  d  
29 taken by nauplii stages and  $\sim 8.5$  d by copepodite stages (Yoon et al., 1998). Collinearity  
30 between all explanatory variables was checked. Temperature was not considered in the  
31 models, because it changed similarly in all the bags (Paul et al., 2015). The model  
32 simplifications were done manually in backward stepwise manner by removing the non-



1 significant effects and by using Akaike's information criterion (AIC). We report t- or z-  
2 statistics (EH) of the retained fixed effects. To separate the effect of hatching environment  
3 from maternal environment, EH and DI were divided by the corresponding values measured  
4 in the Baltic Sea water. The ratio of Mesocosm EH (or DI) / Baltic EH (or DI) >1 indicates  
5 that eggs hatch or develop better in the maternal conditions (Mesocosm water), whereas the  
6 ratio <1 indicates that eggs hatch or develop better in the Baltic Sea water. The effect of  
7 maternal environment ( $f\text{CO}_2$ , TPC (<55  $\mu\text{m}$ ) and C:N) on the ratio was tested with LMM,  
8 where the ratio of Mesocosm EH / Baltic EH and Mesocosm DI / Baltic DI were used as  
9 response variables;  $f\text{CO}_2$ , TPC (<55  $\mu\text{m}$ ) and C:N as fixed explanatory variables; and repeated  
10 measure of the mesocosms over time as a random factor. The model simplifications were  
11 made as above.

12 To test if maternal antioxidant capacity (ORAC) correlates with egg hatching success,  
13 Spearman rank correlation tests were used. Data from Days 3, 10 and 17 were included in the  
14 test (n = 17, EH result for MC 6 in Day 3 is missing) because those are the days when both  
15 ORAC and EH were measured.

16 All the statistical analyses were performed using software R 3.0.2 (R Core Team, 2013), and  
17 the significance level was 0.05.

## 18 **3 Results**

### 19 **3.1 Egg production, prosome length, antioxidant capacity and egg hatching** 20 **success**

21 *Acartia* sp. egg production (EPR) increased in all mesocosms between Day 3 and Day 10, but  
22 decreased after that, reaching very low rates (1-2 eggs copepod<sup>-1</sup> d<sup>-1</sup>) on Days 24 and 45 (Fig.  
23 1a). Neither food quantity (TPC, <55  $\mu\text{m}$ ), food quality (C:N, <55  $\mu\text{m}$ ), nor ocean  
24 acidification ( $f\text{CO}_2$ ) had a statistically significant effect on copepod egg production (Table 2),  
25 even though there seemed to be variations in those parameters between the mesocosms (Table  
26 3).

27 Prosome length (PL) of *Acartia* sp. females increased during the first week of the study;  
28 however there seemed to be some differences between the mesocosms already on Day 3,  
29 which was not included in the analysis (Fig. 1b). From Day 10 onwards, the smallest *A.*  
30 *bifilosa* adults were found in the mesocosm with the highest  $f\text{CO}_2$  concentration (Fig. 1b).

1  $f\text{CO}_2$ , but also TPC (<55  $\mu\text{m}$ ) had a statistically significant negative impact on copepod body  
2 size (Table 2).

3 Antioxidant capacity (ORAC) of the female copepods increased from Day 3 to Day 10 in all  
4 mesocosms (Fig. 1c). Interestingly, on Day 3 ORAC was highest in the three mesocosms with  
5 highest  $f\text{CO}_2$  treatment, whereas on Day 31 the situation was opposite and ORAC was lowest  
6 in the three mesocosms with highest  $f\text{CO}_2$  (Fig. 1c). Despite this, only TPC (<55  $\mu\text{m}$ ) had a  
7 statistically significant effect on ORAC; ORAC decreases with increasing TPC (Table 2).

8

9 The overall egg hatching success (EH) was high throughout the study; over 80 % of the  
10 *Acartia* sp. eggs hatched. As seen for EPR, PL, and ORAC, EH also increased from Day 3 to  
11 Day 10 in all mesocosms (Fig. 1d). Variance in the EH between the four samplings was  
12 highest in the mesocosms with highest  $f\text{CO}_2$ , whereas EH varied the least and remained >90  
13 % in both control mesocosms (MC1, MC5). In spite of this, only TPC (<55  $\mu\text{m}$ ) had a  
14 statistically significant negative effect on EH (Table 4). Eggs that were produced in MCs 3, 5,  
15 6 and 7 had fairly similar hatching success in Baltic water, whereas hatching success of eggs  
16 that were produced in MCs 1 (control) and 8 (the highest  $f\text{CO}_2$ ) was alternately either lower or  
17 higher than in the other MCs (Fig. 1e).

### 18 **3.2 Egg hatching and nauplii development in mesocosm vs. Baltic Sea** 19 **conditions**

20 Neither the maternal food quantity (TPC) nor the quality (C:N) had a statistically significant  
21 effect on offspring quality (EH and DI) in the egg transplant experiment (Table 5). The  $f\text{CO}_2$   
22 was the only detected variable in the maternal environment that influenced the ratio of EH  
23 and DI between mesocosm and Baltic conditions.

24 Egg hatching success for eggs hatching in the mesocosm water differed from eggs hatching in  
25 the Baltic water. On Days 3 and 10, hatching success was higher in the mesocosm water for  
26 the control (MC1, MC5) and for low  $f\text{CO}_2$ -treatment bags (MC7, MC6), whereas eggs  
27 produced in high  $f\text{CO}_2$ -treatment bags (MC3, MC8) showed higher hatching in the Baltic  
28 water (Fig. 2a). Thus, there may be a threshold  $f\text{CO}_2$  for hatching success at high  $f\text{CO}_2$ .  
29 However, on Days 17 and 24 the  $f\text{CO}_2$  treatment did not have a clear effect on hatching  
30 success. Nevertheless,  $f\text{CO}_2$  had a statistically significant negative effect on the ratio of EH  
31 mesocosm / Baltic, meaning that egg hatching was higher in the Baltic water than in the

1 maternal environment when the maternal environment had a high  $f\text{CO}_2$  (Table 5). When  
2 maternal environment had low  $f\text{CO}_2$  the situation was vice versa. The level of  $f\text{CO}_2$  also had a  
3 significant negative effect on the DI mesocosm / Baltic ratio (Fig. 2b; Table 5).

### 4 **3.3 Correlations between antioxidant capacity and offspring quality**

5 Copepod antioxidant capacity (ORAC) was correlated significantly with copepod egg  
6 hatching success. The relationship between the two variables is positive and stronger for eggs  
7 developing in the mesocosm water ( $\rho = 0.75$ ,  $p < 0.001$ ) than for eggs developing in the  
8 Baltic water ( $\rho = 0.62$ ,  $p = 0.007$ ) (Fig. 3).

## 9 **4 Discussion**

10 In this study, conducted in semi-natural mesocosm environments, reproduction of the *Acartia*  
11 sp. copepod showed high phenotypic buffering against acidification, i.e., the species was able  
12 to maintain similar egg production rate and high egg hatching success in all  $f\text{CO}_2$  conditions.  
13 Nevertheless, we found a significant negative effect of ocean acidification on adult female  
14 size. Even more interestingly, we found signs of a possible threshold at high  $f\text{CO}_2$  for  
15 offspring development, above which adaptive maternal effects cannot alleviate the negative  
16 effects of acidification on egg hatching and nauplii development (Fig. 2). However, we did  
17 not find support for the third hypothesis that poor food quantity (lower TPC) and quality  
18 (higher C:N) would weaken the maternal effect by deteriorating the condition of the mother.  
19 Conversely, higher food quantity (TPC  $< 55 \mu\text{m}$ ) correlated negatively with egg hatching  
20 success, adult female size and antioxidant capacity, whereas C:N ratio did not correlate with  
21 any of the measured variables significantly. Copepods were possibly food limited in all the  
22 mesocosms, especially after Day 17 due to a sharp decline in Chl *a* concentrations and in  
23 phytoplankton community size structure (Paul et al., 2015). Dominance of picophytoplankton  
24 that is too small to be consumed by copepods could be the reason for the observed negative  
25 effects of food quantity, and that may also have masked the food quality effect. Also, after  
26 Day 17 egg production rate was so low that it was practically impossible to find differences in  
27 egg production between the mesocosms. Finally, we found a positive correlation between  
28 maternal antioxidant capacity and egg hatching success, suggesting that the female  
29 antioxidant defence might also protect the embryo from oxidative stress.

1 The fact that *Acartia* sp. egg production and egg hatching were unaffected by high  $f\text{CO}_2$  but  
2 the egg transplant experiment revealed that development was slower for nauplii at high  $\text{CO}_2$   
3 supports the importance of looking beyond egg production and egg hatching, which is also  
4 pointed out by Pedersen et al. (2014b). They concluded that the first endogenously feeding  
5 nauplii stages of *Calanus finmarchicus* are more sensitive to  $\text{CO}_2$ -induced acidification than  
6 eggs or later nauplii stages (Pedersen et al. 2014b). Longer developmental times in high  
7  $\text{CO}_2$ /low pH have been observed in crustaceans, echinoderms and molluscs (Cripps et al.,  
8 2014a and references therein). Weydmann et al. (2012) also reported a significant  
9 developmental delay for *Calanus glacialis* eggs when exposed to highly acidified conditions.  
10 Pedersen et al. (2014a) observed that development of C4 copepodites of *C. finmarchicus* was  
11 delayed by 8.9 days in high  $\text{CO}_2$  treatments in comparison to the control condition, when also  
12 the previous generation had been exposed to the same conditions.

13 We expected maternal effects to be most obvious in a high stress situation (high  $f\text{CO}_2$   
14 treatments), as seen for three-spined sticklebacks in a study testing the effects of global  
15 warming (Shama et al., 2014). Instead, egg hatching was higher and nauplii development  
16 faster in the maternal environment than in the Baltic water, when the maternal environment  
17 had a low  $f\text{CO}_2$  (low stress). In high  $f\text{CO}_2$  maternal environment the opposite response was  
18 observed, thus indicating that maternal effects are in fact weak and cannot compensate for the  
19 higher  $f\text{CO}_2$  levels that correspond to near-future levels or that the eggs are damaged by the  
20 high  $f\text{CO}_2$ . This suggests that *Acartia* sp. and its reproduction are after all somewhat sensitive  
21 to ocean acidification. However, the effects were not as clear over the following weeks as in  
22 the beginning of the study, which may be due to an overall low egg number and large  
23 variation in hatching after Day 17, or due to acclimation of the copepods to the treatment  
24 conditions. In addition, the maternal effects seemed to weaken over time. This could be due to  
25 weakening condition of the mothers. In the absence of fish predators, zooplankton density,  
26 especially *Bosmina* sp. (cladocerans), increased strongly in the mesocosms (Lischka et al.,  
27 2015). Senescence and food limitation were thus plausible problems for copepods, and a  
28 likely cause of weakening maternal provisioning. In addition, conditions in the Baltic Sea  
29 changed after Day 17 due to an upwelling event, which caused an increase in  $f\text{CO}_2$  and  
30 decrease in pH (Paul et al., 2015). This might have made the Baltic conditions less favourable  
31 for copepod egg development and evened out the differences between high  $f\text{CO}_2$  mesocosms  
32 and the Baltic conditions.

1 A few studies have highlighted the importance of testing for transgenerational effects to avoid  
2 over- or underestimation of the effects of ocean acidification on copepods. Thor and Dupont  
3 (2015) found decreasing egg hatching of *Pseudocalanus acuspes* with increasing  $p\text{CO}_2$ . In  
4 addition, transgenerational effects alleviated the negative effects on egg production and  
5 hatching of the second generation when the mothers had been acclimatised to the same  
6 treatment. Also, a reciprocal transplant experiment showed that the effect was reversible and  
7 an expression of phenotypic plasticity (Thor and Dupont, 2015). Contrary to the current study,  
8 Pedersen et al. (2014a) found no effect of the  $\text{CO}_2$  environment on egg hatching or  
9 development of pre-feeding nauplii stages N1 and N2 in their multigenerational study using  
10 *C. finmarchicus*. However, the development time of larger nauplii and copepodite stages was  
11 increased by  $p\text{CO}_2$ , although the development delay was not detected in the following  
12 generation (Pedersen et al., 2014a). Vehmaa et al. (2012) studied combined effects of ocean  
13 acidification and warming, and found indications that negative effects on *Acartia* sp.  
14 reproductive success can partly be combated with maternal effects. The used pH treatments (-  
15 0.4 from ambient) were at the same level as the low  $f\text{CO}_2$ -treatments in this study (MC6,  
16 MC7), which makes the results of the two studies consistent.

17 The measurements of female copepod antioxidant capacity were done in order to provide  
18 possible additional information of the maternal provisioning on the offspring. A preferable  
19 practice in oxidative stress studies is to measure several of the four components consisting of  
20 free radical production, antioxidant defences, oxidative damage, and repair mechanisms  
21 (Monaghan et al., 2009). In the current study we only have the estimate for the defences,  
22 antioxidant capacity (ORAC) measurements, which makes our conclusions slightly more  
23 uncertain. However, an earlier study with the same species has indicated that at intermediate  
24 stress levels an upregulation of the antioxidant system enhances protection against oxidative  
25 damage, but at higher stress, the pro-oxidants may exceed the capacity of the antioxidant  
26 system and lead to oxidative damage (Vehmaa et al., 2013). In this study, upregulated  
27 antioxidant defence seemed to have a positive effect on offspring quality, as indicated by the  
28 positive correlation between female ORAC and egg hatching success. Higher ORAC in the  
29 two highest  $f\text{CO}_2$  mesocosms in the beginning of the study could be a sign of an upregulated  
30 antioxidant system in a sudden stressful situation, whereas the lowest ORAC in the high  $f\text{CO}_2$   
31 treatments at day 31 (Fig. 1c) could be caused by prolonged stress and exhausted antioxidant  
32 defence. The change from positive to negative effect in the course of the study could explain

1 why  $f\text{CO}_2$  did not show a significant correlation with ORAC, whereas food quantity (TPC  
2  $<55 \mu\text{m}$ ) did.

3 Ismar et al. (2008) showed that *Acartia* spp. development can be either slow or altered by  
4 certain algal groups causing death before the first copepodite or reproductive stage. A non-  
5 optimal diet could explain why higher food quantity would cause smaller adult female size,  
6 lower egg hatching success or lower antioxidant capacity. *Skeletonema*-diatoms had fairly  
7 high abundance in the mesocosms during the first days of the experiment when egg hatching  
8 success was lowest in every mesocosm, but then declined rapidly. Diatom-dominated  
9 phytoplankton composition has been shown to cause low copepod egg hatching success in the  
10 field (Miralto et al., 1999). Another quality aspect is the size and shape of the food, which  
11 may make it difficult to ingest or assimilate. From day 16 onwards, over 50% of chlorophyll *a*  
12 was in picophytoplankton ( $<2 \mu\text{m}$ ) (Paul et al., 2015), which is too small for *Acartia*  
13 consumption (Rollwagen Bollens and Penry, 2003). Since we did not study what the  
14 copepods preyed upon we can only speculate on diet quantity and quality. Satiated food  
15 conditions can strengthen the maternal or transgenerational effects. The transgenerational  
16 effects were of minor importance for hatching success in *C. finmarchicus* when exposed to  
17 long term high  $\text{CO}_2$  and food limited conditions (Pedersen et al., 2014a). Long term stress and  
18 food limitation could thus also be the reason for weakening maternal effects in the current  
19 study.

20 We found body size (prosome length) to be negatively affected by high  $\text{CO}_2$ . The result seems  
21 to be mostly driven by the mesocosm with the highest  $f\text{CO}_2$  (MC 8), where the adult *Acartia*  
22 sp. copepods were smallest on all the four sampling times that were included in the analysis  
23 (Days 10, 17, 24 and 45) (Fig. 1b). It takes  $\sim 8.5$  days for a sixth stage nauplius of *A. bifilosa*  
24 to develop through the five copepodite stages and reach adulthood at  $17^\circ\text{C}$  (Yoon et al.,  
25 1998). According to the Bělehrádek's temperature function it takes 12–15 days for VI nauplii  
26 to reach adulthood at  $9\text{--}11^\circ\text{C}$  (Bělehrádek, 1935; McLaren, 1966). The constants used in the  
27 equation ( $\alpha=1008$ ,  $a=-8.701$ ) were the same as used in Dzierzbicka-Glowacka et al. (2009) for  
28 the Baltic Sea *Acartia* spp. It is thus possible that the copepods could have developed through  
29 several stages causing the differences in prosome length between the treatments on Day 10.  
30 Lowered pH may have increased copepods' energy requirements and if energy is reallocated  
31 towards maintaining homeostasis, their somatic growth can be reduced. Pedersen et al.  
32 (2014a) found *C. finmarchicus* body size to be inversely related to  $p\text{CO}_2$ . They also found

1 higher respiration rate under more acidified conditions, and claimed that increased energy  
2 expenditure via rising respiration and consecutive decreasing growth and reproduction could  
3 lower the energy transfer to higher trophic levels and thus hamper the productivity of the  
4 whole ecosystem (Pedersen et al., 2014a). This is especially alarming when considering the  
5 projected climate warming, since copepod size is negatively correlated with temperature  
6 (Foster et al., 2011). In addition to temperature, food quantity and quality can affect the  
7 copepod body size (Hart and Bychek, 2011), and create surprising combined effects with  
8 acidification. Garzke et al. (2016) reported an indirect positive effect of  $p\text{CO}_2$  on copepod  
9 body size, which was explained by higher food availability when acidification acted as a  
10 fertilizer for phytoplankton. Temperature and food also interact because temperature affects  
11 the respiration and metabolism, thus the satisfying diet depends on temperature (Boersma et  
12 al., 2016). If high  $\text{CO}_2$  treatment (MC 8) caused a developmental delay in maturation, as  
13 could be interpreted from the prosome length results (Fig. 1b), the maturation would have  
14 occurred at a different temperature than in other mesocosms and possibly in non-optimal food  
15 conditions. Anyway, higher food quantity and quality would be expected to increase copepod  
16 size, contrary to our results. It is therefore possible that the used food quantity (TPC <55  $\mu\text{m}$ )  
17 and quality estimates (C:N <55  $\mu\text{m}$ ) do not fully describe the diet that *Acartia* sp. was  
18 consuming in the mesocosms.

19 Adult copepods have in general shown robustness against acidification (Mayor et al., 2012,  
20 McConville et al., 2013), whereas eggs and nauplii appear to be more sensitive (Cripps et al.,  
21 2014b; Fitzer et al., 2012). In addition, there seems to be notable differences in sensitivity  
22 between species. Nauplii production, adult female fatty acid content and antioxidant capacity  
23 (ORAC) of *Eurytemora affinis* were not affected by  $f\text{CO}_2$  in the current mesocosm campaign  
24 (Almén et al., 2016). Similarly, Lewis et al. (2013) found differences in ocean acidification  
25 sensitivity between the species *Oithona similis* and *Calanus* spp. (*C. glacialis* and *C.*  
26 *hyperboreus*). They argued that *O. similis* is more sensitive to future ocean acidification than  
27 *Calanus* spp., because *O. similis* remains in the surface waters whereas *Calanus* spp. migrates  
28 vertically, and encounters wider  $p\text{CO}_2$  ranges daily than *O. similis* (Lewis et al., 2013). The  
29 same applies to *Acartia* sp. and *E. affinis* in our study area. Although *Acartia* spp. is exposed  
30 to natural variability in pH environment due to daily variations as well as due to staying at  
31 greater depths during the day (low pH in deep water), it does not reside as deep as *E. affinis*  
32 (Almén et al., 2014) and may therefore show higher sensitivity than *E. affinis* during the  
33 current mesocosm campaign (Almén et al., 2016).

1 The results obtained for *Acartia* sp. reproduction in the current study seem to contradict the  
2 results obtained for the *Acartia* sp. abundance determined in the mesocosms. Although our  
3 results indicate that *Acartia* sp. reproduction is in fact sensitive to ocean acidification, no  
4  $f\text{CO}_2$  effect was found for the abundance of this species (Lischka et al., 2015). It is possible  
5 that 45 days was not long enough to detect small negative effects of  $\text{CO}_2$  on copepod size, egg  
6 hatching and nauplii development, to be reflected in copepod abundance. In addition,  
7 especially in the beginning of the study *Acartia* eggs in the mesocosms might have ended up  
8 in the sediment trap before hatching due to slow development at low temperature, which  
9 might have made it difficult to detect differences in *Acartia* abundance between the  
10 mesocosms. On a longer time scale, small acidification induced delays in offspring  
11 development could translate into negative effects for the copepod population, and further on  
12 energy transfer within the pelagic food web. In addition, warming will probably enhance the  
13 sensitivity of the species towards ocean acidification (Vehmaa et al., 2012, 2013).

14

## 15 **5 Conclusions**

16 Our results support the idea that it is important to look beyond egg production as hatching and  
17 development can be more sensitive to ocean acidification. Parental effects will likely be  
18 important in mediating some of the negative effects of ocean acidification. For *Acartia* sp.,  
19 the transgenerational (maternal) effects may alleviate negative impacts of ocean acidification  
20 but potentially only under exposure to medium levels of  $\text{CO}_2$ . We did not find support for the  
21 hypothesis suggesting that poorer food quantity and quality would weaken the maternal effect  
22 by deteriorating the condition of the mother, which could be due to the overall food limitation  
23 especially during the latter half of the study or the fact that our estimates of food quantity and  
24 quality did not describe the diet in a satisfactory manner. Nevertheless, maternal antioxidant  
25 defence seems to correlate positively with offspring egg hatching success. Overall, these  
26 results indicate that *Acartia* sp. could in fact be affected by  $\text{CO}_2$  levels predicted for the year  
27 2100 (IPCC, 2007). However, it is important to remember that this study shows how today's  
28 copepods would react to tomorrow's world; thus these results do not take into account the  
29 possible effects of evolutionary adaptation. Transgenerational effects can buffer short-term  
30 detrimental effects of ocean acidification and thus give time for genetic adaptation and  
31 consequently assist persistence of populations under climate change.

32



1 **Author contributions**

2 A.V. planned the experiment; A.V., A.-K.A., J.E.-Ö., A.B. conducted the laboratory  
3 experiment; A.V. performed the statistical analyses; A.P. analysed TPC and C:N; S.F  
4 analysed ORAC; U.R. coordinated the whole project; A.V. and A.-K.A. shared responsibility  
5 of writing the manuscript with contributions from all co-authors.

6

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## 1 **References**

- 2 Almén, A-K., Vehmaa, A., Brutemark, A., and Engström-Öst, J.: Coping with climate  
3 change? Copepods experience drastic variations in their physicochemical environment on a  
4 diurnal basis, *J. Exp. Mar. Biol. Ecol.*, 460, 120–128, doi:10.1016/j.jembe.2014.07.001, 2014.
- 5 Almén, A-K., Vehmaa, A., Brutemark, A., Bach, L., Lischka, S., Stuhr, A., Furuhaugen, S.,  
6 Paul, A., Bermúdez, R., Riebesell, U., and Engström-Öst, J.: Negligible effects of ocean  
7 acidification on *Eurytemora affinis* (Copepoda) offspring production,  
8 *Biogeosciences*, 13, 1037–1048, doi:10.5194/bg-13-1037-2016, 2016.
- 9 Bates, D., Maechler, M., Bolker, B., and Walker, S.: lme4: Linear mixed-effects models using  
10 Eigen and S4, R package version 1.1-7, available at: [http://CRAN.R-](http://CRAN.R-project.org/package=lme4)  
11 [project.org/package=lme4](http://CRAN.R-project.org/package=lme4), Last access: 11 November 2014.
- 12 Bělehrádek, J.: Temperature and living matter, *Protoplasma Monograph*, No. 8 Borntrager,  
13 Berlin, 1935.
- 14 Boersma, M., Mathew, K.A., Niehoff, B., Schoo, K.L., Franco-Santos, R.M., and Meunier,  
15 C.: Temperature driven changes in the diet preference of omnivorous copepods: no more meat  
16 when it's hot?, *Ecol. Lett.*, 19, 45–53, doi: 10.1111/ele.12541, 2016.
- 17 Bron, J.E., Frisch, D., Goetze, E., Johnson, S.C., Lee, C.E., and Wyngaard, G.A.: Observing  
18 copepods through a genomic lens, *Front. Zool.*, 8, 22, doi:10.1186/1742-9994-8-22, 2011.
- 19 Chevin, L.-M., Collins, S., and Lefèvre, F.: Phenotypic plasticity and evolutionary  
20 demographic responses to climate change: taking theory out to the field, *Funct. Ecol.*, 27,  
21 966–979, doi:10.1111/j.1365-2435.2012.02043.x, 2013.
- 22 Cripps, G., Lindeque, P., and Flynn, K. J.: Have we been underestimating the effects of ocean  
23 acidification in zooplankton?, *Glob. Change Biol.*, 20, 3377–3385, doi:10.1111/gcb.12582,  
24 2014a.
- 25 Cripps, G., Lindeque, P., and Flynn, K. J.: Parental exposure to elevated pCO<sub>2</sub> influences the  
26 reproductive success of copepods, *J. Plankton Res.*, 36, 1165–1174,  
27 doi:10.1093/plankt/fbu052, 2014b.
- 28 Cushing, D.H.: Plankton production and year-class strength in fish populations: an update of  
29 the match/mismatch hypothesis, *Adv. Mar. Biol.*, 26, 249-292, 1990.

- 1 Dzierzbicka-Glowacka, L., Lemieszek, A., and Zmijewska, M.I.: Parameterisation of a  
2 population model for *Acartia* spp. in the southern Baltic Sea. Part 1. Development time,  
3 *Oceanologia*, 51, 165–184, 2009.
- 4 Elser, J.J., and Hassett, R.P. A stoichiometric analysis of the zooplankton-phytoplankton  
5 interaction in marine and freshwater ecosystems, *Nature*, 3070, 211–213, 1994.
- 6 Fitzer, S.C., Caldwell, G.S., Close, A.J., Clare, A.S., Upstill-Goddard, R.C., and Bentley,  
7 M.G.: Ocean acidification induces multi-generational decline in copepod naupliar production  
8 with possible conflict for reproductive resource allocation, *J. Exp. Mar. Biol. Ecol.*, 418–419,  
9 30–36, doi:10.1016/j.jembe.2012.03.009, 2012.
- 10 Foley, C.J., Ryan, D.J., and Höök, T.O.: Length reduction of larval yellow perch and  
11 freshwater amphipods in RNA*later* solution, *N. Am. J. Fish. Manage.*, 30, 1143–1148, DOI:  
12 10.1577/M10-035.1, 2010.
- 13 Foster, J., Hirst, A.G., and Atkinson, D.: How do organisms change size with changing  
14 temperature? The importance of reproductive method and ontogenic timing, *Funct. Ecol.*, 25,  
15 1024–1031, doi:10.1111/j.1365-2435.2011.01852.x, 2011.
- 16 Garzke, J., Hansen, T., Ismar, S.M.H., Sommer, U.: Combined effects of ocean warming and  
17 acidification on copepod abundance, body size and fatty acid content, *PLoS ONE*, 11,  
18 e0155952, doi:10.1371/journal.pone.0155952, 2016.
- 19 Hart, R.C., and E.A., Bychek: Body size in freshwater planktonic crustaceans: an overview of  
20 extrinsic determinants and modifying influences of biotic interactions, *Hydrobiologia*, 668,  
21 61–108, doi:10.1007/s10750-010-0400-y, 2011.
- 22 IPCC (Intergovernmental Panel on Climate Change): *Climate Change 2007: Synthesis Report*.  
23 Fourth assessment report. Available: <http://www.ipcc.ch>, (last access: 27 July 2015), 2007.
- 24 Ismar, S.M.H., Hansen, T., and Sommer, U.: Effect of food concentration and type of diet on  
25 *Acartia* survival and naupliar development, *Mar. Biol.*, 154, 335–343, doi:10.1007/s00227-  
26 008-0928-9, 2008.
- 27 Katajisto, T., Viitasalo, M., and Koski, M.: Seasonal occurrence and hatching of calanoid  
28 eggs in sediments of the northern Baltic Sea, *Mar. Ecol.–Prog. Ser.*, 163, 133–143, 1998.
- 29 Knuckey, R.M., Semmens, G.L., Mayer, R.J., and Rimmer, M.A.: Development of an optimal  
30 microalgal diet for the culture of the calanoid copepod *Acartia sinjiensis*: Effect of algal

1 species and feed concentration on copepod development, *Aquaculture*, 249, 339–51,  
2 doi:10.1016/j.aquaculture.2005.02.053, 2005.

3 Koski, M., and H. Kuosa: The effect of temperature, food concentration and female size on  
4 the egg production of the planktonic copepod *Acartia bifilosa*, *J. Plankton Res.*, 21, 1779–  
5 1789, 1999.

6 Kurihara, H., and A. Ishimatsu: Effects of high CO<sub>2</sub> seawater on the copepod (*Acartia*  
7 *tsuensis*) through all life stages and subsequent generations, *Mar. Pollut. Bull.*, 56, 1086–  
8 1090, doi: 10.1016/j.marpolbul.2008.03.023, 2008.

9 Kurihara, H., Shimode, S., and Shirayama, Y.: Effects of CO<sub>2</sub> concentration on the egg  
10 production rate and early development of two marine copepods (*Acartia steueri* and *Acartia*  
11 *erythraea*), *Mar. Poll. Bull.*, 49, 721–727, 2004.

12 Lewis, C.N., Brown, K.A., Edwards, L.A., Cooper, G., and Findlay, H.S.: Sensitivity to ocean  
13 acidification parallels natural pCO<sub>2</sub> gradients experienced by Arctic copepods under winter  
14 sea ice, *Proc. Natl. Acad. Sci. U.S.A.*, 110, E4960–E4967,  
15 www.pnas.org/cgi/doi/10.1073/pnas.1315162110, 2013.

16 Lischka, S., Bach, L. T., Schulz, K.-G., and Riebesell, U.: Micro- and mesozooplankton  
17 community response to increasing CO<sub>2</sub> levels in the Baltic Sea: insights from a large-scale  
18 mesocosm experiment, *Biogeosciences Discuss.*, 12, 20025–20070, doi:10.5194/bgd-12-  
19 20025-2015, 2015.

20 Mayor, D. J., Matthews, C., Cook, K., Zuur, A. F., and Hay, S.: CO<sub>2</sub>-induced acidification  
21 affects hatching success in *Calanus finmarchicus*, *Mar. Ecol. Prog. Ser.*, 350, 91–97, 2007.

22 Mayor, D.J., Everett, N.R., and Cook, K.B.: End of century ocean warming and acidification  
23 effects on reproductive success in a temperate marine copepod, *J. Plankton Res.*, 34, 258–262,  
24 doi:10.1093/plankt/fbr107, 2012.

25 McConville, K., Halsband, C., Fileman, E.S., Somerfield, P.J., Findlay, H.S., and Spicer, J.I.:  
26 Effects of elevated CO<sub>2</sub> on the reproduction of two calanoid copepods, *Mar. Pollut. Bull.*, 73,  
27 428–434, doi.org/10.1016/j.marpolbul.2013.02.010, 2013.

28 McLaren, I.A.: Predicting development rate of copepod eggs, *Bioll. Bull.*, 131, 457-469,  
29 1966.

- 1 Miralto, A., Barone, G., Romano, G., Poulet, S.A., Ianora, A., Russo, G.L., Buttino, I.,  
2 Mazzarella, G., Laabir, M., Cabrini, M., and Giacobbe, M.G.: The insidious effects of  
3 diatoms on copepod reproduction, *Nature*, 402, 173–176, 1999.
- 4 Monaghan, P., Metcalfe, N.B., and Torres, R.: Oxidative stress as a mediator of life history  
5 trade-offs: mechanisms, measurements and interpretation, *Ecol. Lett.*, 12, 75–92,  
6 doi:10.1111/j.1461-0248.2008.01258.x, 2009.
- 7 Niemi, Å.: Ecology of phytoplankton in the Tvärminne area, SW coast of Finland. II. Primary  
8 production and environmental condition in the archipelago and the seas zone, *Acta Botanica*  
9 *Fennica*. 105:1-73, 1975.
- 10 Omstedt, A., Humborg, C., Pempkowiak, J., Perttilä, M., Rutgersson, A., Schneider, B.,  
11 Smith, B.: Biogeochemical Control of the Coupled CO<sub>2</sub>–O<sub>2</sub> System of the Baltic Sea: A  
12 Review of the Results of Baltic-C, *AMBIO* 43, 49–59. doi:10.1007/s13280-013-0485-4,  
13 2014.
- 14 Ou, B. X., Hampsch-Woodill, M. and Prior, M.: Development and validation of an improved  
15 oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, *J. Agr.*  
16 *Food. Chem.*, 49, 4619-4626, 2001.
- 17 Paul, A.J., Bach, L.T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E.P.,  
18 Hellemann, D., Trense, Y., Nausch, M., Sswat, M., and Riebesell, U.: Effect of elevated CO<sub>2</sub>  
19 on organic matter pools and fluxes in a summer Baltic Sea plankton community,  
20 *Biogeosciences*, 12, 6181–6203, doi:10.5194/bg-12-6181-2015, 2015.
- 21 Pedersen, S.A., Håkedal, O.L., Salaberria, I., Tagliati, A., Gustavson, L.M., Jenssen, B.M.,  
22 Olsen, A.J., and Altin, D.: Multigenerational exposure to ocean acidification during food  
23 limitation reveals consequences for copepod scope for growth and vital rates, *Environ. Sci.*  
24 *Technol.*, 48, 12275–12284, doi:10.1021/es501581j, 2014a.
- 25 Pedersen, S.A., Våge, V.V., Olsen, A.J., Hammer, K.M., and Altin, D.: Effects of elevated  
26 carbon dioxide (CO<sub>2</sub>) concentrations on early developmental stages of the marine copepod  
27 *Calanus finmarchicus* Gunnerus (Copepoda: Calanoidae), *J. Toxicol. Env. Heal. A*, 77, 535–  
28 549, doi:10.1080/15287394.2014.887421, 2014b.

1 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team: nlme: Linear and Nonlinear  
2 Mixed Effects Models, R package version 3.1-118, available at: [http://CRAN.R-](http://CRAN.R-project.org/package=nlme)  
3 [project.org/package=nlme](http://CRAN.R-project.org/package=nlme) (last access: 26 June 2015), 2014.

4 R Core Team, R: A language and environment for statistical computing, R Foundation for  
5 Statistical Computing, Vienna, Austria, available at: <http://www.R-project.org/> (last access:  
6 27 March 2014), 2013.

7 Reusch, T.B.H.: Climate change in the oceans: evolutionary versus phenotypically plastic  
8 responses of marine animals and plants, *Evol. Appl.*, 7, 104–122, doi:10.1111/eva.12109,  
9 2014.

10 Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büden- bender, J., Deckelnick,  
11 M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Mucbe, R., and Schulz,  
12 K. G.: Technical Note: A mobile sea-going mesocosm system – new opportunities for ocean  
13 change research, *Biogeosciences*, 10, 1835–1847, doi:10.5194/bg-10-1835-2013, 2013.

14 Rollwagen Bollens, G.C., and Penry, D.L.: Feeding dynamics of *Acartia* spp. copepods in a  
15 largen, temperate estuary (San Francisco Bay, CA), *Mar. Ecol.–Prog. Ser.*, 257, 139–158,  
16 2003.

17 Rubolini, D., Romano, M., Bonisoli Alquati, A., and Saino, N.: Early maternal, genetic and  
18 environmental components of antioxidant protection, morphology and immunity of yellow-  
19 legged gull (*Larus michahellis*) chicks, *J. Evol. Biol.*, 19, 1571–1584, doi:10.1111/j.1420-  
20 9101.2006.01121.x, 2006.

21 Shama, L.N.S., Strobel, A., Mark, F.C., and Wegner, K.: Transgenerational plasticity in  
22 marine sticklebacks: maternal effects mediate impacts of a warming ocean, *Funct. Ecol.*, 28,  
23 1482–1493, doi:10.1111/1365-2435.12280, 2014.

24 Sharp, J.: Improved analysis for particulate organic carbon and nitrogen from seawater,  
25 *Limnol. Oceanogr.*, 19, 984-989, 1974.

26 Steele, J.H.: The structure of marine ecosystems, Harvard University Press, Cambridge, 1974.

27 Sterner, R.W., and D.O., Hessen: Algal nutrient limitation and the nutrition of aquatic  
28 herbivores, *Annu. Rev. Ecol. Syst.*, 25, 1–29, 1994.

- 1 Thor, P., and S., Dupont: Transgenerational effects alleviate severe fecundity loss during  
2 ocean acidification in a ubiquitous planktonic copepod, *Glob. Change Biol.*, 21, 2261–2271,  
3 doi:10.1111/gcb.1281, 2015.
- 4 Tomanek, L.: Proteomics to study adaptations in marine organisms to environmental stress, *J.*  
5 *Proteomics*, 105, 92–106, doi:10.1016/j.jprot.2014.04.009, 2014.
- 6 Vehmaa, A., Brutemark, A., and Engström-Öst, J.: Maternal effects may act as an adaptation  
7 mechanism for copepods facing pH and temperature changes, *PLoS ONE*, 7, e48538.  
8 doi:10.1371/journal.pone.0048538, 2012.
- 9 Vehmaa, A., Hogfors, H., Gorokhova, E., Brutemark, A., Holmborn, T., and Engström-Öst,  
10 J.: Projected marine climate change: effects on copepod oxidative status and reproduction,  
11 *Ecol. Evol.*, 13, 4548–4557, doi:10.1002/ece3.839, 2013.
- 12 West-Eberhard, M.J.: *Developmental plasticity and evolution*, Oxford University Press, New  
13 York, 2003.
- 14 Weydmann, A., Søreide, J.E., Kwasniewski, S., and Widdicombe, S.: Influence of CO<sub>2</sub>-  
15 induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*, *J. Exp.*  
16 *Mar. Biol. Ecol.*, 428, 39–42, doi:10.1016/j.jembe.2012.06.002, 2012.
- 17 Whiteley, N.M.: Physiological and ecological responses of crustaceans to ocean acidification,  
18 *Mar. Ecol.–Prog. Ser.*, 430, 257–271, doi: 10.3354/meps09185, 2011.
- 19 Yoon, W.D., Shim, M.B., and Choi, J.K.: Description of the developmental stages in *Acartia*  
20 *bifilosa* Giesbrecht (Copepoda: Calanoida), *J. Plankton Res.*, 20, 923–942, 1998.
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1 Tables

2 Table 1. The structure of the full LMM or GLMM models that were used to test effects of  
 3 ocean acidification, food quantity, and food quality on copepod egg production (EPR), egg  
 4 hatching success (EH), prosome length (PL), antioxidant capacity (ORAC), the ratio of EH  
 5 mesocosm / EH Baltic, and the ratio of nauplii development index (DI) mesocosm / DI Baltic.  
 6 The sampling days that were included in each of the models are listed. Repeated measures of  
 7 same mesocosm bags was used as a random effect in all the models, because copepods that  
 8 come from the same bags are more alike than copepods from different bags.

9

Response variable	Fixed effects	Effect tested	Days included in the model					
			3	10	17	24	31	45
<b>EPR (LMM)</b>	<i>f</i> CO <sub>2</sub>	Ocean acidification	x	x	x	x		x
	TPC (<55 μm)	Food quantity						
	C:N (<55 μm)	Food quality						
<b>EH (GLMM)</b>	<i>f</i> CO <sub>2</sub>	Ocean acidification	x	x	x	x		
	TPC (<55 μm)	Food quantity						
	C:N (<55 μm)	Food quality						
<b>PL (LMM)</b>	<i>f</i> CO <sub>2</sub>	Ocean acidification		x	x	x		x
	TPC (<55 μm)	Food quantity						
	C:N (<55 μm)	Food quality						
<b>ORAC (LMM)</b>	<i>f</i> CO <sub>2</sub>	Ocean acidification	x	x	x			x
	TPC (<55 μm)	Food quantity						
	C:N (<55 μm)	Food quality						
<b>EH MC/Baltic (LMM)</b>	<i>f</i> CO <sub>2</sub>	Ocean acidification	x	x	x	x		
	TPC (<55 μm)	Food quantity						
	C:N (<55 μm)	Food quality						
<b>DI MC/Baltic (LMM)</b>	<i>f</i> CO <sub>2</sub>	Ocean acidification	x	x	x	x		
	TPC (<55 μm)	Food quantity						
	C:N (<55 μm)	Food quality						

10

11



1 Table 2. T-statistics of the retained fixed effects in the linear mixed effect models testing the  
 2 effects of TPC (<55 $\mu$ m), C:N and  $f$ CO<sub>2</sub> on egg production rate (EPR), female prosome length  
 3 (PL) and female antioxidant capacity (ORAC). Repeated measures of same mesocosm bags  
 4 was used as a random effect in all the models, because copepods that come from the same  
 5 bags are more alike than copepods from different bags.

6

<b>Response variable</b>	<b>Fixed effect</b>	<b>Estimate</b>	<b>DF</b>	<b>t</b>	<b>p-value</b>
<b>EPR</b>	TPC <55 $\mu$ m	0.21 $\pm$ 0.14	23	1.54	0.137
<b>PL</b>	$f$ CO <sub>2</sub>	-0.000027 $\pm$ 0.000011	16	-2.39	0.030
	TPC <55 $\mu$ m	-0.0037 $\pm$ 0.0017	16	-2.21	0.042
<b>ORAC</b>	TPC <55 $\mu$ m	-0.0045 $\pm$ 0.0021	22	-2.17	0.041

7

8

1 Table 3. Ranges of  $f\text{CO}_2$ ,  $\text{TPC}<55 \mu\text{m}$ , and  $\text{C:N}< 55 \mu\text{m}$  that were used as explanatory  
2 variables in the full LMM and GLMM models. 3-day averages (measured within the latest  
3 three days of the sampling day) were used in testing the effects of the explanatory variables  
4 on copepod egg production (EPR), antioxidant capacity (ORAC), and egg hatching success  
5 (EH), whereas average of all measurements since the start of the experiments until the  
6 sampling day were used when testing the effects of the explanatory variables on copepod size  
7 (PL). Variations in  $f\text{CO}_2$ ,  $\text{TPC} <55 \mu\text{m}$ , and  $\text{C:} <55 \mu\text{m}$  in the course of the study are  
8 presented in Paul et al. (2015).

	$f\text{CO}_2$ ( $\mu\text{atm}$ )		$\text{TPC}<55 \mu\text{m}$		$\text{C:N} <55 \mu\text{m}$	
	3-d average	Average since	3-d average	Average since	3-d average	Average since
		Day 1		Day 1		Day 1
<b>MC 1</b>	267–477	267–365	15.1–31.6	21.4–31.6	5.51–8.43	7.26–8.03
<b>MC 3</b>	745–1201	884–1121	17.4–29.7	20.4–29.7	6.94–8.36	7.79–8.20
<b>MC 5</b>	275–481	274–368	15.8–24.5	19.2–24.8	7.24–8.57	7.24–7.59
<b>MC 6</b>	663–991	683–896	16.5–34.3	21.0–34.3	7.14–8.25	7.60–7.81
<b>MC 7</b>	390–565	390–497	17.5–30.0	21.4–29.9	6.92–8.25	7.43–7.74
<b>MC 8</b>	874–1525	1117–1413	17.4–26.3	21.6–26.3	7.16–8.53	7.59–7.93

9

1 Table 4. Z-statistics of the retained fixed effects in the GLMM testing the effect of  $f\text{CO}_2$ , TPC  
2 (<55  $\mu\text{m}$ ) and C:N on egg hatching success (EH). Repeated measures of same mesocosm bags  
3 was used as a random effect in the model, because copepods that come from the same bags  
4 are more alike than copepods from different bags.

5

<b>Response variable</b>	<b>Fixed effect</b>	<b>Estimate</b>	<b>z</b>	<b>p-value</b>
<b>EH</b>	$f\text{CO}_2$	-0.00062±0.00032	1.94	0.052
	TPC <55 $\mu\text{m}$	-0.09557±0.02505	3.82	<0.001

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1 Table 5. T-statistics of the retained fixed effects in the LMMs testing the effect of  $f\text{CO}_2$ , TPC  
 2 ( $<55 \mu\text{m}$ ) and C:N on ratio of egg hatching success (EH) mesocosm / EH Baltic and nauplii  
 3 development index (DI) mesocosm / DI Baltic. Ratio  $>1$ : higher EH or DI in the mesocosm  
 4 water (maternal environment) than in the Baltic Sea water, ratio  $<1$ : lower EH or DI in the  
 5 mesocosm water (maternal environment) than in the Baltic Sea water. Repeated measures of  
 6 same mesocosm bags was used as a random effect in both models, because copepods that  
 7 come from the same bags are more alike than copepods from different bags.

8

Response variable	Fixed effect	Estimate	DF	t	p-value
EH mesocosm / EH Baltic	$f\text{CO}_2$	$-0.000061 \pm 0.000028$	16	-2.20	0.043
DI mesocosm / DI Baltic	$f\text{CO}_2$	$-0.000145 \pm 0.000067$	16	-2.15	0.047

9

10

1 Figures.

2 Fig. 1. Development of *Acartia bifilosa* a) egg production, b) prosome length (average  $\pm$  s.e.),  
3 c) antioxidant capacity, and d) egg hatching success in the mesocosms, and e) egg hatching  
4 success in Baltic water when eggs are produced in mesocosms in the course of the study. The  
5  $f\text{CO}_2$  ( $\mu\text{atm}$ ) values represent the average in Days 1–43 (Paul et al., 2015).

6

7 Fig. 2. Development of the ratio of a) egg hatching success (EH) mesocosm / EH Baltic and  
8 b) nauplii development index (DI) mesocosm / DI Baltic during the study. Ratio  $>1$ : higher  
9 EH or DI in the mesocosm water (maternal environment) than in the Baltic Sea water, ratio  
10  $<1$ : lower EH or DI in the mesocosm water (maternal environment) than in the Baltic Sea  
11 water. Note that because of different development times, the DI values are not comparable  
12 between the days. The  $f\text{CO}_2$  ( $\mu\text{atm}$ ) values represent the average in Days 1–43 (Paul et al.,  
13 2015).

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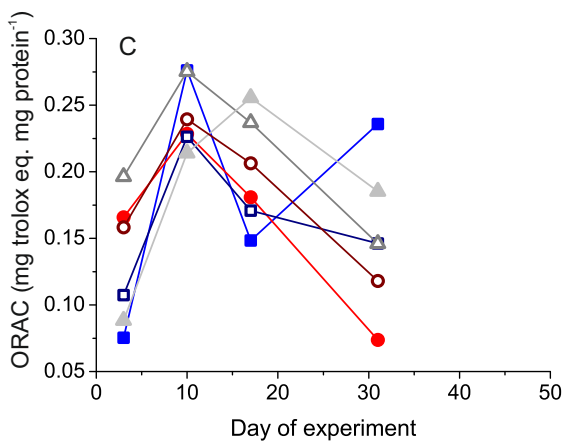
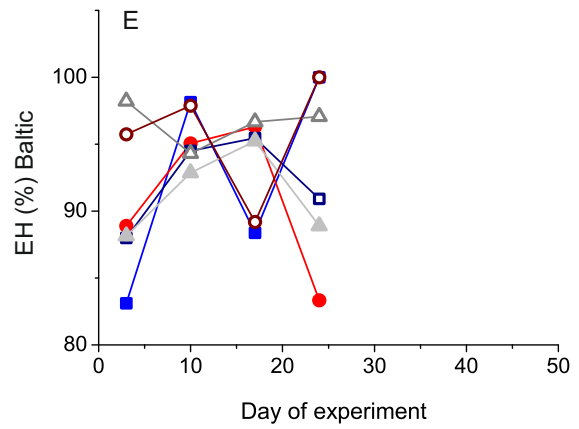
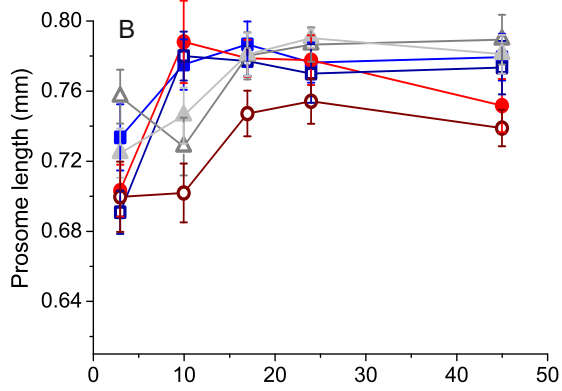
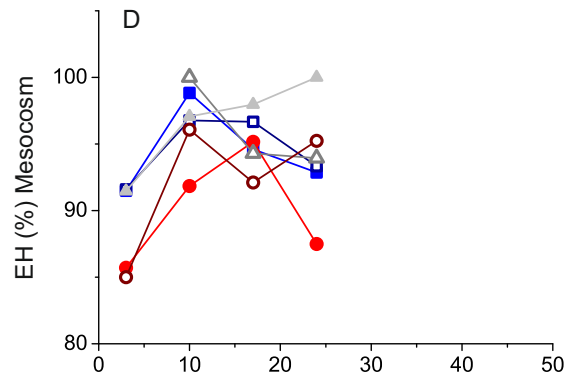
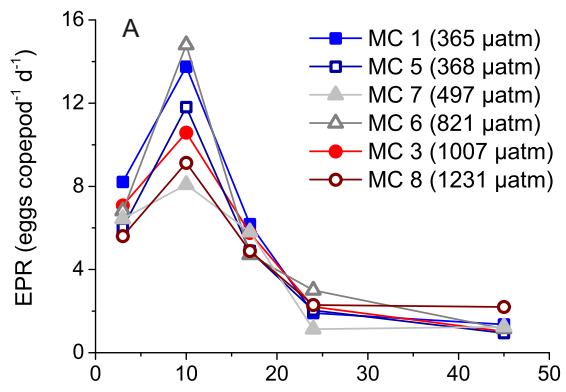
15 Fig. 3. Correlations of copepod egg hatching success (EH) with maternal antioxidant capacity  
16 (ORAC).

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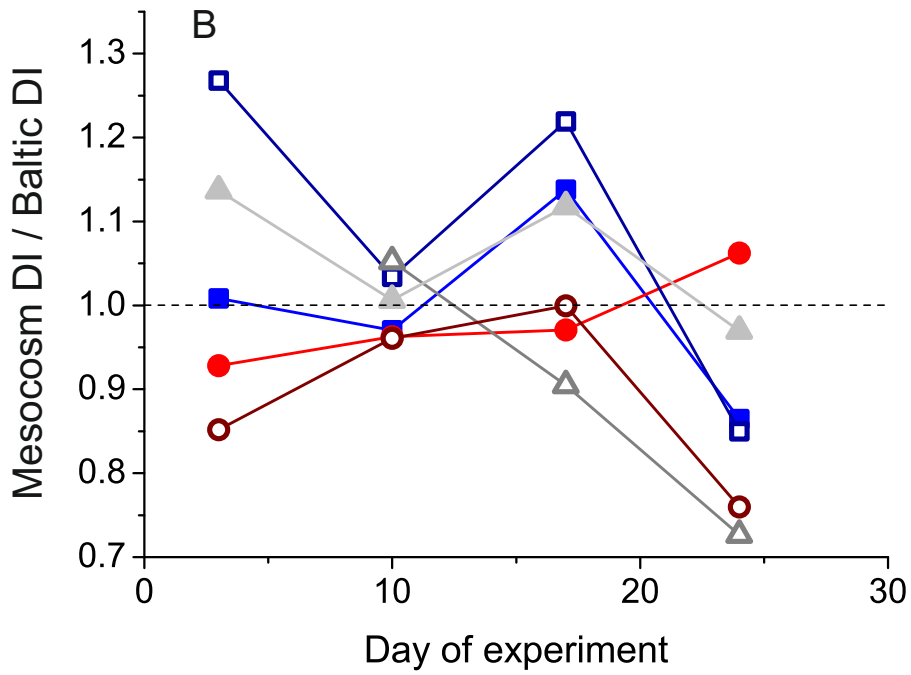
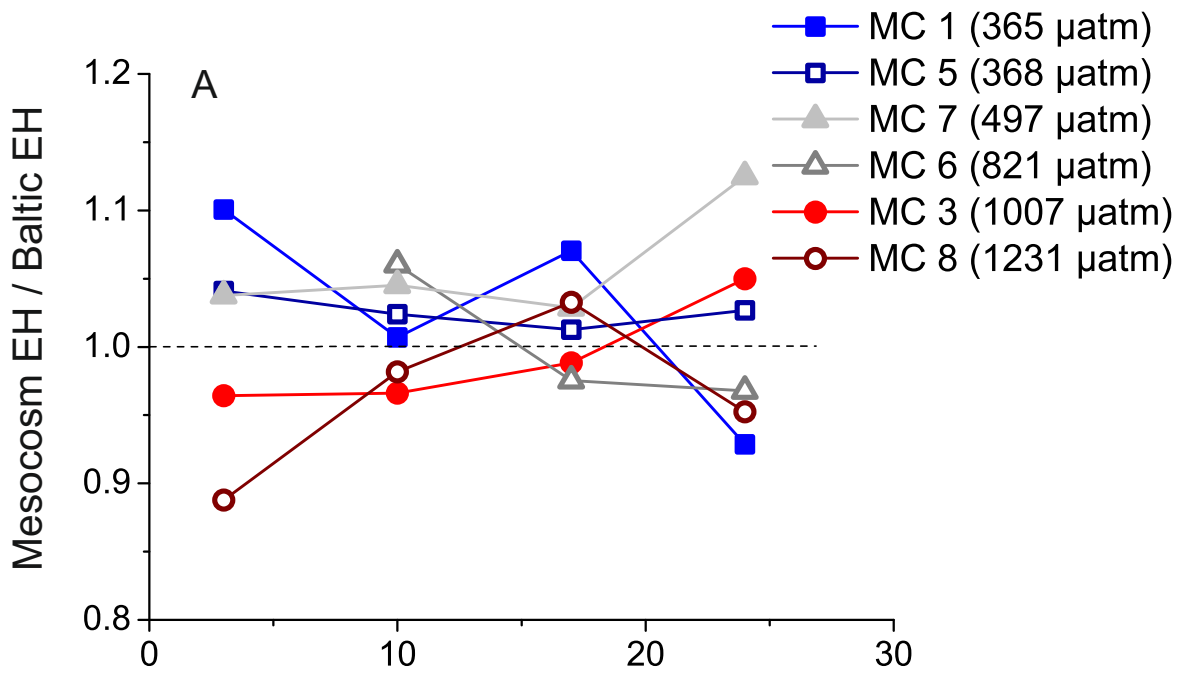
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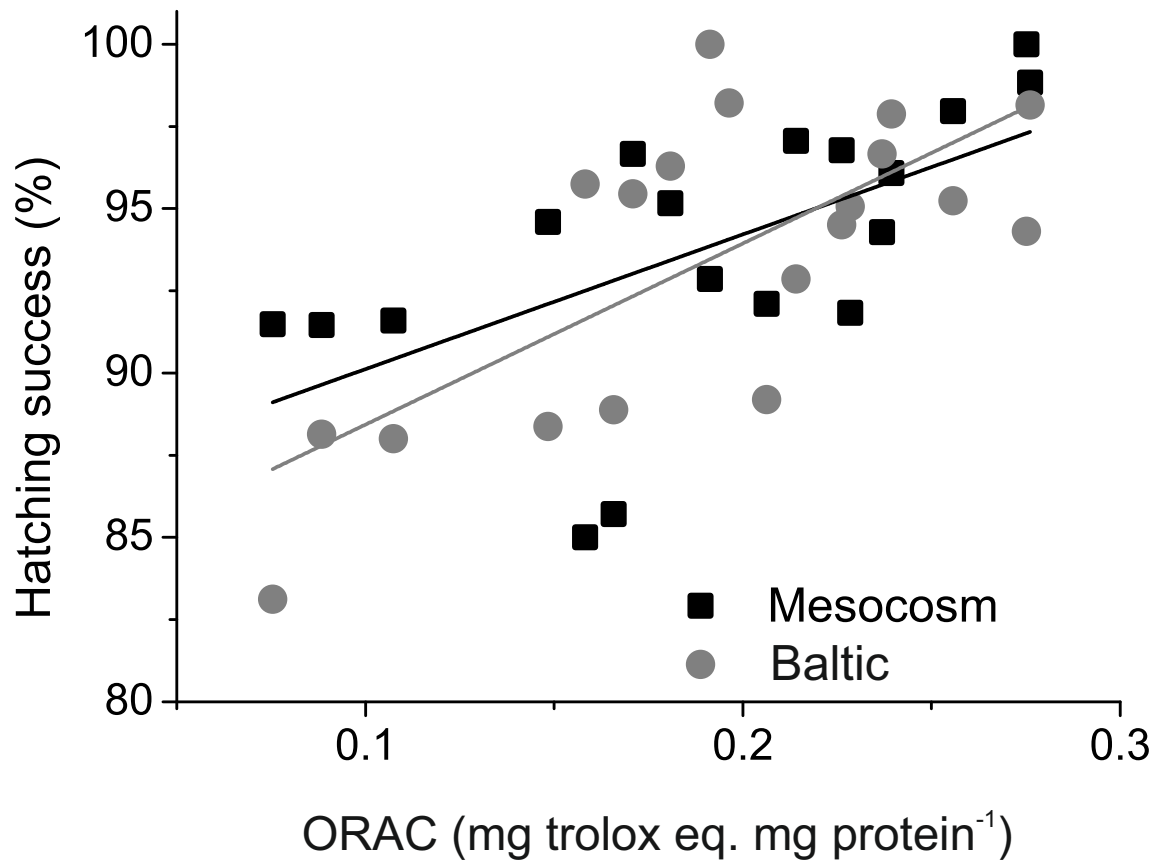
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