Chronic exposure of the North Atlantic copepod *Calanus finmarchicus* (Gunnerus, 1770) to CO$_2$-acidified seawater; effects on survival, growth and development

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Abstract

The impact of chronic exposure to CO$_2$-acidified seawater on survival, growth and development was investigated in the North Atlantic copepod *Calanus finmarchicus*. Using a custom developed microcosm system fertilized eggs and subsequent development stages were exposed to normal seawater (390 ppm CO$_2$) or one of three different levels of CO$_2$-induced acidification (3300, 7300, 9700 ppm CO$_2$). Following the 28 day exposure period survival was found to be unaffected by exposure to 3300 ppm CO$_2$, but significantly reduced at 7300 and 9700 ppm CO$_2$. Also, the proportion of copepodite stages IV to VI observed in the different treatments was significantly affected in a manner that may indicate a CO$_2$-induced retardation of the rate of ontogenetic development. Morphometric analysis revealed a significant increase in size (prosome length) and lipid storage volume in stage IV copepodites exposed to 3300 ppm CO$_2$ and reduced size in stage III copepodites exposed to 7300 ppm CO$_2$. Together, the findings indicate that a $p$CO$_2$ level $\leq$ 2000 ppm (the highest CO$_2$ level expected within year 2300) will probably not directly affect survival in *C. finmarchicus*. Long-term experiments at more moderate CO$_2$ levels are however necessary before the possibility that growth and development may be affected below $\leq$ 2000 ppm CO$_2$ can be ruled out.

1 Introduction

Burning of fossil fuels, altered use of land areas and other anthropogenic activities have contributed to raise the mean atmospheric concentration of carbon dioxide (CO$_2$) from a preindustrial level of around 280 ppm to its present level of $\sim$ 390 ppm CO$_2$ (Solomon et al., 2007). The increasing atmospheric CO$_2$ level has been identified as an explanation for the global warming phenomenon that has been observed during the last decade (Solomon et al., 2007). Approximately 30 % of the anthropogenic CO$_2$ has so far been absorbed by the oceans where it lowers the seawater pH through the production of carbonic acid (Sabine et al., 2004) – a process commonly referred...
to as ocean acidification (OA). As a result of this process the middle pH of ocean surface water (8.2) is already lowered by 0.1 pH units compared to preindustrial time, corresponding to a 30% increase in the concentration of H\(^+\)-ions. Worst-case scenario estimates based on carbon cycle models predict a CO\(_2\) level of 970 ppm by the end of the century (A1FI, Houghton et al., 2001), and possibly up to 1900 ppm in year 2300 (Caldeira and Wickett, 2003), which corresponds to a mean surface seawater pH of 7.8 and 7.4, respectively. Even up to 8000 ppm CO\(_2\) has been put forward as a “worst-case” scenario in year 2300 (Caldeira and Wickett, 2005). The rate of change in the atmospheric CO\(_2\) concentration and ocean pH experienced over the last century is up to a hundred times faster than any change observed during the past 650,000 years (Siegenthaler et al., 2005). There is a growing concern that the stress due to the rising CO\(_2\) level could be magnified by this rapid change, possibly resulting in serious consequences for the marine biota (Monaco Declaration, 2009). Some of this concern comes from studies of fossil records that indicate that previous periods of intense ocean acidification, e.g. at the end of the Paleocene, coincided with the mass extinction event of that time (Jackson, 2010).

Carbon capture and storage (CCS) is currently considered to represent one of the most promising alternatives to mitigate CO\(_2\) emissions (Metz et al., 2005). Since the implementation of international legislation concerning sub-seabed CO\(_2\) storage in Europe (EU, 2009; London Protocol, 2006) industrial scale projects are currently undertaken at several locations. Sub-seabed storage is considered to be a relatively safe method to dispose of CO\(_2\), but risk assessments suggest that a leak may occur within the next 1000 yr, through processes such as cap rock failure, where up to 2% of the stored CO\(_2\) could be lost to the water column (Turley et al., 2004). A leakage from such sub-seabed storages will probably affect a relatively limited area, but the CO\(_2\)-levels in the affected water column could reach levels that are orders of magnitudes higher than the most pessimistic ocean acidification scenarios, with potentially dramatic consequences for the organisms inhabiting the affected area.
The initial concerns raised on OA addressed the possible implications of the reduction in free \( \text{CO}_3^{2-} \)-ions on the formation of calcium-containing structures in calcifying organism. Indeed, many of the calcareous species investigated have been found to be highly vulnerable to elevated levels of \( \text{CO}_2 \) (Talmage and Gobler, 2009; Dupont et al., 2008; Comeau et al., 2009; Fabry et al., 2008). However, in addition to affecting the calcification process, elevated levels of \( \text{CO}_2 \) have also been found to affect various aspects of the normal physiology of marine organisms, such as gene expression (Todgham and Hofmann, 2009), and the energy budgets (Melzner et al., 2011; Stumpp et al., 2011). Negative effects of elevated \( p\text{CO}_2 \) on reproductive endpoints such as sperm mobility, fertilization- and hatching success have been observed in a number of species (Egilsdottir et al., 2009; Ellis et al., 2009; Havenhand et al., 2008; Kurihara and Shirayama, 2004; Kurihara et al., 2004; Mayor et al., 2007; Morita et al., 2009; Parker et al., 2009). Also, alterations in growth rate have been observed in many of the investigated species (Clark et al., 2009; Dupont et al., 2008; Findlay et al., 2009; Kurihara and Shirayama, 2004; Kurihara et al., 2004, 2008; Parker et al., 2009; Talmage and Gobler, 2009). Since many of these physiological processes are relevant to non-calcifying organisms, it is important to also investigate the responses of different members of this group to \( \text{CO}_2 \)-induced acidification.

Copepods are small crustaceans that play a vital role in marine food webs (Shek and Liu, 2010). However, their exoskeleton is non-calcified (Fitzer et al., 2012), and only a limited number of studies have investigated the vulnerability to elevated \( \text{CO}_2 \) levels among species from this group. The information available so far indicates considerable stage- and interspecific difference with regard to sensitivity to elevated \( \text{CO}_2 \) levels. While some species seem to tolerate \( \text{CO}_2 \) levels that are well above 2000 ppm (the level expected for year 2300), others such as *Acartia tsuensis* (Ito) (Kurihara and Ishimatsu, 2008), have been showed to be negatively affected at \( \text{CO}_2 \) levels \( \leq 2300 \) ppm. Recently, a study on the harpacticoid copepod *Tisbe battagliai* (Volkmann-Rocco) revealed negative effect on hatching success and survival at \( \text{CO}_2 \) levels well
below 1000 ppm (Fitzer et al., 2012), nuancing the perception that copepods are generally resistant to elevated levels of CO$_2$.

The copepod investigated in the present study, *Calanus finmarchicus* (Gunnerus), seasonally dominates the zooplankton biomass in the surface waters of the northern North Sea and the North Atlantic (Planque and Batten, 2000; Conover, 1988). The dominance of the northern *Calanus* species has been linked to specific life history traits which involve avoidance of predators and temporary scarcity of food during autumn and winter by descending down towards the seafloor, to a depth down to 1500 m, where the late juvenile stages enter into a quiescent state, before re-emerging in the surface water in time for the algae spring bloom (Edvardsen et al., 2006). Through synthesis and accumulation of lipids the *Calanus* species are able to concentrate energy, and they therefore constitute an important energy link between the phytoplankton and higher trophic level predators including many fish species (Runge, 1988; Beaugarand and Kirby, 2010) and seabirds (Kwasniewski et al., 2012). Through the production of fecal pellets *C. finmarchicus* together with the other calanoid copepods also constitutes a dominant part of the total vertical carbon flux in the ocean (Bathmann et al., 1987).

The total *Calanus* biomass in the North Sea and North Atlantic has reportedly declined by approximately 70% between the 1960s and the post 1990s, a reduction that is considered to reflect regional warming (Edwards et al., 2006, 2012). Due to the importance of *C. finmarchicus* in the marine food webs of northern waters, and its significance for maintenance of commercial fish stocks, negative effects of elevated $p$CO$_2$ and climatic changes on the species could have wide-reaching socioeconomic consequences.

Difficulties with successful rearing under laboratory conditions combined with relative long generation times have so far limited studies examining the potential effects of elevated CO$_2$ on *Calanus* species to short term experiments on wild-caught individuals where the focus have been on endpoints such as the hatching success of eggs and early nauplii survival. Using this approach Mayor et al. (2007) found no effects on egg production when wild-caught females were exposed to 8000 ppm CO$_2$, at the
same time as the hatching success of the eggs incubated under the same conditions was severely reduced (only 4% survival). In a similar experiment, where more moderate CO₂ levels were applied, no significant effect on hatching success was observed when eggs from wild-caught *Calanus helgolandicus* (Claus) females were incubated in seawater with 1000 ppm CO₂ (e.g. a level that may be reached within the end of the century) (Mayor et al., 2012). Recently, Weydmann et al. (2012) reported that egg production in wild-caught *Calanus glacialis* (Jaschnov) females were unaffected by a pH level of 7.6 and 6.9 (corresponding to a pCO₂ of ~1000 and ~7000 ppm at the in situ temperature used, respectively), but reduced hatching success was observed among the eggs that were incubated at pH 6.9. In wild-caught *Calanus sinicus* (Brodsky), no effect on adult survival and egg production rate was observed during an eight day long incubation period in seawater with a CO₂ level of up to 10 000 ppm (Zhang et al., 2011).

The aim of the present study was to provide data on the long-term effects of elevated pCO₂ in a *Calanus* species. The effects on hatching success, mortality and ontogenetic development were integrated by chronically exposing fertilized *C. finmarchicus* eggs and subsequent developmental stages under controlled laboratory conditions to normal seawater (~390 ppm CO₂), or one of three different levels of CO₂-induced acidification (3300, 7300, 9700 ppm CO₂), over a 28 day period.

2 Methods

2.1 Seawater and exposure facilities

Natural seawater for the experiment was supplied through an inshore sub-sea pipeline in Trondheimfjorden (Norway), collecting water from about 70 m depth. Prior to use the seawater was filtered through a sand-filter and temperature and gaseous saturation adjusted in the integrated water treatment system available at NTNU SeaLab research facility, which include using a combination of heavy aeration and sprinkling over biofilm carriers (Kaldnes Miljøteknologi, Norway) in polyethylene holding tank (6 m³), to obtain...
mature water. Before entering the experimental system the water was finally filtered to 1 µm by inline filters.

All experiments were carried out in a temperature-controlled room maintained at 9–10 °C at the research facility of NTNU Centre of Fisheries and Aquaculture (SeaLab).

2.2 Calanus material

*Calanus* eggs used in the present investigation were produced by females from the culture running at NTNU Centre of Fisheries and Aquaculture (SeaLab) (Hansen et al., 2007). The females were transferred to a 50 L polyethylene tank where newly laid eggs were collected from the floor after 12 h incubation using a siphon. Prior to use the collected eggs were gently concentrated using a plankton sieve (mesh size 64 µm) submerged in water.

2.3 Description of the experimental system

A custom flow-through microcosms system was developed to include 12 two liter incubation chambers (borosilicate bottles, Schott) that were maintained in a horizontal position by a rack system (Fig. 1a). Both the inlet and outlet for the continuous addition of seawater to the bottles went through custom developed flask stoppers. A PEEK tube extending from the stopper and further into the bottle created circulation by distributing the inflowing water between three small holes (Ø 0.5 mm), serving as nozzles producing circulation by gentle jet streams. A nylon mesh cloth (mesh size 64 µm, Nitex), mounted in association with the outlet, served as a screen that retained the animals within the bottles (Fig. 1b). The water level in the bottles was determined by a water level controller at the outlet of the system. Two narrow-bored glass tubes (ID 2 mm) mounted through the lids, extending from the headspace in the bottles to the outside, maintained normal ambient air pressure within the bottles. Seawater, pre-equilibrated with different CO₂ enriched air mixtures, was added to each bottle using a 12 channel peristaltic pump (Watson Marlow, model 202) at a constant flow rate of 2.5 mL min⁻¹. All
wetted parts of the setup were made from borosilicate glass and high-grade polymers known to be safe to the animals.

2.4 Preparation of the different CO\textsubscript{2} mixtures and equilibration in columns

A gas mixing system was developed for the present study. Briefly, 5000, 10 000 and 15 000 ppm CO\textsubscript{2}, in addition to ambient air with 390 ppm (control), was obtained by mixing pressurized air and CO\textsubscript{2} gas (100\%, AGA). The appropriate gas flow, necessary to produce the different mixtures, was attained by using fine bore polyethylene tubes of different lengths and diameter (ID 0.28/0.38 mm) as gas flow restrictors. The equal pressure of the two gases, which is a prerequisite for the mixing principle described above, was obtained using a custom valve developed on the principle described by Parsons et al. (1992). The water was equilibrated to the control and the three CO\textsubscript{2} enriched air mixtures using custom developed counter current equilibration columns. The vertical columns consisted of an outer- (60 \times 16 \times 15 \text{cm} (\text{L} \times \text{OD} \times \text{ID})) and smaller inner acrylic tube (60 \times 6 \times 5 \text{cm} (\text{L} \times \text{OD} \times \text{ID})) mounted within the outer tube (Fig. 1c). The water in the columns was maintained at a constant level using in-house developed floating regulator valves. A submersible aquarium pump (Micro-Jet MC 450, Aquarium systems) was used to lift water from the outer tube to the elevated inlet of the inner tube. The elevated water column of the inner tube that was maintained by the pumping activity caused a gravimetrically determined downward flow of water that drained back to the outer tube through lateral holes near the basis of the inner tube, producing a constant circulation. An air stone (lime wood, Aqua Medic) mounted near the basis of the inner tube introduced small bubbles of the different air mixtures that ascended, counter-current to the descending water current, thus providing a favorable condition for the equilibration process.
2.5 Feeding

The copepods were fed a mixture of three species of micro algae (*Rhodomonas baltica* Karsten, *Dunaliella tertiolecta* Bucher, and *Isochrysis galbana* Parke) during the entire experiment. A carefully prepared algae stock suspension was continuously added to the water stream between the equilibration columns and their respective incubation bottles, at a flow rate of 0.075 mL min⁻¹, using a four-channel peristaltic pump (Watson Marlow model 202) fitted with marprene tubing. This maintained a stable density of algae in the exposure water corresponding to a total nominal carbon concentration of 600 µg L⁻¹. The three alga species contributed equally in terms of carbon content.

2.6 Experimental procedure

Batches of 240 newly laid eggs were sorted under a stereo dissection microscope (Leica MZ125, Leica Microsystems, Wetzlar, Germany) and transferred to each incubation chamber (bottle) using a glass Pasteur pipette. The eggs and subsequent stages were exposed for a total period of 28 days to four different *pCO₂* levels (390, 3300, 7300 and 9700 ppm), and the whole experiment was performed at 10°C at a 16:8 day-night cycle. Three replicate chambers of each *CO₂* treatment were included. The incubation bottles were exchanged with clean ones on a weekly basis to reduce the buildup of bacterial microfilm on the inside of the walls. The procedure involved moving the bottles to an upright inverted position, followed by a lowering of the water level by gently tapping the water into a receiving bottle using gravity feed through fine bore tubes (flow restriction). This procedure confined the copepods in a small volume of water above the outlet nylon mesh screen in the bottle stopper. The used bottle could now be detached and replaced by a clean one while reducing the disturbance of the animals to a minimum. The clean bottle was finally gently filled up through the outlet, again using gravity feed. To secure water quality continuity the “old” water collected during the draining procedure was reused.
2.7 Determination of mortality, stage distribution and morphometry

Following 28 days of exposure the animals were transferred from their respective incubation bottles and inspected using a Leica MZ125 dissecting microscope (Leica Microsystems, Wetzlar, Germany). Pictures of all animals were captured with a digital still-video camera (Sony DWF-sx900, Sony Corporation, Tokyo, Japan), operated by Fire-i software (Unibrain Inc., San Ramon CA, USA). Morphometric characteristics of the animals were measured manually on scaled captured images by the use of the software Image J (National Institutes of Health, Bethesda MD, USA). Length of prosome, urosome, total body length, area of the lipid storage and area of the prosome was measured with the aid of a graphical tablet (Wacom Cintiq 12WX, Wacom Co., Ltd., Saitama, Japan). Volume of lipid storage sack and prosome were calculated according to Miller et al. (1998) from the area and length of the lipid sack and prosome, respectively. Copepodite stage and sex of the adults were determined based on the number and shape of the urosome segments (Marshall and Orr, 1972; Mauchline, 1998).

2.8 Carbonate system determination

Weekly measurements were performed to monitor pH and temperature in the incubation bottles. The pH was determined potentiometrically (PHM240 pH-meter with a pHC2401-electrode and a T201 temperature sensor, Radiometer Analytical) using the NBS scale. The pH-meter was calibrated with IUPAC precision pH buffer 4.005 and 7.000 (Radiometer Analytical). Total alkalinity was determined by titration according to the method described by Anderson and Robinson (1946). Seawater carbonate species were calculated using the CO2SYS software (Pierrot et al., 2006) with the dissociation constants for NBS scale of Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Measured values and derived species are presented in Table 1.
2.9 Statistical treatment

Prior to statistical analysis data on mortality and morphometric parameters were arc-sin- and log transformed, respectively. Deviation from homogenous variation was examined using Levines test. Statistical comparisons of the different treatments were performed using one way ANOVA followed by Dunnet’s post hoc test to identify significant differences between control and the elevated CO$_2$ levels. Due to violation of the assumption of homogeneous variation stage percentages were analyzed using Kruskall-Wallis test. No post hoc test was applied here due to low power. The level of significance was set to 0.05 in all tests. All statistical analysis were performed using the statistical package SPSS.

3 Results

The overall mortality among the animals during the 28 day experiment period was 49.0 % in the control treatment (Fig. 2). The treatment that received seawater acidified by 3300 ppm CO$_2$ (47.9 %) showed no significant difference in mortality compared to the control. In the treatment that received seawater acidified by 7300 ppm CO$_2$ there was a significant increase in the mortality up to 73.8 % ($p < 0.05$) when compared to the control. With the exception of two single nauplii found in one of the replicate bottles, no animals developed under the highest CO$_2$ treatment (9700 ppm CO$_2$), resulting in an overall mortality of 99.7 % ($p < 0.05$).

After 28 days of exposure stage V copepodites dominated numerically in controls and the groups exposed to 3300 ppm CO$_2$ and constituted 82.4 ($\pm 2.6$) and 62.7 ($\pm 15.8$) % of the animals in the two groups, respectively (Fig. 3). Exposure to elevated $p$CO$_2$ had a significant effect on the relative proportion of stage CIV–CVI, but for stage III no apparent effect was observed. Increasing CO$_2$ level caused a significant higher percentage of CIV, while the proportion of the later stages, CV and CVI was reduced ($p < 0.05$),
resulting in CIV being the dominant stage in the groups exposed to 7300 ppm. No males were present among all the CVI at day 28.

For the control treatment mean prosome length of stage III, IV and V was 1.25 (±0.02), 1.46 (±0.03) and 1.91 (±0.03) mm, respectively (Fig. 4). The lowest level of CO₂-acidified seawater (3300 ppm) caused a 5.6 % increase of the prosome length of the stage IV copepodites (p < 0.05), while exposure to 7300 ppm CO₂ caused a 11.2 % reduction of the prosome length in stage III copepodites compared to the control (p < 0.05).

The fat content of the copepodites in the control treatment increased with increasing developmental stages and constituted 2.1 (±1.5), 2.9 (±1.7), and 7.9 (±1.7) % in stage III, IV and VI, respectively (Fig. 4). The only significant effect of CO₂ exposure observed was a 2.3 fold increase in the fat content of stage IV copepodites exposed to 3300 ppm.

4 Discussion

The results from the present study on the survival of *C. finmarchicus* egg cohorts raised in seawater with different degree of CO₂ acidification are well in line with the results that have been reported previously in similar, but more short-term studies, on *Calanus* species. Exposure to high levels of CO₂-induced acidification has previously been shown to reduce the survival in terms of low hatching success. The hatching success of *C. finmarchicus* (Mayor et al., 2007) and *C. glacialis* (Weydmann et al., 2012) eggs was severely reduced by incubation in seawater acidified with ~8000 and ~7000 ppm CO₂, respectively. Within this range of CO₂ levels the present study also revealed a strong and significant reduction of the survival in the raised cohorts; survival was reduced by ~50 % in the treatment with 7300 ppm, while no animals developed at 9700 ppm CO₂ (except two individuals which survived arrested as nauplii in one of the three replicates). This suggest that ~10 000 ppm CO₂ may represent the upper limit for successful hatching and continued development of fertilized eggs in *C. finmarchicus*. Such a level of CO₂-induced acidification is within the range of what may be relevant to
episodes of leakage from sub seabed storage sites for CO₂ (Vetter and Smith, 2005). Due to the overwintering strategy of *C. finmarchicus* near the seafloor (Edvardsen et al., 2006), a CO₂ leakage from such a storage site it is likely to negatively affect the dia-pausing animals. However, since most of the potential leakage pathways from CO₂ stores are considered most likely to lead to relatively low flux emissions (Holloway, 2007), the affected area is therefore assumed to be relatively limited and any negative impacts on *C. finmarchicus* and other members of the fauna is therefore likely to be only local.

Previous short-term studies on wild-caught animals have reported results indicating that *Calanus* species may be relatively robust to the most pessimistic ocean acidifi-cation scenarios expected within the end of this century (∼1000 ppm CO₂). Incuba-tion in seawater acidified with ∼1000 ppm CO₂ had no significant effect on survival, in terms of hatching success, in eggs from wild-caught females of both *C. helgolandicus* (Mayor et al., 2012) and *C. glacialis* (Weydmann et al., 2012). The results from the present long-term study on cohorts of *C. finmarchicus* eggs revealed no appar-ent effect on survival after 28 days of exposure to seawater acidified with 3300 ppm CO₂. However, recent studies have shown that some copepods may be negatively affected at pCO₂ levels that are in the range of what could occur within the end of this century (∼1000 ppm CO₂). A multigenerational study of the harpacticoid cope-pod *T. battag利亚i* showed that naupliar production was negatively affected by pH levels as high as 7.82 (≈400–470 ppm CO₂) (Fitzer et al., 2012). In light of this the results from the present and more short-term studies (Mayor et al., 2007, 2012; Weydmann et al., 2012) *Calanus* species may rank among the more tolerant copepods with re-gard to CO₂-induced seawater acidification. Collectively, the results so far available on *Calanus* species suggest that CO₂ levels ≤2000 ppm (the worst case CO₂ level predicted for the year 2300) is not likely to directly affect the survival of individuals from this genus.

Early life stages have been suggested to be the most vulnerable part of the life cy-cle with retards to elevated pCO₂ in marine organisms in general (Dupont et al., 2008;
Kurihara, 2008). Indeed, adult copepods have been found to be much more resistant to elevated levels of $p$CO$_2$ than eggs and nauplii (Kurihara et al., 2004; Pascal et al., 2010; Mayor et al., 2007). It has been proposed that the negative effect of elevated $p$CO$_2$ on hatching success of eggs may be caused by a reduction of intracellular pH (Kurihara, 2008). Also, the shift in energy source from endogenous yolk to exogenous food represents a critical phase that may explain much of the high mortality rate observed among the early life stages (Takahashi and Ohno, 1996). Additional stress from elevated $p$CO$_2$ levels could make this transition an even tighter bottleneck for the successful development in these animals. The only information available regarding the relative sensitivity of the early copepod stages (e.g. eggs v.s. early nauplii stages) comes from a study on Acartia erythraea (Giesbrecht) where a significant reduction in nauplii survival and hatching success was observed at 5400 and 10 400 ppm, respectively, suggesting that the first nauplii stages may be more sensitive to elevated $p$CO$_2$ than the eggs (Kurihara et al., 2004). If this is the case results from egg hatching experiments could underestimate the sensitivity to CO$_2$-acidified seawater, and nauplii survival would perhaps provide a more realistic picture of sensitivity among copepods. The results on survival observed in the present study reflect the sensitivity of the most sensitive developmental stage(s), but the experimental design does not allow the relative contribution of the different life stages to the overall mortality to be identified. More knowledge on the relative sensitivity of eggs and early nauplii stages is required since this information is of vital importance when trying to assess the sensitivity to CO$_2$-induced acidification in marine species.

Although the present study indicate that survival in C. finmarchicus may be relatively robust to $p$CO$_2$ levels $\leq$ 2000 ppm, it should be noted that the use of fertilized eggs in both the present and other studies (e.g. Mayor et al., 2012; Weydmann et al., 2012) could potentially mask any negative effect of CO$_2$-induced acidification on fertilization processes. Indeed, near future CO$_2$ levels have been found to affect fertilization processes in other invertebrate species, including the sea urchin Heliocidaris erythrogramma Valenciennes (Havenhand et al., 2008). Also, the two multigenerational...
studies on copepods available so far (Kurihara and Isimatsu, 2008; Fitzer et al., 2012), which have included in situ fertilization, showed reduced survival at pCO$_2$ levels $\leq$ 2300 ppm. Similar studies, spanning multiple generations and incorporating in situ fertilization, should also be conducted on $C. finmarchicus$ before finally concluding on the sensitivity to pCO$_2$ levels $\leq$ 2000 ppm.

The relative contribution of copepodite stages IV, V and VI to the total population in the different treatments were significantly affected in a manner that suggests a retardation of the development rate with increasing pCO$_2$ (Fig. 3). To our knowledge this is the first time effects of elevated pCO$_2$ on ontogenetic development has been reported in $Calanus$ spp. The relationship was relatively weak in the sense that no significant differences could be identified in the post hoc-tests, following the ANOVA. By the way of comparison, stage distribution was not significantly affected by long-term exposure to 2300 ppm CO$_2$ in a multiple generation study on $A. tsuensis$ (Kurihara et al., 2008). Retardation in the development may have consequences for the survival since a delayed development can lead to animals staying in more vulnerable stages for longer periods (Lopez, 1996). The reduction in development rate observed for $C. finmarchicus$ in the present study could be related to extra energetic costs associated with the induction of compensatory mechanisms in an effort to maintain a normal internal environment. This hypothesis is supported by studies that have shown that extra energy is used for compensatory responses against CO$_2$-induced stress, leaving less energy to support key biological processes such as growth and development (Wood et al., 2008; Beniash et al., 2010; Stumpp et al., 2011). The fact that only a moderate effect of CO$_2$ exposure on development rate was observed in the present study may be due to the use of ad libitum feeding conditions. This may potentially have reduced any negative effects related to a reduction of the energy budgets of the animals. Indeed, negative effects of CO$_2$ exposure on calcification were recently found to be potentiated by low algae concentration in the blue mussel $Mytilus edulis$ L., and were linked to an overall reduction of the energy budgets in the animals (Melzner et al., 2011).
In addition to development, exposure to CO$_2$ in the present study was also found to have a profound effect on stage specific morphometric characters. Exposure to 7300 and 3300 ppm CO$_2$ had opposite effects on stage specific body length (prosome length) and fat content (volume %). While exposure to 3300 ppm caused a significant increase in both length and fat content in CIV copepods, a reduced body length was apparent among stage III copepodites at 7300 ppm CO$_2$ (Fig. 4). The increase in prosome length and fat content among stage IV copepodites does not necessarily imply a positive effect of 3300 ppm CO$_2$ on performance of the animals, but is more likely an indirect consequence of a CO$_2$-induced expansion the stage duration of this copepodite. Fitzer et al. (2012) observed a marked reduction of the body length (∼25 %) in *T. battagliai*, developing under pH 7.67 (∼600 ppm CO$_2$). Recently, CO$_2$ exposure was also found to induce developmental delay in the sea urchin *Strongylocentrotus purpuratus* (Stimpson), and was linked to a reduction in the scope for growth caused by elevated metabolic rate (Stumpp et al., 2011). The authors also observed negative effects on morphological characters, but attributed these differences to an indirect effect of the delayed development (Stumpp et al., 2011).

Zooplanktons like *C. finmarchicus* are highly dependent on a fine-tuned match between their own and phytoplankton blooming events. Thus, even a moderate alteration in full life cycle developmental time, as observed in the present study, could induce a mismatch between the timing of the phytoplankton bloom and the reproduction cycle, which could ultimately have a large negative impact on the recruitment. This problem could be potentiated by associated increase in seawater temperatures. Svensson et al. (2005) demonstrated that large year to year fluctuations in spring temperatures could lead to the mismatching of larval release with phytoplankton blooming, and thus reduce the recruitment. Combination of ocean acidification and other types of stress (e.g. rising temperature, environmental contaminants) could result in more severe effects at the population- and ecosystem level than indicated from the present experiment. Although limited, studies combining ocean acidification scenarios with other stressors are starting to appear. No interaction between exposure to 1000 ppm CO$_2$...
and different temperatures (8, 10 and 12 °C) were observed on egg survival in *C. helgolandicus* (Mayor et al., 2012). In a study on the harpacticoid copepod *Amphiascoides atopus* (Lotufo and Fleeger) antagonistic effects of ocean acidification on the toxicity of Cu$^{2+}$-ions were observed, possibly due to the competition between H$^+$-ions and free Cu$^{2+}$-ions for binding sites at lower pH-levels (Pascal et al., 2010).

The microcosm system developed for the present study was capable of maintaining stable exposure conditions during the 28 day long experiment. However, the moderate volume/surface ration of the exposure bottles, combined with settling algae, made it necessary to include weekly cleaning procedures for all the experimental units. The fact that no males developed in any of the groups was probably related to the modest volume (2 L) of the bottles used for the exposure of the animals. Limited container size and/or density have previously been reported to negatively affect the proportion of developing males in *C. finmarchicus* (Campell et al., 2001). Future experiments incorporating multiple generations in a similar system should therefore use larger experimental units to secure development of males, and successful fertilization.

5 Conclusions

Although some reservations with regards to the potential negative effects on fertilization processes must be taken, the absence of any apparent effect on hatching and survival at 3300 ppm CO$_2$ makes it seem unlikely that a CO$_2$ level of ≤ 2000 ppm (worst case scenario for the end of year 2300) will directly affect the hatching success or later survival in *C. finmarchicus*. However, since effects on processes like growth and development were observed in the treatment that received seawater that were acidified by 3300 ppm CO$_2$ we cannot exclude the possibility that these processes also may be affected by a pCO$_2$ level ≤ 2000 ppm, especially if the acidification were to be combined with other forms of stress such as rising seawater temperatures or environmental contaminants. If development rate and growth is affected this could have severe impact on the recruitment due to the critical importance of timing of production cycle with alga
bloom events. This question would best be addressed through multigenerational studies where several stress factors are combined.

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References


Fitzer, S. C., Caldwell, G. S., Close, A. J., Clare, A. S., Upstill-Goddard, R. C., and Bentley, M. G.: Ocean acidification induces multi-generational decline in copepod naupliar pro-


Table 1. Carbonate system speciation in the experimental treatments. Total dissolved inorganic carbon ($C_T$), $pCO_2$ and calcium carbonate saturation state for aragonite and calcite ($\Omega_{Ca}$, $\Omega_{Ar}$) were calculated from pH and total alkalinity ($A_T$).

<table>
<thead>
<tr>
<th>pH</th>
<th>$A_T$</th>
<th>$S$</th>
<th>$T$</th>
<th>$pCO_2$</th>
<th>$C_T$</th>
<th>$\Omega_{Ca}$</th>
<th>$\Omega_{Ar}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.20 ± 0.01</td>
<td>2353</td>
<td>33</td>
<td>10</td>
<td>365 ± 9</td>
<td>2150 ± 4</td>
<td>3.60 ± 0.07</td>
<td>2.29 ± 0.04</td>
</tr>
<tr>
<td>7.31 ± 0.04</td>
<td>2353</td>
<td>33</td>
<td>10</td>
<td>3332 ± 282</td>
<td>2464 ± 15</td>
<td>0.53 ± 0.04</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>6.97 ± 0.05</td>
<td>2353</td>
<td>33</td>
<td>10</td>
<td>7281 ± 868</td>
<td>2650 ± 39</td>
<td>0.25 ± 0.03</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>6.85 ± 0.03</td>
<td>2353</td>
<td>33</td>
<td>10</td>
<td>9651 ± 597</td>
<td>2755 ± 26</td>
<td>0.19 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
</tbody>
</table>
Fig. 1. (A) Schematic outline of the experimental setup, including equilibration columns for preparation of the different CO$_2$-enriched air mixtures, gas mixer, alga addition and exposure bottles. (B) Illustration of the bottles used for the exposure, including water inlet and -outlet, air holes. (C) Illustration of the columns used for equilibration of the water, including outer- and inner tube, submersible pump, air stone introducing the different CO$_2$ enriched air mixtures into the water.
Fig. 2. Mortality recorded after 28 days of exposure to different levels of CO₂-acidified seawater. The bars show mean ± 1 std, and significant differences between control (390 ppm) and exposed groups are indicated by asterisks (*).
Fig. 3. Stage distribution (percentage) of the copepodites exposed to different levels of CO$_2$-acidified seawater. Three replicate groups at each exposure level. The bars show mean ± 1 std. Significant differences ($p < 0.05$) between the experimental groups are indicated by asterisks (*).
Fig. 4. Stage-specific prosome length and fat content (volume %) of the animals following 28 days exposure to different levels of CO$_2$-acidified seawater. The bars indicate mean ±1 std, and significant differences ($p < 0.05$) between control and exposed groups are indicated by asterisks (*).