ICES Journal of Marine Science

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

ICES Journal of Marine Science (2016), 73(3), 927–936. doi:10.1093/icesjms/fsv226

Contribution to Special Issue: 'Towards a Broader Perspective on Ocean Acidification Research'

Original Article

Ocean acidification does not alter grazing in the calanoid copepods *Calanus finmarchicus* and *Calanus glacialis*

Nicole Hildebrandt[1]*, Franz J. Sartoris[1], Kai G. Schulz[2,3], Ulf Riebesell[2], and Barbara Niehoff[1]

[1]*Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, Bremerhaven 27570, Germany*
[2]*GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, Kiel 24105, Germany*
[3]*Centre for Coastal Biogeochemistry, School of Environmental Science and Management, Southern Cross University, PO Box 157, Lismore, NSW 2480, Australia*

*Corresponding author: tel: +49 471 4831 1810; fax: +49 471 4831 1149; e-mail: nicole.hildebrandt@awi.de

Hildebrandt, N., Sartoris, F. J., Schulz, K. G., Riebesell, U., and Niehoff, B. Ocean acidification does not alter grazing in the calanoid copepods *Calanus finmarchicus* and *Calanus glacialis*. – ICES Journal of Marine Science, 73: 927–936.

Received 30 June 2015; revised 4 November 2015; accepted 5 November 2015; advance access publication 13 December 2015.

It is currently under debate whether organisms that regulate their acid–base status under environmental hypercapnia demand additional energy. This could impair animal fitness, but might be compensated for via increased ingestion rates when food is available. No data are yet available for dominant *Calanus* spp. from boreal and Arctic waters. To fill this gap, we incubated *Calanus glacialis* at 390, 1120, and 3000 µatm for 16 d with *Thalassiosira weissflogii* (diatom) as food source on-board RV *Polarstern* in Fram Strait in 2012. Every 4 d copepods were subsampled from all CO_2 treatments and clearance and ingestion rates were determined. During the SOPRAN mesocosm experiment in Bergen, Norway, 2011, we weekly collected *Calanus finmarchicus* from mesocosms initially adjusted to 390 and 3000 µatm CO_2 and measured grazing at low and high pCO_2. In addition, copepods were deep frozen for body mass analyses. Elevated pCO_2 did not directly affect grazing activities and body mass, suggesting that the copepods did not have additional energy demands for coping with acidification, neither during long-term exposure nor after immediate changes in pCO_2. Shifts in seawater pH thus do not seem to challenge these copepod species.

Keywords: *Calanus*, clearance rate, CO_2, food uptake, ingestion rate.

Introduction

Anthropogenic CO_2 emissions, which recently amounted to ~34 billion tons year^{-1} (Friedlingstein *et al.*, 2010), have increased the atmospheric CO_2 concentration since pre-industrial times from 280 to ~400 µatm (Tans, 2014). About 30% of the emitted CO_2 is absorbed by the world's oceans (Solomon *et al.*, 2007), making them the second largest sink for human-made CO_2. The CO_2 uptake changes seawater chemistry, resulting in decreasing seawater pH and carbonate ion concentrations (Raven *et al.*, 2005). Model calculations based on the IPCC emissions scenario IS92a (Alcamo *et al.*, 1995) projected that CO_2 concentrations might increase to ~750 µatm by 2100 and to almost 2000 µatm by the year 2300 (Caldeira and Wickett, 2003), with a corresponding decline in surface ocean pH by ~0.4 units until the end of the century and by a maximum of 0.77 units until 2300, a process usually referred to as "ocean acidification" (OA).

OA has the potential to severely affect the performance of marine organisms. Metabolic rates can be depressed at elevated CO_2 concentrations (Reipschläger and Pörtner, 1996; Rosa and Seibel, 2008; Seibel *et al.*, 2012), possibly triggered by a lack of or incomplete compensation for increasing pCO_2 in extracellular fluids due to environmental hypercapnia (Reipschläger and Pörtner, 1996; Pörtner *et al.*, 1998). Food uptake and growth may then be impaired and mortality may increase (Michaelidis *et al.*, 2005; Miles *et al.*, 2007; Appelhans *et al.*, 2012; Vargas *et al.*, 2013). For species that are able to actively regulate their internal acid–base balance, such as crustaceans (Widdicombe and Spicer, 2008), it is discussed that additional energy has to be allocated to maintaining the acid–base status, and therefore fitness is lowered at elevated seawater pCO_2 (Wood *et al.*, 2008; Thomsen and Melzner, 2010; Melzner *et al.*, 2011; Appelhans *et al.*, 2012, Saba *et al.*, 2012). Therefore, negative effects seem to be particularly distinct when food is

limited (Melzner *et al.*, 2011; Appelhans *et al.*, 2012), while, when food supply is sufficient, feeding rates may increase to meet the additional energy demands, as suggested for krill (Saba *et al.*, 2012) and the calanoid copepod *Centropages tenuiremis* (Li and Gao, 2012).

Calanoid copepods are key players in pelagic marine environments and often dominate zooplankton communities (Longhurst, 1985). Studies on the sensitivity of copepods to future OA show mixed responses. Levels of OA that are predicted to occur until 2300 were detrimental in four species. Dupont and Thorndyke (2008) reported that generation times in *Acartia tonsa* increased under elevated pCO_2. Cripps *et al.* (2014) showed for the same species that hatching success and nauplii survival are also sensitive to OA. In *A. spinicauda* and *C. tenuiremis*, survival of females as well as egg hatching rates was negatively affected (Zhang *et al.*, 2011), and in *Pseudocalanus acuspes*, egg production rates significantly decreased (Thor and Dupont, 2015). In all other species studied so far, parameters such as growth, egg production, hatching success and survival of nauplii, copepodites and adults were only affected at CO_2 concentrations $\geq$5000 μatm, if at all (e.g. Kurihara *et al.*, 2004; Zhang *et al.*, 2011; Mayor *et al.*, 2012; McConville *et al.*, 2013; Pedersen *et al.*, 2013; Isari *et al.*, 2015). Under controlled laboratory conditions, food uptake of calanoid copepods in response to elevated pCO_2 has only been studied in two species so far. In *C. tenuiremis*, grazing rates decreased at elevated pCO_2 (1000 μatm) during the 1 d of exposure; however, no significant effects were found thereafter (Li and Gao, 2012). In *P. acuspes*, effects of OA (~800 and 1400 μatm CO_2) on ingestion rates were non-linear, and also dependent on the geographical region and food concentration (Thor and Oliva, 2015).

In our study, we focus on two *Calanus* species, i.e. *C. finmarchicus* (Gunnerus, 1770) and *Calanus glacialis* Jaschnov (1955). Both are important components of the lipid-based foodweb of the Arctic (Falk-Petersen *et al.*, 2007 and references therein). *Calanus finmarchicus* is a North Atlantic species and is transported into the Arctic Ocean via the West Spitsbergen Current, into the Barents Sea with the North Cape Current and into Davis Strait with the West Greenland Current (e.g. Jaschnov, 1970; Conover, 1988). *Calanus glacialis* has its origin in the Arctic shelf regions and penetrates southward into Fram Strait with the East Greenland Current (Jaschnov, 1970; Conover, 1988). In areas where the warm Atlantic water submerges under the cold Arctic water, both species co-occur (Conover, 1988). During spring and summer, they inhabit surface waters where they feed on phytoplankton and accumulate large lipid stores (e.g. Marshall and Orr, 1955; Lee, 1974; Pasternak *et al.*, 2001). In late summer, they descend to deeper waters and enter a diapause to sustain the food scarce period in winter (e.g. Tande *et al.*, 1985; Hirche, 1998). The natural seawater pH the copepods experience during their seasonal migrations ranges from ~8.2 in surface waters (Charalampopoulou *et al.*, 2008) to ~7.9 in deep waters (Jutterström *et al.*, 2010).

Previous studies on the effects of OA on mortality, body mass, metabolic rates, and reproductive output of *C. finmarchicus* and *C. glacialis* indicate that copepodites V (CV) and females are robust to CO_2 levels predicted for the next centuries (Mayor *et al.*, 2007; Weydmann *et al.*, 2012; Hildebrandt *et al.*, 2014). Feeding has not been studied under controlled laboratory conditions yet; however, Pedersen *et al.* (2013) published a study on *C. finmarchicus* that were raised from eggs to adults at CO_2 concentrations of 3300–9700 μatm and showed that fat contents in CV grown at control and elevated pCO_2 were similar. We may thus hypothesize that the food uptake was not lowered by high CO_2 concentrations. It remains open, however, whether *C. finmarchicus* compensated for additional energy demands due to OA stress with an increase in grazing rates (Saba *et al.*, 2012). At more realistic surface seawater CO_2 concentrations ($\leq$1420 μatm), de Kluijver *et al.* (2013) examined trophic interactions during a 30-day mesocosm study in an Arctic fjord, using ^{13}C as a tracer. Their study found reduced rates of ^{13}C incorporation in *Calanus* spp. in high pCO_2 mesocosms, indicating that grazing rates decrease with increasing pCO_2. However, as the food quality, i.e. the algal community composition in the mesocosms, changed with the CO_2 concentration (Brussaard *et al.*, 2013; Schulz *et al.*, 2013), the decrease in grazing could reflect indirect effects of OA.

To elucidate whether *Calanus* spp. ingest more food to compensate for additional energy demands or whether ingestion rates decrease when exposed to OA, we performed controlled laboratory experiments at different pCO_2, feeding the copepods with monoalgal food (*Thalassiosira weissflogii*). Part of our study was conducted within the SOPRAN 2011 mesocosm experiment in Bergen, Norway. Here, we weekly sampled *C. finmarchicus* from mesocosms initially adjusted to 390 and 3000 μatm CO_2 and exposed the copepods of each group to both, high and low pCO_2 conditions to study immediate responses to changing pCO_2 as well as long-term effects of exposure to high pCO_2. In addition, we incubated *C. glacialis* at 390, 1120, and 3000 μatm CO_2 for 16 d in the cold-rooms on-board RV *Polarstern* and repeatedly conducted grazing experiments.

Methods

Experiments during the SOPRAN mesocosm study 2011

Sampling

In May and June 2011, experiments were conducted within the framework of the SOPRAN mesocosm experiment at Espegrend, the Marine Biological Station of the University of Bergen, Norway. Nine KOSMOS (Kiel Offshore Mesocosms for Future Ocean Simulations) mesocosms of 25 m length and 2 m diameter were deployed in the Raunefjord at N 60° 15.87′, E 005° 12.33′ for 6 weeks. For technical details on the mesocosms and their deployment, see Riebesell *et al.* (2013). Briefly, each mesocosm enclosed ~75 m^3 of fjord water containing natural plankton <3 mm. Larger mesozooplanktonic and nektonic organisms were excluded by a mesh (3 mm mesh size) that covered the openings of the mesocosm bags during deployment, as these organisms occur only in low numbers and are patchily distributed. On the lower end of each mesocosm, a sediment trap was installed to collect settling material. Ambient fjord water was aerated with pure CO_2 and added to seven of the mesocosms in five steps over 5 d to adjust the pH to ~390, ~560, ~840, ~1120, ~1400, ~2000, and ~3000 μatm CO_2, while two mesocosms were kept at *in situ* pCO_2 (~280 μatm). For our experiments, only copepods from the mesocosms with 390 (control) and 3000 μatm CO_2 (high pCO_2) were used. The pH (reported on the total scale) in the control mesocosm ranged between 8.0 and 8.1 throughout the experiment. In the high CO_2 mesocosm, the initial pH after CO_2 manipulation was ~7.2. However, due to outgassing and biological activities, it increased over time, especially in the upper water column. At the end of the experiment, the pH was 7.8 at the surface and 7.4 at depth. On Day 14, nutrients (~5 μmol l^{-1} NO_3, ~0.16 μmol l^{-1} PO_4) were added to the mesocosms to induce a phytoplankton bloom.

Once a week zooplankton was sampled with an Apstein net (mesh size 55 μm). Sampling was performed in the fjord on Day 0 and thereafter in the nine mesocosms. Sampling depth was

limited to 23 m in order not to resuspend material from the sediment traps. Within 1 h, the plankton samples were brought to a cold room adjusted to 10°C, which was the approximate *in situ* temperature at that time.

Preparation of experimental water and food algae

Filtered seawater was adjusted to target values of 390 (control) and 3000 µatm CO_2 (high CO_2) by mixing with filtered CO_2 saturated fjord water. The pH was monitored with a pH electrode (Mettler Toledo InLab Routine Pt1000, connected to a pH meter WTW pH 3310), which was calibrated with NIST buffers (pH 6.865 and 9.180). Then the pH was converted to total scale (pH_T) using TRIS-based reference material (Batch no. 4, A. Dickson, Scripps Institution of Oceanography). Dissolved inorganic carbon (DIC) in the experimental water was measured with a Technicon Analyzer TrAAcs 800 (Seal Analytical GmbH, Germany). pH_T and DIC were then used to calculate DIC using the programme CO2SYS (Lewis and Wallace, 1998). Mean pH values of control and high CO_2 treatments were 7.9 (592 ± 42 µatm pCO_2) and 7.2 (3202 ± 521 µatm pCO_2), respectively (Table 1). Temperature and salinity were determined with a conductivity meter WTW Cond340i (Table 1). Monocultures of the diatom *T. weissflogii* were grown in f/2 medium (Guillard, 1975) at 10 °C under constant light as food for the copepods.

Determination of grazing rates

About 60 *C. finmarchicus* copepodites V (CV) were sorted from the control and high CO_2 mesocosm sample, respectively. To eliminate the effect of possibly different feeding conditions in the mesocosms on grazing rates during the experiments, the copepods were pre-incubated for 1 d at 10°C in filtered seawater adjusted to the respective CO_2 concentration and containing *T. weissflogii* in excess.

For the determination of grazing rates, high and control CO_2 water was inoculated with *T. weissflogii* at a concentration of 4000 cells ml^{-1}. Thirty copepods each from pre-incubation at control and pre-incubation at high pCO_2 were transferred to 1 l bottles (10 copepods $bottle^{-1}$) containing seawater of the respective pCO_2. To test whether immediate changes in CO_2 concentration affect the food uptake, 30 copepods from pre-incubation at control seawater pH were placed in three 1 l bottles containing high CO_2 water, and 30 copepods from the pre-incubation at high pCO_2 were transferred to 1 l bottles with control seawater. Three additional bottles for each CO_2 concentration were prepared that contained seawater with algae but no copepods. These bottles were run as a blank to correct the grazing rates of the copepods for algal growth. After transfer of algal suspensions and copepods, all bottles were immediately sealed airtight and mounted to a plankton wheel for 17.5 to 22 h in the dark. After the experiment, the copepods were sorted from all bottles, rinsed in distilled water and stored in groups of two to six in tin caps at −20°C to measure C and N content. Subsamples of 3 × 100 ml of the experimental water for each CO_2 treatment were filtered on GF/F filters and frozen to determine the chlorophyll *a* (chl *a*) concentration before and at the end of the experiments.

Prosome length of *C. finmarchicus* was measured under a stereomicroscope with an ocular micrometre (error ± 5 µm) in 36 individuals sampled from the fjord at the beginning of the study.

On-board incubation experiment

Copepod sampling and incubation

The experiment with *C. glacialis* was conducted in July 2012 during a cruise with RV *Polarstern* to Fram Strait (ARK-XXVII/1 and 2). Copepods were sampled with vertical bongo net hauls (200 and 300 µm mesh size, 0–250 m) at approximately N 79° 40.3′, W 011° 59.3′ (PS 80/91) and immediately brought to a cooling container adjusted to 0°C. About 1350 *C. glacialis* CV were sorted from the samples and acclimated to laboratory conditions in filtered seawater enriched with *T. weissflogii* for 2 d.

Preparation of incubation water and food algae

Filtered seawater was adjusted to 1120 (intermediate) and 3000 µatm (high) CO_2 by bubbling with gas mixtures from a gas cylinder (purchased from Air Liquide Deutschland GmbH, Düsseldorf). Untreated filtered seawater was used as a control (390 µatm). The resulting pH values (Table 1) differed at least by 0.3 units between control and intermediate and between intermediate and high CO_2 treatments. There was no possibility to measure DIC samples onboard, and as storage of such samples for a period of several months, until the return of RV *Polarstern* to Bremerhaven, is not recommended, we did not measure parameters other than pH. However, preliminary tests have shown that bubbling with the used air/CO_2 mixes yield the desired seawater pCO_2. *T. weissflogii* was cultured according to the experiment in 2011, but at temperatures of 10 and 18°C. Before it was fed to the copepods, the algal suspension was adjusted to the incubation temperature.

CO_2 incubation

The copepods were transferred to 6 l glass bottles and incubated at control, intermediate and high CO_2 for 16 d. Three bottles, each containing 150 *C. glacialis*, were set up for each CO_2 treatment. The copepods were fed daily with *T. weissflogii* at a concentration of 8000 cells ml^1 to prevent food limitation as these large bottles could not be mounted on a plankton wheel and therefore part of the algae sank to the bottom. Every 4 d, the incubation water was completely changed and pH, salinity, and temperature were measured. pH values increased on average by 0.19, 0.13, and 0.06

Table 1. Water parameters during the determination of grazing rates (a) and during the CO_2 incubations on RV *Polarstern* in 2012 (b).

	Species	CO_2 treatment (µatm)	Temp. (°C)	Salinity (psu)	pH (total scale)	*N*
(a)	*C. finmarchicus*	390	10.0 ± 0.3	32.4 ± 0.2	7.86 ± 0.03	4
	C. finmarchicus	3000	9.9 ± 0.2	32.4 ± 0.4	7.16 ± 0.07	4
	C. glacialis	390	1.1 ± 0.7	32.4 ± 1.2	7.97 ± 0.06	5
	C. glacialis	1120	1.0 ± 0.7	32.9 ± 0.5	7.62 ± 0.04	5
	C. glacialis	3000	1.0 ± 0.7	32.7 ± 0.7	7.21 ± 0.05	5
(b)	*C. glacialis*	390	1.4 ± 0.3	33.2 ± 0.4	7.86 ± 0.13	24
	C. glacialis	1120	1.3 ± 0.4	33.1 ± 0.4	7.54 ± 0.09	24
	C. glacialis	3000	1.3 ± 0.4	33.3 ± 0.4	7.21 ± 0.07	24

Values are presented as mean ± SD. Temp., temperature; *N*, number of measurements throughout the experiments/incubations.

units in between water exchanges at 390, 1120, and 3000 μatm CO_2, respectively. When the water was changed, 10 copepods from each bottle were removed to determine grazing rates. Thus, the number of copepods in the bottles decreased over time. At start and at the end of the CO_2 incubation, 36–66 copepods from each CO_2 treatment were measured under a stereomicroscope with an ocular micrometre (prosome length) then deep-frozen individually for C and N measurements.

Determination of grazing rates

Thirty copepods from each of the three different CO_2 treatments were transferred to three 1 l bottles (10 copepods bottle^{-1}) with seawater of the respective pCO_2. *T. weissflogii* was added at a concentration of 2000 cells ml^{-1}. Along with three blanks for each CO_2 concentration (see above), the bottles were mounted to a plankton wheel for 21.5–25 h. Subsamples for chl *a* measurements as well as copepod C and N samples were taken as described above.

Sample analysis

To determine the chl *a* concentration in the incubation bottles and blank bottles at the start and the end of the grazing experiments, the algal cells on the GF/F filters were first disrupted in 90% acetone using ultrasound (Branson Sonifier 250, Heinemann, Schwäbisch Gmünd, Germany). Then, the chl *a* was extracted from the algal cells for 2 h in the dark at 5°C. After centrifugation at 4500 rpm for 15 min at 0°C, the chl *a* fluorescence in the supernatant was determined with a Turner fluorometer (TD-700, Turner Designs, Sunnyvale, CA, USA) at 665 nm before and after adding two drops of 1 N HCl. Clearance rates (CRs) and ingestion rates (IRs) of the copepods were then calculated after Frost (1972).

On some days, initial chl *a* concentrations were significantly different among CO_2 treatments (*C. finmarchicus*: Day 8; *C. glacialis*: Days 1 and 8). In order not to bias the results of the grazing experiments, we excluded the grazing rates that were measured on these days from our analyses.

To determine carbon and nitrogen content, the copepods in the tin caps were dried at 60°C for 48 h then combusted with subsequent gas chromatographic separation by an elemental analyser (Euro EA, HEKAtech GmbH, Wegberg, Germany). Acetanilide was used as a standard.

Statistics

Statistical analyses were performed with SigmaStat 3.5 (Systat Software, Inc.). A *t*-test or a one-way ANOVA followed by a *post hoc* Holm-Sidak test was performed to identify differences in grazing and body mass between copepods from different CO_2 treatments at each experimental day. A Spearman rank-order correlation was used to identify changes in body carbon and nitrogen during the experiments, and to test for correlation between CR/IR and the initial chl *a* content in the incubation water to determine whether our food concentrations limited grazing rates. Data were considered significantly different at a $p < 0.05$. Results are presented as mean ± standard deviation.

Results

Mean initial chl *a* concentrations ranged between 7.8 and 9.6 μg l^{-1} in the grazing experiments with *C. finmarchicus* CV from Bergen and between 3.9 and 6.2 μg l^{-1} in the experiments on-board RV *Polarstern* with *C. glacialis* CV (Table 2). The concentrations were significantly different among days but not among the pCO_2 treatments at a specific day. At the end of the grazing experiment, chl *a* concentrations were still high at 5.6–9.5 (*C. finmarchicus*) and 1.4–4.3 μg l^{-1} (*C. glacialis*).

In *C. finmarchicus* collected from the Raunefjord on Day 0 of the mesocosm experiment, clearance and ingestion rates at control pCO_2 were 2.2 ± 0.7 ml copepod^{-1} h^{-1} and 0.018 ± 0.005 μg chl *a* copepod^{-1} h^{-1}, respectively (Figure 1). On Days 15, 22, and 28, *C. finmarchicus* were collected from the control (390 μatm) and high pCO_2 mesocosm (initially 3000 μatm), and grazing rates were determined at the respective CO_2 concentrations. Mean CR (Figure 1a) and IR (Figure 1b) were similar to the control values measured at Day 0. The pCO_2 generally did not affect the grazing rates. Only during the experiment on Day 28, CR and IR were significantly lower in copepods from the control than from the high pCO_2 mesocosm (Table 3). Neither CR nor IR correlated with the initial

Table 2. Chlorophyll *a* concentration (mean ± SD, $n = 3$) in the experimental water at the start and at the end of grazing experiments with *Calanus* spp. as well as in blank bottles.

			Chlorophyll *a* (μg l^{-1})		
Species	**Day**	**CO_2 treatment**	**Start**	**End**	**Blank (end)**
C. finmarchicus	0	390	9.6 ± 0.3	9.5 ± 0.9	12.0 ± 3.6
	15	390	7.9 ± 0.1	5.6 ± 0.6	8.5 ± 1.4
	15	3000	7.8 ± 0.1	6.0 ± 0.4	8.8 ± 0.4
	22	390	9.0	5.8 ± 0.7	8.6 ± 2.5
	22	3000	8.7	6.6 ± 1.1	9.8 ± 0.7
	28	390	9.3	9.5 ± 0.4	11.0 ± 0.6
	28	3000	9.4	9.0 ± 1.5	12.0 ± 0.5
C. glacialis	4	390	3.9 ± 0.3	1.4 ± 0.3	3.6 ± 0.3
	4	1120	4.1 ± 0.2	1.7 ± 0.3	3.9 ± 0.1
	4	3000	3.9 ± 0.2	1.6 ± 0.4	3.7 ± 0.3
	12	390	4.7 ± 0.2	3.1 ± 0.4	5.2 ± 0.3
	12	1120	5.1 ± 0.3	3.7 ± 0.5	5.6 ± 0.4
	12	3000	4.7 ± 0.2	4.0 ± 0.6	5.2 ± 0.2
	16	390	6.0 ± 0.2	3.7 ± 0.7	5.9 ± 0.7
	16	1120	6.2 ± 0.5	4.3 ± 0.1	6.2 ± 0.1
	16	3000	5.4 ± 0.6	3.1 ± 0.5	5.9 ± 0.4

On Days 22 and 28 (*C. finmarchicus*), there was only one start measurement for chlorophyll *a*.

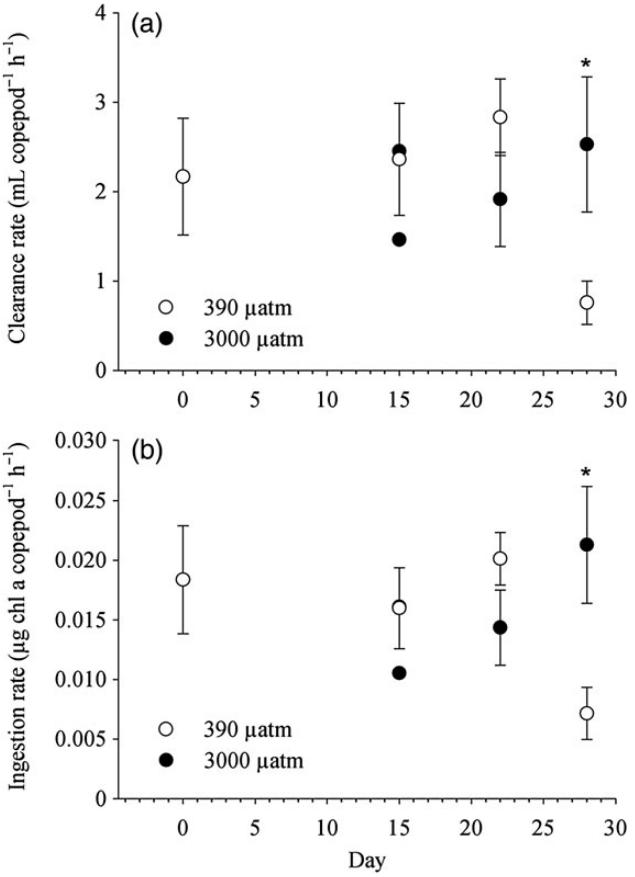

Figure 1. Long-term response of *C. finmarchicus* CV to OA: clearance rate (a) and ingestion rate (b) at control (white circles) and high pCO_2 (black circles). Data on Day 0 present only control measurements at low pCO_2, conducted before the adjustment of the pH in the mesocosms. Data are presented as mean ± standard deviation. Asterisk marks significant differences between CO_2 treatments.

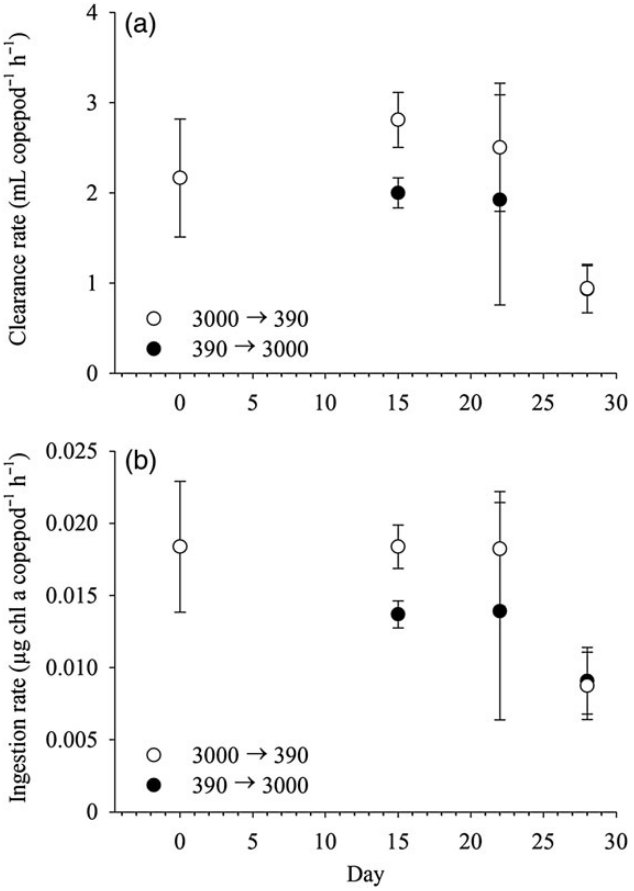

Figure 2. Immediate response of *C. finmarchicus* CV to OA: clearance rate (a) and ingestion rate (b) of copepods originating in the high CO_2 mesocosm that were incubated at control pCO_2 (white circles) and of copepods originating in the control mesocosm that were incubated at high pCO_2 (black circles). The data on Day 0 present only control measurements at low pCO_2, conducted before the adjustment of the pH in the mesocosms. Data are presented as mean ± standard deviation, except for Day 15, where only two data points were available for the high pCO_2 treatment. Here, both data points are shown separately.

Table 3. Results of the one-way ANOVAs to test for differences in grazing rates of *C. finmarchicus* and *C. glacialis* among different CO_2 treatments.

Species	Parameter	Day	d.f.	*F*	*p*	Power
C. finmarchicus	CR	15	3	1.985	0.205	0.172
		22	3	1.064	0.417	0.057
		28	**3**	**10.701**	**0.004**	**0.955**
	IR	15	3	2.342	0.160	0.222
		22	3	1.254	0.353	0.080
		28	**3**	**12.954**	**0.002**	**0.983**
C. glacialis	CR	4	2	0.212	0.815	0.050
		12	2	2.244	0.187	0.178
		16	2	3.626	0.093	0.335
	IR	4	2	2.134	0.200	0.166
		12	2	2.435	0.168	0.200
		16	2	2.586	0.155	0.217

Values presented in bold indicate significant differences in grazing rates among CO_2 treatments. CR, clearance rates; IR, ingestion rates. In *C. finmarchicus*, the analysis compares data from both long-term and immediate response groups.

chl *a* content in the incubation water ($p > 0.05$), indicating that food concentrations did not limit grazing.

Also sudden changes in pCO_2 did not affect the grazing activity (Figure 2, Table 3). In copepods transferred from control to high CO_2 and *vice versa*, CR and IR at Days 15 and 22 were again similar to the rate measured on Day 0, whereas lower rates (CR: 0.9 ml copepod^{-1} h^{-1}, IR: 0.009 µg chl *a* copepod^{-1} h^{-1}) were measured on Day 28. Again, no correlation was found between CR or IR and the initial chl *a* content ($p > 0.05$).

The mean C and N contents of *C. finmarchicus* CV (2.4 ± 0.1 mm prosome length) from the control mesocosms were 161 and 19 µg, respectively (Table 4), and did not change significantly over time (Table 5). In copepods from the high CO_2 mesocosm, the C and N contents were significantly lower when compared with copepods from the control mesocosm on Days 8 and 15 (*t*-test, $0.001 \leq p \leq 0.008$). On the last two sampling days, however, C and N had increased, and no differences in body mass were found between copepods from high and control mesocosms (Tables 4 and 5).

In *C. glacialis* CV from the Fram Strait (Figure 3), mean clearance rates were 1.4–4.7 ml copepod^{-1} h^{-1}, and ingestion rates varied from 0.006 to 0.013 µg chl *a* copepod^{-1} h^{-1} independent from CO_2 conditions (Table 3). There was no correlation between IR and the initial chl *a* concentration, while for copepods incubated

Table 4. Carbon and nitrogen contents of *C. finmarchicus* CV sampled from control and high CO_2 mesocosms.

	Control CO_2			High CO_2		
Day	C (µg)	N (µg)	*n*	C (µg)	N (µg)	*n*
0	151 ± 34	19 ± 2	19	151 ± 34	19 ± 2	19
8	167 ± 26	19 ± 2	11	134 ± 17	17 ± 1	8
15	167 ± 26	19 ± 2	12	123 ± 23	16 ± 2	12
22	162 ± 12	19 ± 1	12	158 ± 12	18 ± 1	12
28	164 ± 25	18 ± 2	12	169 ± 26	19 ± 2	12

Values are presented as mean ± standard deviation. C, carbon content; N, nitrogen content; *n*, number of samples. For each sample, 2–6 copepods (usually 5) were pooled. In the control CO_2 group, all copepods initially sampled from the control mesocosm are included (i.e. also copepods from the immediate response group which were transferred to high pCO_2 during the grazing experiments). Likewise, in the high CO_2 group, all copepods initially sampled from the high CO_2 mesocosm are represented.

Table 5. Spearman rank-order correlation analyses between carbon and nitrogen content in *C. finmarchicus* CV and time during 28 d of the SOPRAN mesocosm experiment 2011.

[CO_2] (µatm)	Parameter	Correlation coefficient	*p*
390	C	0.167	0.180
3000	C	0.256	**0.043**
390	N	0.019	0.881
3000	N	0.139	0.275

Values presented in bold indicate significant correlations between C and N content and time.
[CO_2], CO_2 concentration; C, carbon content; N, nitrogen content.

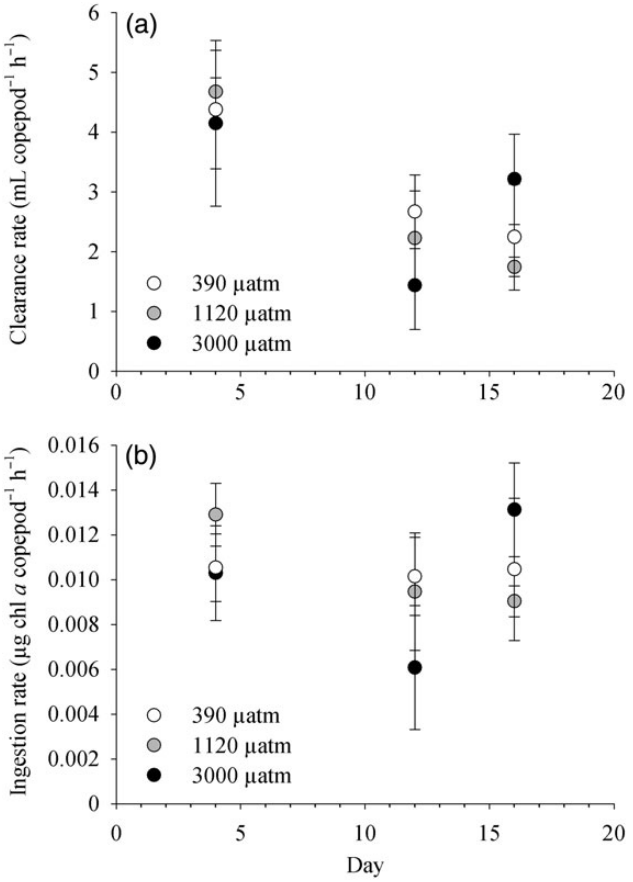

Figure 3. Response of *C. glacialis* CV to OA: clearance rate (a) and ingestion rate (b) at control (white circles), intermediate (grey circles), and high pCO_2 (black circles). Data are presented as mean ± standard deviation.

at 390 and 1120 µatm CO_2, a negative correlation between CR and chl *a* was found ($p = 0.009$). This indicates that food uptake was not limited during the grazing experiments but copepods at 390 and 1120 µatm CO_2 had to filter more water to ingest the same amount of algae compared with copepods from 3000 µatm.

The C and N contents of *C. glacialis* (3.5 ± 0.1 mm prosome length) at the start of the incubation were 646 and 56 µg, respectively (Table 6). Over time, the N content increased significantly by 30–40% at all CO_2 concentrations. Also the carbon content increased, however, significantly only in copepods kept at 1120 and 3000 µatm CO_2 (Table 7). The pCO_2 did not have significant effects on body mass, with two exceptions. On Day 15, the C content in copepods from 390 µatm was significantly lower when compared with the other CO_2 treatments (Holm-Sidak test, $p \leq 0.004$), whereas on Day 16 copepods from 1120 µatm had significantly more body carbon than those from 3000 µatm CO_2 ($p = 0.011$).

Discussion

Ocean acidification (OA) has been shown to influence feeding rates of marine organisms. In the common sea star *Asterias rubens*, for example, which does not compensate for elevated extracellular pCO_2 under environmental hypercapnia, feeding rates are lower when exposed to OA than under normocapnic conditions (Appelhans *et al.*, 2012). Also in larval sea urchins, i.e. *Strongylocentrotus droebachiensis* and *Dendraster excentricus*, and in larvae of the gastropod *Concholepas concholepas*, food uptake was significantly impaired by elevated seawater pCO_2 (Dupont and Thorndyke, 2008; Chan *et al.*, 2011; Vargas *et al.*, 2013). Other species regulate the extracellular pH, such as the shore crab *Carcinus maenas*. Feeding in this species was reduced at high when compared with low pCO_2 during a 10-week incubation experiment, but not in short-term feeding assays (Appelhans *et al.*, 2012). The authors suggested that during long-term exposure additional energy had to be allocated to acid–base regulation, which affected energy demanding processes related to feeding such as digestion or prey handling (Appelhans *et al.*, 2012). In contrast, the Antarctic krill *Euphausia superba* ingested more food at elevated pCO_2 (Saba *et al.*, 2012), and this study discussed that the increase in feeding compensated for elevated energetic needs due to acid–base regulation. Li and Gao (2012) came to a similar conclusion in their study on the copepod *Centropages tenuiremis*, although the differences in grazing rates were not significant. In *Pseudocalanus acuspes* from the Svalbard area, elevated CO_2 concentrations led to increased ingestion rates at intermediate and high food concentrations; however, respiration rates were not significantly affected by pCO_2 (Thor and Oliva, 2015).

Calanus glacialis was incubated at relatively stable CO_2 concentrations throughout our experiments, simulating a realistic scenario that is projected to occur in about the year 2100 (1120 µatm) and an extreme scenario (3000 µatm) that is not realistic for surface waters (Caldeira and Wickett, 2003). *Calanus finmarchicus* were sampled from large-scale mesocosms which were initially adjusted to 390 and 3000 µatm CO_2. In the high CO_2 mesocosm, the pH increased by 0.2–0.6 units over time, depending on the water depth, and thus, *C. finmarchicus* in this mesocosm was not kept at a stable pCO_2.

Table 6. Prosome length, carbon, and nitrogen contents of *C. glacialis* CV incubated at different CO_2 concentrations.

	390 µatm CO_2			1120 µatm CO_2			3000 µatm CO_2		
Day	**C (µg)**	**N (µg)**	***n***	**C (µg)**	**N (µg)**	***n***	**C (µg)**	**N (µg)**	***n***
0	646 ± 168	56 ± 12	36	646 ± 168	56 ± 12	36	646 ± 168	56 ± 12	36
1	789 ± 124	69 ± 9	**9**	674 ± 105	63 ± 7	**9**	687 ± 55	63 ± 4	**10**
4	704 ± 137	64 ± 9	**6**	743 ± 134	71 ± 11	**6**	629 ± 94	61 ± 8	**9**
8	713 ± 74	70 ± 5	**8**	736 ± 63	71 ± 6	**6**	702 ± 108	62 ± 22	**9**
12	719 ± 93	72 ± 9	**9**	747 ± 60	66 ± 22	**9**	672 ± 104	65 ± 8	**8**
15	693 ± 159	71 ± 18	44	788 ± 140	77 ± 17	41	783 ± 132	76 ± 16	43
16	725 ± 85	73 ± 7	**9**	778 ± 58	78 ± 7	**9**	685 ± 67	72 ± 8	**9**

Values are presented as mean ± standard deviation.
C, carbon content; N, nitrogen content; *N*, number of samples; usually 3–4 copepods were pooled for analyses (*n* highlighted in bold); single copepods measurements are indicated by regular numbers.

Table 7. Spearman rank-order correlation analyses between carbon and nitrogen content in *C. glacialis* CV and time during 16 d of incubation on-board RV *Polarstern* in 2012.

[CO_2] (µatm)	**Parameter**	**Correlation coefficient**	***p***
390	C	0.117	0.200
1120	C	0.391	**<0.001**
3000	C	0.299	**<0.001**
390	N	0.471	**<0.001**
1120	N	0.625	**<0.001**
3000	N	0.596	**<0.001**

[CO_2], CO_2 concentration; C, carbon content; N, nitrogen content. Values presented in bold indicate significant correlations between C and N content and time.

There were, however, marked differences in pH between control and high CO_2 mesocosms throughout the study, which allowed for detecting possible changes in feeding activity due to OA.

Ingestion rates are generally sigmoidally related to the food concentration (e.g. Kiørboe *et al.*, 1982; Urban-Rich *et al.*, 2004). Thus, a compensation for elevated energy demands via increased feeding rates is likely only detected under non-limiting food concentrations, as was the case during our study. The final chl *a* content in the incubation bottles at the end of the grazing experiments with *C. finmarchicus* ranged between 5.6 and 9.5 µg l^{-1} (Table 2), which is higher than typically found during spring phytoplankton blooms in western Norwegian coastal areas and the Norwegian Sea (Meyer-Harms *et al.*, 1999; Larsen *et al.*, 2004; Husa *et al.*, 2014) and also above the peak of the artificially induced phytoplankton bloom in the mesocosms which ranged between 3 and 5 µg l^{-1} chl *a*. During the shipboard experiments with *C. glacialis*, final chl *a* concentrations were between 1.4 and 4.3 µg l^{-1} (Table 2), which is similar to bloom conditions in Arctic waters (Wu *et al.*, 2007; Cherkasheva *et al.*, 2014). Furthermore, in both species, IR were not significantly correlated with the chl *a* content, while correlations between CR and chl *a* were either negative or not significant. This also indicates that algae were provided in excess and the copepods were not food limited throughout the grazing experiments. In the 6 l CO_2 incubation bottles on-board RV *Polarstern*, we did not monitor chl *a*. However, we provided algal concentrations which were ~4 times higher than during the incubation for measuring grazing rates while the copepod densities were at maximum only 2.5 times higher when compared with the grazing experiments. In addition, C and N contents of *C. glacialis* increased and we therefore believe that the copepods were not food limited during the long-term incubation.

High food concentrations, on the other hand, might bear the risk that grazing rates of the copepods are already at or close to the maximum physiological feeding capacity. This would hinder the copepods to increase their feeding rates as a response to OA stress. However, Urban-Rich *et al.* (2004) found ingestion rates of up to 0.04 µg chl *a* copepod^{-1} h^{-1} for *C. finmarchicus* CIV and CV while the rates during our experiment did not exceed 0.027 µg chl *a* copepod^{-1} h^{-1}. Also for *C. glacialis*, higher CR and IR than measured in our study were reported (Tande and Båmstedt, 1985). We thus believe that, despite high food supply, the copepods would have been able to increase their grazing rates if environmental hypercapnia had led to additional energy demands.

Our data, however, indicate that elevated seawater pCO_2 did not affect the grazing activity of *C. finmarchicus* and *C. glacialis*, neither after sudden changes in pCO_2 nor after 2–4 weeks of exposure. We found significant differences in grazing between copepods from control and high pCO_2 treatments only on one occasion and there was no consistent trend, neither to higher nor lower grazing activity under OA scenarios. All copepods from control and high pCO_2 treatments were fed with diatoms, i.e. *Thalassiosira weissflogii*, which were grown at control conditions. The copepods thus received food organisms of the same quality. De Kluijver *et al.* (2013) inferred from a calculation of the amount of incorporated ^{13}C during a mesocosm study in an Arctic fjord that the food uptake of *Calanus* spp. decreased at elevated CO_2 concentrations. Their approach, however, addressed both direct and indirect effects of OA, as CO_2 affected the algal community composition in the mesocosms (Brussaard *et al.*, 2013; Leu *et al.*, 2013; Schulz *et al.*, 2013) and therefore the food quality for the copepods.

When ingestion rates are similar, differences in body weight may serve as another indicator for changes in energy demands due to elevated pCO_2, i.e. the copepod body mass may increase to a lesser extent or even decrease if coping with OA stress is energetically costly. In our laboratory experiments, however, the body mass of *C. glacialis* kept at low, medium, and high CO_2 levels was not significantly different and, thus, there was no indication for additional energy demands. Only during the mesocosm experiments, we found significantly lower body mass in *C. finmarchicus* CV from the high pCO_2 mesocosm on 2 d (Days 8 and 15). This may be attributed to lower food quality in high pCO_2 mesocosms as the phytoplankton community changed with pCO_2 (J. R. Bermúdez Monsalve (GEOMAR), pers. comm.). At day 14, nutrients were added, which induced a phytoplankton bloom (KGS and UR, unpublished data). The food availability thus improved in all mesocosms and this could explain why there were no differences in body mass at later sampling dates.

In conclusion, we did not find direct effects of elevated pCO_2 on the copepods grazing rates and body mass, neither in *C. finmarchicus* nor in *C. glacialis*, suggesting that the energy demand of these two *Calanus* species does not increase when exposed to future OA. The mechanisms responsible for the tolerance to OA in *C. finmarchicus* and *C. glacialis*, as was also reported in other studies (Mayor *et al.*, 2007; Weydmann *et al.*, 2012; Niehoff *et al.*, 2013; Hildebrandt *et al.*, 2014), remain unknown. Just recently, a seasonal study on *C. glacialis* has shown that this species strongly regulates its extracellular acid–base status, exhibiting extracellular pH (pH_e) values as low as 5.5 during winter (i.e. during diapause) and high pH_e (7.9) during summer (Freese *et al.*, 2015). Similar mechanisms were also reported from two diapausing Antarctic copepod species (Sartoris *et al.*, 2010; Schründer *et al.*, 2013). Compensating for comparably small shifts in seawater pH due to environmental hypercapnia might therefore not be a challenge for these copepods.

Acknowledgements

We thank J. Büdenbender, D. Freese, and all scientists on site for their help in copepod sampling and sorting during the SOPRAN mesocosm study. Furthermore, we acknowledge the support of S. Koch Klavsen and A. Ludwig in chlorophyll *a* measurements during the mesocosm study and of M. Bullwinkel who measured DIC. N. Knüppel and the officers and crew of RV *Polarstern* are thanked for their help in copepod sampling in Fram Strait. E. Allhusen kindly provided algae cultures. Two anonymous reviewers are thanked for their valuable comments which have improved our manuscript.

Funding

Financial support was provided by the Federal Ministry of Education and Research (BMBF) through the projects SOPRAN (FKZ 03F0611A) and BIOACID (FKZ 03F0608).

References

Alcamo, J., Bouwman, A., Edmonds, J., Grübler, A., Morita, T., and Sugandhy, A. 1995. An evaluation of the IPCC IS92 emission scenarios. In Climate Change 1994, pp. 247–304. Ed. by J. T. Houghton, L. G. Meira Filho, J. Bruce, H. Lee, B. A. Callander, E. Haites, N. Harris, *et al.* Cambridge University Press, Cambridge, New York, Melbourne. 339 pp.

Appelhans, Y. S., Thomsen, J., Pansch, C., Melzner, F., and Wahl, M. 2012. Sour times: seawater acidification effects on growth, feeding behaviour and acid-base status of *Asterias rubens* and *Carcinus maenas*. Marine Ecology Progress Series, 459: 85–97.

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A., and Riebesell, U. 2013. Arctic microbial community dynamics influenced by elevated CO_2 levels. Biogeosciences, 10: 719–731.

Caldeira, K., and Wickett, M. E. 2003. Anthropogenic carbon and ocean pH. Nature, 425: 365.

Chan, K. Y. K., Grünbaum, D., and O'Donnell, M. J. 2011. Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. The Journal of Experimental Biology, 214: 3857–3867.

Charalampopoulou, A., Poulton, A. J., Tyrrell, T., and Lucas, M. 2008. Surface seawater carbonate chemistry, nutrients and phytoplankton community composition on a transect between North Sea and Arctic Ocean. doi:10.1594/PANGAEA.763990.

Cherkasheva, A., Bracher, A., Melsheimer, C., Köberle, C., Gerdes, R., Nöthig, E.-M., Bauerfeind, E., *et al.* 2014. Influence of the physical environment on polar phytoplankton blooms: a case study in the Fram Strait. Journal of Marine Systems, 132: 196–207.

Conover, R. J. 1988. Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. Hydrobiologia, 167/168: 127–142.

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

De Kluijver, A., Soetaert, K., Czerny, J., Schulz, K. G., Boxhammer, T., Riebesell, U., and Middelburg, J. J. 2013. A ^{13}C labeling study on carbon fluxes in Arctic plankton communities under elevated CO_2 levels. Biogeosciences, 10: 1425–1440.

Dupont, S., and Thorndyke, M. C. 2008. Ocean acidification and its impact on the early life-history stages of marine animals. CIESM Workshop Monographs, 36: 89–97.

Falk-Petersen, S., Pavlov, V., Timofeev, S., and Sargent, J. R. 2007. Climate variability and possible effects on arctic food chains: The role of *Calanus*. In Arctic Alpine Ecosystems and People in a Changing Environment, pp. 147–166. Ed. by J. B. Ørbæk, R. Kallenborn, I. Tombre, E. N. Hegseth, S. Falk-Petersen, and A. H. Hoel. Springer, Berlin, Heidelberg. 434 pp.

Freese, D., Niehoff, B., Søreide, J. E., and Sartoris, F. J. 2015. Seasonal patterns in extracellular ion concentrations and pH of the Arctic copepod *Calanus glacialis*. Limnology and Oceanography, 60: 2121–2129.

Friedlingstein, P., Houghton, R. A., Marland, G., Hackler, J., Boden, T. A., Conway, T. J., Canadell, J. G., *et al.* 2010. Update on CO_2 emissions. Nature Geoscience, 3: 811–812.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnology and Oceanography, 17: 805–815.

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. In Culture of Marine Invertebrate Animals, pp. 26–60. Ed. by W. L. Smith, and M. H. Chanley. Plenum Press, New York. 338 pp.

Hildebrandt, N., Niehoff, B., and Sartoris, F. J. 2014. Long-term effects of elevated CO_2 and temperature on the Arctic calanoid copepods *Calanus glacialis* and *C. hyperboreus*. Marine Pollution Bulletin, 80: 59–70.

Hirche, H.-J. 1998. Dormancy in three *Calanus* species (*C. finmarchicus*, *C. glacialis* and *C. hyperboreus*) from the North Atlantic. Archiv fur Hydrobiologie Special Issues Advances in Limnology, 52: 359–369.

Husa, V., Kutti, T., Ervik, A., Sjøtun, K., Hansen, P. K., and Aure, J. 2014. Regional impact from fin-fish farming in an intensive production area (Hardangerfjord, Norway). Marine Biology Research, 10: 241–252.

Isari, S., Zervoudaki, S., Saiz, E., Pelejero, C., and Peters, J. 2015. Copepod vital rates under CO_2-induced acidification: a calanoid species and a cyclopoid species under short-term exposures. Journal of Plankton Research, 37: 912–922.

Jaschnov, W. A. 1970. Distribution of *Calanus* species in the seas of the northern hemisphere. Internationale Revue der Gesamten Hydrobiologie und Hydrographie, 55: 197–212.

Jutterström, S., Anderson, L. G., Bates, N. R., Bellerby, R., Johannessen, T., Jones, E. P., Key, R. M., *et al.* 2010. Arctic Ocean data in CARINA. Earth System Science Data, 2: 71–78.

Kiørboe, T., Møhlenberg, F., and Nicolajsen, H. 1982. Ingestion rate and gut clearance in the planktonic copepod *Centropages hamatus* (Lilljeborg) in relation to food concentration and temperature. Ophelia, 21: 181–194.

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

Larsen, A., Fonnes Flaten, G. A., Sandaa, R.-A., Castberg, T., Thyrhaug, R., Erga, S. R., Jacquet, S., *et al.* 2004. Spring phytoplankton bloom dynamics in Norwegian coastal waters: microbial community succession and diversity. Limnology and Oceanography, 49: 180–190.

Lee, R. F. 1974. Lipid composition of the copepod *Calanus hyperboreas* from the Arctic ocean. Changes with depth and season. Marine Biology, 26: 313–318.

Leu, E., Daase, M., Schulz, K. G., Stuhr, A., and Riebesell, U. 2013. Effect of ocean acidification on the fatty acid composition of a natural plankton community. Biogeosciences, 10: 1143–1153.

Lewis, E., and Wallace, D. W. R. 1998. CO2SYS – program developed for the CO_2 system calculations. Report ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, TN, USA. 21 pp.

Li, W., and Gao, K. 2012. A marine secondary producer respires and feeds more in a high CO_2 ocean. Marine Pollution Bulletin, 64: 699–703.

Longhurst, A. R. 1985. The structure and evolution of plankton communities. Progress in Oceanography, 15: 1–35.

Marshall, S. M., and Orr, A. P. 1955. On the biology of *Calanus finmarchicus* VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. Journal of the Marine Biological Association of the United Kingdom, 34: 495–529.

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

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

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

Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S. N., *et al.* 2011. Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. PLoS ONE, 6: e24223.

Meyer-Harms, B., Irigoien, X., Head, R., and Harris, R. 1999. Selective feeding on natural phytoplankton by *C. finmarchicus* before, during, and after the 1997 spring bloom in the Norwegian Sea. Limnology and Oceanography, 44: 154–165.

Michaelidis, B., Ouzounis, C., Paleras, A., and Pörtner, H. O. 2005. Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. Marine Ecology Progress Series, 293: 109–118.

Miles, H., Widdicombe, S., Spicer, J. I., and Hall-Spencer, J. 2007. Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. Marine Pollution Bulletin, 54: 89–96.

Niehoff, B., Schmithüsen, T., Knüppel, N., Daase, M., Czerny, J., and Boxhammer, T. 2013. Mesozooplankton community development at elevated CO_2 concentrations: results from a mesocosm experiment in an Arctic fjord. Biogeosciences, 10: 1391–1406.

Pasternak, A., Arashkevich, E., Tande, K., and Falkenhaug, T. 2001. Seasonal changes in feeding, gonad development and lipid stores in *Calanus finmarchicus* and *C. hyperboreus* from Malangen, northern Norway. Marine Biology, 138: 1141–1152.

Pedersen, S. A., Hansen, B. H., Altin, D., and Olsen, A. J. 2013. Medium-term exposure of the North Atlantic copepod *Calanus finmarchicus* (Gunnerus, 1770) to CO_2-acidified seawater: effects on survival and development. Biogeosciences, 10: 7481–7491.

Pörtner, H. O., Reipschläger, A., and Heisler, N. 1998. Acid-base regulation, metabolism and energetics in *Sipunculus nudus* as a function of ambient carbon dioxide level. Journal of Experimental Biology, 201: 43–55.

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., *et al.* 2005. Ocean acidification due to increasing atmospheric carbon dioxide. Policy document 12/05, The Royal Society, London, UK. 60 pp.

Reipschläger, A., and Pörtner, H. O. 1996. Metabolic depression during environmental stress: the role of extracellular versus intracellular pH in *Sipunculus nudus*. The Journal of Experimental Biology, 199: 1801–1807.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., *et al.* 2013. Technical note: a mobile sea-going mesocosm system – new opportunities for ocean change research. Biogeosciences, 10: 1835–1847.

Rosa, R., and Seibel, B. A. 2008. Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proceedings of the National Academy of Sciences of the United States of America, 105: 20776–20780.

Saba, G. K., Schofield, O., Torres, J. J., Ombres, E. H., and Steinberg, D. K. 2012. Increased feeding and nutrient excretion of adult Antarctic krill, *Euphausia superba*, exposed to enhanced carbon dioxide (CO_2). PLoS ONE, 7: e52224.

Sartoris, F. J., Thomas, D. N., Cornils, A., and Schnack-Schiel, S. B. 2010. Buoyancy and diapause in Antarctic copepods: the role of ammonium accumulation. Limnology and Oceanography, 55: 1860–1864.

Schründer, S., Schnack-Schiel, S. B., Auel, H., and Sartoris, F. J. 2013. Control of diapause by acidic pH and ammonium accumulation in the hemolymph of Antarctic copepods. PLoS ONE, 8: e77498.

Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., *et al.* 2013. Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide. Biogeosciences, 10: 161–180.

Seibel, B. A., Maas, A. E., and Dierssen, H. M. 2012. Energetic plasticity underlies a variable response to ocean acidification in the pteropod, *Limacina helicina antarctica*. PLoS ONE, 7: e30464.

Solomon, S., Quin, D., Manning, M., Alley, R. B., Berntsen, T., Bindoff, N. L., Chen, Z., *et al.* 2007. Technical summary. In Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, pp. 20–91. Ed. by S. Solomon, D. Quin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, *et al.* Cambridge University Press, Cambridge and New York. 996 pp.

Tande, K. S., and Båmstedt, U. 1985. Grazing rates of the copepods *Calanus glacialis* and *C. finmarchicus* in arctic waters of the Barents Sea. Marine Biology, 87: 251–258.

Tande, K. S., Hassel, A., and Slagstad, D. 1985. Gonad maturation and possible life cycle strategies in *Calanus finmarchicus* and *Calanus glacialis* in the northwestern part of the Barents Sea. In Marine Biology of the Polar Regions and Effects of Stress on Marine Organisms, pp. 141–155. Ed. by J. S. Gray, and M. E. Christiansen. John Wiley & Sons Ltd, New York. 660 pp.

Tans, P. 2014. Trends in Atmospheric Carbon Dioxide. NOAA/ESRL, Boulder, CO, USA. http://www.esrl.noaa.gov/gmd/ccgg/trends/ (last accessed 23 November 2015).

Thomsen, J., and Melzner, F. 2010. Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis*. Marine Biology, 157: 2667–2676.

Thor, P., and Dupont, S. 2015. Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. Global Change Biology, 21: 2261–2271.

Thor, P., and Oliva, E. O. 2015. Ocean acidification elicits different energetic responses in an Arctic and a boreal population of the copepod *Pseudocalanus acuspes*. Marine Biology, 162: 799–807.

Urban-Rich, J., McCarty, J. T., and Shailer, M. 2004. Effects of food concentration and diet on chromophoric dissolved organic matter accumulation and fluorescent composition during grazing experiments with the copepod *C.* finmarchicus. ICES Journal of Marine Sciences, 61: 542–551.

Vargas, C. A., de la Hoz, M., Aguilera, V., San Martin, V., Manríquez, P. H., Navarro, J. M., Torres, R., *et al.* 2013. CO_2-driven ocean acidification reduces larval feeding efficiency and change food selectivity in the mollusc *Concholepas concholepas*. Journal of Plankton Research, 35: 1059–1068.

Weydmann, A., Søreide, J. E., Kwasniewski, S., and Widdicombe, S. 2012. Influence of CO2-induced acidification on the reproduction

of a key Arctic copepod *Calanus glacialis*. Journal of Experimental Marine Biology and Ecology, 428: 39–42.

Widdicombe, S., and Spicer, J. I. 2008. Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? Journal of Experimental Marine Biology and Ecology, 366: 187–197.

Wood, H. L., Spicer, J. I., and Widdicombe, S. 2008. Ocean acidification may increase calcification rates, but at a cost. Proceedings of the Royal Society B, 275: 1767–1773.

Wu, Y., Peterson, I. K., Tang, C. C. L., Platt, T., Sathyendranath, S., and Fuentes-Yaco, C. 2007. The impact of the sea ice on the initiation of the spring bloom in the Newfoundland and Labrador Shelves. Journal of Plankton Research, 29: 509–514.

Zhang, D., Li, S., Wang, G., and Guo, D. 2011. Impacts of CO_2-driven seawater acidification on survival, egg production rate and hatching success of four marine copepods. Acta Oceanologica Sinica, 30: 86–94.

Handling editor: Stéphane Plourde