ICES Journal of Marine Science

ICES Journal of Marine Science (2016), 73(3), 650–658. doi:10.1093/icesjms/fsv078

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

Original Article

Lack of evidence for elevated CO_2-induced bottom-up effects on marine copepods: a dinoflagellate – calanoid prey – predator pair

Stamatina Isari[1,2]*, Soultana Zervoudaki[1], Janna Peters[3], Georgia Papantoniou[4], Carles Pelejero[2,5], and Enric Saiz[2]

[1]*Institute of Oceanography, Hellenic Centre for Marine Research, PO 712, 46.7 km Avenue Athens-Sounio, 19013 Anavyssos, Athens, Greece*
[2]*Institut de Ciències del Mar (CSIC), Pg. Marítim de la Barceloneta 37-49, 08003 Barcelona, Catalonia, Spain*
[3]*Institute for Hydrobiology and Fisheries Science, Hamburg University, Grosse Elbstrasse 133, 22767 Hamburg, Germany*
[4]*Department of Biology, University of Athens, Panepistimioupoli, Ilisia, 15701 Athens, Greece*
[5]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Catalonia, Spain*

**Corresponding author: tel: +30 2291076374; fax: +30 2291076347; e-mail: misari@hcmr.gr.*

Isari, S., Zervoudaki, S., Peters, J., Papantoniou, G., Pelejero, C., and Saiz, E. Lack of evidence for elevated CO_2-induced bottom-up effects on marine copepods: a dinoflagellate – calanoid prey – predator pair. – ICES Journal of Marine Science, 73: 650–658.

Received 16 January 2015; revised 8 April 2015; accepted 9 April 2015; advance access publication 6 May 2015.

Rising levels of atmospheric CO_2 are responsible for a change in the carbonate chemistry of seawater with associated pH drops (acidification) projected to reach 0.4 units from 1950 to 2100. We investigated possible indirect effects of seawater acidification on the feeding, fecundity, and hatching success of the calanoid copepod *Acartia grani*, mediated by potential CO_2-induced changes in the nutritional characteristics of their prey. We used as prey the autotrophic dinoflagellate *Heterocapsa* sp., cultured at three distinct pH levels (control: 8.17, medium: 7.96, and low: 7.75) by bubbling pure CO_2 via a computer automated system. *Acartia grani* adults collected from a laboratory culture were acclimatized for 3 d at food suspensions of *Heterocapsa* from each pH treatment (ca. 500 cells ml^{-1}; 300 μg C l^{-1}). Feeding and egg production rates of the preconditioned females did not differ significantly among the three *Heterocapsa* diets. Egg hatching success, monitored once per day for the 72 h, did not reveal significant difference among treatments. These results are in agreement with the lack of difference in the cellular stoichiometry (C : N, C : P, and N : P ratios) and fatty acid concentration and composition encountered between the three tested *Heterocapsa* treatments. Our findings disagree with those of other studies using distinct types of prey, suggesting that this kind of indirect influence of acidification on copepods may be largely associated with interspecific differences among prey items with regard to their sensitivity to elevated CO_2 levels.

Keywords: *Acartia grani*, bottom-up, copepods, dinoflagellates, food quality, *Heterocapsa*, ocean acidification, pH.

Introduction

Climate-driven changes in the marine environment have been largely associated with the anthropogenic rise of the atmospheric CO_2 concentration, projected to increase by a factor of 1.1–2.4 by the end of the century (IPCC, 2013; levels in 2011: 391 ppm). Elevated CO_2 in the atmosphere is driving, in addition to global warming, a concurrent change in the seawater carbonate chemistry and an associated pH drop that could reach up to 0.4 pH units from years 1950 to 2100 (IPCC, 2013), widely known as ocean acidification (OA).

A significant amount of data have been obtained in the last decade related to the OA impact on several groups of marine organisms [as reviewed by Fabry *et al.* (2008); Doney *et al.* (2009); Dupont *et al.* (2010); Hofmann *et al.* (2010); Koch *et al.* (2013)]. Among plankton, research interest has mainly focused on calcifying primary producers, given the anticipated impact of high CO_2/low pH on the processes of photosynthesis and calcification (e.g. Riebesell *et al.*, 2000; Van de Waal *et al.*, 2013). Copepods, comprising the major group of secondary producers in the planktonic foodweb, have been typically considered relatively tolerant of a

lowering in seawater pH due to the lack of calcite/aragonite formations in their exoskeleton. This has been verified in several occasions, in studies where the copepod physiological performance was tested at CO_2 levels projected at the end of the century (Zhang *et al.*, 2011; Mayor *et al.*, 2012; Weydmann *et al.*, 2012; McConville *et al.*, 2013; Zervoudaki *et al.*, 2014) or even in long-time exposures at CO_2 levels rising far beyond the near-future projections (Kurihara and Ishimatsu, 2008; Hildebrandt *et al.*, 2014). Sublethal effects may be of relevance under extreme acidification regimes related with deep ocean CO_2 sequestration (e.g. Kurihara *et al.*, 2004; Mayor *et al.*, 2007; Zhang *et al.*, 2011; Cripps *et al.*, 2014a). Some recent studies, however, report a high sensitivity of copepods even at near-future OA levels (Lewis *et al.*, 2013; Thor and Dupont, 2015), and others suggest that the most experimental works might have underestimated the actual direct effect of OA on copepods due to the inadequacy of the experimental approaches used (Cripps *et al.*, 2014a, b).

In addition to reconsidering potential biases in the estimation of the direct OA effects on copepods, future research should also take into deeper account the fact that CO_2-induced changes in seawater chemistry may influence copepod performance not only in a direct way (i.e. by changing the acid–base regulation of their physiological and metabolic functions), but also indirectly through foodweb "bottom-up" effects. Climate-related changes in the quantitative and qualitative properties of lower trophic level organisms may be transferred to upper trophic levels through the food chain, but this indirect OA effect has been only poorly investigated for copepods. Food quality is a determinant factor for copepod performance (e.g. Jónasdóttir, 1994; Koski *et al.*, 1998; Jones *et al.*, 2002; Klein Breteler *et al.*, 2005; Isari *et al.*, 2013) and interestingly, a couple of recent works have emphasized a negative indirect OA effect on copepods (e.g. a decrease in egg production or/and growth and development) through a deterioration of the nutritional quality of their prey when grown under high CO_2 (Rossoll *et al.*, 2012; Schoo *et al.*, 2013).

There are several evidences for CO_2-induced changes in algal quality (Burkhardt and Riebesell, 1997; Burkhardt *et al.*, 1999; Urabe *et al.*, 2003; Rossoll *et al.*, 2012; Schoo *et al.*, 2013), but the response patterns show a profound species/strain-dependence. The underlying physiological mechanisms and metabolic pathways for this variability are not yet well understood, but the exposure of algae to high CO_2 levels is often accompanied by a downregulation of the genes associated with cellular CO_2-concentrating mechanisms (CCMs; e.g. Ratti *et al.*, 2007; Crawfurd *et al.*, 2011; Van de Waal *et al.*, 2013, 2014); the CCMs are responsible for the increase of free CO_2 concentration near the RuBisCO (ribulose1,5-bisphosphate carboxylase/oxygenase) enzyme, involved in the carbon fixation (Giordano *et al.*, 2005; Hopkinson *et al.*, 2011). Such shifts in gene expression and protein production are assumed to save energy and elemental resources and can be further expressed through changes in algal physiology and elemental and biochemical properties (Giordano *et al.*, 2005). Ultimately, the final outcome will depend on the efficiency of RuBisCO and CCMs in each species (Reinfelder, 2011).

In this study, in contrast to the flagellate and diatom preys used by Schoo *et al.* (2013) and Rossoll *et al.* (2012), respectively, we assess the bottom-up effects of OA on copepods offering as a prey a dinoflagellate species. Dinoflagellates constitute a major prey for copepods in most of the oceans (Saiz and Calbet, 2011); in addition, this group is expected to particularly benefit under increased CO_2 levels, due to their less efficient RuBisCO (form II) and their high dependence on CCMs (Reinfelder, 2011). Our hypothesis is that a higher CO_2 supply under OA conditions could facilitate the growth and improve the nutritional quality of dinoflagellates, and therefore enhance copepod productivity. To test our hypothesis, we cultured the armored dinoflagellate *Heterocapsa* sp. at three pCO_2/pH levels representative of the projected values at the end of the century (IPCC, 2013), then examined the response of the copepod *Acartia grani* in terms of feeding on dinoflagellate and reproduction.

Methods

Target copepod species

Eggs from a culture of the calanoid copepod *A. grani* maintained at the Institut de Ciències del Mar (CSIC) were collected, hatched, and reared to adulthood at 18 ± 1°C and a salinity of 37.4. Throughout their development, the copepods were fed *ad libitum* a suspension of the Cryptophyte alga *Rhodomonas salina* grown exponentially in f/2 medium. Only adult female copepods that had matured within the previous week were used in the experiments.

Dinoflagellate culture conditions

The marine autotrophic dinoflagellate *Heterocapsa* sp. was grown in batch culture amended with nutrients (f/2 medium; Guillard, 1975), and 250-ml aliquots of exponentially growing cells (ca. 60 000 cells ml^{-1}) were inoculated into 13-l Nalgene polycarbonate bottles containing 5 l of autoclaved 0.2-μm filtered seawater (salinity 37.4). These algal cultures were exposed to three distinct pH_{NBS} (National Bureau of Standards pH scale) levels (control: 8.17, medium: 7.96, and low: 7.75 pH units), adjusted by injections of pure CO_2 through a solenoid valve controlled automatically by an Aqua-medic™ computer automated system with a plastic shafted pH electrode. During the night-time, CO_2-free air was bubbled in the cultures (using a home-made filter filled with soda lime, Sigma Aldrich) to compensate for the decrease of pH due to algal respiration. The small number and size of the bubbles produced by the gas/air injections precluded any harmful mechanical effect on the algae. Algal cultures were maintained for a period of 16 days under these conditions in a cold room (18 ± 1°C) with 18 : 6 h light : dark cycle (150 μmol m^{-2} s^{-1}), and cell number and volume were daily monitored *in vivo* with an Multisizer III Coulter Counter. Growth rate (μ, d^{-1}) was calculated as the slope of the least-squares linear regression of ln(*Ni*) vs. time, where *Ni* was the cell concentration at time *i*. The division rate (k, doublings d^{-1}) was calculated as $k = \mu/\ln 2$. For the first 6 d, the algal cultures were grown exponentially until reaching a concentration of 10 000 cells ml^{-1}, sufficient to provide enough algae for the experiments with copepods. Thereafter, and based on the estimated algal growth rate, the cultures were kept in a semi-continuous phase at a stable culture biomass of ca. 10 000 cells ml^{-1} by daily removing a certain volume of culture and its replacement with f/2 medium.

Seawater chemistry

Throughout the experiment, we took the seawater pH readings of our computer automated system in our experimental treatments every 30 min. The accuracy of the pH measurements performed by the electrodes connected to the automated system (which we never removed from the closed culture bottles to avoid contamination) was daily verified, immediately after culture sampling, with an Metrohm 826 pH meter fitted with a glass electrode (6.0257.600, Aquatrode plus, Metrohm), equipped with temperature sensor, and

previously calibrated with buffer solutions of pH_{NBS} 7 and 9. Salinity was measured daily using an YSI-30 M/10FT probe, and total alkalinity (TA) was analysed on day 13 by potentiometric titration (Perez and Fraga, 1987; Perez *et al.*, 2000). Calculations of the other parameters of the carbonate system were performed from TA and pH using the CO_2SYS program (Pierrot *et al.*, 2006), with dissociation constants for carbonate determined by Mehrbach *et al.* (1973) and refit by Dickson and Millero (1987), whereas the dissociation constants of sulphuric acid were taken from Dickson (1990) and total boron from Lee *et al.* (2010).

Algal elemental (C : N : P) and fatty acid compositions

We assessed the elemental (C, N, and P) and fatty acid (FA) compositions of the algae grown under the different CO_2/pH conditions on the 15th day of growth of the algal culture. Triplicate (30 ml for C–N analysis, 20 ml for P analysis) and tetraplicate (40 ml for FAs) aliquots of known cell concentration were filtered onto pre-combusted 25-mm Whatman GF/C filters (450°C, 6 h). Samples for C and N analysis were dried for 24 h at 60°C and kept in a desiccator until analysis (FLASH 2000 Thermo Scientific CHNS analyser). Filters for particulate phosphorus and FAs were frozen at −80°C immediately after filtration. For phosphorus determination, filters were subjected to orthophosphate acid persulphate oxidation and posterior analysis as inorganic phosphorus (Grasshoff *et al.*, 1999). For the FA analysis, lipid extraction and conversion of acyl groups into fatty acid methyl ester derivatives (FAMES) were performed after Peters *et al.* (2013) using modified protocols of Folch *et al.* (1957) and Kattner and Fricke (1986). Total lipids were extracted using ultrasonic disruption of the cells in dichloromethane : methanol (2 : 1, v/v) and a washing procedure with aqueous KCl solution (0.88%). For quantification of FAMES, tricosanoic acid was added as an internal standard before extraction. Acyl groups were converted into FAMEs using methanolic sulphuric acid (3%) at 80°C for 4 h. After adding Milli-Q water, FAMEs were extracted by washing the polar phase three times with hexane. FAMES and fatty alcohols were separated by gas chromatography (column DB-FFAP, programmable temperature vaporizer injector, and solvent vent mode), detected by flame ionization, and identified by comparing retention times with those derived from standards of known composition. Only compounds with >0.2% of total FAs and alcohols were included in the dataset. Statistical data analyses of FAs were performed with the 12 most important FAs (defined as those with values >1% of tFA), either as absolute or relative content (in the latter case, after arcsine square root transformation).

Copepod experiments

The impact of the three different diets (algae grown at the respective CO_2/pH treatments) on the feeding and reproductive output of the copepod *A. grani* was assessed after a 3-d preconditioning period at the specific diets. Food suspensions at 500 cells ml^{-1} (ca. 300 $\mu g\ C\ l^{-1}$) at each CO_2/pH level were prepared by diluting the respective algal stock cultures with filtered seawater (FSW) pre-equilibrated at the respective pH, either by bubbling CO_2 (medium and low pH treatments) or CO_2-free air (control treatment). Pyrex screw-cap bottles (2300 ml) were filled with the suspensions and groups of 20 females and 12 males (to ensure fertilization) were added for preconditioning. During this period, the copepods were daily transferred to fresh suspensions (discarding dead animals if present). After the conditioning period, four replicated groups (4 × Cop) of five females were placed in 625 ml Pyrex screw-cap bottles filled with the respective food suspensions; five additional bottles with food suspensions but without the addition of copepods served as initial (1 × Init) and control (4 × Contr) bottles. Initial bottles were sacrificed immediately at the beginning of the incubation, and subsamples withdrawn and preserved with 2% Lugol's acetic solution; the remaining bottles were incubated for 24 h at 18°C under the light conditions where the algae had been growing to determine the copepod feeding and egg production rates on each respective diet. After the incubation time, the pH of the bottle contents was first checked (no substantial change was observed), then they were carefully poured onto a 200- and 20-μm meshes to collect the females and the eggs, respectively. The number and condition of the copepods were checked (mortality was negligible), and subsamples of the food suspensions were preserved as described above. A proportion (40%) of the eggs laid in each bottle was transferred into Petri dishes filled with FSW equilibrated at the parental incubation pH, sealed with plastic covers (no bubbles left), and left undisturbed to determine hatching success; the rest of the eggs for each bottle were preserved in Lugol's acetic solution for posterior counting. The number of unhatched eggs was monitored daily for 3 d, and the hatching rate was calculated. To follow the pH throughout the hatching incubation, pH measurements were taken every 24 h in separate Petri dishes filled with pCO_2 equilibrated FSW (but no eggs), sealed similarly to the ones used for the hatching test.

Algal concentration in the feeding experiment samples was determined microscopically, and clearance and ingestion rates were computed according to Frost (1972). Assuming that C and N content of eggs were 0.046 and 0.0091 $\mu g\ egg^{-1}$, respectively (Kiørboe *et al.*, 1985), and calculating the egg phosphorus content applying the Redfield N : P ratio (16 : 1 similar to 16.5 : 1 of *A. grani* females from our culture), we estimated the respective elemental copepod gross-growth efficiencies (GGEs; %) for each treatment by dividing the egg production and ingestion rates expressed at the respective elemental units (carbon: GGE_C; nitrogen: GGE_N; phosphorus: GGE_P).

Results

Seawater carbonate chemistry

Overall, the level of pH in the algal cultures was maintained rather stable throughout the entire period (Figure 1), except for the medium pH treatment that exhibited larger deviations (control: 8.17 ± 0.02; medium pH: 7.93 ± 0.07; low pH: 7.75 ± 0.02; n = 16 d; NBS scale). Alkalinity, which was measured only on the 13th day of the experiment, was found similar between treatments (Table 1). The corresponding pCO_2 calculated from pH and

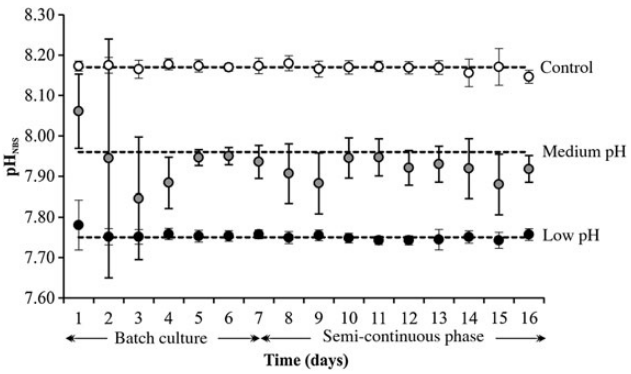

Figure 1. Daily average pH in the three *Heterocapsa* sp. cultures during the experimental period. The error bars indicate the *SD* in each 24 h period (n = 48 pH readings).

Table 1. Parameters of the seawater carbonate system in each pH treatment of the *Heterocapsa* sp. cultures on the 13th day of the experiment, the only day in which alkalinity was measured (averaged values with *SD* in parenthesis).

pH treatment	pH_{NBS}	pH_T	TA	pCO_2	χCO_2	DIC	HCO_3^-	CO_3^{2-}	Ω_A
Control	8.17 (0.02)	8.05 (0.02)	2454 (0.73)	414 (0.60)	423 (0.13)	2174 (0.67)	1962 (0.60)	199 (0.06)	3.025 (0.001)
Medium	7.93 (0.04)	7.81 (0.04)	2487 (0.80)	798 (0.26)	814 (0.27)	2328 (0.77)	2175 (0.72)	127 (0.04)	1.930 (0.001)
Low	7.74 (0.03)	7.62 (0.03)	2492 (0.19)	1296 (0.10)	1323 (0.11)	2410 (0.19)	2281 (0.18)	86 (0.01)	1.307 (0.000)

Total alkalinity (TA, μmol kg^{-1}-SW; $n = 3$), pH_{NBS} ($n = 48$), salinity (37.4), and temperature (18.5°C) were used to calculate the rest of the parameters using the CO_2SYS program (Pierrot *et al.*, 2006): pCO_2, partial pressure of CO_2 (μatm, $n = 3$); χCO_2, mole fraction of CO_2 in dry air (ppm, $n = 3$); DIC, dissolved inorganic carbon (μmol kg^{-1}-SW; $n = 3$); HCO_3^-, bicarbonate ion concentration (μmol kg^{-1}-SW; $n = 3$); CO_3^{2-}, carbonate ion concentration (μmol kg^{-1}-SW; $n = 3$), Ω_A, saturation state of seawater with respect to aragonite ($n = 3$). pH_T, pH in the total scale ($n = 48$) is also reported for better comparison with other studies, and it was calculated using the $SWCO_2$ package (Hunter, 2007).

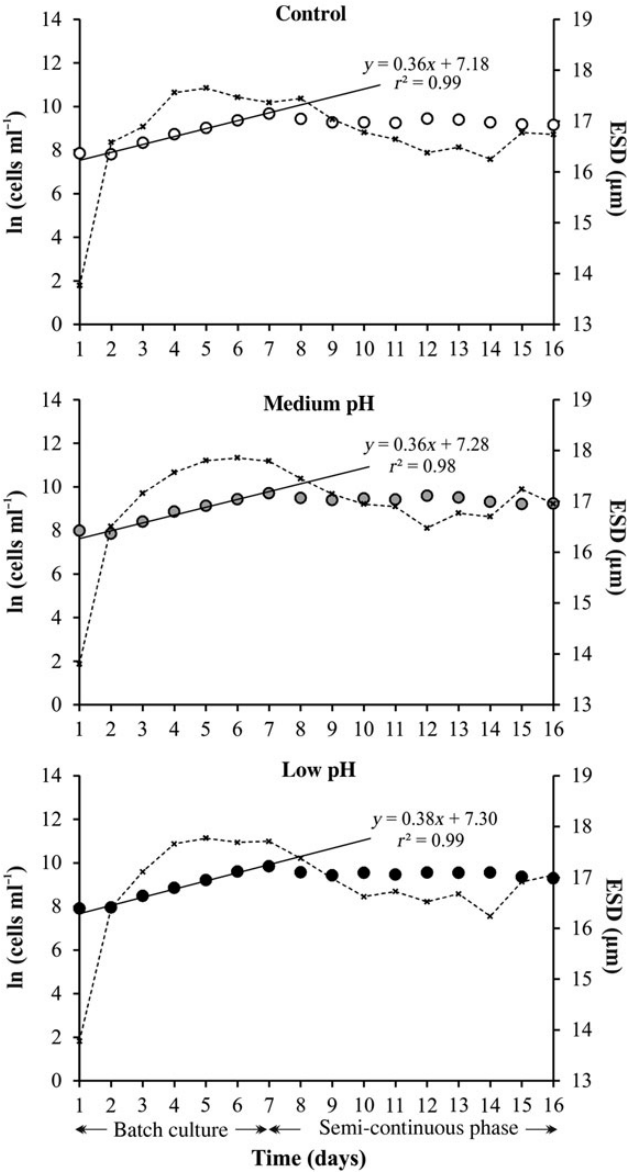

Figure 2. Cell concentration (circles) and ESD (crosses and dotted lines) of *Heterocapsa* sp. cultures grown at the three selected pCO_2/pH conditions. The slope of regression line corresponds to the growth rate for each treatment.

alkalinity reached 798 and 1296 μatm in the medium and low pH treatments, respectively, fitting well possible values projected for the end of the century (IPCC, 2013).

Algal growth, and elemental and FA composition

The growth rates (d^{-1}) of *Heterocapsa* sp. were similar among the pH treatments (control: 0.36, medium pH: 0.36, and low pH: 0.38; Figure 2), with no signs of cell damaging due to the CO_2/air bubbling. Given a ca. 2-d generation time, the total experimental period of 16 d corresponded to eight cell generations, which should be sufficient to condition the organisms to the experimental conditions. Throughout the algal growth period, the cell size varied similarly among the pH treatments (Figure 2). During the copepod incubation, which corresponded to the last 4 d of algal culture, the average equivalent spherical diameter (ESD, μm) of the cells was similar (control: 16.6 ± 0.24, medium pH: 16.9 ± 0.24, and low pH: 16.7 ± 0.36).

The elemental characteristics of the cells grown at the different pH treatments are summarized in Table 2. Cells cultured in the medium pH conditions had slightly higher biovolume (9%) and elemental content (carbon: 8%, nitrogen: 10%, and phosphorus: 3%) compared with the control treatment, whereas the molar elemental ratios (C : N, C : P, and N : P) did not differ significantly. Cells grown at the low pH conditions presented similar elemental composition characteristics to those of the control treatment. The comparison between the cells grown in medium and low pH conditions revealed that the former had slightly higher biovolume, nitrogen content, and lower C : N than the latter (Table 2). Regarding the FA composition of *Heterocapsa* sp., the saturated 16 : 0 and polyunsaturated FAs (PUFAs) were the main contributors, accounting for 18 and 63% of total FAs, respectively. Among PUFAs, the 18:4(n-3) (12% of total FA, 7–8 pg $cell^{-1}$), 18:5(n-3) (18–19% of total FA, 11–12 pg $cell^{-1}$), as well as 22:6(n-3) (20% of total FA, 12–13 pg $cell^{-1}$) isomers comprised together ca. 61–62% of the total FA (Table 3). The overall variation in FA composition between treatments was very small. Low but significant differences occurred in the relative amount as well as content of monounsaturated FAs (MUFAs) in the low pH treatment, caused by lower 16:1(n-7) and 18:1(n-9) isomer levels. The total FA content as well as the 18:3(n-3) and 22:6(n-3) isomer contents were significantly higher in the medium pH treatment, but the differences were, however, of low magnitude (~10% of the respective value).

Copepod feeding, reproduction, and egg hatching success

The average food concentration was ca. 500 cells ml^{-1} in all treatments ($F = 0.95$, $p > 0.05$; control: 512 ± 22, medium pH: 531 ± 18, and low pH: 532 ± 29). Neither feeding rates nor egg production rates of the copepod *A. grani* presented any significant differences among the three pH treatments (clearance rates 28–30 ml cop^{-1} d^{-1}: $F = 0.29$, $p = 0.76$; ingestion rates 15 895–15 812 cells cop^{-1} d^{-1}: $F = 0.31$, $p > 0.05$; egg production rates 49–54 eggs cop^{-1} d^{-1}: $F = 1.81$, $p > 0.05$; Figure 3). Although ingestion rates in terms of elemental

Table 2. Cell biovolume, elemental composition (C: carbon, N: nitrogen, and P: phosphorus), and molar elemental ratios of the distinct *Heterocapsa* sp. cultures grown at the three selected pH levels (control: 8.17, medium: 7.96, and low: 7.75).

					t-test		
	Control	**Medium**	**Low**	***F***	**Control vs. medium**	**Control vs. low**	**Medium vs. low**
Biovolume (μm^3)	2468.3^{a} (65.6)	2678.5^{b} (2.4)	2528.7^{a} (39.9)	19.2^{***}			
pg C $cell^{-1}$	581.7^{a} (24.7)	626.6^{b} (13.8)	$592.7^{a,b}$ (8.4)	5.6^{*}			
pg N $cell^{-1}$	82.1^{a} (2.5)	90.1^{b} (0.8)	80.2^{a} (1.3)	29.0^{**}			
pg P $cell^{-1}$	17.8^{a} (1.0)	18.4^{b} (0.9)	$16.2^{a,b}$ (0.7)	5.3^{ns}			
C : N (molar)	8.3 (0.2)	8.1 (0.1)	8.6 (0.1)		$0.99^{n.s.}$	$-2.24^{n.s.}$	-5.73^{**}
C : P (molar)	84.3 (5.0)	87.8 (4.6)	94.4 (4.0)		$-0.89^{n.s.}$	$-2.73^{n.s.}$	$-1.88^{n.s.}$
N : P (molar)	10.2 (0.6)	10.8 (0.6)	11.0 (0.5)		$-1.36^{n.s.}$	$-1.76^{n.s.}$	$-0.31^{n.s.}$

Differences among treatments were assessed with one-way ANOVA and Tukey *post hoc* tests (*F*: ANOVA statistics, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s.: not significant) as well as Student's *t*-test. Numbers in parentheses correspond to the *SD* and superscripts indicate the homogenous groups according to the Tukey *post hoc* test.

Table 3. FA content (pg $cell^{-1}$) and relative FA composition (% of total FA) for *Heterocapsa* sp. cultures grown at the three selected pH levels (control: 8.17, medium: 7.96, and low: 7.75).

pg $cell^{-1}$	**Control**	**Medium pH**	**Low pH**	***F***
14:0	2.5 (0.4)	3.1 (0.4)	3.1 (0.9)	$1.3^{n.s.}$
16:0	10.6 (0.8)	11.9 (0.5)	10.8 (0.9)	$3.7^{n.s.}$
18:0	1.3 (0.1)	1.4 (0.2)	1.2 (0.1)	$2.6^{n.s.}$
16:1(n-7)	$0.9^{a,b}$ (0.1)	1.1^{b} (0.3)	0.6^{a} (0.1)	8.4^{**}
16:1(n-9)	0.8 (0.1)	0.8 (0.0)	0.7 (0.1)	$1.4^{n.s.}$
18:1(n-7)	1.3^{a} (0.0)	1.4^{a} (0.1)	1.0^{b} (0.0)	146.6^{***}
18:1(n-9)	1.9^{a} (0.1)	2.0^{a} (0.1)	1.6^{b} (0.1)	35.8^{***}
18:2(n-6)	$4.0^{a,b}$ (0.2)	4.3^{b} (0.3)	3.8^{a} (0.1)	8.2^{**}
18:3(n-3)	2.2^{a} (0.1)	2.5^{b} (0.1)	2.1^{a} (0.1)	20.3^{***}
18:4(n-3)	7.2^{a} (0.3)	8.0^{b} (0.5)	7.2^{a} (0.4)	6.1^{*}
18:5(n-3)	11.1 (0.5)	12.3 (0.7)	11.1 (0.6)	$4.9^{n.s.}$
22:6(n-3)	11.6^{a} (0.3)	13.2^{b} (0.5)	11.9^{a} (0.6)	77.7^{**}
tFA	58.2^{a} (2.9)	64.9^{b} (2.6)	57.6^{a} (3.3)	7.6^{*}
MUFAs	4.9^{a} (0.3)	5.3^{a} (0.2)	3.9^{b} (0.2)	44.3^{***}
PUFAs	36.8^{a} (1.3)	41.0^{b} (2.1)	36.7^{a} (1.8)	7.9^{*}
% of total FA				
14:0	4.3 (0.5)	4.7 (0.5)	5.3 (1.2)	$1.8^{n.s.}$
16:0	18.2 (0.6)	18.4 (0.2)	18.8 (0.6)	$1.6^{n.s.}$
18:0	2.2 (0.1)	2.2 (0.2)	2.1 (0.2)	$0.1^{n.s.}$
16:1(n-7)	$1.5^{a,b}$ (0.1)	1.7^{a} (0.5)	1.1^{b} (0.1)	7.2^{*}
16:1(n-9)	1.3 (0.1)	1.2 (0.1)	1.2 (0.1)	$1.4^{n.s.}$
18:1(n-7)	2.3^{a} (0.1)	2.1^{b} (0.0)	1.7c (0.1)	79.1^{***}
18:1(n-9)	3.3^{a} (0.1)	3.1^{a} (0.1)	2.8^{b} (0.2)	13.3^{**}
18:2(n-6)	7.0^{a} (0.1)	6.6^{b} (0.2)	6.5^{b} (0.2)	7.9^{*}
18:3(n-3)	$3.7^{a,b}$ (0.1)	3.8^{a} (0.1)	3.6^{b} (0.1)	6.6^{*}
18:4(n-3)	12.4 (0.3)	12.3 (0.3)	12.4 (0.2)	$0.2^{n.s.}$
18:5(n-3)	19.1 (0.6)	18.9 (0.5)	19.3 (0.5)	$0.7^{n.s.}$
22:6(n-3)	19.9 (0.6)	20.3 (0.2)	20.6 (0.9)	$1.2^{n.s.}$
MUFAs	9.1^{a} (0.4)	8.7^{a} (0.7)	7.2^{b} (0.3)	17.2^{***}
PUFAs	63.3 (1.1)	63.1 (1.2)	63.6 (1.7)	$0.2^{n.s.}$

FA >1% of total FA (tFA) as well as values for tFA, total MUFAs, and total PUFAs are presented. Differences among treatments were assessed with one-way ANOVA and Tukey *post hoc* tests (*F*: ANOVA statistics, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s.: not significant). Numbers in parentheses correspond to the *SD* and superscripts indicate the homogenous groups according to the Tukey *post hoc* test.

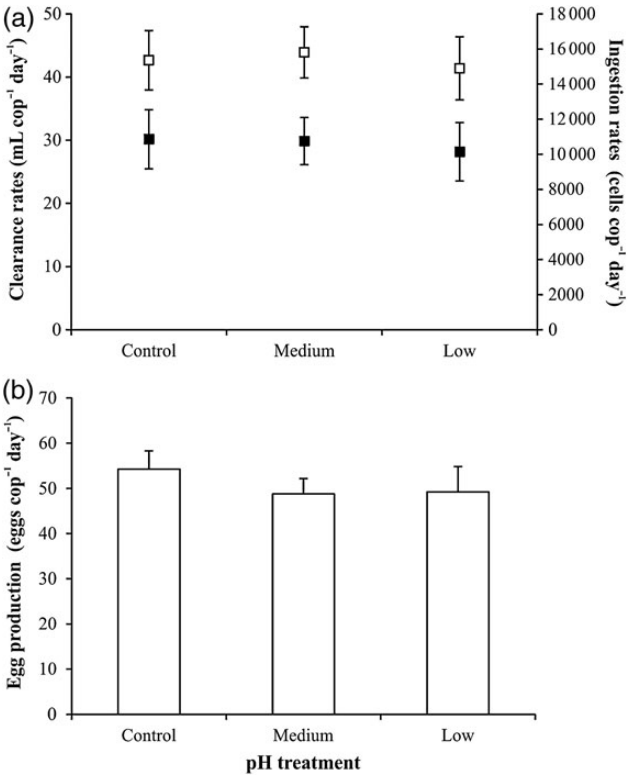

Figure 3. (a) Copepod feeding rates (clearance: black squares, ingestion: white squares) and egg production rates (b) in the different CO_2/pH treatments. Error bars indicate the *SD*.

units (C, N, and P) were slightly higher for the medium pH treatment (6–13%), those differences were not statistically significant neither in terms of carbon ($F = 1.48$, $p > 0.05$) and nitrogen ($F = 2.83$, $p > 0.05$) nor in terms of phosphorus ($F = 1.29$, $p > 0.05$; Figure 4). GGEs for each element were generally lower in the medium treatment (Figure 4). Those differences were more profound for GGE_N ($F = 5.72$, $p < 0.05$; 17–21% reduction compared with control and low pH treatments), whereas they were of marginal statistical significance for GGE_C and GGE_P ($F = 4.56$, $p = 0.043$ and $F = 4.44$, $p = 0.045$, respectively). Egg hatching increased with time in all treatments approaching ca. 95% of hatching success after 72 h. No significant differences among pH treatments were found in the cumulative hatching success percentage at the end of the incubation time ($F = 1.30$, $p > 0.05$).

Discussion

Until recently, the study of the impact of OA on planktonic copepods has mainly focused on assessing potential direct effects,

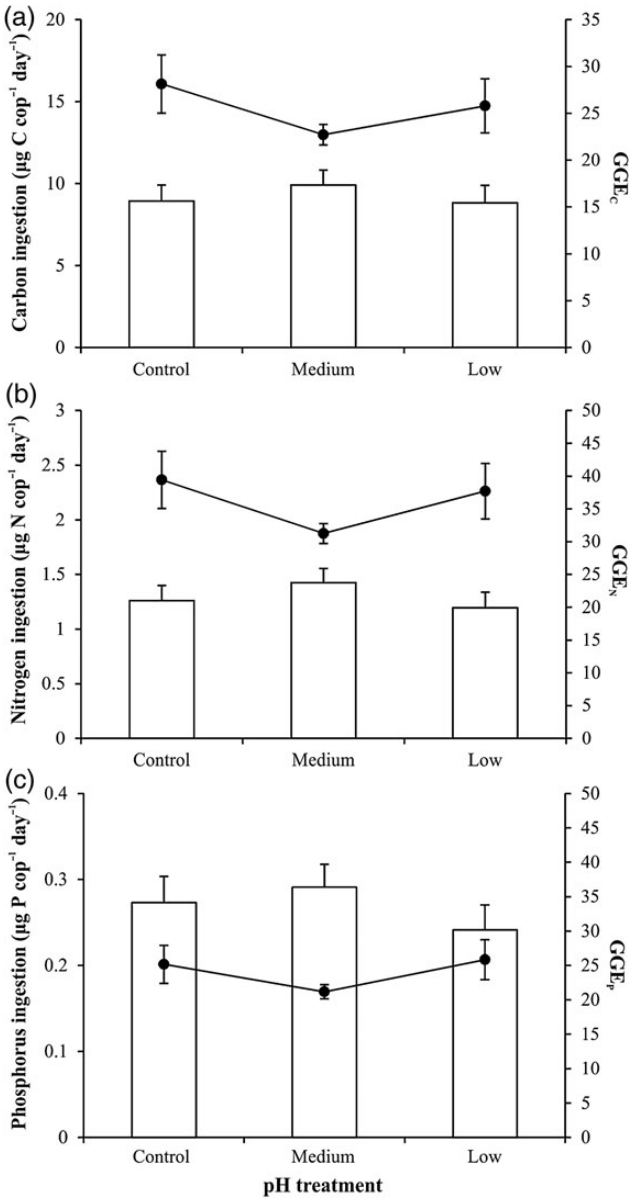

Figure 4. Ingestion rates (white bars) in the different CO_2/pH treatments in terms of (a) carbon, (b) nitrogen, and (c) phosphorus. Corresponding GGE for its element are given in black circles. Error bars indicate the *SD*.

often revealing the high tolerance of this group of organisms even under pCO_2 levels falling beyond near-future projections (e.g. Kurihara and Ishimatsu, 2008; Hildebrandt *et al.*, 2014). Interestingly, Rossoll *et al.* (2012) and Schoo *et al.* (2013) pointed out the possibility of "bottom-up" indirect effects on copepods at realistic acidification scenarios projected by the end of the century. On the one hand, Rossoll *et al.* (2012) observed a significant reduction in egg production and somatic growth of *Acartia tonsa* associated with a decrease in both the total FA concentration and the relative amount of PUFAs of the diatom *Thalassiosira pseudonana* grown under acidified conditions. On the other hand, and also for *A. tonsa*, Schoo *et al.* (2013) reported delayed development and a stage-dependent impact on respiration and excretion rates of the copepod associated with a decrease in the nutritional quality (expressed as higher C : N and C : P ratios) of its prey, the flagellate *R. salina*, grown under increased pCO_2 levels.

Although the algae used for the copepod experiments in our study were cultured at pCO_2 levels comparable or even higher to those of the two above-mentioned studies, we did not find any clear evidence of a change in the cell nutritional characteristics among experimental treatments (at least in terms of elemental and FA composition; other biochemical compounds, e.g. proteins and vitamins, have not been measured). In instances in which we detected statistically significant differences, the extent of such dissimilarity was very small, and most likely it would not affect substantially the nutritional quality of the copepod diet. In fact, drastic changes in the elemental ratios or FA content of prey seem to be required to detect considerable impacts in the reproductive output (Rossoll *et al.*, 2012; Isari *et al.*, 2013) and development (Rossoll *et al.*, 2012; Schoo *et al.*, 2013) of copepods.

Indeed, the slight and mostly insignificant variation in the elemental and biochemical properties of the prey offered in our experiments did not seem to have any statistically significant impact in the copepod feeding and egg production rates and hatching success. Nevertheless, the estimated GGEs for the distinct elements were generally lower (12–21%) in the medium pH treatment. However, those differences were most likely associated with the divergences in *Heterocapsa* sp. size among pH treatments and not with nutritionally mediated effects *per se*. During the copepod experiments, the biovolume of the cells grown at medium pH increased 10% compared with the others; given the similarity in copepod daily food consumptions on a cell basis (cells cop^{-1} d^{-1}) among treatments, this biovolume variation resulted in a higher elemental intake for the medium pH treatment (µg element cop^{-1} d^{-1}), which was not accompanied by an increase in the egg production output. The reason for such biovolume difference remains uncertain, but could be associated with the relatively higher pH variation observed in the medium pH culture. It has been documented that distinct environmental stressors may have an impact in the cell wall structure of algae and further impair their digestibility by predators (Van Donk and Hessen, 1995; Van Donk *et al.*, 1997). Cell material and related structures in algae have been also argued to constrain digestion and assimilation efficiency of copepods, decreasing their reproductive success (Dutz *et al.*, 2008). Therefore, it could be hypothesized that a larger variability in the pH growth conditions may induce changes in the morphological cell wall properties and affect digestibility and assimilation by copepods. And in fact, larger variability in pH is expected, in the future, not only in seawater from its decrease in buffering capacity but also at the exterior surface of marine organisms (Flynn *et al.*, 2012).

Since the exact elemental content of the eggs was not measured, a slightly higher C, N, and P content of the eggs produced in the medium pH treatment due to the higher copepod dietary elemental intake could be also possible. Although this may compensate for the calculated lower GGE in that treatment, the similarity observed in the egg hatching success among treatments can be most likely interpreted as indicating the lack of any quality difference (e.g. elemental difference) in the eggs laid.

Based on previous knowledge of the lack of direct OA effect on congeneric species even during multigenerational experiments (Kurihara and Ishimatsu, 2008), *a priori* our study assumed that no direct effect on the vital rates of *A. grani* would be imposed under our experimental pCO_2/pH levels. Some recent studies by Cripps *et al.* (2014a, b), however, have reported a reduction in the

reproductive output of *A. tonsa* at acidification levels and duration of exposure comparable to ours. Given the similarity in nutritional quality among the prey offered to *A. grani* in our experiments, the results we obtained (lack of any effects) can nevertheless be interpreted as further experimental evidence about the lack of direct impact of OA on our target copepod species, thus supporting our initial assumption. However, the observed absence of OA bottom-up effects on copepod performance under the dinoflagellate diet offered here did not lend support to our initial hypothesis.

Changes in seawater pCO_2 concentration may greatly impact several algal physiological processes and biochemical synthetic pathways; however, the final impact seems to be highly dependent on the algal group and may also vary even on a species basis (Riebesell, 2004). Dinoflagellate physiology is considered particularly sensitive, due to the low CO_2 affinity of their form II-RuBisCO and, therefore, the high dependence on the enzymatic machinery involved in maximizing CO_2 acquisition [the CCMs and also the carbonic anhydrases (CAs), which catalyse the interconversion of HCO_3^- and CO_2]. It could thus be argued that an increased CO_2 supply would lead to a beneficial downregulation in the expression of CCMs/CAs-related genes and might be positively reflected in the cellular physiology and biochemical composition of the dinoflagellate group (Giordano *et al.*, 2005), therefore ending up in positive bottom-up impact under OA conditions. Our findings, however, did not confirm this expectation since growth and cellular characteristics of *Heterocapsa* sp. under the pCO_2/pH scenarios tested were similar, although other dinoflagellate studies have indeed showed that elevated pCO_2 availability may result in a decreased activity of the CCM and CA activities (Ratti *et al.*, 2007; Van de Waal *et al.*, 2013; Van de Waal *et al.*, 2014) and an increase in the growth rates (Ratti *et al.*, 2007). Unfortunately, our planned experiment did not include the determination of CCM and CA activities of *Heterocapsa* sp., something that may have shed some light onto the physiological mechanisms behind the pattern found. It is noticeable, however, that a recent study by Van de Waal *et al.* (2014), carried out under elevated pCO_2 scenarios comparable to ours, indeed reported a downscaling of CCMs in the dinoflagellate *Alexandrium tamarense*, but such physiological change actually did not translate into changes in the algal growth and elemental composition, in line with the lack of effects found in our study. It seems likely that shifts in the gene expression and suppression of the enzymatic systems may be often associated with only subtle elemental and energy gain, insufficient to be reflected to growth, elemental, and biochemical composition.

An additional point to be considered regarding the effects of OA on algal physiology is that the sensitivity to changes in CO_2 supply may be also dependent in the ability for active uptake of bicarbonate (HCO_3^-) for a particular algal species. In this sense, Rost *et al.* (2006) reported a lesser dependence on CO_2 uptake for algae which were able to directly uptake bicarbonate. In that particular case, elemental ratios of algal cells might be less sensitive to changes in CO_2 supply, whereas a higher sensitivity would be expected for groups that predominantly use CO_2 (Burkhardt *et al.*, 1999; Fu *et al.*, 2008). Species-specific variability in CCM capabilities have been recorded in the genus *Heterocapsa*, with *H. oceanica* depicting a high dependence on free CO_2 and limited capacity for direct bicarbonate uptake (Dason *et al.*, 2004), whereas *H. triquetra* relies not only on diffusive CO_2 supply, but may also acquire HCO_3^- (Rost *et al.*, 2006). Therefore, it is uncertain if the lack of relevant effects of OA on *Heterocapsa* sp. in our experiments are the result of either the capability of this species to directly uptake bicarbonate (i.e. less dependence on CO_2 uptake) or that the pH levels in our experiments, within realistic predictions, were not sufficiently low to provide energy and elemental benefits that could influence other physiological processes.

The underlying algal physiological mechanisms and metabolic pathways that lead to the deterioration of the prey nutritional quality in the previous copepod studies (Rossoll *et al.*, 2012; Schoo *et al.*, 2013) seem unclear. The diatom cultured by Rossoll *et al.* (2012), *T. pseudonana*, showed a distinct FA profile when grown under elevated pCO_2 but, unfortunately, no further information was provided about the culture conditions (growth rates and cell size) and the elemental ratios under the different treatments. Remarkably, Crawfurd *et al.* (2011) found that the same diatom species, when subjected to a 3-month continuous culture at increased CO_2, showed a suppression of the genes involved with the CO_2 acquisition and had cellular C : N ratios closer to the Redfield ratio (i.e. better nutritional quality), which would be contradictory with the hypothesis of negative bottom-up effects. On the other hand, Schoo *et al.* (2013) reported that the cryptophyte *R. salina* maintained under elevated pCO_2 either in continuous chemostat or batch cultures had lower nutritional quality (higher molar ratios) that negatively affected copepod development. These authors, however, based on previous findings by Malzahn *et al.* (2007), made the assumption that algal cells with higher molar elemental ratios (high pCO_2 grown—*Rhodomonas*) would be a prey of better quality from a biochemical perspective (i.e. increased FA concentration and relative contribution of unsaturated FAs). Thus, one may wonder what would be the actual impact of a high pCO_2-*Rhodomonas* diet on other biological processes of copepods, i.e. reproductive success, and whether the stoichiometric characteristics or the FA properties of the prey would be the main nutritional drivers. It also remains unclear how the combined bottom-up effect on distinct copepod vital rates (e.g. development and reproduction) would ultimately influence copepod populations in nature.

The present study clearly points towards a lack of bottom-up effects mediated by OA in our particular predator–prey pair under realistic future scenarios, questioning the generalization of the results reported in previous copepod studies on this topic (Rossoll *et al.*, 2012; Schoo *et al.*, 2013). The physiological response of algae under OA conditions (combined with other climate change and human-related pressures, e.g. changes in inorganic nutrient supply) will not be uniform among taxa (Flynn *et al.*, 2015). Changes in phytoplankton competitive interactions and species succession are also anticipated (Flynn *et al.*, 2015), and it is thus evident that any potential indirect bottom-up effect on copepods in nature will be highly dependent on their prey-specific characteristics. To extrapolate laboratory results to nature and evaluate the OA foodweb implications, future research should integrate algal physiological approaches, describing the modes of carbon acquisition mechanisms of key algal species to better delimit their physiology, biochemistry, and their interactions.

Acknowledgements

The authors thank Kaiene Griffell and Àngel López-Sanz for assistance in the experimental work, Afrodite Adroni for the C–N analysis, Joachim Lütke for the support with the lipid analyses, and Xavier Leal for his technical help. Special thanks go to Jörg Dutz for his guidance on the experimental set-up and discussion on the topic as well as on three anonymous reviewers who helped in the improvement of the manuscript with their constructive

comments. The participation of ES and CP was helped from funding by the Spanish Ministry of Economy and Competitivity through projects CTM2011-23480 and CTM2012-32017, respectively. This work was supported by the project CROA (LS8-1893), implemented within the framework of the Action "Supporting Postdoctoral Researchers" of the Operational Program "Education and Lifelong Learning" (Action's Beneficiary: General Secretariat for Research and Technology), and is co-financed by the European Social Fund (ESF) and the Greek State.

References

Burkhardt, S., and Riebesell, U. 1997. CO_2 availability affects elemental composition (C:N:P) of the marine diatom *Skeletonema costatum*. Marine Ecology Progress Series, 155: 67–76.

Burkhardt, S., Zondervan, I., and Riebesell, U. 1999. Effect of CO_2 concentration on C:N:P ratio in marine phytoplankton: A species comparison. Limnology and Oceanography, 44: 683–690.

Crawfurd, K. J., Raven, J. A., Wheeler, G. L., Baxter, E. J., and Joint, I. 2011. The response of *Thalassiosira pseudonana* to long-term exposure to increased CO_2 and decreased pH. PLoS ONE, 6: e26695.

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

Cripps, G., Lindeque, P., and Flynn, K. 2014b. Parental exposure to elevated pCO_2 influences the reproductive success of copepods. Journal of Plankton Research, 36: 1165–1174.

Dason, J. S., Huertas, I. E., and Colman, B. 2004. Source of inorganic carbon for photosynthesis in two marine dinoflagellates. Journal of Phycology, 40: 285–292.

Dickson, A. G. 1990. Standard potential of the reaction: $AgCl(s) + 1/2\ H_2(g) = Ag(s) + HCl(aq)$, and the standard acidity constant of the ion HSO_4^- in synthetic seawater from 273.15 to 318.15 K. Journal of Chemical Thermodynamics, 22: 113–127.

Dickson, A. G., and Millero, F. J. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Research Part I-Oceanographic Research Papers, 34: 1733–1743.

Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. 2009. Ocean acidification: The other CO_2 problem. Annual Review of Marine Science, 1: 169–192.

Dupont, S., Ortega-Martínez, O., and Thorndyke, M. 2010. Impact of near-future ocean acidification on echinoderms. Ecotoxicology, 19: 449–462.

Dutz, J., Koski, M., and Jónasdóttir, S. H. 2008. Copepod reproduction is unaffected by diatom aldehydes or lipid composition. Limnology and Oceanography, 53: 225–235.

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

Flynn, K. J., Blackford, J. C., Baird, M. E., Raven, J. A., Clark, D. R., Beardall, J., Brownlee, C., *et al.* 2012. Changes in pH at the exterior surface of plankton with ocean acidification. Nature Climate Change, 2: 510–513.

Flynn, K. J., Clark, D. R., Mitra, A., Fabian, H., Hansen, P. J., Glibert, P. M., Wheeler, G. L., *et al.* 2015. Ocean acidification with (de)eutrophication will alter future phytoplankton growth and succession. Proceedings of the Royal Society B, 282: 20142604.

Folch, J., Lees, M., and Sloane-Stanley, G. H. 1957. A simple method for isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226: 497–509.

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.

Fu, F. X., Zhang, Y., Warner, M. E., Feng, Y., Sun, J., and Hutchins, D. A. 2008. A comparison of future increased CO_2 and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum*. Harmful Algae, 7: 76–90.

Giordano, M., Beardall, J., and Raven, J. A. 2005. CO2 concentrating mechanisms in algae: Mechanisms, environmental modulation, and evolution. Annual Review of Plant Biology, 56: 99–131.

Grasshoff, K., Kremling, K., and Ehrhardt, M. 1999. Methods of seawater analysis. Wiley-VCH, Weinheim, New York, Chiester, Brisbane, Singopore, Toronto.

Guillard, R. 1975. Culture of phytoplankton for feeding marine invertebrates. *In* Culture of Phytoplankton for Feeding Marine Invertebrates, pp. 26–60. Ed. by W. L. Smith, and M. H. Chanley. Plenum Press, New York, USA.

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 *Calanus hyperboreus*. Marine Pollution Bulletin, 80: 59–70.

Hofmann, G. E., Barry, J. P., Edmunds, P. J., Gates, R. D., Hutchins, D. A., Klinger, T., and Sewell, M. A. 2010. The effect of ocean acidification on calcifying organisms in marine ecosystems: An organism-to-ecosystem perspective. Annual Review of Ecology, Evolution, and Systematics, 41: 127–147.

Hopkinson, B. M., Dupont, C. L., Allen, A. E., and Morela, F. M. M. 2011. Efficiency of the CO_2-concentrating mechanism of diatoms. Proceedings of the National Academy of Sciences of the United States of America, 108: 3830–3837.

Hunter, K. A. 2007. $XLCO_2$–Seawater CO_2 Equilibrium Calculations Using Excel Version 2. University of Otago, New Zealand. http://neon.otago.ac.nz/research/mfc/people/keith_hunter/software/swco2/.

IPCC 2013. Summary for policymakers. *In* Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, pp. 3–29. Ed. by T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley. Cambridge University Press, Cambridge, United Kingdom; New York, NY, USA.

Isari, S., Antó, M., and Saiz, E. 2013. Copepod foraging on the basis of food nutritional quality: Can copepods really choose? PLoS ONE, 8: e84742.

Jónasdóttir, S. 1994. Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. Marine Biology, 121: 67–81.

Jones, R. H., Flynn, K. J., and Anderson, T. R. 2002. Effect of food quality on carbon and nitrogen growth efficiency in the copepod *Acartia tonsa*. Marine Ecology Progress Series, 235: 147–156.

Kattner, G., and Fricke, H. S. G. 1986. Simple gas-liquid chromatography method for simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. Journal of Chromatography A, 361: 263–268.

Kiørboe, T., Mshlenberg, F., and Hamburgefl, K. 1985. Bioenergetics of the planktonic copepods *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Marine Ecology Progress Series, 26: 85–97.

Klein Breteler, W. C. M., Schogt, N., and Rampen, S. 2005. Effect of diatom nutrient limitation on copepod development: Role of essential lipids. Marine Ecology Progress Series, 291: 125–133.

Koch, M., Bowes, G., Ross, C., and Zhang, X. H. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Global Change Biology, 19: 103–132.

Koski, M., Breteler, W. K., and Schogt, N. 1998. Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Marine Ecology Progress Series, 170: 169–187.

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

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.

Lee, K., Kim, T. W., Byrne, R. H., Millero, F. J., Feely, R. A., and Liu, Y. M. 2010. The universal ratio of boron to chlorinity for the North Pacific and North Atlantic oceans. Geochimica et Cosmochimica Acta, 74: 1801–1811.

Lewis, C. N., Brown, K. A., Edwards, L. A., Cooper, G., and Findlay, H. S. 2013. Sensitivity to ocean acidification parallels natural pCO_2 gradients experienced by Arctic copepods under winter sea ice. Proceedings of the National Academy of Sciences of the United States of America, 110: E4960–E4967.

Malzahn, A. M., Aberle, N., Clemmesen, C., and Boersma, M. 2007. Nutrient limitation of primary producers affects planktivorous fish condition. Limnology and Oceanography, 52: 2062–2071.

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., Findlay, H. S., and Spicer, J. I. 2013. Effects of elevated CO_2 on the reproduction of two calanoid copepods. Marine Pollution Bulletin, 73: 428–434.

Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowicz, R. M. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography, 18: 897–907.

Perez, F. F., and Fraga, F. 1987. A precise and analytical procedure for alkalinity determination. Marine Chemistry, 21: 169–182.

Perez, F. F., Rios, A. F., Rellan, T., and Alvarez, M. 2000. Improvements in a fast potentiometric seawater alkalinity determination. Ciencias Marinas, 26: 463–478.

Peters, J., Dutz, J., and Hagen, W. 2013. Trophodynamics and life-cycle strategies of the copepods *Temora longicornis* and *Acartia longiremis* in the Central Baltic Sea. Journal of Plankton Research, 35: 595–609.

Pierrot, D., Lewis, E., and Wallace, D. W. R. 2006. MS Excel Program Developed for CO_2 System Calculations. ORNL/CDIAC-105, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN.

Ratti, S., Giordano, M., and Morse, D. 2007. CO_2-concentrating mechanisms of the potentially toxic dinoflagellate *Protoceratium reticulatum* (Dinophyceae, Gonyaulacales). Journal of Phycology, 43: 693–701.

Reinfelder, J. R. 2011. Carbon concentrating mechanisms in eukaryotic marine phytoplankton. Annual Review of Marine Science, 3: 291–315.

Riebesell, U. 2004. Effects of CO_2 enrichment on marine phytoplankton. Journal of Oceanography, 60: 719–729.

Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M. M. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature, 407: 364–366.

Rossoll, D., Bermúdez, R., Hauss, H., Schulz, K. G., Riebesell, U., Sommer, U., and Winder, M. 2012. Ocean acidification-induced food quality deterioration constrains trophic transfer. PLoS ONE, 7: e34737.

Rost, B., Riebesell, U., and Sültemeyer, D. 2006. Carbon acquisition of marine phytoplankton: Effect of photoperiod length. Limnology and Oceanography, 51: 12–20.

Saiz, E., and Calbet, A. 2011. Copepod feeding in the ocean: Scaling patterns, composition of their diet and the bias of estimates due to microzooplankton grazing during incubations. Hydrobiologia, 666: 181–196.

Schoo, K. L., Malzahn, A. M., Krause, E., and Boersma, M. 2013. Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore. Marine Biology, 160: 2145–2155.

Thor, P., and Dupont, S. 2015. Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. Global Change Biology, in press; doi:10.1111/gcb.12815.

Urabe, J., Togari, J., and Elser, J. J. 2003. Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. Global Change Biology, 9: 818–825.

Van de Waal, D. B., Eberlein, T., John, U., Wohlrab, S., and Rost, B. 2014. Impact of elevated pCO_2 on paralytic shellfish poisoning toxin content and composition in *Alexandrium tamarense*. Toxicon, 78: 58–67.

Van de Waal, D. B., John, U., Ziveri, P., Reichart, G. J., Hoins, M., Sluijs, A., and Rost, B. 2013. Ocean acidification reduces growth and calcification in a marine dinoflagellate. PLoS ONE, 8: e65987.

Van Donk, E., and Hessen, D. O. 1995. Reduced digestibility of UV-B stressed and nutrient-limited algae by *Daphnia magna*. Hydrobiologia, 307: 147–151.

Van Donk, E., Lürling, M., Hessen, D. O., and Lokhorst, G. M. 1997. Altered cell wall morphology in nutrient-deficient phytoplankton and its impact on grazers. Limnology and Oceanography, 42: 357–364.

Weydmann, A., Søreide, J. E., Kwasniewski, S., and Widdicombe, S. 2012. Influence of CO_2-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*. Journal of Experimental Marine Biology and Ecology, 428: 39–42.

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

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