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CO_2-driven ocean acidification reduces larval feeding efficiency and changes food selectivity in the mollusk *Concholepas concholepas*

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We present experimental data obtained from an experiment with newly hatched veliger larvae of the gastropod *Concholepas concholepas* exposed to three pCO_2 levels. Egg capsules were collected from two locations in northern and central Chile, and then incubated throughout their entire intra-capsular life cycle at three nominal pCO_2 levels, ~400, 700 and 1000 ppm (i.e. corresponding to ~8.0, 7.8 and 7.6 pH units, respectively). Hatched larvae were fed with natural food assemblages. Food availability at time zero did not vary significantly with pCO_2 level. Our results clearly showed a significant effect of elevated pCO_2 on the intensity of larval feeding, which dropped by >60%. Incubation also showed that pCO_2-driven ocean acidification (OA) may radically impact the selectivity of ingested food by *C. concholepas* larvae. Results also showed that larvae switched their clearance rate based on large cells, such as diatoms and dinoflagellates to tiny and highly abundant nanoflagellates and cyanobacteria as pCO_2 levels increased. Thus, this study reveals the important effect of low pH conditions on larval feeding behavior, in terms of both ingestion magnitude and selectivity. These findings support the notion that larval feeding is a

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key physiological process susceptible to the effects of OA.

KEYWORDS: ocean acidification; larval feeding; natural food supply; diatoms; nanoflagellates

INTRODUCTION

Increasing carbon dioxide (CO_2) emissions are predicted to acidify the oceans and cause dramatic changes in ocean chemistry driven by the absorption of CO_2 from the atmosphere into the oceans through a process known as ocean acidification (OA) (Feely *et al.*, 2004). The impact of this process on marine ecosystems has only recently been recognized as a human-induced stressor, with potentially serious impacts for the sustainability and management of many economically important marine resources (Miller *et al.*, 2009). Within the various phyla particularly vulnerable to OA, calcifying organisms, such as marine mollusks, are exceptionally vulnerable (Byrne, 2011). They have been recognized for their great economic value and as a food source for humans (Leiva and Castilla, 2001). Therefore, any impacts of OA on sensitive life-history traits of these organisms will potentially lead to adverse ecological and economic impacts.

In recent years, numerous studies have demonstrated the negative effects of OA on marine mollusks, including reduced growth and calcification rates (Gazeau *et al.*, 2007; Miller *et al.*, 2009), reduced fertilization success (Byrne *et al.*, 2010), compromised induced defenses (Bibby *et al.*, 2007) and impairment of immune function (Bibby *et al.*, 2008). Although feeding could be one of the key physiological processes affected by OA, there have been few reports of the effect on feeding behavior in both adult and/or larval stages of marine mollusks (Stumpp *et al.*, 2011; Barton *et al.*, 2012). Feeding processes during early life stages of marine invertebrates are likely to be more sensitive to OA than in adults (Findlay *et al.*, 2008, 2010). Moreover, OA has been shown to induce morphological changes in larval invertebrates with significant implications for feeding performance and food selectivity (Chan *et al.*, 2011). In consequence, since it is well known that food quantity and quality greatly influences larval survival, development and growth (Boidron-Métairon, 1995), the influence of OA on feeding may explain observed impacts on these physiological traits and on other energy dependent processes including calcification.

Here, we selected an important species of the rocky intertidal and sub-tidal communities of the Chilean coast, in the Southern Pacific Ocean, the carnivorous gastropod known as "*loco*" (Chilean abalone), *Concholepas concholepas* (Brugière, 1789) (Castilla, 1999). *Concholepas concholepas* is an economically and ecologically important component of the rocky intertidal and sub -tidal communities along the Chilean coast (Castilla, 1999). After 1 to 2 months of intra-capsular development, the veliger larvae hatch from clumps of benthic egg capsules at about 240–260 μm protoconch length (DiSalvo, 1988). The competent larval stage (i.e. larvae ready to settle) is reached after about 3 months at a protoconch size between 1600 and 1900 μm (DiSalvo, 1988), which corresponds to the time lapse between maximal abundance of egg capsules and peak abundance of competent larvae in the water (Manríquez and Castilla, 2001). Then, they require from 3 to 12 months of planktonic life to reach competence and settling on the rocky shore (Gallardo, 1973). We conducted incubation experiments to investigate the effect of CO_2-driven OA on larval feeding behavior of *C. concholepas* feeding on natural food assemblages. Through this experimental approach, we address two questions: (i) is there any significant evidence of the effects of OA on the clearance and ingestion rates of newly hatched veliger larvae originating from egg capsules maintained under contrasting conditions of pCO_2? (ii) Is there evidence for changing food selectivity from hatched larvae under pCO_2-driven OA effects? The answers to these questions have at least two major implications for the general understanding of OA effects on economically important benthic resources with complex life cycles: (i) newly hatched larvae of *C. concholepas* might fulfill their nutritional requirements for development under high-pCO_2 conditions through changes in their ability to feed on different particle sizes and qualities, and (ii) changes in prey selectivity at high-CO_2 conditions might have important implications for assessing the position of larval invertebrates within trophic food webs.

METHOD

Collection of egg capsules

The experiments with newly hatched larvae of *C. concholepas* were conducted on two separate occasions: (a) 30–31 October 2010 (Exp I and II) and (b) 15–17 January 2011 (Exp III and IV; Table I). Newly hatched larvae of *C. concholepas* were obtained from egg capsules recently laid by females maintained in captivity in aquariums in central

Table I. Average (± SE) conditions of carbonate system parameters during incubation of egg capsules and feeding experiments conducted with newly hatched veliger larvae of C. concholepas *during the rearing period (June to October 2011): pH (total scale), Total Alkalinity (TA in μmol kg^{-1}), partial pressure of CO_2 (levels of pCO_2 in seawater in μatm), carbonate ion concentration (CO_3^{2-} in μmol kg^{-1}), saturation states of the water with respect to aragonite minerals (Ω_{arag})*

	Experiments I and II (31 October 2010)			Experiments III and IV (15–16 January 2011)		
CO_2 system parameters	400	700	1000	400	700	1000
Temperature (°C)	12.98 ± 0.04	13.00 ± 0.01	13.00 ± 0.01	15.42 ± 0.02	16.02 ± 0.01	16.06 ± 0
Salinity (psu)	33.23 ± 0.07	33.13 ± 0.53	33.35 ± 0.20	31.47 ± 0.01	31.47 ± 0.01	31.47 ± 0.01
pH@25°C (pH units)	7.898 ± 0.02	7.674 ± 0.03	7.516 ± 0.08	7.819 ± 0.002	7.669 ± 0.001	7.530 ± 0.003
pH *in situ* (pH units)	8.077 ± 0.21	7.845 ± 0.03	7.676 ± 0.08	7.995 ± 0.01	7.795 ± 0.00	7.649 ± 0.01
TA (μmol Kg^{-1})	2186.0 ± 14.33	2188.40 ± 15.63	2124.4 ± 50.95	2226.83 ± 2.12	2189.80 ± 7.25	2170.05 ± 4.02
pCO_2 *in situ* (μatm)	353.64 ± 19.87	648.82 ± 52.27	968.74 ± 183.71	434.12 ± 87.57	723.81 ± 38.25	1028.0 ± 62.30
[CO_3^{2-}] *in situ* (μmol Kg^{-1})	144.50 ± 6.49	90.96 ± 5.38	63.18 ± 12.98	140.53 ± 27.20	93.48 ± 7.89	69.91 ± 8.27
Ω_{arag}	2.22 ± 0.10	1.40 ± 0.08	0.97 ± 0.20	2.19 ± 0.43	1.46 ± 0.12	0.99 ± 0.13

The different experimental levels of CO_2 in our experimental system and in rearing containers were achieved and maintained during the entire experimental period by active injection of mixed CO_2 and air. The experimental nominal treatments considered three different CO_2 levels: 400 ppm (present), 700 (2100 years*) and 1000 (2200 years*).
*Based on rate of change in pH predicted by the IS92a climate change scenario (IPCC Special report on emissions scenarios (Caldeira and Wickett, 2003).

Chile (Las Cruces, 33°30′S, 71°38′W; Exp I) and southern Chile (Calfuco, 39°78′S, 73°39′W; Exp II, III and IV). At Calfuco, as soon as they were laid, the egg capsules were identified and a few days later when the female had moved away from the oviposition site, they were transferred to rearing conditions. A similar procedure was carried out at Las Cruces, but once egg capsules were accessible, they were transported under wet conditions to Calfuco.

Egg capsule conditioning and experimental conditions

Under rearing conditions, four groups of ca. 10 egg capsules were assigned to 0.5 L plastic beakers placed on top of a 280 L head space container in which three nominal levels of pCO_2 were set: 400, 700 and 1000 ppm, hereafter referred to as low, medium and high levels of pCO_2. The pCO_2 levels chosen for the medium and high levels were close to predicted levels for the worst case scenarios for 2100 and 2200, respectively (IPCC, Caldeira and Wickett, 2003). In consequence, although feeding measurements were conducted over a short time scale, exposure to different CO_2 levels involved a biologically relevant time scale, encompassing the entire period of development for eggs and non-feeding larval intracapsular stages until hatchling (i.e. 6–8 weeks), simulating the effect of more persistent pCO_2 driven OA, than short-term exposure to acidic waters driven by the natural variability in the nearshore (i.e. days, Torres *et al.*, 2011).

For the medium and high CO_2 treatments, CO_2 concentrations were modified by equilibrating the seawater with air containing different CO_2 concentrations, as per Findlay *et al.* (Findlay *et al.*, 2008). Air/CO_2 mixtures were produced using a bulk flow technique, where known flows of dry air (i.e. by compressing atmospheric air, 117 psi and passing it through a 1 μm particle) and ultra-pure (i.e. research grade) CO_2 gas were supplied, via a mass flow controller (MFC), and mixed before equilibration with sea water. Air flow in MFC was set manually to 5 L min^{-1} for both treatments and CO_2 flow was set manually to 1.33 ml min^{-1} and 4.25 ml min^{-1}, to produce CO_2 treatments of approximately 700 and 1000 ppm, respectively. The CO_2 of blended gas was monitored to allow fine regulation of CO_2 through MFCs to reach each target pCO_2 in seawater. During the experiments, seawater pH was monitored in each tank every 3 days in a 25 mL cell thermostatted at 25.0 ± 0.1°C for standardization, with a Metrohm® pH meter using a glass combined double junction Ag/AgCl electrode following the DOE potentiometric method (DOE, 1994). Temperature and salinity were monitored during incubations using a small CTDO (Ocean Seven 305 Plus). Temperature averaged 13°C in spring and from 15.4 to 16°C during summer experiments (Table I). Total Alkalinity (AT) was also measured using the automated potentiometric titration method (Haraldsson *et al.*, 1997). The pH, AT, phosphate (Strickland and Parsons, 1968), dissolved silicate (Strickland and Parsons, 1968), temperature and salinity data were used to calculate the rest of carbonate system parameters and the saturation stage of Omega Aragonite (Ω_{arag}) using CO_2SYS software (Lewis and Wallace, 1998) set with Mehrbach solubility constants (Mehrbach *et al.*, 1973) refitted by Dickson and Millero (Dickson and Millero, 1987).

An electric pump was placed inside each header container and was used to pump equilibrated seawater into each rearing beaker. A small opening (1 cm in diameter) was made within the upper part of each rearing beaker and sealed with 100 μm mesh size to allow seawater overflow and to prevent larval loss if hatching took place during the rearing. Seawater overflow was accumulated within a plastic container in which the four rearing beakers were placed and then conducted by gravity through a silicone pipe into the header container. Once a week, the rearing containers were washed and the egg capsules were carefully cleaned with the aid of a soft paintbrush. Mature egg capsules bearing near-hatch larvae were easily recognizable by their brownish coloration (Manríquez and Castilla 2001), which under our rearing conditions were obtained after about 2 months. The detection of newly hatched larvae in the rearing containers marked the end of the intra-capsular period (Exp I, Las Cruces = ~6.5 weeks; Exp, II, III, and IV, Calfuco = ~8 weeks).

Feeding experiments

Once larvae hatched from egg capsules, they were removed from the rearing containers and placed in a small Petri dish with 0.5 μm filtered seawater. Under a stereo microscope, larvae were carefully removed and transferred to feeding experiment conditions. For larval feeding estimates, seawater containing the natural-food assemblage used in our experiments was collected from the first 5 m of the water column off Calfuco in a clean plastic bucket. Seawater was then filtered through a 200 μm net to remove the majority of grazers and large debris, but maintaining natural food assemblages. Then seawater was stored in 10 L tanks and subsequently also equilibrated for 6–8 h to low, medium and high CO_2 levels until the bottle incubation experiment (Table I).

Larvae of *C. concholepas* selected for the experiments were pipetted into 500 mL acid-washed polycarbonate bottles filled with the filtered and equilibrated seawater from the three different pCO_2 treatments and including natural food assemblages. For all four experiments, there were no significant differences in food supply and composition among the three different pCO_2 treatments once we start the feeding experiment; ANOVA tests, Exp. I ($F_{2,87} = 2.6$, $P > 0.05$), Exp. II ($F_{2,87} = 2.9$, $P > 0.05$), Exp. III ($F_{2,87} = 3.1$, $P > 0.05$) and Exp. IV ($F_{2,87} = 1.9$; $P > 0.05$) (Fig. 1A). Therefore, the physiological effect of elevated pCO_2 in the natural phytoplankton assemblage was not observed during this short period of equilibration. Care was taken to avoid air bubbles in the bottles. Three control bottles without larvae and three bottles with 30 newly hatched larvae (0.06 ind. mL^{-1}) for each

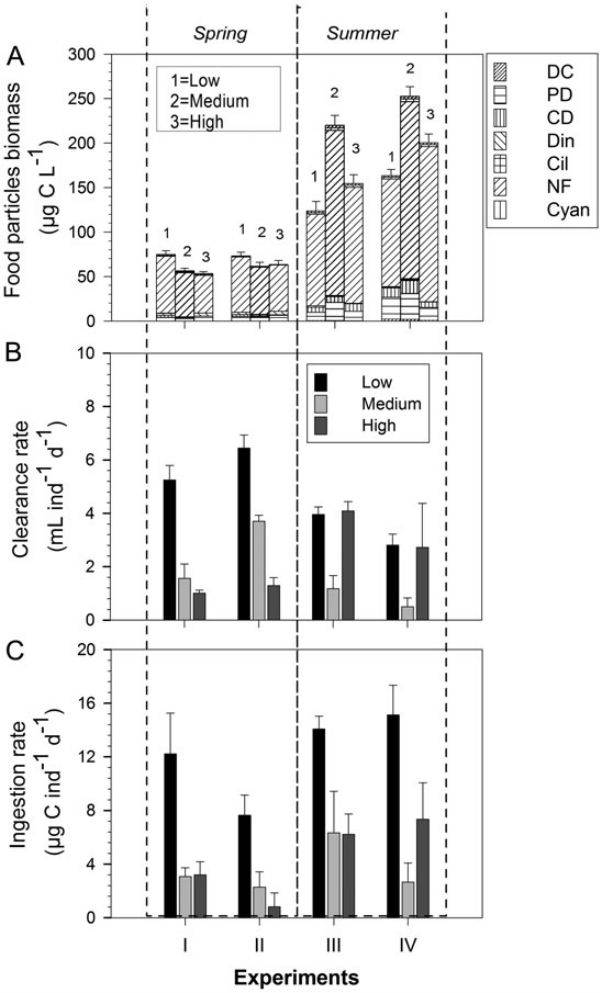

Fig. 1. (**A**) Comparison of the initial biomass before CO_2 equilibration for different plankton groups available for feeding of newly hatched larvae during spring (left) and summer (right) feeding experiments; error bars belong to total plankton biomass. (**B**) Clearance and (**C**) ingestion rates in four experiments including three CO_2 treatments.

treatment (low, medium and high pCO_2 levels) were incubated for approximately 24 h, and periodically rotated by hand to avoid particle sedimentation. Bottles were immersed in a container with a flow-through seawater system used for maintaining temperature fluctuation during a given experiment within one degree (15 ± 1°C). In all experiments, 60 mL subsamples from the corresponding pCO_2-adjusted water stock of each treatment were immediately preserved with 2% acid Lugol's solution for cell counts and biomass estimation (T_0). At the end of the incubation period, 60-mL subsamples from both control and experimental bottles were taken and preserved in acid Lugol's solution to determine cell concentration. The remaining volume was gently poured through a 20 μm sieve in order to check that incubated

larvae were healthy and actively filtering after the incubation period.

Cell counts and larval ingestion estimates

For the enumeration of cyanobacteria and nanoflagellates (NF), subsamples were filtered through a 0.8 μm polycarbonate membrane filter and stained with Proflavine (0.033% W/V in distilled water) according to Haas (Haas, 1982) and fixed with glutaraldehyde (as above) for subsequent analysis. Both bacteria and nanoflagellates were counted with an inverted microscope OLYMPUS IX-51, equipped with UV model U-MWU2 (width band pass 330−385 nm) and FITC model U-MWB2 (width band pass 450−480 nm) filter sets. Autotrophic NF cells were identified by autofluorescence. Biovolumes were converted to carbon using the equation in Chrzanowski and Simek (Chrzanowski and Simek, 1990), and then by multiplying biovolumes with a carbon:volume conversion factor of 220 fg C μm^{-3} (Borsheim and Bratbak, 1987). Large cells were counted under the same inverted microscope. Subsamples of 50 mL were allowed to settle for 24 h in Utermöhl sedimentation chambers before diatoms, dinoflagellates and ciliates were identified, counted and measured under the microscope. Plasma volumes were calculated (Edler, 1979) and averaged from a minimum of 50 cells $species^{-1}$. Biovolumes of ciliates were calculated assuming conical shapes with length to diameter ratios of 1.25 for ciliates <50 μm and 2 for ciliates >50 μm (Tiselius, 1989). Carbon to plasma volume ratios of 0.11 pg C μm^{-3} for diatoms (Edler, 1979), 0.3 and 0.19 pg C μm^{-3} for heavily thecate and athecate dinoflagellate forms, respectively (Lessard unpublished data fide Gifford and Caron, 2000) and 0.148 pgC μm^{-3} for ciliates (Ohman and Snyder, 1991) were applied.

Clearance (CR) and ingestion rates (IR), measured as cell removal, were calculated according to Frost (Frost, 1972) modified by Marin *et al.* (Marín *et al.*, 1986), and for the following groups: cyanobacteria (Cyan), nanoflagellates (NF), dinoflagellates (Din), ciliates (Cil), pennate (PD), solitary centric (CD) and chain forming diatoms (DC). Clearance and ingestion rates were calculated only when the differences in prey concentration between control and experimental bottles proved to be significant (*t*-test: $P < 0.05$). Field cell availability (i.e. concentration and biomass) of protozoan and phytoplankton in each CO_2 treatment were estimated from the T_0 subsample before the incubation. Cell concentration and biomass were determined using the same methodology outlined above.

Statistical analyses

Composition, abundance and biomass of natural food assemblages were compared among seasons and pCO_2 treatments through two-way ANOVA tests. When significant differences were found, either among experiments or pCO_2 treatments, a LSD *post hoc* test identified the source of such differences. A similar approach was adopted to evaluate two larval feeding behaviors (i.e. clearance and ingestion rates), regarding the experiments and pCO_2 treatments. Simple correlation tests were performed to include the effect of food availability (i.e. composition and abundance) on larval feeding behavior at different pCO_2 concentrations.

RESULTS

The experimental setup and average environmental and carbonate chemistry parameters are shown in Table I. Data were pooled separately for spring and summer experiments; mostly due to the fact that incubation water was the same for Exp I and II with larval stages from different locations, as well as Exp III and IV with larval stages from Calfuco, but conducted on two subsequent dates. During Exp I and II in the spring 2010, seawater temperature was around 12°C and pH was maintained at 8.077, 7.845 and 7.676, corresponding to pCO_2 values of approximately 353.6, 648.8 and 968.7 μatm CO_2, respectively. During the experiments in summer 2011, seawater temperature increased in ∼3°C and larvae were exposed to pH values of 7.959, 7.795 and 7.649, corresponding to pCO_2 levels of 496, 751 and 1072 μatm CO_2 during both the entire intracapsular development and feeding Exp III and IV (Table I). Saturation states for Aragonite varied significantly among all the different treatments (ANOVA, $F_{2,12} = 107.3$, $P < 0.0001$), and during the spring incubations (Exp I and II) ranged from supersaturation ($\Omega_{arag} = 2.22$) to slightly undersaturated ($\Omega_{arag} = 0.97$), whereas during summer experiments (Exp III and IV), Ω_{arag} varied between 1.89 and 0.99 (Table I).

The natural phytoplankton and protozoan assemblages provided as initial food supply varied in abundance among experiments and represented the natural spectrum of particle sizes and composition to which larvae are exposed (Vargas *et al.*, 2006). Therefore, our results under laboratory conditions are good indicators of potential larval feeding behavior under natural scenarios with different pH and pCO_2 levels in seawater (Fig. 1A). Food availability in our experiments differed in concentration, but specific composition did not vary radically.

During the study period, the major contribution to the total biomass was from small cells, mostly NF (40 to 190 μg C L^{-1}; Fig. 1A). Food offered varied significantly among seasons (ANOVA test, $F_{3,\ 116} = 4.4$, $P = 0.005$), and the lowest carbon biomass was observed during the spring experiments (Exp I and II, Fig. 1A). In contrast, food availability reached roughly 250 μg C L^{-1} during the summer experiments (Exp III and IV), coinciding with NF dominating the food supply, but also with a substantial contribution of PD (i.e. mostly *Navicula* spp. and *Pinnularia* spp.; Fig. 1A).

Mean size of newly hatched larvae from egg capsules reared under contrasting pCO_2 levels did not show differences in larval size (i.e. 252 ± 8; 243 ± 11 and 257 ± 9 μm at low, medium and high pCO_2 levels, respectively). Estimates from the different experiments with natural food assemblages clearly showed a significant reduction (>60%) in larval feeding upon increasing CO_2 levels in seawater especially on the ingestion rates estimates (Fig. 1C). An ANOVA test showed significant effects of pCO_2 levels on clearance and ingestion rates for all four experiments (one-way ANOVA, $P \leq 0.001$). Nevertheless, both CR and IR were significantly different between seasons for larval stages from Calfuco (ANOVA test, $F_{1,21} = 15.7$, $P = 0.001$, and $F_{1,21} = 36.7$, $P = 0.0001$, for CR and IR, respectively), but significant differences for experiments conducted in different seasons upon pCO_2 changes were only observed for CR estimates (two-way ANOVA, with significant "season × pCO_2" interaction, $F_{2,21} = 27.7$, $P < 0.0001$) (Table II).

During the experiments conducted in summer, we observed a reduction in CR in the medium CO_2 treatment, followed by an increase in CR in the high pCO_2 treatment (Fig. 1B). It is highly likely that such a reduction in CR at intermediate pCO_2 levels is a response to slightly higher food availability in this experimental treatment, resulting in reduced clearance. In any case, the effect of increasing pCO_2 was evident when analyzing results from IR estimates in Exp III (Fig. 1C), observing the same response pattern to higher pCO_2 levels as spring experiments. However, the exception to the pattern described above was Exp IV, where both CR and IR were relatively higher in the increased pCO_2 treatment.

Incubation in our experimental system also indicated that CO_2-driven OA may radically impact which kind of food particle was cleared and ingested by these gastropod larvae. In order to clarify the effect of pCO_2 on larval feeding behavior, CR anomalies were estimated as, CR on each prey group at high pCO_2 levels (medium or high pCO_2 treatment) minus CR on the corresponding prey item at the control pCO_2 level (low CO_2), and averaged for spring and summer experiments (Fig. 2). Thus, such estimates showed that in general CR decreased for most

Table II. Results of two-way ANOVA for testing variation in (A) clearance and (B) ingestion rates in newly hatched larvae of C. concholepas *from southern Chile (Calfuco), incubated under increased levels of* pCO_2 *in seawater and in two different seasons (spring and summer)*

Source of variation	DF	SS	MS	*F*	*P*-value
(A)					
Season	1, 21	5601.9	5601.9	15.7	**0.0010**
pCO_2	2, 21	18220.6	10429.2	29.1	**<0.0001**
Season × pCO_2	2, 21	19826.4	9913.2	27.7	**<0.0001**
(B)					
Season	1, 21	87 914	87 914	36.7	**<0.0001**
pCO_2	2, 21	224 284	87 268	36.5	**<0.0001**
Season × pCO_2	2, 21	14 381	7190	3.0	0.0710

Significant effects are showed in bold.

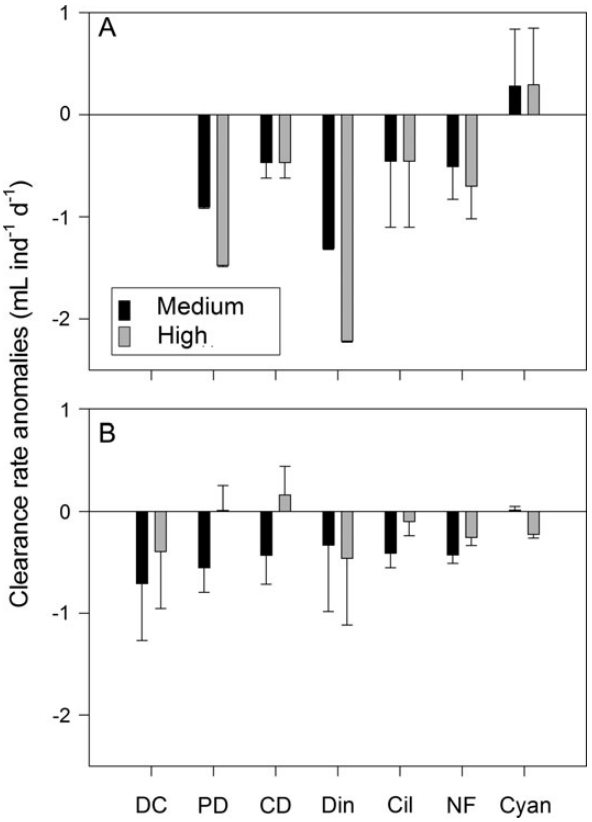

Fig. 2. Clearance rates anomalies (± SD) of *C. concholepas* newly hatched larvae computed to changes in prey selectivity due the increasing CO_2 levels (700 and 1000 ppm) averaged for: (**A**) spring (Exp I and II) and (**B**) summer experiments (Exp III and IV). Negative values indicate a decrease in the clearance of a particular plankton group in relation to control conditions (400 ppm).

prey groups under medium and high pCO_2 conditions. During spring experiments, this reduction was more intense for large cells, such as dinoflagellates and diatoms (CD and PD), whereas a positive anomaly was observed for small cyanobacteria (Fig. 2A) (LSD *post hoc* test, $P = 0.001$). In summer experiments, there was also a pattern of decrease in CR from large to small cells (Fig. 2B), with a significantly lower reduction in CR for small NF and cyanobacteria. A relationship between the percentage of food items available and those actually ingested by *C. concholepas* larvae also demonstrates a slight shift in food particle selection related to pCO_2 changes (Fig. 3). In both experimental series (i.e. spring and summer), large and scarce PD and Din were the basic components of the larval diet at low pCO_2 conditions (Fig. 3A and B). Although previous food items remained in the diet, along with a subtle contribution of CD, especially during summer experiments, cyanobacteria became more important in the daily food ratio, as pCO_2 increased up to medium and high levels (Fig. 3C–F). Feeding on cyanobacteria at increased pCO_2 levels was only evident when food availability was low in spring experiments (Fig. 3C and E). Small NFs were also slightly more preferred in some replicate bottles during summer experiments (Exp III and IV) (Fig. 3E). Similar results were also observed during summer experiments, when at medium CO_2 level NF were a little more preferred than at low CO_2 level (Fig. 3D).

DISCUSSION

Monitoring the removal of particles from the water over time is a common technique for measuring clearance of planktonic organisms (Vargas *et al.*, 2006). However, most particle depletion experiments have used artificial or monoculture algal diets (e.g. Strathmann *et al.*, 1992). CR estimates for *C. concholepas* larvae in the present study

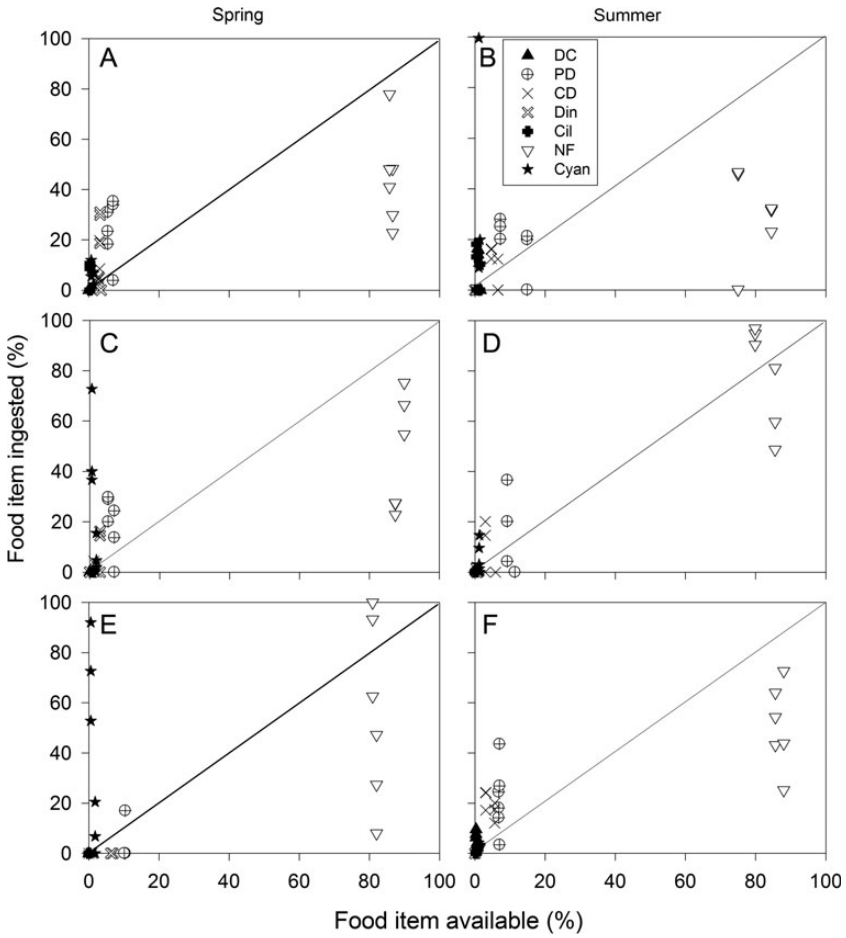

Fig. 3. Results of the selective feeding index of *C. concholepas* newly hatched larvae exposed to three nominal CO_2 levels: (**A** and **B**) low, 400 ppm, (**C** and **D**) medium, 700 ppm, (**E** and **F**) high, 1000 ppm. Left panel shows spring experiments (Exp I and II) and right panel shows summer experiments (Exp III and IV). Symbols above the fitted curve represent active selective feeding behavior.

were in the mean range of published values for other mollusk species, both on autotrophic and heterotrophic prey (from 0.4 up to 7 mL ind^{-1} day^{-1}). Baldwin and Newell (Baldwin and Newell, 1991) found that veligers of the eastern oyster *Crassostrea virginica* cleared autotrophic ^{14}C-labeled cells between 0.2 and 30 μm at 2 mL ind.$^{-1}$ day^{-1}, and heterotrophic H^3-labeled prey (bacteria and phagotrophic protozoans) at ~0.05 mL ind.$^{-1}$ day^{-1} (Baldwin and Newell, 1991). However, Vargas *et al.* (Vargas *et al.*, 2006) had previously reported high CR estimates in *C. concholepas* larvae of up to 100 mL ind.$^{-1}$ day^{-1}, based on chlorophyll estimates and cell counts of both autotrophic and heterotrophic cells. Nevertheless, differences in CR may be associated with several factors, including field food concentration, incubation temperature and, more importantly, differences in larval body size. Indeed, all feeding structures, such as length of the prototrochal ciliary band, prototrochal cilia and the angular velocity of the cilia scale with larval body size (Strathmann and Liese, 1979).

During the last few years, several studies have reported the impacts of OA on the growth and development of early life stages of shellfish (Miller *et al.*, 2009). However, to date there are few reports regarding the effect of OA on feeding behavior of larval invertebrates. It is evident from our experiments that an important effect of CO_2-driven OA is the radical change in larval feeding behavior, both in terms of quantity and quality of food ingested. To our knowledge, this is one of the first studies exploring the consequences of different pH conditions on feeding efficiency in gastropod larvae. However, the underlying mechanism by which OA impacts the larval feeding process remains unclear. It is well known that pH depression dramatically affects calcification in marine invertebrate larval stages and adults (Gazeau *et al.*, 2007; Arnold *et al.* 2009) and $CaCO_3$ structures have vital functions for calcified larvae, including the feeding process itself (Simkiss and Wilbur, 1989). On the other hand, hypercapnia can also lead to additional metabolic costs for many marine organisms (Stumpp *et al.*, 2011), as well as the effect of uncompensated extracellular pH (Stumpp *et al.*, 2012), which can also affect the filtration rate in delicate larval stages. Changing patterns of particle selection under the different treatments suggest that morphological traits of hatched larvae from egg capsules exposed to long-term high pCO_2 levels could be affected. However, larvae from each treatment did not display significant differences in mean larval size, which agrees with the lack of significant differences in larval size at hatch under the same three pCO_2 levels found in a study designed to evaluate the consequences of OA on developing and morphologic post-hatching traits (Manriquez *et al.*, in preparation). Nevertheless, differences in velar margin and length of the preoral cilia might affect CR in veliger larvae (Strathmann *et al.*, 1972). In consequence, longer preoral cilia in larvae from the control treatment could be more efficient in capturing particles in the larger size ranges (e.g. diatoms) (Strathmann and Liese, 1979). Furthermore, particle selection is also affected by the ability of larvae to transport particles within the food grove to the mouth (Chaparro *et al.*, 2002), and transport velocities could also be different at contrasting pCO_2 and pH levels. In any case, our results support the notion that feeding is one of the key physiological processes affected by OA.

These findings could have significant implications for larval recruitment, since it is well known that food quality and quantity can drastically impact early life stages of planktotrophic larvae (Boidron-Métairon, 1995; Phillips and Gaines, 2002). From an energetic point of view, ingesting larger cells is probably more advantageous for veliger larvae than ingesting smaller ones (Raby *et al.*, 1997). Switching diet from large diatoms with high polyunsaturated fatty acids (PUFA; e.g. Brett and Müller-Navarra, 1997) to small NF and cyanobacteria of scarce nutritional value may be also responsible for differences in larval growth and survival (Huntley and Boyd, 1984; Olson and Olson, 1989). Moreover, recently Rossoll *et al.* (Rossoll *et al.*, 2012) have shown that OA may affect the nutritional quality of phytoplankton cells which indeed may cascade up to grazers such as invertebrate larvae, with additional consequences for larval growth. Indeed, prolonged periods of high pCO_2 affecting the quality and quantity of food selected may also influence the duration of larval development, metamorphosis success (Boidron-Métairon, 1995) and performance of post-settlers during this critical life stage (Phillips and Gaines, 2002).

Our results highlight the negative effects of OA on the larval feeding process of an economically important marine resource under projected rising atmospheric CO_2 scenarios. Moreover, coastal areas are typically exposed to periodic pulses of corrosive high pCO_2 and low pH waters. For instance, egg capsules and larvae in nearshore areas are typically exposed to persistent acid water inputs with lower Ω_{arag} values, as a result of interactions with freshwater discharges (Salisbury *et al.*, 2008). Coastal upwelling events along the eastern boundary region enhance the surface intrusion of deep supersaturated CO_2 waters, which also expose marine invertebrates to low oxygen and aragonite undersaturated waters (Feely *et al.*, 2008). However, in the experimental setup of our study, we considered the context of permanent exposure under pCO_2-driven OA scenarios involving an incubation encompassing the entire period of development for eggs and non-feeding larval intracapsular stages. In spite

of the fact that this incubation period encompasses a biologically relevant timescale, our approach also must confront the inherent decoupling between experimental periods and the timescale at which the OA process is projected to occur in nature. This simplistic view disregards that other relevant process such species acclimation, adaptation and selective breeding may also operate leading to uncertainties about biological responses of species in a future high CO-ocean. In addition, OA in nature will act simultaneously with other climate-related variables, including the extent of low oxygen minimum zone in coastal areas and global temperature rise (Doney *et al*., 2012). The interaction between these co-occurring stressors implies even worse scenarios and it might have a larger effect on the metabolic process of marine invertebrates (e.g. Pörtner, 2008).

Thus, having in mind these inherent difficulties of OA research, our results suggest that the larval stages of marine invertebrates are vulnerable to exposure to such unfavorable conditions nearshore in the intertidal zone, which may be exacerbated under future OA scenarios. The gastropod *C. concholepas* supports small-scale fisheries that rely on natural stocks (Leiva and Castilla, 2001). Therefore, any negative impacts associated with OA that affect sensitive life history traits of this species may produce significant socio-economic and ecological disruptions to the ecosystem and to the essential ecosystem services that it provides for humans.

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REFERENCES

Arnold, K. E., Findlay, H. S., Spicer, J. L. *et al*. (2009) Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). *Biogeosciences*, **6**, 1747–1754.

Baldwin, B. S. and Newell, R. I. E. (1991) Omnivorous feeding by planktotrophic larvae of the eastern oyster *Crassostrea virginica*. *Mar. Ecol. Prog. Ser.*, **78**, 285–301.

Barton, A., Hales, B., Waldbusser, G. G. *et al*. (2012) The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: implications for near-term ocean acidification effects. *Limnol. Oceanogr.*, **57**, 698–710.

Bibby, R., Cleall-Harding, P., Rundle, S. D. *et al*. (2007) Ocean acidification induced defenses in the intertidal gastropod *Littorina littorea*. *Biol. Lett.*, **3**, 699–701.

Bibby, R., Widdicombe, S., Parry, H. *et al*. (2008) Effects of ocean acidification on the immune response of the blue mussel *Mytilus edulis*. *Aquat. Biol.*, **2**, 67–74.

Boidron-Métairon, I. (1995) Larval nutrition. In McEdward, L. (ed.), *Ecology of Marine Invertebrate Larvae*. CRC Press Inc, Washington D.C., USA, pp. 223–248.

Borsheim, K. Y. and Bratbak, G. (1987) Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. *Mar. Ecol. Prog. Ser.*, **36**, 171–175.

Brett, M. T. and Müller-Navarra, D. C. (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshw. Biol.*, **38**, 483–499.

Byrne, M. (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanogr. Mar. Biol.*, **49**, 1–42.

Byrne, M., Soars, N., Selvakumaraswamy, P. *et al*. (2010) Sea urchin fertilization in a warm, acidified ocean and high pCO_2 ocean across a range of sperm densities. *Mar. Environ. Res.*, **69**, 234–239.

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

Castilla, J. C. (1999) Coastal marine communities: trends and perspectives from human-exclusion experiments. *Trends Ecol. Evol.*, **14**, 280–283.

Chan, K. Y. K., Grünbaum, D. and O'Donnell, M. J. (2011) Effects o ocean-acidification-induced morphological changes on larval swimming and feeding. *J. Esp. Biol.*, **214**, 3857–3867.

Chaparro, O. R., Soto, A. E. and Bertran, C. E. (2002) Velar characteristics and feeding capacity of encapsulated and pelagic larvae of *Crepidula fecunda* Gallardo 1979 (Gastropoda, Calyptraeidae). *J. Shellfish Res.*, **21**, 233–237.

Chrzanowski, T. H. and Simek, K. (1990) Prey-size selection by freshwater flagellated protozoa. *Limnol. Oceanogr.*, **35**, 1429–1436.

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 Res.*, **34**, 1733–1743.

DiSalvo, L. H. (1988) Observations on the larval and postmetamorphic life of *Concholepas concholepas* (Bruguiere 1789) in laboratory culture. *The Veliger*, **30**, 358–368.

Doney, S. C., Ruckelshaus, M., Duffy, J. E. *et al*. (2012) Climate change impacts on marine ecosystems. *Annu. Rev. Mar. Sci.*, **4**, 11–37.

Edler, L. (1979) Recommendations for marine biological studies in the Baltic Sea. *Baltic* Mar. Biol., **5**, 1–38.

Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M. *et al*. (2008) Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science*, **320**, 1490–1492.

Feely, R. A., Sabine, C. L., Lee, K. *et al.* (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science*, **305**, 362–366.

Findlay, H. S., Kendall, M. A., Spicer, J. I. *et al.* (2008) A novel microcosm system for investigating the impacts of elevated carbon dioxide and temperature on intertidal organisms. *Aquat. Biol.*, **3**, 51–62.

Findlay, H. S., Kendall, M. A., Spicer, J. I. *et al.* (2010) Post-larval development of two intertidal barnacles at elevated CO_2 and temperature. *Mar. Biol.*, **157**, 725–735.

Frost, B. W. (1972) Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.*, **17**, 805–815.

Gallardo, C. (1973) Desarrollo intracapsular del *Concholepas concholepas* (Bruguière) (Gastropoda, Muricidae). *Mus. Nac. Hist. Nat. Publ. Ocas.* (In Spanish), **16**, 3–16.

Gazeau, F., Quiblier, C., Jansen, J. *et al.* (2007) Impact of elevated CO_2 on shellfish calcification. *Geophys. Res. Lett.*, **34**, L07603.

Gifford, D. J. and Caron, D. A. (2000) Sampling, preservation, enumeration and biomass of marine protozooplankton. In Harris, R. P., Wiebe, P. H., Lenz, J., Skjoldal, H. R. and Huntley, M. (eds), *ICES Zooplankton Methodology Manual*. Academic Press, London, U.K, pp. 193–221.

Haas, L. W. (1982) Improved epifluorescence microscopy for observing planktonic micro-organisms. *Ann. Instit. Oceanogr.*, **58**, 261–266.

Haraldsson, C. L., Anderson, G., Hassellöv, M. *et al.* (1997) Rapid, high-precision potentiometric titration of alkalinity in ocean and sediment pore waters. *Deep-Sea Res.*, **44**, 2031–2044.

Huntley, M. E. and Boyd, C. (1984) Food-limited growth of marine zooplankton. *Am. Nat.*, **124**, 455–478.

Leiva, G. and Castilla, J. C. (2001) A review of the world gastropod fishery: evolution of catches, management and the Chilean experience. *Rev. Fish Biol. Fish.*, **11**, 283–300.

Lewis, E. and Wallace, D. W. R. (1998) CO_2SYS–Program developed for the CO_2 system calculations. Carbon Dioxide. Inf. Anal. Center; Report ORNL/CDIAC-105.

Manríquez, P. H. and Castilla, J. C. (2001) Significance of marine protected areas in central Chile as seeding grounds for the gastropod *Concholepas concholepas*. *Mar. Ecol. Prog Ser.*, **215**, 201–211.

Marín, V., Huntley, M. E. and Frost, B. W. (1986) Measuring feeding rates of pelagic herbivores: analysis of experimental design and methods. *Mar. Biol.*, **93**, 49–58.

Mehrbach, C., Culberson, C. H., Hawley, J. E. *et al.* (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.*, **18**, 897–907.

Michaelidis, B., Ouzounis, C., Paleras, A. *et al.* (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Mar. Ecol. Prog Ser.*, **293**, 109–118.

Miller, A. W., Reynolds, A. C., Sobrino, C. *et al.* (2009) Shellfish face uncertain futures in high ρCO_2 world: influence of acidification in oyster larvae calcification and growth in estuaries. *PLoS ONE*, **4**, 108.

Ohman, M. D. and Snyder, R. A. (1991) Growth kinetics of the omnivorous oligotrich ciliate *Strombidium* sp. *Limnol. Oceanogr.*, **36**, 922–935.

Olson, R. R. and Olson, M. H. (1989) Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success?. *Annu. Rev. Ecol. Syst.*, **20**, 225–247.

Phillips, N. E. and Gaines, S. D. (2002) Spatial and temporal variability in size at settlement of intertidal mytilid mussels from around Pt. Conception, California. *Invert. Reprod. Dev.*, **41**, 171–177.

Pörtner, H-O. (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Mar. Ecol. Prog Ser.*, **373**, 203–217.

Raby, D., Mingelbier, M., Dodson, J. *et al.* (1997) Food-particle size and selection by bivalve larvae in a temperate embayment. *Mar. Biol.*, **127**, 665–672.

Rossoll, D., Bermúdez, R., Hauss, K. *et al.* (2012) Ocean acidification-induced food quality deterioration constrains trophic transfer. *PLoS ONE*, **7**, e34737. doi:10.1371/journal.pone.0034737.

Salisbury, J., Green, M., Hunt, C. *et al.* (2008) Coastal acidification by rivers: a new threat to shellfish?. *Eos, Trans. Am. Geophys. Union*, **89**, 513–513.

Simkiss, K. and Wilbur, K. M. (1989) *Biomineralization. Cell Biology and Mineral Deposition*. Academic Press, Oxford University Press, New York.

Strathmann, R. R., Fenaux, L. and Strathmann, M. F. (1992) Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of non feeding larvae. *Evolution*, **46**, 972–986.

Strathmann, R. R., Jahn, T. L. and Fonseca, J. R. C. (1972) Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trocophore. *Biol. Bull.*, **142**, 505–519.

Strathmann, R. R. and Liese, E. (1979) On feeding and clearance rates of molluscan veligers. *Biol. Bull.*, **157**, 524–535.

Strickland, J. D. H. and Parsons, T. R. (1968) A practical handbook of seawater analysis. *Fish. Res. Board Can. Bull.*, **167**, 311 pp.

Stumpp, M., Trübenbach, K., Brennecke, D. *et al.* (2012) Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification. *Proc. Natl Acad. Sci. USA*, **109**, 18192–18197.

Stumpp, M., Wren, J., Melzner, F. *et al.* (2011) CO_2 induced seawater acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, **160**, 331–340.

Tiselius, P. (1989) Contribution of aloricate ciliates to the diet of Acartia clausi and Centropages hamatus in coastal waters. *Mar. Ecol. Prog Ser.*, **56**, 49–56.

Torres, R., Pantoja, S., Harada, N. *et al.* (2011) Air-sea CO_2 fluxes along the coast of Chile: from CO_2 outgassing in central northern upwelling waters to CO_2 uptake in southern Patagonian fjords. *J. Geophys. Res.*, **116**, C09006. doi:10.1029/2010JC006344, 2011.

Vargas, C. A., Manríquez, P. H. and Navarrete, S. A. (2006) Feeding by larvae of intertidal invertebrates: assessing their position in pelagic food webs. *Ecology*, **87**, 444–457.