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High pCO_2 levels affect metabolic rate, but not feeding behavior and fitness, of farmed giant mussel *Choromytilus chorus*

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ABSTRACT: Benthic habitats such as intertidal areas, sandy or rocky shores, upwelling zones, and estuaries are characterized by variable environmental conditions. This high variability of environmental stressors such as temperature, salinity, and pH/pCO_2 levels have been shown to impose restrictions on organismal performance. The giant mussel *Choromytilus chorus* forms intertidal and subtidal mussel beds in estuarine zones associated with fjords occurring in southern Chile and is an important aquacultural resource in Patagonia. In this study, we estimated the sensitivity of physiological traits and energy balance of *C. chorus* juveniles exposed to 3 pCO_2 treatments (500, 750, and 1200 µatm) for 30 d. Results showed that in acidified, high pCO_2 conditions, *C. chorus* juveniles had increased metabolic rates; however, other physiological traits (clearance and ingestion rates, ammonia excretion, absorption efficiency, growth rate, biomass production, net calcification, and dissolution rates) were not affected. These results suggest that when subjected to acidification, the adaptive response of *C. chorus* triggers tradeoffs among physiological traits that favor sustained feeding and growth in order to combat increased metabolic stress. As has been reported for other marine organisms, chronic exposure to variable pH/pCO_2 in their native habitats, such as estuarine zones, could explain the differential acclimatization capacity of giant mussels to cope with the increase in pCO_2. Additionally, the fact that the mussels did not suffer from mortality indicates that increased pCO_2 levels may have chronic, but not lethal, effects on this species under these experimental conditions.

KEY WORDS: Estuaries · pH · Physiological traits · Acclimation · Phenotypic plasticity · Aquaculture

INTRODUCTION

The increasing concentration of carbon dioxide (CO_2) in the atmosphere has caused major changes in the global climatic system. Nearly one-third of total anthropogenic CO_2 emission has been absorbed by the ocean (Sabine et al. 2004), triggering a chemical process called ocean acidification (OA) (Caldeira & Wickett 2003, Orr et al. 2005, Gattuso et al. 2015), a global stressor with widespread consequences for calcifying organisms (Cooley & Doney 2009, Nienhuis et al. 2010). Due to the long-term projected

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changes in carbonate system parameters (pCO_2, pH, alkalinity, etc.) in the ocean and the differences in temporal scales operating over populations and organisms, a description of organismal responses as evidence of CO_2-sensitivity of the selected study models has recently been proposed, showing impacts of OA on species abundance and distribution (MacElhany 2017). Studies carried out with bivalve species have shown clear CO_2-sensitivity in several physiological traits. For instance, higher pCO_2 levels have been shown to negatively affect net calcification rates (e.g. *Mytilus chilensis*; Duarte et al. 2015) by increasing the dissolution rates of many calcifying organisms (Doney et al. 2009). Furthermore, high pCO_2 levels have been shown to alter the metabolic rate and growth of *M. galloprovincialis* (e.g. Michaelidis et al. 2005) and *M. chilensis* (e.g. Navarro et al. 2016). Similarly, OA has been shown to negatively affect the feeding rates of *M. chilensis* (e.g. Navarro et al. 2013) and physiological traits and the health of *M. edulis* (Beesley et al. 2008, Thomsen et al. 2013). Despite this, it has been proposed that species inhabiting environments where naturally increased pCO_2 levels occur regularly (i.e. lower pH or more acidic conditions) should be better adapted to cope with future ocean acidification (Thomsen et al. 2013, Lardies et al. 2014, Duarte et al. 2014, 2015). For example, Fernández-Reiriz et al. (2012) reported no apparent change in clearance, ingestion, and respiration rates of juvenile *M. galloprovincialis* from highly alkaline coastal waters and exposed to increased pCO_2 levels. Furthermore, Thomsen et al. (2013) showed that *M. edulis* may be able to cope with high pCO_2 levels when food supply is ensured. In addition, it has recently been demonstrated that some of the above effects of OA may be modified by concurrent exposure to other stressors, such as changes in salinity (Freitas et al. 2017), reduced food supply (Thomsen et al. 2013, Ramajo et al. 2016), and warming (Harvey et al. 2013, Lagos et al. 2016). In general, these studies suggest that exposure to a wide range of environmental conditions and naturally high and variable pH/pCO_2 conditions (Thomsen et al. 2013, Duarte et al. 2015) explain the variability and even neutral responses of species to experimental pCO_2 manipulations (Vargas et al. 2017). Thus, it has been proposed that the species inhabiting these types of environments may have physiological and metabolic adaptations (Thomsen et al. 2013, Duarte et al. 2014, Lardies et al. 2017) to face conditions of elevated pCO_2. Overall, biological responses to high pCO_2 conditions, especially by calcifying species, are variable and complex, species-specific (Miller et al. 2009, Wang et al. 2015, Vargas et al. 2017), and strongly dependent on the environmental variability experienced by species in their local habitats (Osores et al. 2017).

Bivalves dominate the macrofauna of many estuaries and coastal environments and have been shown to play an important ecological role in benthic ecosystems (Vihtakari et al. 2013, Duarte et al. 2015, Gazeau et al. 2015, Osores et al. 2017). Additionally, bivalves provide ecosystem services to aquaculture industries (Dame 2011, Lemasson et al. 2017). In Chile, approximately 60% of mussel production occurs in northern Patagonia (Avendaño et al. 2017). In this geographical zone, significant sequestration of atmospheric pCO_2 occurs in the surface seawater, and precipitation introduces acidic freshwater to the fjord ecosystems (Torres et al. 2011, Vargas et al. 2018). As such, these areas are vulnerable to progressive acidification, which could potentially impact the structure and function of the Patagonian ecosystem, as well as the aquacultural activities performed there (Navarro et al. 2013).

Recent studies performed in southern Chile have focused on the physiological responses of cultured *M. chilensis* when exposed to high pCO_2 conditions (Navarro et al. 2013, 2016, Duarte et al. 2014, 2015, Osores et al. 2017). However, less effort has been made to determine the effects of elevated pCO_2 on the giant mussel *Choromytilus chorus*. This species forms dense mussel beds in subtidal and intertidal habitats (Barria et al. 2012) and is distributed from Callao (Peru) to the Strait of Magellan and the Beagle channel in southern Chile (Bellolio et al. 1996). Along this wide distributional range, *C. chorus* inhabits coastal areas subject to variable marine and estuarine conditions (Navarro 1988), the mixing of which regulates the pH and carbonate saturation state in these ecosystems (see Duarte et al. 2013). Despite being an important commercial species in Chile, in which production increased from 339 t of biomass in 2012 to 2090 t in 2014 (SERNAPESCA 2014), the pCO_2 sensitivity of the physiological traits and energy balance of *C. chorus* have not been addressed. Previous studies carried out with *C. chorus* have mainly focused on farming (Avendaño et al. 2017), feeding behavior (e.g. Ibarrola et al. 2012), accumulation of toxic compounds (Toro et al. 2003), and the effects of salinity on physiological ecology (Navarro 1988). Therefore, the aim of this study was to evaluate, under laboratory conditions, the effects of high pCO_2 levels on physiological traits and energy balance of *C. chorus*. We hypothesized that the exposure of *C. chorus* to fluctuating pH/pCO_2 in its

natural environment would allow this species to physiologically acclimate to elevated pCO_2 conditions. The evaluation of physiological integration of this economically important marine organism may provide crucial information to elaborate adaptation strategies that might benefit the aquaculture industry in Chile under future ocean acidification scenarios.

MATERIALS AND METHODS

Animal collection

During the 2016 austral winter season, juvenile *Choromytilus chorus* individuals (mean ± SE: 41.53 ± 0.41 mm) were randomly collected from suspended cultures (permanently submerged) at Laraquete (Arauco Gulf, 37° 10′ S; 73° 11′ W), southern Chile. The samples were immediately deposited into 3 thermoboxes (l × w × h: 100 × 100 × 40 cm; with 20 to 25 mussels thermobox^{-1}) containing seawater from the same site (ca. 14°C), with constant aeration. The thermobox samples were transported to the Bioengineering Laboratory at the Universidad Adolfo Ibañez, Santiago for 10 d of acclimation. During acclimation, the animals were maintained with a natural photoperiod and running seawater (ca. 14°C), with pH (8.0) and salinity (~30 psu) conditions similar to those found in Laraquete during the winter season (see Vargas et al. 2017). Furthermore, food availability was not restricted; the mussels were fed daily with *Isochrysis galbana* equal to 5% of the animals' body weight (no flow-through). Following the acclimation period, the environmental conditions were maintained during the experimental period.

Experimental setup

After the acclimation period, the mussels were randomly assigned to aquaria and exposed to 1 of 3 pCO_2 treatments: current pCO_2 conditions (control = 500 µatm pCO_2; see Vargas et al. 2017), and 2 increased levels of acidification (750 and 1200 µatm pCO_2), as projected for future global atmospheric CO_2. Each treatment was replicated 3 times, and each replicate contained 6 experimental animals which were identified using bee tags. The pCO_2 levels in seawater were selected taking into account the rate of change projected for atmospheric CO_2 by the year 2200, consistent with the IPCC A2 emission scenario (Caldeira & Wickett 2003, Orr et al. 2005, Doney et al. 2009) and the current level of pCO_2 reported for the coast of Chile (Vargas et al. 2017). To achieve the 3 different pCO_2 levels (treatments) (see Table 1), we used a laboratory-based pCO_2-equilibration system similar to that used in previous studies (see Torres et al. 2013, Duarte et al. 2014). In brief, for the control treatment (pCO_2 = 500 µatm), pure atmospheric air was blended with pure CO_2 and bubbled directly into the experimental aquaria using mass flow controllers (MFCs) (Aalborg®). In order to obtain high pCO_2 conditions (750 and 1200 µatm pCO_2), we blended dry air with pure CO_2 to the target concentration using MFCs for air and CO_2. This blend was then bubbled into the corresponding containers. The experimental containers were constantly bubbled with the corresponding CO_2 concentration and each replicate was manipulated independently. These increases of pCO_2 in the seawater resulted in a corresponding drop in pH (~0.2 units for 750 µatm pCO_2; ~0.5 units for 1200 µatm pCO_2). The pH of the control (500 µatm pCO_2) remained at ~8.0 units (see Table 1).

The mussels were maintained in their respective pCO_2 treatments for 30 d. During the experiments, the pH and total alkalinity (A_T) of the water were monitored every 2 d. The pH samples were collected in 50 ml syringes and immediately transferred to a 25 ml temperature-controlled cell maintained at 25.0 ± 0.1°C for standardization. Here, the pH was measured using a Metrohm® pH meter with a glass combined double junction Ag/AgCl electrode (Metrohm model 6.0258.600) calibrated with the Metrohm® pH 4 (6.2307.200), pH 7 (6.2307.210), and pH 9 (6.2307.220) standard buffers. The pH values are reported on the NBS scale. Samples for A_T were poisoned with 50 µl of saturated $HgCl_2$ solution and stored in 500 ml borosilicate bottles (Pyrex, Corning®) with ground-glass stoppers lightly coated with Apiezon L® grease and stored in the dark at room temperature. Additionally, temperature and salinity were monitored daily during the experimental period using a portable Salinometer (Salt6+, Oakton®; accuracy: ±1% and ±0.5°C, respectively). A_T was determined using the open-cell titration method (Dickson et al. 2007) using an automatic alkalinity titrator (Model AS-ALK2, Apollo SciTech) equipped with a combination pH electrode (8102BNUWP, Thermo Scientific) and temperature probe (Star ATC, Thermo Scientific) connected to a pH meter (Orion Star A211, Thermo Scientific). All samples were analyzed at 25°C (±0.1°C) and temperature-regulated using a water bath (Lab Companion CW-05G). Accuracy was controlled using certified reference material (CRM, supplied by A. Dickson, University Cali-

fornia San Diego) and the A_T repeatability was 2 to 3 µmol kg^{-1} on average. Temperature, salinity, pH, and A_T data were used to calculate the carbonate system parameters (pCO_2, CO_3^{2-}). Analyses were performed using CO2SYS software in MS Excel (Pierrot et al. 2006) set with Mehrbach solubility constants (Mehrbach et al. 1973) refitted by Dickson & Millero (1987). The $KHSO_4$ equilibrium constant determined by Dickson (1990) was used for all calculations (see Table 1).

Physiological rates

Clearance and ingestion rates

Immediately after completion of the experiment period (i.e. 30 d), we determined the clearance and ingestion rates in a static system homogenized by aeration using a food concentration of 25×10^6 cells of *I. galbana* l^{-1}. Each mussel was placed individually in an experimental chamber (0.5 l glass bottles, Duran®). The decrease in the number of particles was analyzed over a period of 2 h using an Elzone 180XY particle counter equipped with a 120 mm aperture tube. A control experimental chamber (i.e. glass bottle without experimental mussels) was used to discard particle loss by sedimentation. Subsequently, the clearance rates (l h^{-1} $mussel^{-1}$) were calculated according to Coughlan (1969). Organic ingestion rates were calculated as the product of the clearance rate and organic material contained in the diet as described in our previous studies (see Navarro et al. 2013, 2016).

Absorption efficiency

Absorption efficiency (AE) was measured using Conover's (1966) method, based on the relationship between the organic and inorganic matter ingested as food and excreted as fecal material. This method assumes that absorption affects only the organic portion of the food. AE was calculated according to the following equation:

$$AE = [(F' - E') / (1 - E') F'] \times 100$$

where F' = the proportion of organic matter in the food, and E' = the proportion of organic matter in the feces. In order to obtain fecal pellets, 6 mussels from each replicate and pCO_2 treatment were maintained in the same experimental chamber (see above). The feces over the course of 24 h were collected and frozen until subsequent laboratory analysis. Food and fecal samples were filtered through pre-ashed, pre-weighed Whatman GF/F filters (47 mm diameter), rinsed with an isotonic ammonium formate solution, dried to a constant weight at 60°C, weighed, combusted at 450°C for 4 h, and weighed again to estimate the organic and inorganic fractions. Absorption rate was calculated as the product of the organic ingestion rate and AE.

Ammonia excretion and metabolic rate

Each mussel (n = 6 $replicate^{-1}$ $treatment^{-1}$) was placed in a sealed glass bottle (0.5 l) containing filtered (0.40 µm) seawater (0.35 ml). One additional experimental chamber for each treatment containing filtered seawater without mussels was used as a control. The containers were filled with seawater from the corresponding experimental pCO_2 levels (i.e. 500, 750, and 1200 µatm) and were maintained at 14°C by submerging them in thermoregulated water baths. After 2 h, water samples (5 ml) from each container were taken and analyzed for ammonia–nitrogen by the phenol-hypochlorite colorimetric method of Solórzano (1969). Excretion rate values are expressed in µg NH_4-N.

On Days 15 and 30 of the experiment, mussels were maintained in the aquaria for 24 h without food and then subjected to metabolic rate measurements (as oxygen consumption, in mg O_2 h^{-1} g^{-1}) by using a Presens Mini Oxy-4 respirometer. The experimental animals were placed individually in respirometry chambers filled with 131 ml of seawater collected from the corresponding pCO_2 treatments (see above), ~30 psu salinity, and oxygen-saturation through air bubbling (15 to 20 min) before measuring began. The measurements were performed at 14°C using an automated temperature chiller. In each chamber, dissolved oxygen was quantified every 15 s over the course of at least 45 min. Oxygen sensors were previously calibrated in anoxic water using a saturated solution of Na_2SO_3 and in water 100% saturated with oxygen using bubbled air. The same chambers and experimental conditions, without animals, were used for controls, and the oxygen concentration did not decay more than 3%. Oxygen decay due to background noise was deducted from the individual measurements. Once oxygen consumption was complete, the pH of the treated seawater in the respirometric chamber was measured with using a Metrohm® pH meter as described above.

Growth rate and biomass

The growth rates of juvenile *C. chorus* individuals were estimated from changes in maximum shell length (mm d^{-1}; to the nearest 0.01 mm using a Mitutoyo® caliper) recorded on Days 1 and 30 of the experiment. The final biomass (i.e. metabolically active tissue) was determined using the condition index estimated as the difference between the total and buoyant weight. This index was expressed as a percentage of the total weight during the time of the experiment (see Lardies et al. 2017). In addition, calcification and dissolution rates (see also Gazeau et al. 2015) were estimated from changes in the buoyant weight of individual mussels and from changes in empty shell weight recorded on Days 1 and 30 of the experiment, using an analytical balance (see Lagos et al. 2016 for details). Increases in buoyant weight serve as proxies for shell growth because buoyancy is equivalent to the calcification rate and is not affected by the amount of seawater and tissue weight inside the animal (see Palmer 1982).

Scope for growth

We also estimated the scope for growth (SFG), which represents the energy status of an animal and gives insight into the individual physiological components that affect the growth rate (Navarro et al. 2013, 2016, Zhang et al. 2015). SFG was calculated according to Widdows (1985) after converting all physiological rates described previously to energy equivalents (J h^{-1}): 1 ml O_2 = 19.9 J; 1 µg NH_4-N = 0.0249 J (Elliott & Davison 1975), and 1 mg of organic material of *I. galbana* = 18.75 J (Whyte 1987).

Data analysis

To avoid pseudo-replication, the values of the variables measured in each mussel from each replicate (aquaria) were averaged. The physiological responses of *C. chorus* exposed to the 3 pCO_2 treatments (500, 750, and 1200 µatm pCO_2) were analyzed using ANOVA models and Kruskal-Wallis tests. The clearance and organic ingestion rates, absorption rates, biomasses, net calcification and dissolution rates, ammonia excretion, growth rates, and SFG of *C. chorus* in the different pCO_2 treatments were compared using 1-way ANOVAs. In addition, a repeated-measures ANOVA was used to evaluate the temporal effects (i.e. Days 15 and 30) of metabolic rate on individuals exposed to the pCO_2 treatments. Because the data obtained in the absorption efficiency did not meet the assumptions of ANOVA, a Kruskal-Wallis test was used to compare the absorption efficiency of individuals exposed to different pCO_2 treatments. The normality and homoscedasticity of the data were tested using Kolmogorov-Smirnov and Bartlett tests, respectively (Zar 1999). All analyses were carried out using the software Minitab v.14, and differences were considered significant at $p < 0.05$.

RESULTS

The seawater conditions in which *Choromytilus chorus* were maintained during the experiments are summarized in Table 1. The pH values decreased in the 3 treatments (500, 750, and 1200 µatm pCO_2) from 8.027 ± 0.04 to 7.599 ± 0.005, respectively (Table 1). The carbonate in the seawater decreased with increasing pCO_2, whereas salinity (~30 psu), temperature (~14°C) and A_T (~1725 µmol kg^{-1}) remained similar between the 3 pCO_2 treatments. Representative of conditions recorded in estuarine zones in fjords in southern Chile, low A_T values were observed for all treatments (<2000 µmol kg^{-1}) (Vargas et al. 2017).

Table 1. Average (±SE) conditions of carbonate system parameters during the experiment conducted with juvenile *Choromytilus chorus*. pH is reported on the NBS scale; A_T: total alkalinity; pCO_2: partial pressure of CO_2 levels in seawater; CO_3^{2-}: carbonate ion concentration; Ω_{arag}, $\Omega_{calcite}$: saturation states of the water with respect to aragonite and calcite minerals, respectively

CO_2 system parameters	500 (µatm)		750 (µatm)		1200 (µatm)	
	Mean	SE	Mean	SE	Mean	SE
pH at 25°C	8.027	0.040	7.842	0.009	7.599	0.005
pH *in situ*	8.163	0.037	7.975	0.008	7.697	0.005
Salinity (psu)	30.334	0.409	30.313	0.026	30.286	0.048
Temperature (°C)	14.028	0.022	14.005	0.019	13.956	0.003
A_T (µmol kg^{-1})	1681.541	12.658	1696.554	17.250	1797.441	9.450
CO_3 (µM kg^{-1} SW)	118.166	7.723	78.296	2.178	45.737	0.512
pCO_2 (µatm)	431.371	24.245	615.314	20.164	1172.565	6.659
$\Omega_{calcite}$	2.900	0.189	1.922	0.053	1.123	0.013
$\Omega_{aragonite}$	1.839	0.120	1.218	0.034	0.712	0.008

Physiological rates

There was a subtle average increase in the clearance rate of *C. chorus* as pCO_2 increased; however, differences in clearance rates were not significant (1-way ANOVA: $F_{2,50}$ = 1.69, p = 0.19; Fig. 1a). A similar pattern was found for the organic ingestion rates; pCO_2 increases had no effect on this physiological trait (1-way ANOVA: $F_{2,50}$ = 1.69, p = 0.19; Fig. 1b). In addition, absorption efficiency, absorption rate and ammonia excretion were not significantly affected by increased pCO_2 levels (Kruskal-Wallis, p = 0.05, Fig. 2a; 1-way ANOVA: $F_{2,47}$ = 1.65, p = 0.20, Fig. 2b; 1-way ANOVA: $F_{2,53}$ = 1.19, p = 0.31, Fig. 2c), respectively. In contrast, juvenile *C. chorus* mussels exposed to the highest levels of pCO_2 (1200 µatm) had metabolic rates that were significantly higher than those of the control (500 µatm) and the other elevated pCO_2 treatment (750 µatm). In all treatments, metabolic rates were significantly higher after 30 d of exposure compared to after 15 d of exposure (interaction time × pCO_2: $F_{1,53}$ = 45.47, p < 0.001; Fig. 2d).

Given high pCO_2 conditions (1200 µatm), the net calcification and dissolution rates increased slightly, but these increments were not significantly different from the other elevated pCO_2 treatment (1-way ANOVA: $F_{2,47}$ = 0.57, p = 0.57; Fig. 3a) or from the control (1-way ANOVA $F_{2,47}$ = 0.81, p = 0.48; Fig. 3b). In addition, biomass percentage or condition index and growth rate of mussels did not show significant impact when exposed to increased pCO_2 levels (1-way ANOVA: $F_{2,53}$ = 0.01, p = 0.99, Fig. 3c; $F_{2,53}$ = 0.51, p = 0.60, Fig. 3d, respectively).

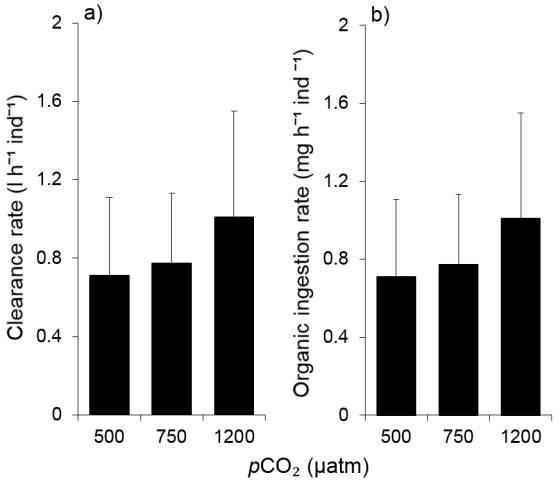

Fig. 1. (a) Clearance rates and (b) organic ingestion rates of the mussel *Choromytilus chorus* exposed to contrasting pCO_2 levels. Bars correspond to means ±1 SE

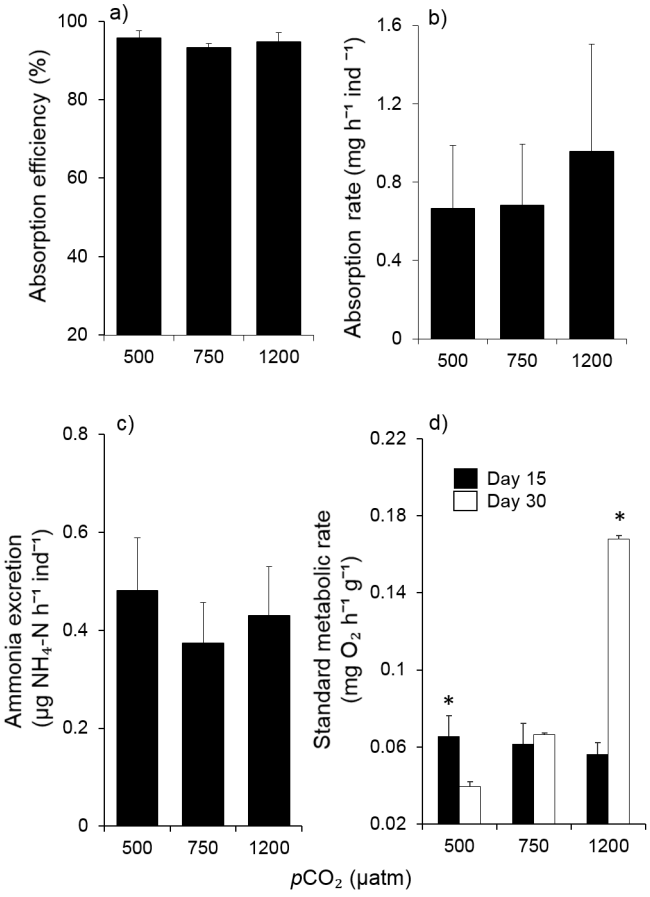

Fig. 2. (a) Absorption efficiency, (b) absorption rates, (c) ammonia excretion, and (d) metabolic rates of the mussel *Choromytilus chorus* exposed to contrasting pCO_2 levels. Bars correspond to means ±1 SE. Asterisk indicates significant differences among treatments (Tukey's HSD test)

The SFG index was positive for the 3 pCO_2 treatments. On average, the SFG of juvenile mussels exposed to the highest pCO_2 levels were highest. However, differences in SFG values among treatments were not significant (1-way ANOVA: $F_{2,45}$ = 2.05, p = 0.14; Fig. 4).

DISCUSSION

Our study showed that the metabolic rates of juvenile *Choromytilus chorus* mussels were higher with increased pCO_2 levels in seawater. Despite this, pCO_2 level had no effect on feeding performance (clearance and ingestion rates), ammonia excretion, absorption efficiency, growth rate, net calcification and dissolution rates, or biomass during the experimental period. In this study, no juveniles in any of the experimental treatments suffered mortality, which

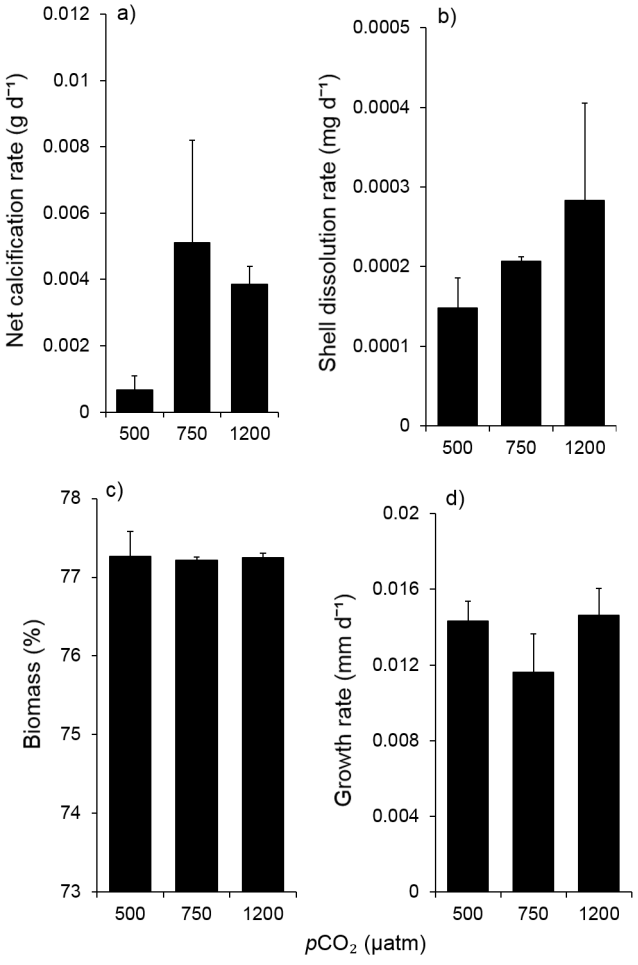

Fig. 3. (a) Net calcification, (b) shell dissolution rates, (c) biomass, and (d) growth rates of the mussel *Choromytilus chorus* exposed to contrasting pCO_2 levels. Bars correspond to means ±1 SE

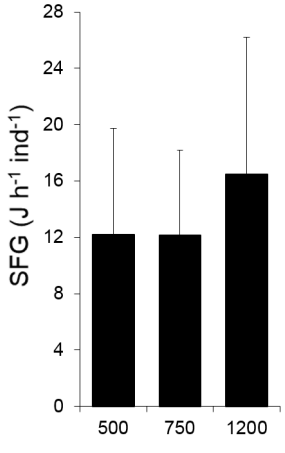

Fig. 4. Scope for growth (SFG) of the mussel *Choromytilus chorus* exposed to contrasting pCO_2 levels. Bars correspond to means ±1 SE

indicates that the projected increase of pCO_2 levels in the seawater had some chronic, but not lethal, effects under these experimental conditions. Similar results have been reported in other studies of bivalves from estuarine and upwelling zones (e.g. Duarte et al. 2014, Lagos et al. 2016, Lardies et al. 2017) and also in other mussel species that appear resilient to elevated pCO_2 (e.g. Thomsen & Melzner 2010, Mackenzie et al. 2014, Clements et al. 2018). Furthermore, these results are aligned with our hypothesis that the high environmental variability experienced by *C. chorus* in their local habitats (e.g. subtidal and intertidal beds, estuaries, and coastal upwelling) would allow these animals to respond to changing environmental conditions, such as changes in pCO_2. In fact, the contrasting responses of marine organisms to high pCO_2 levels have been attributed to the different natural conditions to which the animals are naturally exposed (Vargas et al. 2017). While significant metabolic stress was observed, increased pCO_2 conditions did not affect the energy balance (i.e. SFG) or biomass production of the studied juvenile mussels under these experimental conditions.

Clearance and ingestion rates were not significantly affected by increased pCO_2, which contrasts with previous studies that reported negative effects on bivalve feeding performance with increased pCO_2 (Vargas et al. 2015, Gray et al. 2017). For instance, Navarro et al. (2013) showed that the feeding rate of the mussel *Mytilus chilensis* was negatively affected by elevated levels of pCO_2 (pH 7.57) after 35 d of exposure. Furthermore, other studies recorded suppression of feeding activity in *M. galloprovincialis* and *M. edulis* (Michaelidis et al. 2005). In addition, it has been reported that high pCO_2 levels reduced the feeding activity of the mussel *M. californianus* (Gray et al. 2017). Despite these results, high pCO_2 levels (i.e. 1000 µatm) have also shown positive or no effect on feeding performance in bivalves (e.g. Fernández-Reiriz et al. 2012, Lardies et al. 2017). For instance, Fernández-Reiriz et al. (2012) showed that clearance and ingestion rates of the mussel *M. galloprovincialis* were unaffected in high pCO_2 conditions. These results are in agreement with our study, where elevated pCO_2 levels had no effect on the feeding performance of juvenile *C. chorus*. Other studies have shown that differences in exposure time are relevant when assessing the physiological responses (e.g. feeding behavior) of marine organisms (Navarro et al. 2013, Range et al. 2014). Thus, the conclusions made here should be considered with caution, as it remains unknown if the physiological responses of this organism would vary over time.

Our results showed that the metabolic rate of *C. chorus* exposed to high pCO_2 levels (1200 µatm) was significantly higher than that measured in the intermediate (750 µatm) and control (500 µatm) pCO_2 treatments. Metabolic rate was significantly higher after 30 d compared to after 15 d of exposure. Increases in the metabolic rates of mussels exposed to high pCO_2 levels have been found for other bivalves and may be a requirement to maintain intracellular pH and cellular homeostasis (e.g. Lardies et al. 2014, 2017). For example, Parker et al. (2012) showed that the metabolic rate of *Saccostrea glomerata* was higher when animals were exposed to high levels of pCO_2. In contrast, Fernández-Reiriz et al. (2011) found significantly decreased oxygen consumption in juveniles of the clam *Ruditapes decussatus* over the course of 87 d. The period of exposure used in our study (30 d) and the timing of the measurements of metabolic rates should have been sufficient to detect significant effects of increased pCO_2 on juvenile *C. chorus*. Notwithstanding, here we observed increases in the metabolic rate of *C. chorus* only at the end of the experiment, after 30 d of exposure but not after 15 d of exposure. In addition, the results obtained here should be interpreted with caution (see Zhang et al. 2015), as longer exposure times might produce more drastic effects (Dupont et al. 2013). Namely, there may be enough energy to cope with the additional costs for short-term exposure to elevated pCO_2 conditions, but if exposure is prolonged, reduction in performance may be observed. Thus, our experimental results are not sufficient to determine if the compensatory physiological performance (i.e. increased metabolic rate) observed in *C. chorus* under elevated pCO_2 levels is sustainable for longer periods of time, or if it would affect the fitness of this mussel species.

There are few studies of the effects of high pCO_2 on AE of marine organisms (see Navarro et al. 2013, 2016, Zhang et al. 2015 for examples). For instance, Navarro et al. (2013) reported that elevated pCO_2 (1200 µatm) significantly reduced the AE of *M. chilensis*, and that those negative effects produced deficiencies in the digestive systems. In contrast, Fernández-Reiriz et al. (2012) reported that high pCO_2 conditions (i.e. pCO_2 = ~1900 and 3700 µatm) increased the AE of *M. galloprovincialis*, while Wang et al. (2015) show that elevated pCO_2 levels (~2000 and 4400 µatm) had no significant effect on the mussel *M. coruscus*. In general, these studies suggest that the AE responses of bivalves subjected to different pCO_2 conditions are species-specific. Our results showed that pCO_2 levels did not significantly affect the AE of *C. chorus*. These results may indicate that the digestive system of this species is stable in the given experimental acidification. That is, the chronic exposure to variable pCO_2/pH in its native habitat could also confer tolerance in this species.

The rate of production of ammonia provides a measure of the protein catabolism rate, which can vary with the nutritional and reproductive status of the animal (Griffiths & Griffiths 1987). Several studies have demonstrated that, in spite differences in exposure period, ammonia excretion in bivalves increases in high pCO_2 conditions (Michaelidis et al. 2005, Thomsen & Melzner 2010, Fernández-Reiriz et al. 2011). However, Wang et al. (2015) showed that the excretion rate of *Mytilus coruscus* was reduced when exposed to high pCO_2 conditions. These studies suggest that increases in ammonia excretion in elevated pCO_2 levels or reduced pH could be an intracellular pH regulatory mechanism. Indeed, greater ammonia excretion and protein degradation can promote pH regulation (Boron 2004). We found that ammonia excretion by *C. chorus* was stable and did not significantly differ between pCO_2 treatments. Thus, we suggest that this mussel species, under these experimental conditions, is able to fully compensate for disturbances in its acid–base balance when exposed to high pCO_2 levels. Additionally, tolerance may have resulted from exposure to high pCO_2 in its natural habitat, as has been described for other benthic marine species (Lardies et al. 2014, Duarte et al. 2015, Thomsen et al. 2017, Vargas et al. 2017). However, it is important to emphasize that the lack of effect of elevated pCO_2 on ammonia excretion, absorption efficiency, and feeding rates in *C. chorus* does not necessary preclude changes in other physiological traits. Thus, the maintenance of an increased metabolism could be interpreted as a compensatory mechanism of this species to maintain its growth rate during conditions of increased pCO_2. It is important to emphasize that in our experiment, food availability was not restricted. In addition to salinity, food supply seems to play a major role modulating organismal responses of *C. chorus* by providing the energetic means to support the physiological cost imposed by increased pCO_2 stress (Ramajo et al. 2016).

Net carbonate production is affected by shell deposition and shell dissolution; thus, both processes should be evaluated simultaneously in experiments (Nienhuis et al. 2010). Our results showed that both net calcification and dissolution rates increased slightly in elevated pCO_2 levels, but these rates were not significantly different from that measured in the control pCO_2 treatment (see Fig. 3d). In the control treatment, the shell dissolution rate represented ca.

2.1% of the calcification rate, while for the elevated pCO_2 conditions it represented 4.1% (750 µatm) and 7.3% (1200 µatm) of the calcification rate. Thus, in general, dissolution did not preclude animal growth, but in saturated conditions (1200 µatm) it is expected that the mineral phase of the shell would be eroded, and hence more energy would be required to maintain shell integrity and functionality (e.g. Fitzer et al. 2015). However, our results indicate non-significant differences in the calcification and dissolution rates of. *C. chorus* among pCO_2 treatments, which is consistent with results reported for other mussel species.

In our experiment, the relative biomass production (condition index) and growth rate recorded for juvenile *C. chorus* mussels was not significantly affected by increased pCO_2 levels. Significant decreases in active tissue mass have been found in other bivalve species, such as scallops exposed to high pCO_2 conditions, and these decreases in mass are attributed to the energetic cost of maintaining homeostasis (Lagos et al. 2016, Lardies et al. 2017). Decreases in biomass given high pCO_2 conditions could be explained by energy allocation being diverted away to other key physiological processes such as calcification, acid–base balance, and reproduction (e.g. Findlay et al. 2009, Lagos et al. 2016). However, our results suggest that this mussel species could potentially offset reductions in its energy budget caused by elevated pCO_2 levels. The maintenance of mussel biomass across the pCO_2 treatments used in this study may require high food ingestion, and more energy needs to be canalized to the shell structure (e.g. calcification, growth; Lagos et al. 2016). However, in our study we did not observe significant increases in the feeding rates (i.e. clearance and organic ingestion rates) of animals in the elevated pCO_2, treatments; rather, we found increased metabolism in juvenile mussels exposed to high pCO_2 levels. On the other hand, reduced but variable shell growth has been reported for mollusks subjected to high pCO_2 conditions (e.g. Hiebenthal et al. 2013, White et al. 2013, Lagos et al. 2016). Additionally, Duarte et al. (2014) found that the carbonate deposition and total weight of *M. chilensis* were negatively affected by increased pCO_2. In contrast, Wang et al. (2015) showed that the growth of *M. coruscus* was not affected by high pCO_2 levels.

The SFG index integrates physiological, cellular, and biochemical features of bivalves, providing a quantitative assessment of the energy status and the individual physiological components that affect growth (Navarro et al. 2013). Previous studies demonstrated that the SFG of mollusks is negatively affected by environmental stressors such as salinity, food supply, temperature, and high pCO_2 conditions (Toro et al. 2003,Velasco & Navarro 2003, Navarro et al. 2013). This may result from a reduction in the energy ingested or due to an increase in energy loss. In our study, the SFG values obtained did not significantly differ among pCO_2 treatments. Thus, we suggest that *C. chorus* is capable of physiological acclimatization and is tolerant to increased pCO_2 levels, at least at a temporal scale equivalent to that tested here. Despite this, energetic costs are associated with the metabolic stress of compensation. One possible explanation for this tolerance is that *C. chorus* inhabits a wide range of environmental conditions (e.g. estuaries and the marine environment), involving variable carbonate availability (see Melzner et al. 2013, Vargas et al. 2017) and thus has acclimatized to overcome the negative effects of high pCO_2 conditions.

The results of this study show that *C. chorus* had the capacity to cope with the increase in pCO_2, at least during the period of study. As has been reported for other marine organisms, chronic exposure to variable pH/pCO_2 in their native habitats, such as estuarine zones, would explain this capacity. On the other hand, because the exposition time may modify the responses of this and other species to environmental stressors, future studies should evaluate different exposure times. Finally, understanding how this economically important resource responds to anthropogenic-induce changes in the coastal ocean will help to establish adaptive strategies for the aquaculture industry in Chilean Patagonia.

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