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Intraspecific Variability in the Response of the Edible Mussel *Mytilus chilensis* (Hupe) to Ocean Acidification

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Abstract Ocean acidification (OA) has been shown to affect significantly the net calcification process and growth rate of many marine calcifying organisms. Recent studies have shown that the responses of these organisms to OA can vary significantly among species. However, much less is known concerning the intraspecific variability in response to OA. In this study, we compared simultaneously the responses of two populations of the edible mussel *Mytilus chilensis* (Hupe) exposed to OA. Three nominal CO_2 concentrations (380, 700, and 1,000 μatm of CO_2) were used. Negative effects of CO_2 increase on net calcification rate were only found in individuals from Huelmo Bay. However, no effects were found in individuals from Yaldad Bay. Moreover, OA had not significant effects on the shell dissolution rate in individuals from both localities. This suggests that the negative effect of the OA on the net calcification rate of this species is explained by shell deposition, but not by the shell dissolution processes. We do not know the specific underlying mechanisms responsible for these differences, but some possibilities are discussed. These results highlight that the responses of marine organism to OA can be highly variable even within the same species. Therefore, more studies across the distribution range of the species, considering environmental variability, are needed for a better understanding of the consequences of OA on marine organisms. Finally, because mussels exert influence on their physical and biological surroundings, the negative effects of a CO_2 increase could have significant ecological consequences.

Keywords Ocean acidification · Mussel · Calcification · Growth rate

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Introduction

As a result of human activity, primarily fossil fuel combustion and cement manufacture, the atmospheric concentration of CO_2 has increased significantly during the past century (Houghton et al. 1996, 2001). About half of this anthropogenically produced CO_2 has been absorbed by the oceans (Sabine et al. 2004). Because of the oceanic uptake of CO_2, the marine concentration of CO_3^{2-} and the water pH are currently disturbed, and it is expected that in the future, these changes will become more pronounced (Feely et al. 2008; Wolf-Gladrow et al. 1999). For example, the pH of the ocean is already nearly 0.1 unit lower than the values registered in the pre-industrial era, and it is predicted to decrease by 0.4 units by the end of the century and nearly 0.8 units within the next 300 years (Caldeira and Wickett 2003; Orr et al. 2005) in a process called ocean acidification (OA). This decrease in the pH of the waters will produce a reduction in the concentration of CO_3^{2-} (i.e., saturation state (Ω)), which could fall by about 50 % by the end of the next century (e.g., Orr et al. 2005; Wolf-Gladrow et al. 1999). Both of these processes could potentially have a negative effect on marine calcifying organisms, while the reduction in the concentration of carbonate ions would complicate the formation of biogenic calcium carbonate at low levels of pH, the carbonate in already formed shells might start to dissolve, causing shells to disintegrate (e.g., Caldeira and Wickett 2003; Kleypas et al. 2006; Langdon and Atkinson 2005; Langdon et al. 2000; Leclercq et al. 2000; Orr et al. 2005; Riebesell et al. 2000 Hiebenthal et al. 2013). Additionally, increased CO_2 produces hypercapnic conditions (or acidosis), which have been shown to have negative effects on the physiology, growth, and reproductive success of marine calcifying organisms (Arnold et al. 2009; Berge et al. 2006; Cummings et al. 2011; Kurihara 2008; Michaelidis et al. 2005; Spicer et al. 2007; Widdicombe and Spicer 2008; Vhitakari et al. 2013).

Although most studies have described the negative effects of ocean acidification, recent studies have also shown that the responses of these organisms to the CO_2 increase can vary significantly between species (even between closely related species), with no change, or in some cases, even increased calcification rates in response to decreasing pH levels (due to CO_2 increase) (e.g., Gooding et al. 2009; Gutowska et al. 2008; Iglesias-Rodriguez et al. 2008; Miller et al. 2009; Ries et al. 2009; Parker et al. 2013). For example, laboratory experiments carried out with 18 marine benthic organisms (Ries et al. 2009) showed that 10 of the 18 species reduced their net calcification rates under elevated CO_2; in seven species, the net calcification increased with the intermediate and highest levels of CO_2, and finally, one species (*Mytilus edulis*, very related to *Mytilus chilensis*) did not show any response at all. It has been proposed that the differences in the sensitivity of the organisms and therefore the described mixed responses might depend of the pCO_2 content (or pH) to which they are naturally exposed in the field (Wootton et al. 2008; Hall-Spencer et al. 2008; Gazeau et al. 2010; Thomsen et al. 2010, 2013). Thus, species inhabiting environments with naturally higher CO_2 content (lower pH values) would be better adapted to cope with the ocean acidification process (Cummings et al. 2011; Gazeau et al. 2010; Marshall et al. 2008; Miller et al. 2009; Pascal et al. 2010; Widdicombe et al. 2009). For example, larvae of the euryhaline oyster species *Crassostrea ariakensis*, which inhabit an environment with naturally low pH, did not show problems in growing, calcifying, and developing normally when exposed to elevated CO_2 levels and seawater undersaturated with respect to aragonite (Miller et al. 2009). However, it is also important to emphasize that environments with naturally low pH may exacerbate the negative effects of the ocean acidification (see Salisbury et al. 2008; Cai et al. 2011; Melzner et al. 2013).

Overall, the studies carried out so far have shown that there is a great need to determine whether calcifying organisms will have the capacity to adapt to the CO_2 increase in the ocean (Ilyina et al. 2009). Evaluating the intra- and not only the interspecific variability in the responses to pH decrease will help to better understand this concern. Some efforts in this line have been made (e.g., Havenhand and Schlegel 2009; Parker et al. 2011; Sunday et al. 2011; Range et al. 2012, 2013). For example, Parker et al. (2011), evaluating the effects of OA on the oyster *Saccostrea glomerata*, showed that the response to OA may vary even within the same species. Genetic differences among populations and/or the spatial variability in the water characteristic has been proposed as mechanisms to explain the intraspecific variability in the responses of the organism to OA (Parker et al. 2011; Range et al. 2011). Species of the genus *Mytilus* inhabit a wide range of environmental conditions, and therefore, they are naturally exposed to highly variable pH conditions (e.g. Vihtakari et al. 2013; Thomsen et al. 2013), which would explain in part the intraspecific variability in the response of this group to OA. In Chile, *M. chilensis* has a wide geographic distribution (37° S–73° S) inhabiting from completely marine environments to habitat with estuarine conditions (Krapivka et al. 2007); in consequence, this species is naturally exposed to a wide range of environmental conditions. Therefore, we hypothesized that populations of *M. chilensis* inhabiting two geographically separated localities can show differential responses to OA. Therefore, the aim of this study was to compare the effect of ocean acidification on both $CaCO_3$ net deposition and dissolution rates and on the growth rate in juvenile individuals of the mussel *M. chilensis* (Hupe) collected from two southern Chile populations.

M. chilensis is the most widely cultivated species in Chile and has been recorded (like other mussel species) as having a significant influence on its physical and biological surroundings (Duarte et al. 2006). Consequently, the potential negative

effects produced by ocean acidification on this species could affect not only the ecosystem functioning but also the national economy.

Methods

Animal Collection

Juvenile individuals of *M. chilensis* (~1.5 cm in size and ~350 mg fresh weight) were collected from culture ropes (2 m of depth) in two bays: Yaldad (43° 08′ S, 73° 44′ W) and Huelmo (41° 40′ S, 73° 02 W). Both are tidal inlets located in southern Chile (ca. 40–42° S). Unlike Huelmo Bay, Yaldad Bay receives a constant input of freshwater from the Yaldad river (Cáceres et al. 2008). After collection, the mussels were transported in chilled conditions to the Calfuco Coastal Laboratory (Laboratorio Costero de Calfuco (LCC), ca. 39° S) and placed in plastic containers (30 cm in diameter and 40 cm in height) where they were kept in filtered (0.1 μm), aerated seawater (pH=8.1±0.01, temperature= 13.1±0.01 °C, and salinity=~33 psu). To acclimatize them to experimental conditions, the mussels were kept for 2 weeks and fed daily with microalgae *Isochrysis galbana* (~25× 10^6 cell/L) before the beginning of the experiments.

Experimental Setup

Seawater Acidification System and Measurements

After the acclimatization period, the experimental animals were reared at 380, 700, and 1,000 μatm of CO_2. To achieve the three different CO_2 levels in the seawater (Table 1), we used laboratory-based experimental system implemented to investigate the consequences of ocean acidification on Chilean marine calcifying organisms (Torres et al. 2013; Duarte et al. 2014; Navarro et al. 2013; Manríquez et al. 2013; Vargas et al. 2013). The two highest CO_2 levels represent the worst case scenarios for the end of the years 2050 and 2100 respectively (IPCC 2007; Caldeira and Wickett 2003). During the experiment, the pH, temperature, salinity, and total alkalinity of the water were monitored every 3 days (three replicates) in each header tank (Navarro et al. 2013). The pH measurements were done in a closed 25-ml cell thermostatically controlled at 25.0 °C; pH was measured with a Metrohm 713 pH meter (input resistance $>10^{13}$Ohm, 0.1 mV sensitivity, and nominal resolution 0.001 pH units) and a glass combined double junction Ag/AgCl electrode (Metrohm model 6.0219.100) calibrated with 8.089 Tris buffer (DOE 1994) 25 °C. pH values are therefore reported on the total hydrogen ion scale (DOE 1994). Temperature and salinity were measured using a CTD (Ocean Seven 305 Plus CTD, www.idronaut.it). Total alkalinity was measured using the method of Haraldsson et al. (1997). The pH, A_T, phosphate, dissolved silicate, and hydrographic data were used to calculate the remaining carbonate system parameters and the saturation stage of Omega Aragonite using CO2SYS software (Lewis and Wallace 1998) set with Mehrbach solubility constants (Mehrbach et al. 1973) readjusted by Dickson and Millero (1987).

Net Calcification Rate, Shell Dissolution, and Growth Rate

Mussels were randomly assigned to one of 15 4-L aquaria and exposed to three CO_2 treatments (see above). Each treatment was replicated five times, and each replicate contained three experimental animals from each population, which were identified using bee tags. In addition, two empty shells (one from each population) of *M. chilensis*, previously tagged and weighed, were placed in each container. Empty shells were obtained by separating the soft body of live mussels, which were similar in size to the experimental animals. These shells were cleaned with distilled water to eliminate any organic residue and salt and then dried for 24 h (60 °C) before weighing. The experimental animals and empty shells were selected because shell lengths, buoyant weights, and shell

Table 1 Sea water characteristics (mean±SD) used to maintain *M. chilensis* during the experimental period

CO_2 system parameters	Experimental treatments (nominal levels of CO_2 ppm)		
	380 (current)	700 (year 2050[a])	1,000 (year 2100[a])
pH at 25 °C (pH units)	7.926±0.053	7.719±0.033	7.579±0.038
pH in situ (pH units)	8.054±0.047	7.849±0.031	7.703±0.037
Salinity (psu)	33.60 (0.41)	33.64 (0.46)	33.42 (0.64)
Temperature (°C)	16.31 (0.73)	15.98 (0.70)	15.93 (0.62)
TA (μmol Kg^{-1})	2251±25	2247±13	2294±39
pCO_2 in situ (μatm)	388±49	664±52	979±87
$[CO_3^{2-}]$ in situ (μmol Kg^{-1})	161±18	106±9	79±8
Ωca	3.87±0.43	2.54±0.20	1.91±0.20
Ωar	2.49±0.28	1.63±0.13	1.23±0.13

TA total alkalinity, $[CO_3^{2-}]$ carbonate ion concentration, Ω_{ca} omega calcite, Ω_{ar} omega aragonite

[a] Based on the rate of change in pH predicted by the IS92a climate change scenario (IPCC special report on emissions scenarios, http://www.grida.no/publications/other/ipcc_sr/)

weights did not show significant differences among the different CO_2 treatments compared to the previous experiment. During the experiments, mussels were maintained according to Navarro et al. (2013). The aquaria were maintained without aeration and at constant temperature (13 °C). Seawater was gently changed everyday, with the corresponding CO_2 levels from the seawater acidification system following the methodology proposed by Navarro et al. (2013).

The net calcification rates of the organisms under the three CO_2 treatments were estimated from changes in their buoyant weight (i.e., underwater weight) verified with dry weight measurements of the shells after harvesting (Palmer 1982). To prevent errors due the entrance of air within the closed valves during the measurements of the buoyant weight (see Palmer 1982), each specimen was gently moved from the rearing container to the balance. Following this procedure, only seawater remained within the valves and yields exact buoyant weights of the experimental specimens. Buoyant weight increment is an important proxy for growth because it is equivalent to the calcification rates and is not affected by the amount of seawater and tissue weight (see Palmer 1982) methodology successfully validated for *M. chilensis* (Duarte et al. 2014).

The measurements were made on day 0 (at the beginning of the experiment) and at the end of a 20-day period. Shell dissolution rates, in turn, were estimated by weighing the empty shells of *M. chilensis* in air at the beginning and at the end of the experiment. Live animals and empty shells were weighed to the nearest 0.01 mg. Growth rates were estimated from changes in the total body weight (to the nearest 0.01 mg) of the mussels. These measurements were made along with those for estimating net calcification and dissolution rate. For all measurements, each mussel was carefully removed from the water, immediately weighed under the water (buoyant weight), then gently blotted, and weighed in air (total weight).

Statistical Analysis

To avoid pseudoreplication problems, the variables measured were averaged for the three mussels from each population in each replicate. Two-way ANOVA was used to evaluate whether CO_2 levels, locality, and interaction between factors affected net calcification, shell dissolution rate, growth rate (increase in total weight with time), and survival (Zar 1999). When the analysis showed significant interactions, a one-way ANOVA was carried out for each factor separately in each level from the other factor, followed by Tukey's a posteriori HSD test. When the analysis did not show significant interactions, multiple comparisons were carried out using Tukey's a posteriori HSD test on each factor that showed significant differences (Underwood 1997). Net calcification rate and total weight increase were expressed in milligrams per mussel per day, while shell dissolution was expressed in micrograms per mussel per day. All analyses were carried out using Statistica version 7.0. Assumptions of normality and homoscedasticity for the ANOVA's test were evaluated using the Kolmogorov-Smirnov and Bartlett tests, respectively (Zar 1999).

Results

Water Characteristics

The measured parameters of the seawater are shown in Table 1. Averaging all measurements, the mean (±SD) pH values of the treatments (at 25 °C) were 7.98±0.05, 7.56±0.005, and 7.47±0.05 corresponding to the CO_2 treatments at 380, 700, and 1,000 μatm, respectively (Table 1). The carbonate contents decreased with increase in CO_2, but the seawater was not undersaturated with respect to aragonite and calcite in any of the treatments.

Net Calcification and Shell Dissolution

In specimens from Huelmo Bay, the net calcification (estimated by changes in the buoyant weight) was significantly higher at the control CO_2 treatment than in the intermediate and highest CO_2 treatments, while in this same bay, the net calcification recorded at the intermediate CO_2 concentration was not significantly different from the highest CO_2 treatment (two-way ANOVA; locality: $F_{1,24}=8.71$, $p<0.05$; CO_2 concentration: $F_{2,24}=0.55$, $p>0.05$; locality × CO_2 concentration: $F_{2,18}=5.08$, $p<0.05$; one-way ANOVA: $F_{2,12}=6.22$, $p<0.05$; Fig. 1). No significant effect of CO_2 concentrations on net calcification was recorded in individuals from Yaldad Bay

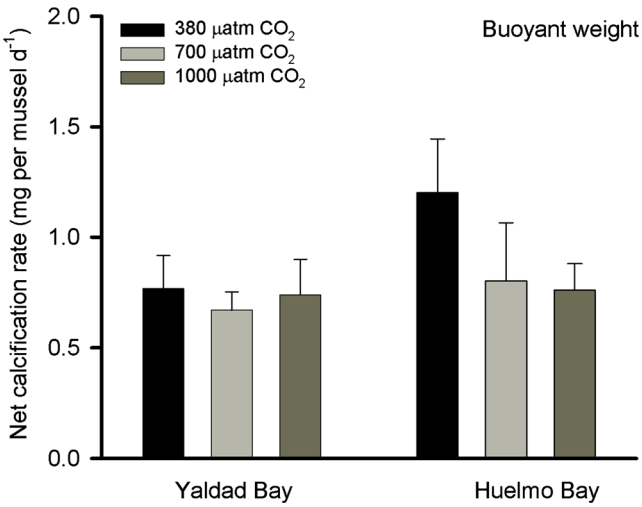

Fig. 1 Net calcification rate (mean±SD) of the individuals of *M. chilensis* from the bays of Yaldad and Huelmo reared under three nominal CO_2 levels (Table 1)

(one-way ANOVA: $F_{2,12}=1.05$, $p>0.05$). There was a location effect, which was driven by higher calcification rate in control animals from Huelmo Bay.

Shell dissolution of *M. chilensis* (estimated by changes in empty shell weight) collected in the different localities and CO_2 treatments are shown in Fig. 2. The shell dissolution rate over the studied period was not affected by CO_2 concentrations. However, a locality effect was detected with values of shell dissolution significantly higher in individuals from Huelmo than from Yaldad Bay (two-way ANOVA; locality: $F_{1,24}=44.19$, $p<0.05$; CO_2 concentration: $F_{2,24}=1.11$, $p>0.05$; locality × CO_2 concentration: $F_{2,24}=0.54$, $p>0.05$; Fig. 2).

Total weight increase (growth rate) under the different CO_2 levels over the duration of the experiment is shown in Fig. 3. Total weight increase of bivalves was significantly higher in individuals from Huelmo Bay. The CO_2 concentration also affected, significantly and negatively, this parameter, so total weight increase was lower in the highest CO_2 concentration than in the control treatment, while total weight increase registered at intermediate CO_2 concentration was not significantly different from the other two treatments (two-way ANOVA; locality: $F_{1,24}=31.70$, $p<0.05$; CO_2 concentration: $F_{2,24}=4.88$, $p<0.05$; locality×CO_2 concentration: $F_{2,24}=1.97$, $p>0.05$; Fig. 3).

Discussion

The short period of exposure used in our study (20 days) was enough to detect significant effects of OA on the net calcification and growth rate of juvenile mussels of *M. chilensis*; such had been shown in a previous study (see Nienhuis et al. 2010), where short (even shorter than this study) exposure time allowed to detect OA acidification effects on marine mollusk. In addition, it is important to emphasize that in a previous study, the responses of this species when exposed for a longer time to high CO_2 levels (60 days; Duarte et al. 2014) were similar to those detected in the present study. This suggests that OA might have significant effects on net calcification in a short period of time.

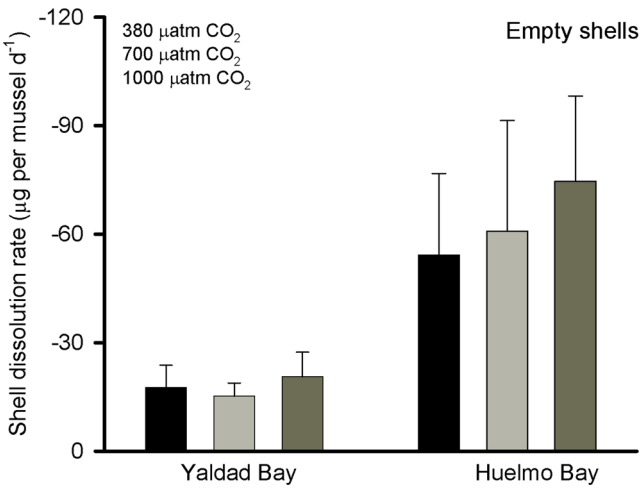

Fig. 2 Dissolution rate (mean±SD) of the individuals of *M. chilensis* from the bays of Yaldad and Huelmo reared under three nominal CO_2 levels (Table 1)

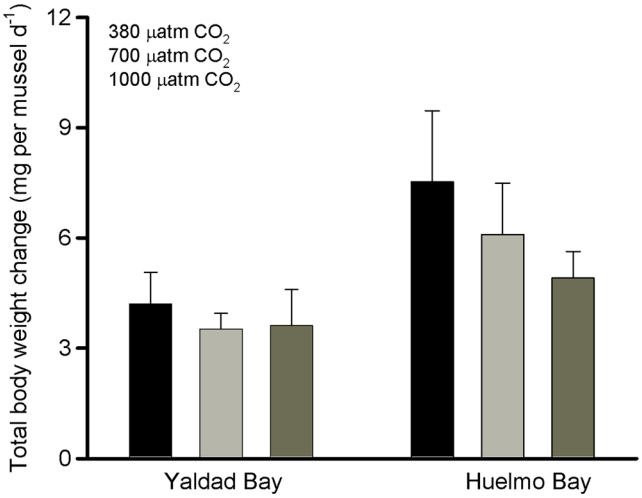

Fig. 3 Total body weight increase (mean±SD) of the individuals of *M. chilensis* from the bays of Yaldad and Huelmo reared under three nominal CO_2 levels (Table 1)

Although the studies carried out to evaluate OA effects have used different methodologies, increasing evidence suggests that the effects of the projected CO_2 increase on calcification and growth rates are species-specific, being in some cases negative (e.g., Duarte et al. 2014; Miller et al. 2009; Nienhuis et al. 2010), in others positive (e.g. Findlay et al. 2009; Iglesias-Rodriguez et al. 2008; Gooding et al. 2009; Gutowska et al. 2010), and in others neutral (e.g. Fabry et al. 2008; Range et al. 2011; Ries et al. 2009). These contrasting responses have also been registered within species closely related to *M. chilensis* (i.e., Mytilid species) (Berge et al. 2006; Gazeau et al. 2007; Michaelidis et al. 2005; Range et al. 2012, 2013, 2013; Thomsen and Melzner 2010; Vhitakari et al. 2013). In this study, we show that *M. chilensis* shows substantial intraspecific variation in response to ocean acidification. Our experiments showed that increased concentration of CO_2 affects negatively the net calcification and growth rate of *M. chilensis*, as it had previously been registered in this species (Duarte et al. 2014). However, in the case of the net calcification rate, only the individuals from Huelmo Bay were affected by OA, but not those of Yaldad Bay for which no significant differences in calcification rate were found among the three CO_2 treatments. It should be noted that the absence of mortality during the experiment indicated that the projected CO_2 levels had chronic, but not lethal, effects on those animals.

Many coastal organisms may already be exposed to waters with high CO_2 levels and subsaturating levels of CO_3^{2-}, similar to those projected to occur in the next century (Talmage and Gobler 2009; Wootton et al. 2008; Hall-Spencer et al. 2008; Pansch et al. 2013; Thomsen et al. 2010, 2013). Recent studies suggest that taxa associated with environments that naturally present high CO_2 levels may have physiological and metabolic adaptations and consequently to be better acclimatized to ocean acidification (e.g., Cummings et al. 2011; Findlay et al. 2009; Kurihara 2008; Pascal et al. 2010; Widdicombe et al. 2009; Sunday et al. 2011). For example, the expression of the chitin synthase (CHS) enzyme, which is key in the synthesis of bivalve shells, was upregulated in individuals of the Antarctic bivalve *Laternula elliptica* exposed to hypercapnic conditions, indicating some degree of adaptation to ocean acidification in this species (Cummings et al. 2011). Similarly, Pascal et al. (2010) recorded that copepods associated with sediments with higher CO_2 concentrations were better adapted to hypercapnic environments than copepods inhabiting sediments with lower CO_2 levels. In this context, the lower susceptibility of the net calcification to ocean acidification recorded in the Yaldad Bay mussels could have resulted from an adaptation of these organisms to the freshwater inputs (from Yaldad River), which may produce naturally lower pH values and low carbonate ion contents (see Feely et al. 2008; Salisbury et al. 2008), as those to which the organisms were experimentally exposed. Our preliminary data would give support to this hypothesis since the pH and the $CaCO_3$ saturation state of water column where mussels are cultured in Yaldad Bay may be substantially lower (pH=25–7.4, Omega Ar <1) compared to Huelmo Bay (pH=25–8.1, Omega Ar >1). We have no evidence to conclude that local adaptation to freshwater inputs at Yaldad River is the ultimate cause for the reported differences. Therefore, we propose this explanation just as a potential mechanism to explain the reported different responses between both populations. More studies comparing the water column characteristics between both bays are needed to support this hypothesis.

A very striking result of this research was that the Yaldad Bay individuals presented to be independent on the CO_2 levels and lower values for the net calcification and growth rate than those recorded in the Huelmo Bay individuals. In other bivalve species, it has been registered that inhabiting in areas with constant freshwater inputs could have a cost for these organisms. For example, Salisbury et al. (2008) recorded that the discharges of the Kennebec River into the Gulf of Maine, which are more acidic than the receptor body, have the potential to negatively affect the survival and settlement of the commercially valuable clam *Mya arenaria*. Similarly, Tunnicliffe et al. (2009) found that populations of the vent mussel *Bathymodiolus brevior*, living in areas with naturally high CO_2 levels and low pH, presented thinner shells and lower daily growth than populations of this species living in waters with pH>7.8. This could be the case for the individuals of Yaldad Bay, although it is necessary, again, to indicate that more data are needed to confirm this (see above). It must be indicated that the lower calcification and growth rate of the Yaldad Bay individuals could also result from natural intraspecific variability between the two populations. However, independent on the factors that account for these differences (out of the scope of this study), the results highlight the importance of considering the intraspecific variability in the responses of the individuals to OA, as previously demonstrated (Range et al. 2013). We strongly agree that laboratory settings may impose limitations since those conditions do not recreate the natural environment. However, in our study, individuals of both populations were exposed to similar laboratory conditions.

Animal net carbonate production is influenced by shell deposition and shell dissolution (Nienhuis et al. 2010). Consequently, both of these processes must be evaluated when the effects of ocean acidification on the net calcification rate are studied (Iglesias-Rodriguez et al. 2008; Nienhuis et al. 2010) since an increase in the deposition rate in order to overcome shell dissolution could disguise the effects of ocean acidification (Iglesias-Rodriguez et al. 2008). The shell dissolution rate of the individuals of both populations did not present significant differences among the three CO_2 treatments, which would indicate two things: (i) the Yaldad Bay individuals did not have to increase their deposition rate to compensate for shell dissolution, thus confirming that ocean acidification did not affect the net calcification rate of this population at all and (ii) the net calcification rate of the Huelmo Bay individuals was not affected by this process, but by the deposition process. This result disagrees with the study of Nienhuis et al. (2010), where elevated CO_2 concentration affected the shell dissolution rate but not the deposition rate in the intertidal snail *Nucella lamellose*. It is important to emphasize that in both populations, the shell dissolution accounted less than 6 % of the total shell weight, and that the experimental seawater was not undersaturated for calcite or aragonite. Therefore, the reported shell lost could be a minor artifact that contributed equally to each treatment and associated with handling during the measurements. Interestingly, this reduction in shell calcification of the Huelmo Bay population was found despite the fact that the seawater in the treatments with higher CO_2 content was saturated with respect to the calcium carbonate measured. Different studies have reported that the calcification rate may decrease even if seawater remains above the saturation state (e.g., Beniash et al. 2010; Ries et al. 2009; Talmage and Gobler 2009). For example, in a recent study, Beniash et al. (2010) showed that exposure to high CO_2 levels resulted in a significant reduction in the shell deposition rate of the oyster *Crassostrea virginica*, and that this reduction occurred in spite of the fact that the seawater was not undersaturated with

respect to calcite. Similarly, another study also reported a significant decrease in larval growth (total length) of the bivalves *Mercenaria mercenaria* and *Argopecten irradians* when exposed to high CO_2 levels even in seawater saturated with respect to calcite (Talmage and Gobler 2009). The calcification rates of the mussel *M. edulis* and the oyster *Crassostrea gigas* decreased in seawater enriched with CO_2, but not supersaturated with respect to calcium carbonate (Gazeau et al. 2007). The calcification rate of other groups, such as foraminifera, coccolithophores, and corals, has also been shown to decrease with low carbonate concentration even in saturated seawaters (Kleypas et al. 2006; Riebesell et al. 2000). In fact, Doney et al. (2009) indicated that for many organisms, the chemical saturation threshold do not relate with the calcification thresholds. Therefore, the supply of carbonates would not explain the negative effects on the net calcification rate of the Huelmo Bay individuals recorded in this study; these calcification problems would therefore most likely be related with hypercapnia or acidosis; this has previously been proposed by Arnold et al. (2009) to explain why the lobster *Homarus gammarus* showed a reduction in the mineral content (calcium and magnesium) of its carapace in treatments with CO_2 acidified sea water even though in these treatments, the seawater was not undersaturated with respect to the calcium carbonate polymorphs measured by the authors.

Conclusions

This study evaluated simultaneously and under similar conditions the impacts of OA on individuals from two populations of the same species over a 20-day time scale. The results of this study corroborate the increasing evidence that shows that substantial intraspecific variation in response to increments of CO_2 levels may also occur, which warns the danger of generalizing the results obtained even within the same species. Only in the higher CO_2 concentrations that the responses of *M. chilensis* were negatively affected, which suggests that, within certain limits, this species can cope with some CO_2 increase. The absence of mortality indicated that the projected CO_2 levels had chronic, but not lethal, effects on the experimental animals. More studies should be focused on comparing directly the responses of different populations to OA and the specific mechanisms (e.g., physiological adaptation) that allow some populations to be less sensitive to this phenomenon.

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