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# Estuarine conditions more than pH modulate the physiological flexibility of mussel *Perumytilus purpuratus* populations

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ABSTRACT

Coasts and their marine biota are exposed to major environmental heterogeneity as a consequence of natural drivers and anthropogenic stressors. Here, individuals of the mussel *Perumytilus purpuratus* from two different geographical populations exposed to contrasting environmental conditions (i.e. estuarine versus open coastal conditions) were used in a reciprocal transplant and a laboratory experiment with the aim of determining the grade of of local adaptation to their native sites, as well as their sensibility to ocean acidification. After characterizing environmentally the coastal habitats, a set of life-history traits and a phenotypic plasticity index were determined for both mussel populations. From the reciprocal transplant experiment, we observed that mussels originally coming from the estuarine habitat exhibited a distinctive performance pattern usually associated with physiological stress (i.e. higher metabolic rates, lower calcification and growth rates) leading to important physiological *trade-offs* and higher levels of phenotypic plasticity. Alternatively, mussels originating from the open coastal site showed lower physiological phenotypic plasticity suggesting a high grade of local adaptation to their habitat. In addition, both populations responded very similarly to lower pH conditions (i.e. increased metabolic rates with no important effects on growth and calcification, and lower physiological phenotypic plasticity). The study results indicated that overall estuarine conditions more than isolated pH changes would be modulating the performance and the level of phenotypic plasticity of the two *P. purpuratus* geographical populations studied. Our study also emphasizes the necessity of characterizing phenotypic plasticity under multiple-driver environments to cast more accurate predictions about the susceptibility of marine biota to future climate stressors such as ocean acidification.

## 1. Introduction

Phenotypic plasticity is the potential of a genotype to render different phenotypes in response to changing environmental conditions (Via, 1993). Indeed, phenotipic plasticity expression is considered as an important adaptive strategy to cope with variable, and sometimes stressful environments (Stearns, 1989; Schlichting and Pigliucci, 1998; Botero et al., 2015; Gaitán-Espitía et al., 2017; Ramajo et al., 2020). Therefore, assessing environmental variability and its role to modulate the phenotypic plasticity and the physiological performance of marine species is key to determine how they will face future ocean conditions entailed by climate change (Calosi et al., 2017; Ramajo et al., 2020).

Coastal areas such as estuaries are suitable places to test phenotypic plasticity as they show heterogeneous environmental conditions at

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multiple scales when comparing with ocean and other open coastal areas (Vargas et al., 2017). River plumes can transport acidic water (Feely et al., 2008), and estuarine zones often show high concentrations of dissolved $CO_2$ ($pCO_2$), lower pH, and undersaturated carbonate conditions (Frankignoulle et al., 1998). Additionally, freshwater inputs by rivers commonly decrease the salinity and total alkalinity ($A_T$) of coastal seawater (Raymond and Cole, 2003; Marshall et al., 2008), although $A_T$-salinity relationship in estuaries can be scattered and variable as a consequence of the spatio-temporal variability of the continental alkalinity and terrestrial processes (e.g. calcification, anaerobic respiration, land use, among others) (e.g. Pérez et al., 2016). The effects of living under higher $pCO_2$ conditions have been extensively studied on marine organisms in the context of ocean acidification (OA) (Kroeker et al., 2013). In general, studies show important harmful effects over a great majority of taxa and organisms (i.e. mainly calcifiers), physiological traits (e.g. growth, calcification), and ecological processes (e.g. abundance) (Hendriks et al., 2010; Kroeker et al., 2011, 2013). Besides, salinity drives important changes, direct and indirectly, on growth, biomineralization, metabolism, survivorship, and reproduction on several invertebrate species (i.e. Wang et al., 2011, 2012, 2013; Wrange et al., 2014; Osores et al., 2017; Ramajo et al., 2016a: Duarte et al., 2018; Grenier et al., 2020). Nevertheless, neutral impacts (or even positive) have been also observed to lower pH/$pCO_2$ (i.e. Ramajo et al., 2016b) and salinity levels (i.e. Wrange et al., 2014). These counterintuitive observations have been attributed to the presence of evolutionary mechanisms (i.e. phenotypic plasticity and/or local adaptation) underpinned by the historical environmental conditions that species experience in their native habitats (i.e. Hendriks et al., 2015; Ramajo et al., 2016a, 2019, 2020; Lagos et al., 2016; Vargas et al., 2017). Indeed, multiple researches have concluded that the phenotypic plasticity and tolerance to climate stressors of multiple species are bounded by the local patterns of environmental variability of the populations from where they were obtained (i.e. Lewis et al., 2013; Wrange et al., 2014; Eriander et al., 2015; Ramajo et al., 2016a,b, 2019; Vargas et al., 2017). In this regard, tools as the reciprocal transplants, as well as common-garden experiments allow comparing dissimilarities on key physiological traits among different geographical populations exposed to dissimilar environmental conditions to identify how well-locally and non-locally adapted are these populations, as well as their level of sensibility to particular climate stressors (Stelkens et al., 2012; Osores et al., 2017; Ramajo et al., 2017).

The mussel *Perumytilus purpuratus* (Lamarck, 1819) locally dominates the primary space of the mid–intertidal zone over vast sections of the coast from Perú to Southern Argentina (Wieters et al., 2012). This broad geographical distribution makes that *P. purpuratus* populations be exposed to large differences in the mean environmental conditions and patterns of variability (e.g. Ramajo et al., 2015). Indeed, the Chilean coast shows a clinal latitudinal gradient of Sea Surface Temperature (SST), overlapped with regional changes in Sea Surface Salinity (SSS) and ocean/atmosphere $CO_2$ fluxes (Strub et al., 1998; Torres et al., 2011; Ramajo et al., 2015). These latitudinal and regional changes are a consequence of the oceanic and atmospheric circulation processes, the latitudinal gradient in solar radiation, wind-driven upwelling events along the central-northern region, as well as the seasonal inflows of freshwater from rivers and glaciers, among others (Strub et al., 1998; Torres et al., 2011; Mayol et al., 2012; Dávila et al., 2002; Lagos et al., 2005, 2008). While SST and SSS show a clear decrease from north to south, the $pCO_2$ surface coastal water tends to increase poleward due to the presence of dissimilar biological (primary productivity/respiration), physical (e.g. upwelling/haline stratification, temperature), and biogeochemical processes (e.g. salinity, $A_T$) (Torres et al., 2011; Mayol et al., 2012; Ramajo et al., 2013, 2015, 2019).

Based on that, hereby using the differential environmental regimes found in the multiple *P. purpuratus* habitats where this species lives allowed us to scale the use of phenotypic plasticity to assess differences in the local adaptation patterns, as well as the sensibility to climate change stressors such as OA. Indeed, our hypothesis sought to corroborate what previously has been suggested, that living under more heterogeneous and physiological demanding environments could reduce the vulnerability of marine species to some climate stressors. To do that, here first we examined the level of phenotypic plasticity and local adaptation of two geographically separated populations of the mussel *P. purpuratus* that experience different environmental conditions (estuarine and open coastal), to then evaluating their sensibility to different pH conditions that simulated the current and future OA scenarios.

## 2. Material and methods

### 2.1. Study sites

Selected *P. purpuratus* populations came from Las Cruces (33°30′S, 71°38′W; hereafter, *central population*) and Calfuco (39°46′S, 73°39′W; hereafter, *southern population*) (see Fig. S1). The central location is influenced by coastal upwelling events during austral spring-summer (Wieters et al., 2003) that imply lower SST (Wieters et al., 2003; Lagos et al., 2007; Broitman et al., 2018), and lower pH conditions (Pérez et al., 2015; Ramajo et al., 2016a). After accounting for upwelling variability, SST and pH at Las Cruces are relatively stable with values that range between 12 and 14 °C and 7.7 to 7.9, respectively (Ramajo et al., 2016a). Following the open-coast location of the central site, salinity is highly stable and higher than 33.5 (Narváez et al., 2004; Ramajo et al., 2016a). Wind-induced equatorward excursions of the freshwater plume of the Maipo river, located about 15 km south, can take place during the upwelling season, in association with high flow periods (Meza et al., 2012), which can modify the salinity and carbonate system parameters over diurnal scales (Ramajo et al., 2016a). In terms of productivity (i.e. chlorophyll-*a*), the coastal phytoplankton community at the central site is dominated by pico and nano-phytoplankton (Vargas et al., 2006), and higher concentrations correspond to spring peaks during upwelling relaxation (Wieters et al., 2003). The southern location, located ca. 800 Km south of central site, is persistently influenced by Valdivia river run-off (see Torres et al., 2013; Aguilera et al., 2013). Valdivia river discharges increase the range of variation of salinity (30.0–34.0), alkalinity (2000–2400 μmol $kg^{-1}$), and pH (8.3–7.8) (Torres et al., 2013). Other environmental factors such as the tidal cycle and the annual temperature cycle amplify SST variability at the southern site (10-15 °C, see Torres et al., 2013). Moreover, primary productivity in central location is highly variable as a result of the input of terrestrial organic matter transported by Valdivia River (Aguilera et al., 2013; Pérez et al., 2016).

### 2.2. Field reciprocal transplant experiment

In order to evaluate the potential local adaptation to their habitats and phenotypic plasticity patterns of the two selected *P. purpuratus* populations, a field reciprocal transplant was performed. Mussels from both study sites were collected by hand during low tide periods in March 2012 from the mid-intertidal from semi-exposed platforms with similar tidal regimes (SHOA 2020). Then, mussels were transferred to the laboratory, labeled with bee tags glued onto the shells allowing their identification through the experiment, and the initial size (maximum length) and buoyant weight were determined. Further, by following a similar experimental design described in Ramajo et al. (2016a), 150 individuals with similar maximum shell length (6.51 ± 0.04 mm; *one-way* ANOVA test: $F_{3,149} = 0.44$, $P = 0.722$) were used. Hereafter, *self-transplant treatment* indicates the treatment where the origin and destination sites of mussels were the same, while the *transplant treatment* corresponds to the treatment where mussels were moved between study sites (i.e. from central to southern location, and vice versa) (see Fig. S2). After 50 days (from April to May 2012), all experimental mussels were removed from the field, transported to the laboratory, and maintained in aerated seawater of each location until their final size and buoyant

weight, as well as their metabolic rates, were recorded. During the experiment, 3% of experimental mussels lost their labels (bee tags), probably due to wave action or traction from byssal threads of other individuals inside the experimental cages. Unfortunately, rough weather conditions at the southern site during the experiment caused the loss of two experimental cages, one assigned to the *self-transplant*, and one assigned to the *transplant treatment*.

### 2.3. Laboratory OA common-garden experiment

To evaluate the susceptibility of both *P. purpuratus* populations to future changes in ocean pH levels (OA), a common-garden experiment was performed (see Fig. S2). Mussels from central and southern locations were collected and subsequently transferred to the laboratory in aerated seawater sourced from their native habitats. After that, mussels were acclimatized for 7 days under similar conditions of temperature, salinity, and $pH_{NBS}$ (14 °C, 33.0 psu and 8.1, respectively), and subsequently exposed for a total of 33 days to seawater at current (control pH treatment = 8.1) and future pH conditions (low pH treatment = 7.6) predicted by 2100 (IPCC, 2007). Three aquaria (9 L volume) were used per pH treatment, and 20 healthy mussels (i.e. no shell damage) with similar size (central: 9.01 ± 0.06 mm; southern: 9.06 ± 0.06 mm; *one-way* ANOVA test: $F_{1,236} = 0.33$, $P = 0.567$) were haphazardly assigned to each replicate. The experimental pH treatments were generated by aerating the seawater of each aquarium with a mix of pure atmospheric air (no $CO_2$) and pure $CO_2$ using a pH controller (IKS Aquastar, Germany). Mussels were daily fed *ad libitum* (>5% of their dry weight, see Martinez et al., 2000) with a mix of *Isochrysis* sp. and *Pavlova* sp. (Phytogold S, Brigtwell, USA). Aquaria were cleaned and re-filled with filtered (10 μm plus UV filter) and pH treated water every 3 days to maintain water quality and stable salinity levels (33.0). The aquaria received a 12 h:12 h light cycle, and the water temperature was maintained at 14±1 °C using a programmed chiller (BOYU, Model L075).

### 2.4. Environmental monitoring at study sites and laboratory experimental conditions

Environmental conditions at both experimental sites (Las Cruces and Calfuco) were monitored weekly from 2010 to 2013 (see Fig. S4). At each site, SST and SSS were monitored using a CTDO (Model OCEAN SEVEN 304, Hydronaut©, Italy). In addition, discrete seawater samples (n = 3) were collected from the intertidal zone at both study sites for $pH_T$ (total scale). The pHmeter was calibrated with TRIS buffers (pH = 8.089) at 25 °C using a thermoregulated water bath and commercial buffers (Metrohm®, Switzerland). Experimental conditions during the OA common-garden experiment were determined twice per week (see Table 2). Two discrete seawater samples from each experimental aquarium were used to determine pH (NBS scale). Experimental temperature and salinity of aquaria were measured using a digital salinometer (Eutech-Salt-6). For both experiments, water pH was always measured within the first 60 min after sample collection to avoid changes in the real values and determined by using a Metrohm 826 pHMobile Meter (Metrohm®, Switzerland) connected to a combined electrode (double juncture). Total Alkalinity ($A_T$) for both experiments were determined by using two discrete water samples stored in 500 mL borosilicate glass bottles (Corning, USA), and fixed with mercuric chloride ($HgCl_2$, 0.2 $cm^3$ of a 50% saturated solution). Bottles were sealed with Apiezon® L grease (Sigma Aldrich, St. Louis, USA) and kept in the laboratory until they were measured. $A_T$ from the central site was estimated using automated potentiometric titration (Haraldsson et al., 1997), while $A_T$ samples from the OA common-garden experiment were determined by using an automatic titration method (open-cell method) (Tritando 808 and Aquatrode plus, Metrohm®) with HCl (Fixanal®) and double endpoint titration to pH 4.45 and 4.41 (NBS scale) following Dickson Sop 3 b (version 3.01). The accuracy of $A_T$ measurements was checked using certified reference seawater supplied by the Scripps Institution of Oceanography (San Diego, CA). Problems with $A_T$ samples from Calfuco (southern site) mandate us to estimate $A_T$ values for this location, which were calculated using the relationship between salinity and $A_T$ recorded for the same site during 2011 ($A_T = 248.9 + 58.9SSS$, n = 94, $R^2 = 0.91$, $P < 0.0001$) (see Fig. S4). For both experiments, the partial pressure of $CO_2$ ($pCO_2$), pH (*in situ*), and saturation states (Ω) of calcite and aragonite were estimated using the CO2SYS software (Pierrot et al., 2006), and the dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987), and $KHSO_4$ (Dickson 1990) (see Fig. 1, Table 2, and Fig. S3).

Additionally, interannual patterns of variability in nearshore oceanographic conditions at both study sites were examined using remote sensing. Satellite images of Chlorophyll-*a* (Chl-*a*, $mg/m^3$) and SST (°C) from the MODIS-Aqua platform were downloaded from the NASA Ocean Color website (http://oceancolor.gsfc.nasa.gov). A level-3, eight-day night-time mapped composites at 4 km spatial resolution was used for the 2003–2015 period of the MODIS mission, with the Chl-*a* data generated using the standard OC3 algorithm (O'Reilly et al., 2000). For each study location, the nearest valid coastal pixel was located and then derived the 8-day 2003–2015 time series by averaging Chl-*a* and SST retrievals over an area of 12 km alongshore and 20 km offshore (i.e. 3 × 5 pixels). The spatial averaging was carried out in order to improve the number of valid observations over the small area considered in the analysis (Broitman et al., 2005) (see Fig. 2 and Fig. S5).

### 2.5. Biological responses

Metabolic rates were determined as oxygen consumption rates, which were quantified at the end of both experiments (reciprocal transplant and OA common-garden). Before the measurements, mussels from the reciprocal transplant experiment were transported to the laboratory and acclimatized at 14 ± 1 °C for 7 days in aerated seawater collected from the same location that individuals were assigned during the experiments. Finally, mussels were acclimated by 48 h before oxygen consumption rate measurements and starved for 24-h by in UV filtered seawater from the same treatment which they were assigned (reciprocal transplant experiment) or seawater of the pH treatment assigned (OA common-garden). The temperature was controlled (14 °C) during measurements using a chiller. Before measurements, mussels were detached from the cluster formed during the acclimatization period which could generate non-desired stress in the experimental mussels potentially affecting the magnitude of the metabolic rates measured (i.e. overestimation). As a solution, the first 10 min of the oxygen consumption measurements were eliminated to estimate the final metabolic rates. Oxygen consumption rates, in mussels from the reciprocal transplant experiment were measured using an oxygen optode connected to a Microx TX3 temperature-compensated oxygen meter (Presens, GmbH, Germany) with a tip diameter of 140 μm using respiration chambers of 20 and 30 ml. In the case of mussels from the OA common-garden experiment, metabolic rates were determined using an optical fiber system (Presens Mini Oxy-4 Respirometer, Germany) and respiration chambers of 25 ml. Between 12 and 14 mussels were measured by treatment for the reciprocal transplant experiment, while for the OA common-garden experiment 6 individuals were measured by replicate (aquarium). Only one mussel was used by chamber during the metabolic measurements for both experiments. Prior to all measurements, oxygen sensors were calibrated with a solution of $Na_2O_3S$ at 5% and aerated water at 0% and 100% air saturation at 14 °C, respectively. Oxygen consumption rates are reported as consumption per gram of animal buoyant weight (i.e. $mgO_2h^{-1}g^{-1}$). Survivorship was monitored at the end of the reciprocal transplant experiment, and daily during the OA common-garden experiment. Growth rates were estimated as the change in maximum shell length between initial and final experimental periods using a Vernier caliper (Mitutoyo, precision ± 0.01 mm). Net calcification rate was calculated using the buoyant weight technique (Davies 1984). Buoyant weight (BW) was converted into dry weight (DW)

according to the equation:

$$DW = \frac{BW}{\left(1 - \frac{\rho_{seawater}}{\rho_{aragonite}}\right)}$$

where, $\rho_{seawater}$ is the seawater density where experimental mussels were weighed (salinity = 33.0, temperature = 14 °C) and $\rho_{aragonite}$ is the density of aragonite (see Ramajo et al., 2016b). In this case, aragonite density (2.93 g $cm^{-3}$) was used as shell density as this is the only $CaCO_3$ polymorph in *P. purpuratus* shells (Taylor et al., 1969; Ramajo et al., 2016a). Finally, net calcification rates were calculated as the change in shell DW between the initial and final of both experiments and normalized by the initial shell DW ($mgCaCO_3 g^{-1} d^{-1}$). Net calcification rates include both precipitation and dissolution rates of shells, which incorporate the net amount of calcium carbonate, organic matrix, and inorganic carbon deposited and lost by the animal over time (i.e. Ramajo et al., 2016b). Finally, some mussels from both experiments (reciprocal transplant and OA common-garden) were euthanized, and the shells were used to estimate the amount of total shell organic matter (i.e. periostracum and, inter and intra-crystalline organic matter). Ten to twenty-five shells for each treatment from both experiments were randomly selected, dried for 24 h at 60 °C,and weighted. Subsequently, dry shells were burned at 500 °C for 5 h and weighted in order to estimate the shell organic matter content (i.e. periostracum and intra and inter-crystalline shell organic matter). In order to establish differences among treatments (*self-transplant* and *transplant*; control and low pH treatments), the organic shell matter of each specimen was standardized by the shell dry weight.

### 2.6. Phenotypic plasticity index

For both experiments, we calculated a percent change for all biological traits measured as an index of phenotypic plasticity. This fractional change allowed us to compare the phenotypic plasticity grade between *central* and *southern P. purpuratus* populations. $PI_{md}$ (plasticity index) was estimated as:

$$PI_{md} = \left[ (Median_{max} - Median_{min}) / Median_{max} \right] \times 100$$

where, $Median_{max}$ – $Median_{min}$ corresponds to median values for each one of the biological traits measured in both experiments for both local populations (see Valladares et al., 2006).

### 2.7. Data analysis

Differences in the initial shell length between individuals of each population for both experiments were evaluated using one-way ANOVA. For the reciprocal transplant experiment, differences in biological responses (growth, net calcification, and shell organic matter) were determined using two-way ANOVA considering individual mussels as replicates, and the origin and destination of the mussels as fixed factors. For OA common-garden experiment, the geographical origin of individuals (central and southern) and the pH treatment (control and low pH) were used as fixed factors. Differences in metabolic rates were evaluated, for both experiments, using a *two-way* ANCOVA and buoyant weight as a covariate. Multiple comparisons were carried out using *a posteriori* Tukey's HSD test. For a discussion regarding pseudo-replication during the reciprocal transplant experiment, see Ramajo et al. (2016a). In order to compare environmental conditions between study sites in terms of pH, SST, SSS, and carbonate systems parameters ($A_T$, $pCO_2$, and Ω), we used paired t–tests and multivariate analysis of variance (MANOVA) with a reclassification test (Discriminant Function Analysis, DFA). Prior to all statistical analyses, data were transformed to satisfy normality and homogeneity of variance assumptions, which were evaluated using Shapiro-Wilk and Levene tests, respectively. Values reported through the text are means ± SE. Tests were performed using JMP software (version 9.0.1).

## 3. Results

### 3.1. Environmental conditions of study sites

On average, from 2010 to 2013, the central site (Las Cruces) showed significantly higher SST (>1.58 ± 0.36 °C), SSS (>1.48 ± 0.23) and $A_T$ (>92 ± 24 μmol $kg^{-1}$) than the southern site (Calfuco). Significant differences were not detected in $pH_T$, $pCO_2$, and carbonate saturation (Ω) between sites (Fig. 1 and Fig. S3). The southern site showed higher variability in SSS (CV = 2.81%) (Fig. 1B, D and Fig. S4) than the central site (CV = 0.45%). On the other hand, the central site showed the highest variability in SST (CV: central = 13.91%; southern = 8.41%) (Fig. 1A and Fig. S3), while $pH_T$ and $pCO_2$ showed similar variability between sites (Fig. 1C and Fig. S3). Calcite (CV: central = 26.28%; southern = 16.44%) and aragonite saturation states (CV: central = 26.63%; southern = 16.54%) showed higher variability at the central than southern site. Multivariate analysis indicated that spatial–temporal variability in SST, SSS, and $A_T$ showed a significant discriminatory power between study sites (MANOVA: Wilks's $\lambda = 0.560$, $P < 0.001$). Reclassification analysis established that 84% of the environmental samples were successfully classified into the corresponding geographical location from where come from. Satellite data from MODIS-Aqua showed warm water conditions (i.e. exceeding 1 standard deviation) around the central Chile location during early March and late April through May 2012, which were alternated by brief cooling events (see Fig. S5). These short-cooling events did not impact Chl-*a* concentrations off the central site, which were extremely low during the austral fall period (from March to May 2012) (Fig. 2A). At the southern location, a similar anomalously warm event was registered during March 2012 (early austral fall), but then cool conditions prevailed until the beginning of austral winter (June–July). Although the Chl-*a* records are incomplete for fall-winter due to persistent cloudiness, the valid satellite retrievals showed elevated Chl-*a* concentrations during the fall period (Fig. 2B).

### 3.2. Reciprocal transplant experiment

Mussels from the central site presented significantly lower metabolic rates (0.703 ± 0.081 $mgO_2 h^{-1} g^{-1}$) than mussels from the southern site (1.298 ± 0.347 $mgO_2 h^{-1} g^{-1}$) (Tukey HSD test: $P < 0.05$). After 50 days, individuals transplanted from the central to the southern site showed no changes in their oxygen consumption rates (Tukey HSD test: $P > 0.05$). In contrast, individuals transplanted from the southern to the central site decreased significantly their oxygen consumption rates (0.508 ± 0.061 $mgO_2 h^{-1} g^{-1}$) to similar levels to those observed in the mussels sourced from the central site (Tukey HSD test: $P > 0.05$, see Fig. 3A). These patterns of variability led to significant differences in metabolism between local populations (*two-way* ANCOVA: Origin, $F_{1,44} = 4.68$, $P = 0.036$) and in the interaction term (Origin x Destination; $F_{1,44} = 4.25$, $P = 0.045$) (Table 1). Growth rates of individuals from the central site (self-transplant treatment) were significantly higher (0.009 ± 0.001 mm $d^{-1}$) than from the southern site (0.004 ± 0.000 mm $d^{-1}$, Tukey HSD test: $P < 0.05$, Fig. 3B). After 50 days, mussels transplanted from the southern to the central site increased significantly their growth rates to values up to 0.014 ± 0.001 mm $d^{-1}$, a rate that was higher than that observed in self-transplanted mussels at the central site (Tukey HSD test: $P < 0.05$). On the contrary, mussels transplanted from central to the southern site decreased their growth rates by almost 50% (0.004 ± 0.000 mm $d^{-1}$), thus matching the growth rates observed in the local population (Tukey HSD test; $P > 0.05$, Fig. 3B). These patterns of variability led to significant differences in growth between destination site (two-way ANOVA: Destination, $F_{1,140} = 232.7$, $P < 0.0001$) and in the interaction term (*two-way* ANOVA: $F_{1,140} = 20.11$, $P < 0.0001$)

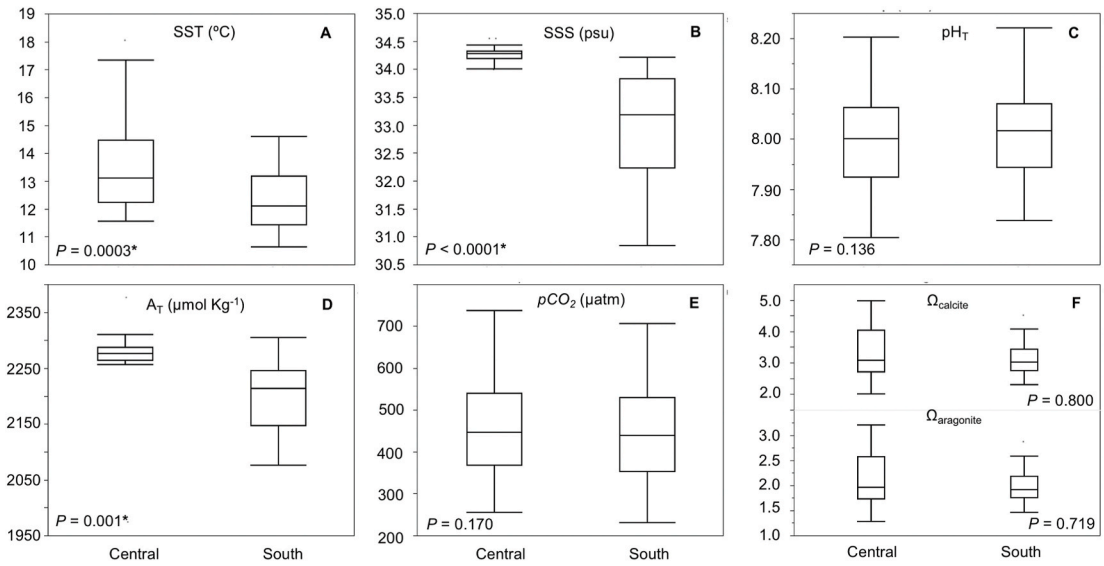


**Fig. 1. Environmental conditions**. Environmental comparison of (A) SST, (B) salinity, (C) $pH_T$, (D) $A_T$, (E) $pCO_2$, and (F) saturation state (Ω) of calcite and aragonite for central and southern sites (Las Cruces and Calfuco, respectively) during 2012. Significance values from paired t-test are shown (α= 0.05). Box boundaries indicate quartiles and whiskers indicate the range of environmental data.

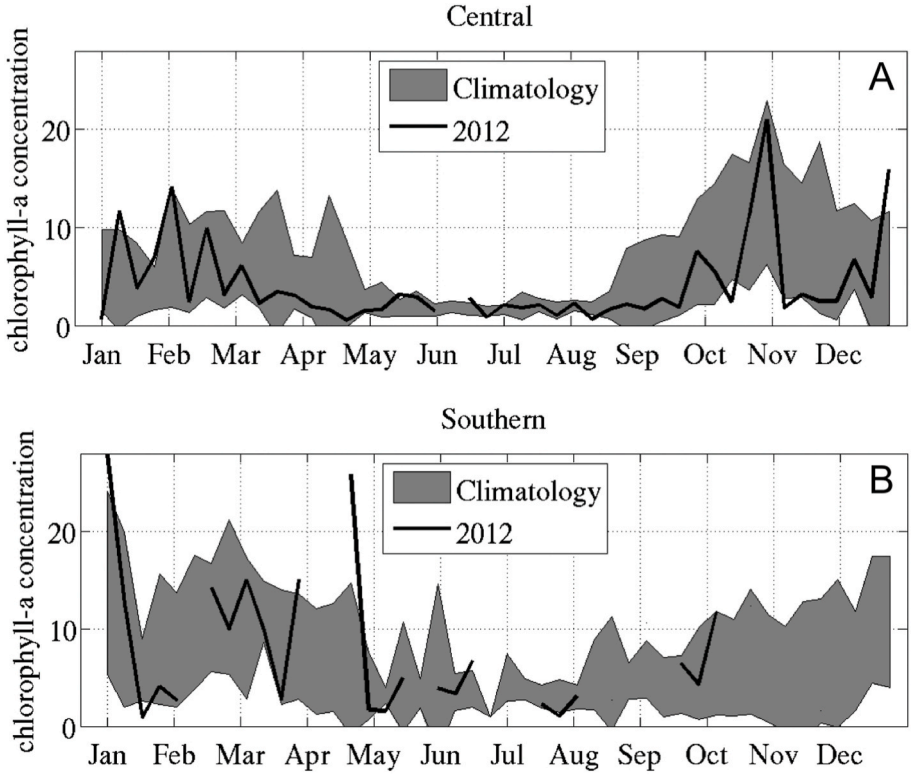


**Fig. 2. Food Availability**. Satellite-derived time series of Chlorophyll-a concentrations of the (A) central and (B) southern sites. The black lines show the values of the 8-day records available for the 2012 study period and the shaded areas represent +/- 1 standard deviation around the climatological (2003-2012) mean.

(Table 1). Net calcification rates were significantly different when we compared local populations (*two-way* ANOVA: $F_{1,140} = 80.75$, $P < 0.0001$, Table 1). Both mussel populations in the *self-transplant* conditions presented significant differences in their net calcification rates. Individuals from the *southern population* showed significantly higher calcification rates (6.3467 ± 0.5615 $mgCaCO_3g^{-1}d^{-1}$) than the *central population* (3.5486 ± 0.2550 $mgCaCO_3g^{-1}d^{-1}$; Tukey HSD test; $P < 0.05$). No significant differences in calcification rates were found between mussels transplanted from the central to the southern site (3.2948 ± 0.3751 $mgCaCO_3g^{-1}d^{-1}$), as well as, in those mussels transplanted from the southern to the central site (7.6072 ± 0.3838 $mgCaCO_3g^{-1}d^{-1}$, Fig. 3C). The total shell organic matter content in mussels from the southern site was significantly higher than in mussels from the central site (Tukey HSD test: $P < 0.05$, Fig. 3D). After 50 days, mussels transplanted from the central to the southern site increased significantly their shell organic matter amount to values similar to those

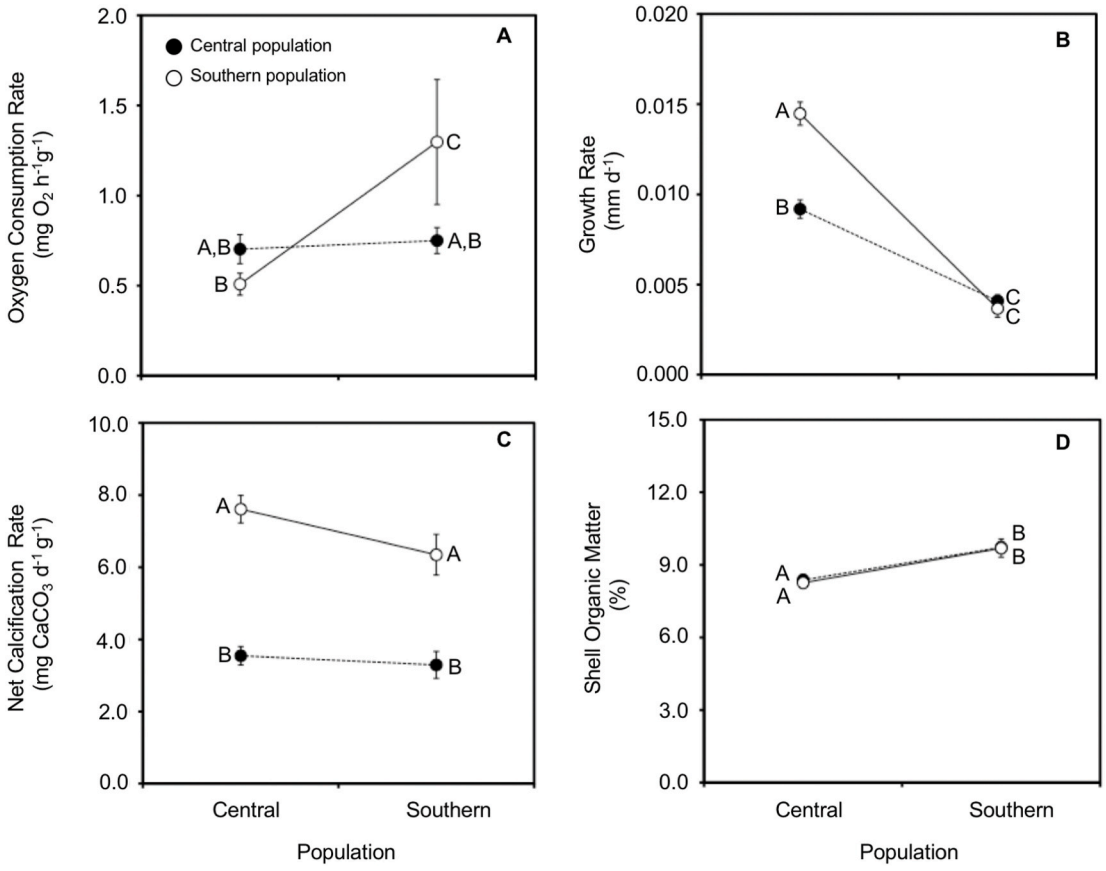


**Fig. 3. Reciprocal Transplant Experiment**. (A) Metabolic rates measured as oxygen consumption ($mgO_2$ $h^{-1}g^{-1}$), (B) growth rate (mm $d^{-1}$), (C) net calcification rate ($mgCaCO_3$ $d_{-1}g_{-1}$), and (D) shell organic matter (%) of *P. purpuratus* individuals exposed to environmental conditions of the central and southern sites under a reciprocal transplant experimental design. Different letters beside each symbol indicate significant differences between experimental treatments evaluated using the Tukey HSD test as a post *hoc comparison*.

registered in mussels from the southern population (9.70 ± 0.37%, Tukey HSD test: $P < 0.05$), while mussels transplanted from southern to central site decreased their content of shell organic matter (8.26 ± 0.21%, Fig. 3D). These patterns of variability lead to significant differences in the percentage of shell organic matter between destination site (*two-way* ANOVA: $F_{1,78} = 40.21$, $P < 0.0001$, see Table 1). Survivorship was not affected by origin or destination site during the experiment (Fig. S6). In terms of the phenotypic plasticity index, we observed higher values for mussels from the southern population for both metabolic and growth rates. However, traits related to shell processes (i.e. net calcification rate and shell organic matter) presented no appreciable differences between both mussel populations (Fig. 4A).

### 3.3. OA common-garden experiment

*P. purpuratus* oxygen consumption rates increased significantly at low pH for both populations (*two-way* ANCOVA: $F_{1,7} = 11.98$, $P = 0.011$), however not significant differences were found between central and southern mussel populations (Table 3 and Fig. 5A). Significant differences in growth rates were observed in the interaction term (*two-way* ANOVA: Origin x pH, $F_{1,8} = 8.40$, $P = 0.020$, see Table 3). Growth rates of experimental mussels from both populations did not differ in the control treatment, and they were not affected by the low pH conditions (Fig. 5B). Net calcification rates were not affected by low pH, however mussels from the *southern population* showed significantly higher net calcification than mussels from the central site (*two-way* ANOVA: Origin, $F_{1,8} = 341.00$, $P < 0.001$) (Table 3, Fig. 5C). In terms of shell organic matter, no significant differences were found between mussels from both populations in both pH treatments, and nor between control and lower pH conditions (Fig. 5D). Survivorship was not affected by pH treatments for both populations (Fig. S6). The phenotypic plasticity index ($PI_{md}$) was higher for metabolic, growth and calcification rates in mussels from central than southern populations after the OA common-garden experiment, while similar values of $PI_{md}$ were observed in shell organic matter for both *P. purpuratus* populations (Fig. 4B).

## 4. Discussion

We detected significant environmental differences between study sites in SST, $A_{T,}$ and SSS, which showed a clear impact over a relevant set of physiological traits, together with the magnitude of phenotypic plasticity expressed by the two populations of *P. purpuratus* considered in our study. From the reciprocal transplant experiment, we observed that individuals sourced from the mussel population residing at the southern location were clearly stressed by their local environmental conditions in comparison with mussels from the *central population*, as evidenced by their increased metabolic rates and their reduced growth rates. On the other hand, the heterogeneous environmental conditions at the southern site led to a higher plasticity index in several physiological traits (i.e. metabolic and growth rates). The common-garden experiment, where only pH conditions were modified (control vs. low pH) while temperature, salinity, and $A_T$ were kept equal and stable during the experiment, showed that low pH has significant impacts on metabolic and growth rates. In terms of phenotypic plasticity, in general, the magnitude of phenotypic plasticity expressed after exposing mussels to lower pH conditions was lower for all traits in comparison with the levels observed after the reciprocal transplant experiment. Contrary to our expectations, mussels from the *central population* showed higher

**Table 1**
Effects of origin (Central and Southern populations) and destination site (self-transplant and transplant treatments) on metabolic, growth, calcification rates, and shell organic matter after 50 days of reciprocal transplant experiment. In bold are showed significant *P*-values at $\alpha \leq 0.05$. Tukey HSD post hoc comparisons of main factors are shown. C: Central population, S: Southern population.

| Source | DF | SS | F | *P*-value | Tukey HSD test |
|---|---|---|---|---|---|
| *$O_2$ consumption ($mgO_2\ h^{-1}$)* | | | | | |
| Buoyant Weight | 1 | 0.104 | 2.16 | 0.149 | |
| Origin Site (OS) | 1 | 0.225 | 4.68 | **0.036** | S > C |
| Destination Site (DS) | 1 | 0.022 | 0.45 | 0.504 | |
| OS × DS | 1 | 0.204 | 4.25 | **0.045** | |
| Error | 44 | 2.117 | | | |
| Total | 48 | 2.564 | | | |
| *Growth Rate ($mm\ d^{-1}$)* | | | | | |
| Origin Site (OS) | 1 | 0.109 | 3.40 | 0.067 | |
| Destination Site (DS) | 1 | 7.507 | 232.7 | **< 0.0001** | S > C |
| OS × DS | 1 | 0.649 | 20.11 | **< 0.0001** | |
| Error | 140 | 4.517 | | | |
| Total | 143 | 13.317 | | | |
| *Net Calcification Rate ($mg\ CaCO_3\ g^{-1}\ d^{-1}$)* | | | | | |
| Origin Site (OS) | 1 | 2.067 | 80.75 | **< 0.0001** | S > C |
| Destination Site (DS) | 1 | 0.077 | 2.99 | 0.086 | |
| OS × DS | 1 | 0.011 | 0.43 | 0.515 | |
| Error | 140 | 3.584 | | | |
| Total | 143 | 6.303 | | | |
| *Shell Organic Matter (%)* | | | | | |
| Origin Site (OS) | 1 | 0.827 | 0.72 | 0.398 | |
| Destination Site (DS) | 1 | 33.130 | 28.79 | **0.000** | S > C |
| OS × DS | 1 | 0.137 | 0.12 | 0.730 | |
| Error | 79 | 90.342 | | | |
| Total | 82 | 123.871 | | | |

**Table 2**
Average (±SE) water conditions and carbonate system parameters of each pH experimental aquarium registered during laboratory common-garden incubations of *P. purpuratus* for 33 days (n = 7). Salinity was stable at 33.0. pH at *in situ* SST (NBS scale), $A_T$ (μmol/kg$^{-1}$), partial pressure of $CO_2$ (μatm), and saturation states of the water for calcite and aragonite.

| Experimental conditions | pH treatment/Aquarium (replicate) | | | | | |
|---|---|---|---|---|---|---|
| | Control pH | | | Low pH | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| SST (°C) | 14.16 (0.08) | 14.17 (0.09) | 14.24 (0.09) | 14.21 (0.05) | 14.21 (0.06) | 14.23 (0.06) |
| $pH_{NBS}$ (*in situ*) | 8.19 (0.01) | 8.23 (0.01) | 8.17 (0.02) | 7.68 (0.03) | 7.71 (0.02) | 7.71 (0.02) |
| $A_T$ (μmol kg$^{-1}$) | 2217 (15) | 2229 (12) | 2224 (15) | 2241 (13) | 2241 (8) | 2247 (7) |
| $pCO_2$ (μatm) | 367 (12) | 330 (12) | 388 (20) | 1356 (91) | 1254 (47) | 1255 (54) |
| $\Omega_{calcite}$ | 3.60 (0.10) | 3.92 (0.10) | 3.49 (0.11) | 1.29 (0.07) | 1.38 (0.05) | 1.37 (0.05) |
| $\Omega_{aragonite}$ | 2.30 (0.06) | 2.51 (0.06) | 2.23 (0.07) | 0.83 (0.04) | 0.87 (0.03) | 0.88 (0.03) |

phenotypic plasticity index ($PI_{md}$) in metabolic, growth, and calcification rates than mussels from the southern site in the OA common-garden experiment. On the other hand, the *southern mussel population* showed higher phenotypic plasticity after the reciprocal transplant experiment. These results emphasize the importance of the complexity of native environmental conditions of organisms as selective drivers of evolutionary potential for adaptation to respond to future ocean conditions, where pH might not be the only driver of changes to consider.

### 4.1. Environmental conditions on the *P. purpuratus* habitat

Our environmental characterization evidenced that the southern site (Calfuco) presented environmental properties typically estuarine (low salinity and low alkalinity), while the central study site (Las Cruces) showed environmental characteristics of a coastal habitat without a significant influence of freshwater inputs (i.e. higher and more stable salinity and alkalinity). Temperature patterns agreed with the well-documented temperature gradient for the Chilean coast (i.e. Mayol et al., 2012), with higher temperatures at the central than the southern site. On the other hand, the central site showed higher variability in terms of SST and carbonate saturation states (calcite and aragonite) in comparison with the southern site. This could be due that the coast of central-northern Chile is exposed to wind-driven upwelling (Aravena et al., 2014) that generates important changes in the SST and seawater biochemistry (i.e. Torres et al., 2011; Mayol et al., 2012). The lack of significant differences in terms of pH and carbonate system parameters between the central and southern sites could be attributed to the acidic freshwater input by river discharge occurring at the southern site, and the lower pH seawater upwelled by upwelling. However, the low frequency of our measurements compared to the typically spatio-temporal variability that characterizes the coastal ocean, as well as the potential impact of biological processes here not measured such as production or respiration, should be also addressed in future studies as they significantly modulate the environmental patterns of coastal habitats. Furthermore, environmental conditions in microhabitat among studied localities may vary in terms of thermal regime, solar irradiance, and tidal emersion times which could impact the physiological measurements. Although, experimental mussels came from similar microhabitats (i.e. semi-exposed rocky platforms from the mid-intertidal zone), and the timing and amplitude of tides exhibit small changes across the studied region in the Chilean coast (SHOA, 2020) our results must be taken with caution because intertidal marine invertebrates possess ecological memory to different intertidal habitats especially in physiological energetics traits (see Labarta et al., 1997; González-Fernández et al., 2015; Arranz et al., 2016). Therefore, in our experiment, the ecological memory might have affected our results being a confounding factor that needs to be addressed in future studies.

### 4.2. Reciprocal-transplant experiment

The direction and magnitude of selection on phenotypic traits are linked to changes in the native environmental conditions, which may explain the significant differences and changes in the subset of biological responses studied for the two local populations of *P. purpuratus* during the reciprocal transplant experiment. Changes in metabolic rates, as well as the associated *trade-offs* observed (growth rates and shell organic matter), are suggesting that estuarine conditions seem to be physiological stressful for *P. purpuratus*, which agrees with previous studies (e.g. Riisgärd et al., 2012; Ramajo et al., 2016b). Although the significant differences detected in terms of SST between both study sites may account for some of the differences observed, the *southern P. purpuratus population* seems to express the habitual physiological response associated with lower salinity environments more than lower temperatures. Salinity is a significant environmental driver determining the spatial distribution of numerous marine species (Wrange et al., 2014), the biomass/production patterns, physiological processes as growth and metabolism (Wing and Leichter, 2011), and shell biomineralogical proprieties (Dickinson et al., 2013). Also, changes in salinity conditions can generate the expression of multiple biological mechanisms (Riisgård et al., 2012) allowing to the species inhabit and respond to variable salinity conditions. Our results agree with previous studies that suggest that under less saline environments, higher metabolic rates are common and frequently accompanied by curtailed growth and reduced body sizes (Tedengren and Kautsky 1986). Indeed, recent experimental studies on the mussel *Mytilus chilensis* has observed that less saline conditions have

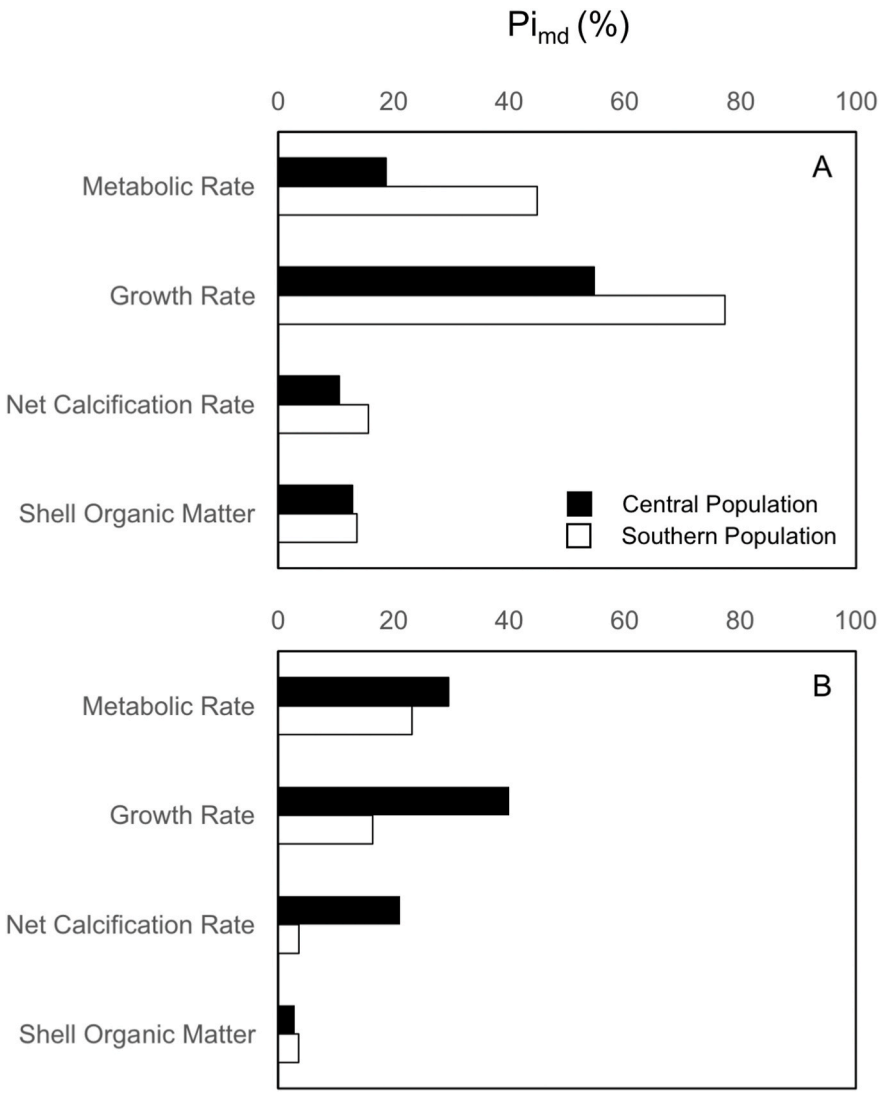


**Fig. 4. Phenotypic Plasticity**. Plasticity Index (%) of physiological traits (metabolic rate, growth rate, and net calcification rate) and mineralogical traits (shell organic matter) of mussels from central and southern *P. purpuratus* populations after the reciprocal transplant (A) and the OA common-garden (B) experiments.

negative impacts on the feeding rates (i.e. clearance rate, absorption efficiency) with significant impacts on the scope for growth (Duarte et al., 2018; Jahnsen-Guzmán et al., 2020) which may also explain the lower growth rates observed on *P. purpuratus* inhabiting southern location. On the other hand, Landes et al. (2015) suggest that higher salinity variance, more than low mean values, may drive increased metabolic rates, facilitate the apparition of *trade-offs,* as well as impact negatively on growth and condition index. In our study, the southern site (Calfuco) presented both environmental attributes: lower mean salinity and larger variability in comparison with the central site indicating that salinity may be the key driver modulating the metabolic pattern observed, as well as the related *trade-offs* detected, in mussels self-transplanted or transplanted to this location.

Higher amounts of total shell organic matter, including periostracum and intra and inter-crystalline shell organic matter, were observed in native mussels from the *southern* than the *central population*. The periostracum and organic shell matrix have a protective role that prevents shell mineral dissolution under lower pH conditions (i.e. Ramajo et al., 2013, 2016a,b). Metabolic costs of producing shell organic matrix are particularly elevated which favors the occurrence of physiological *trade-offs* (Palmer, 1983, 1992; Waldbusser et al., 2013; Ramajo et al., 2015). This may be explaining the higher amount of organic matter recorded in mussels from the southern location that also showed higher metabolic rates and lower growth rates. In addition, pH and salinity are able to affect the chemical composition of periostracum and the amount of shell organic matrix (see Meenakshi et al., 1969; Green et al., 2004; Gutowska et al., 2010; Poulain et al., 2015; Ramajo et al., 2015b). Here, the similar average conditions observed in terms of pH at both study sites and significant differences in terms of salinity would be suggesting that salinity would have a prevalent role to determine the amount of shell organic matter in this species, as it has been recently observed by Grenier et al. (2020) in *Mytilus chilensis*. In addition, a higher amount of shell organic matter recorded for both populations (>9%) could be responsible for the lack of effects on net calcification rates during the reciprocal transplant experiment. Indeed, shell organic matter has a key role in avoiding shell dissolution under non-favorable and stressful environmental conditions (see Harper, 2000).

### 4.3. OA common-garden experiment

In general terms, studies assessing the effects of ocean acidification show that lower pH conditions predicted by the end of this century will affect a large variety of taxa and physiological processes, impacting the fitness of several marine species (Hendriks et al., 2011; Kroeker et al., 2013). However, the direction and magnitude of biological impacts of experimental OA are variable (i.e. Ries et al., 2009; Ries, 2011; Kroeker

**Table 3**
Effects on metabolic, growth and calcification rates and shell organic matter in two different geographical populations (central and southern) exposed to actual (control pH treatment) and future $CO_2$ predictions (low pH treatment) for 33 days. In bold are showed significant *P*-values at $\alpha \leq 0.05$. Results for Tukey pairwise comparisons among levels of the main factors are also shown. C: central population, S: Southern population, Low: Low pH treatment, Control: Control pH treatment.

| Source | DF | SS | F | *P*-value | Tukey HSD test |
|---|---|---|---|---|---|
| *$O_2$ consumption ($mgO_2\ h^{-1}$)* | | | | | |
| Buoyant Weight | 1 | 0.001 | 0.26 | 0.628 | |
| Origin Site (OS) | 1 | 0.005 | 1.04 | 0.343 | |
| pH treatment (pH) | 1 | 0.063 | 11.98 | **0.011** | Low > Control |
| OS ×pH | 1 | 0.000 | 0.06 | 0.817 | |
| Error | 8 | 0.037 | | | |
| Total | 11 | 0.113 | | | |
| *Growth Rate ($mm\ d^{-1}$)* | | | | | |
| Origin Site (OS) | 1 | 0.004 | 0.34 | 0.577 | |
| pH treatment (pH) | 1 | 0.000 | 0.01 | 0.940 | |
| OS ×pH | 1 | 0.095 | 8.40 | **0.020** | Low-S > Low-C |
| Error | 8 | 0.090 | | | |
| Total | 11 | 0.189 | | | |
| *Net Calcification Rate ($mg\ CaCO_3\ g^{-1}\ d^{-1}$)* | | | | | |
| Origin Site (OS) | 1 | 0.959 | 341.00 | **0.000** | S > C |
| pH treatment (pH) | 1 | 0.011 | 3.74 | 0.890 | |
| OS ×pH | 1 | 0.010 | 3.64 | 0.093 | |
| Error | 8 | 0.022 | | | |
| Total | 11 | 1.002 | | | |
| *Shell Organic Matter (%)* | | | | | |
| Origin Site (OS) | 1 | 0.016 | 0.02 | 0.888 | |
| pH treatment (pH) | 1 | 0.542 | 0.72 | 0.422 | |
| OS ×pH | 1 | 0.042 | 0.06 | 0.819 | |
| Error | 8 | 6.047 | | | |
| Total | 11 | 6.647 | | | |

et al., 2013), and many times the lack of effects have been explainedbased on the presence of biological mechanisms to cope with variable and acidified pH environments (Hendriks et al., 2015). However, also evolutionary mechanisms, given by the historical conditions that organisms have experienced across large temporal scales, may explain the absence of negative impacts (Dupont et al., 2013; Ramajo et al., 2016a). Indeed, the expression of phenotypic plasticity seems to be responsible for neutral and positive impacts of experimental OA over several species of bivalves and gastropods, (see Duarte et al., 2013; Lardies et al., 2014). Here, we observed that pH had a relevant role to determine the metabolic and growth rates on both mussel populations. However, contrary to expected, net calcification rates and shell organic matter were not affected by lower pH conditions for both populations remaining similar at the end of the experiment. Metabolic up-regulation is a mechanism to avoid hypercapnia, however important physiological *trade-offs* have been associated to increasing metabolic rates such as lower growth rates (e.g. Ramajo et al., 2016a, 2019). Here, an up-metabolic regulation was observed for both mussel populations under low pH conditions, however no-related changes in their growth rates were observed. This would be suggesting that *P. purpuratus* would have the ability to modify the assignation of the energy budget in order to avoid the negative effects of acidic conditions on growth rates as it has been observed for many other mollusk species (see Kroeker et al., 2013). On the other hand, although we provided an adequate food regime during the experiment (see Martinez et al., 2000) which could support the higher metabolic rates under lower pH conditions (Ramajo et al., 2016a,b), the important differences in the magnitude of growth and calcification rates observed to compare both experiments would be suggesting that the food supply regime used during the OA-common garden experiment would not be equivalent to the one provided by the environment.

### 4.4. Phenotypic plasticity

A previous study (see Briones et al., 2013) characterized the pattern of genetic connectivity of *P. purpuratus* comparing several populations along the Chilean coast (between latitudes 23°S and 33°S). Briones and colleagues found a genetic discontinuity at 28°S delimitating two major gene pools among the study populations. Here, we used two *P. purpuratus* populations south of 28°S (where this genetic discontinuity was detected) which determine that the results obtained from both experiments should be attributed to differences in phenotypic plasticity. It has been suggested that highly variable environments could act as a refuge for organisms to cope with climate change stressors such as ocean acidification (i.e. Hendriks et al., 2013). Indeed, inhabiting variable habitats favor the presence of biological mechanisms, as well as the phenotypic plasticity expression (Hendriks et al., 2015). As it is discussed above, the mussel's populations studied showed different physiological ways to respond to changes in the environmental conditions (natural and experimental conditions). This was evident when comparing the magnitude of the level of physiological plasticity between both mussel populations (central and southern) and experiments (reciprocal transplant vs. OA-common garden). From the reciprocal transplant experiment, we observed a higher plasticity index of the *southern mussel population* in comparison with the *central mussel population*. On the other hand, an opposite pattern was observed after exposing mussels from both populations to lower pH conditions. This would be suggesting that both *P. purpuratus* populations show a different level of local adaptation to their native habitats. Whereas the *central mussel population* seems to be greatly locally adapted to an environment characterized by upwelling processes, the *southern P. purpuratus population* presented a higher ability to counteractenvironmental changes during the reciprocal transplant. On the other hand, the lower phenotypic plasticity index observed after exposing both study populations to lower pH conditions could indicate that the mechanisms to cope with acidic waters are different (or less) than those to cope with a combination of changes in salinity, temperature, and $A_T$. However, our results need to be taken with careful due to the short-term exposure to OA conditions would not be showing the overall physiological picture to future changes in pH. Indeed, this has been explained on the basis that several biological and evolutionary mechanisms that modulate the physiological responses fail to be exhibited (Dupont et al., 2013).

## 5. Conclusions

Our results suggest that chronic exposure to environmental conditions in native habitats have important effects on the level of phenotypic plasticity expressed by different geographical mussel populations along the Chilean coast. Mussel populations experiencing naturally more variable environments, such as estuaries, have evolved to more adaptive plasticity than those inhabiting lower variable environments. Thus, considering intraspecific differences and adaptation patterns across a species' distribution range might provide insights about the vulnerability of species and their populations to climate change stressors. In addition, our study stresses the necessity to consider the overall environmental scenario (multi-driver and multi-stressor) when future predictions about the marine species sensibility will be made.

## Author contributions

LR, SO, MAL, NAL designed both experiments (reciprocal transplant experiment, common-garden experiment). LR and SO performed the common-garden experiment and measured the biological responses of both experiments. SO, PHM, CV, and JN determined the environmental conditions during the reciprocal transplant experiment. BRB collected and analyzed satellite data. LR and SO analyzed the biological and the environmental data. LR prepared the manuscript. All the authors contributed to the manuscript.

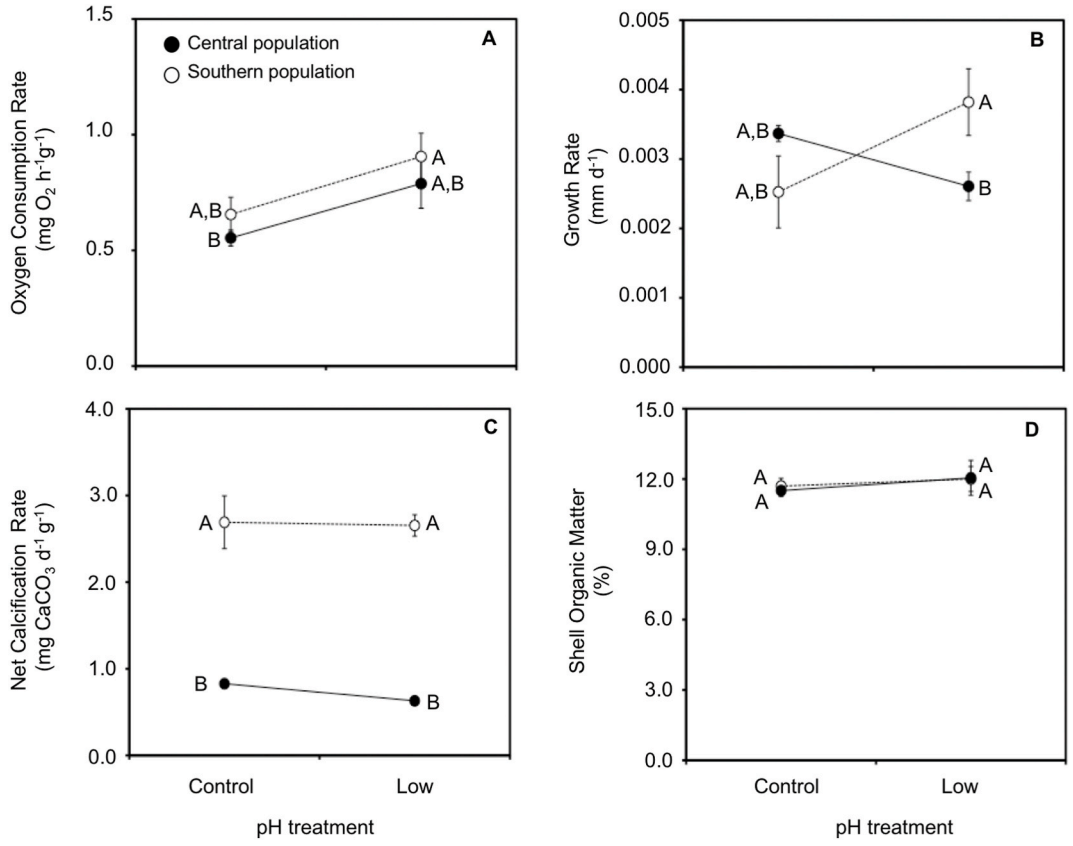


**Fig. 5. OA Common-Garden Experiment**. (A) Metabolic rates measured as oxygen consumption ($mgO_2$ $h^{-1}g^{-1}$), (B) growth rate (mm $d^{-1}$), (C) net calcification rate ($mgCaCO_3$ $d^{-1}g^{-1}$), and (D) shell organic matter (%) of *P. purpuratus* mussels from the central and southern sites exposed to current (control treatment) and future pH predictions (low pH treatment). Different letters beside each symbol indicate significant differences between experimental treatments evaluated using the Tukey HSD test as a *post hoc* comparison.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2020.107098.

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