

Heterogeneous environmental seascape across a biogeographic break influences the thermal physiology and tolerances to ocean acidification in an ecosystem engineer

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Abstract

Aim: Understanding how spatio-temporal environmental variability influences stress tolerance, local adaptation and phenotypic variation among populations is a key challenge for evolutionary ecology and climate change biology. Coastal biogeographic breaks are natural laboratories to explore this fundamental research question due to the contrasting environmental conditions experienced by natural populations across these regions.

Location: In the South East Pacific (SEP) coast, a major break (30°–32°S) is characterized by extreme natural variability in sea surface temperature (SST) and carbonate chemistry parameters related to temporal and spatial dynamics in upwelling events. Calcifying organisms inhabiting this zone are exposed to marked fluctuations and clines in SST that together with naturally acidified waters can impact their metabolism, calcification and fitness, making them particularly prone to the effects of climate change (e.g. ocean acidification, OA). We investigated to what extent the spatial and temporal environmental variability (in SST and seawater carbonate conditions) that characterizes the biogeographic break in the SEP influences intra-specific differences in the thermal ecology and the tolerances to OA of the limpet *Scurria araucana*.

Methods: During two years, we conducted field surveys of limpet populations at sites across the SEP break (27°S, 30°S and 32°S). We collected individuals from each population to test for geographic differences in morphometric (e.g. total buoyancy weight, shell length) and physiological (e.g. oxygen consumption rate, cardiac activity and thermal performance curves; TPC) responses to local environmental conditions (T° and pH/pCO₂) and to simulated OA scenarios.

Results: Populations of *S. araucana* exhibit high tolerance to OA with no signal of geographic influence on this attribute. However, inter-population differences in thermal physiology (metabolic rates and performances) were found across the biogeographic

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break in the SEP coast. Limpets from the central part of the break (30°S) exhibit higher thermal performance compared to limpets from populations at both sides of the break. **Main conclusions:** Variation in SST has a greater effect shaping inter-population differences in thermal physiology of the limpet *S. araucana*. These physiological differences are aligned the thermal heterogenous seascape along the biogeographic break in the SEP. Contrarily, temporal and spatial variation in seawater carbonate conditions does not influence inter-population differences in phenotypic response populations, but an overall high tolerance to OA.

KEYWORDS

Geographic variation, metabolism, molluscs, ocean acidification, phenotypic plasticity, thermal physiology, upwelling

1 | INTRODUCTION

Coastal marine biogeographic breaks are regions where multiple species reach their geographic range limits simultaneously, leading to abrupt transitions in species composition and potentially in species richness (Cox & Moore, 2010; Keith et al., 2015). These discontinuities are often located in areas with prominent topographical features (e.g. coastal/submarine ridges) or sharp environmental changes such as those induced by eddies, ocean fronts, currents and upwelling systems. Across these regions, marine species are exposed to intense biotic and abiotic stressors that influence their local distribution, dispersal, recruitment, physiology and ultimately their survival (Hofmann & Todgham, 2010; Somero, 2010). These biogeographic breaks and transition zones have been described in all oceans. However, the South East Pacific coast offers, perhaps, one of the best studied systems for exploring ecological and evolutionary dynamics of marine organisms at the edges of the species distribution (Barria et al., 2014; Gaitán-Espitia, Bacigalupe, et al., 2017; Gaitán-Espitia et al., 2014; Gaitán-Espitia, Marshall, et al., 2017; Gaitán-Espitia, Villanueva, et al., 2017). Along this coast, a broad transitional zone of species distributions extends between 30°S and 41°S (Camus, 2001), but a narrow area between 30°S and 32°S concentrates the polar or equatorial range edge of several intertidal species (Brante et al., 2012; Broitman et al., 2018; Rivadeneira & Fernandez, 2005). The biogeographic break and transition zone in this region are highly influenced by the interplay of a major topological discontinuity that occurs on the oceanic shelf at 30°S (Hormazabal et al., 2004), the Humboldt Current and the dynamics of the upwelling system (Camus, 2001; Thiel et al., 2007). This interaction creates a heterogeneous seascape that influences spatial and temporal variation in patterns of abundance, life history, morphology and physiology within species and populations of intertidal and benthic organisms (Broitman et al., 2001; Lardies et al., 2010, 2011).

The upwelling system along the South East Pacific coast brings cold deep-sea waters, rich in nutrients and dissolved inorganic carbon (DIC), into the euphotic zone (Torres et al., 2011). The high DIC content of these waters is accompanied by low pH and consequently

a low degree of calcium carbonate saturation ($\Omega_{\text{aragonite/calcite}} < 1$) (Hauri et al., 2013), which makes this region particularly prone to the effects of ocean acidification (OA) (Gruber et al., 2012; Vargas et al., 2017). Cold waters in this naturally acidified coastal system can be corrosive and physiologically challenging, impacting calcification and metabolism of benthic organisms, such as molluscs (Fabry et al., 2008). However, the dynamic of the upwelling system along the biogeographic break of the South East Pacific coast is highly variable across temporal and spatial scales (Torres et al., 2011). Thus, natural populations of benthic organisms inhabiting this region can experience differences in the frequency and intensity of upwelling regimes. This ultimately influences inter-population differences in local adaptation, phenotypic plasticity and organismal responses to environmental stress across a species geographic range, in terms of both temperature and pH (Gaitán-Espitia, Marshall, et al., 2017; Gaitán-Espitia, Villanueva, et al., 2017; Vargas et al., 2017). These geographic differences in phenotypic responses and the associated quantitative genetic variation are the main mechanisms underlying the different physiological tolerances and capacities for rapid phenotypic acclimatization to short-term environmental fluctuations in natural populations (Gaitán-Espitia, Bacigalupe, et al., 2017; Gaitán-Espitia, Villanueva, et al., 2017; Lardies et al., 2014).

On contemporary time-scales, rapid evolution of phenotypic plasticity can be a key mechanism to prevent species extinction as a consequence of anthropogenic stressors such as climate change (e.g. ocean warming and OA) (Fox et al., 2019; Sunday et al., 2014). However, our understanding of the plastic and evolutionary potential of marine organisms in the face of rapid global change is extremely limited (Fox et al., 2019; Kelly & Hofmann, 2013; Munday et al., 2013; Reusch, 2013; Sunday et al., 2014). This restricts our ability to accurately predict how marine biodiversity will respond to ongoing rapid environmental changes, particularly at the geographical and ecological range limits of species (Donelson et al., 2019). Either way, plasticity can be an important source of variation in performances among individuals, potentially influencing short-term selection and the evolutionary trajectories of populations and species (Badyaev & Uller, 2009; Gaitán-Espitia, Villanueva, et al., 2017). Unfortunately,

few investigations have considered the influence of spatio-temporal environmental variation as a driver of geographic differences in phenotypic plasticity among populations of marine organisms under OA (e.g. Broitman et al., 2018; Dupont & Portner, 2013; Gaitán-Espitia, Marshall, et al., 2017; Gaitán-Espitia, Villanueva, et al., 2017; Lardies et al., 2014; Padilla-Gamiño et al., 2016; Rivest et al., 2017). Here, we explored this theoretical framework, assessing the influence of natural variation of sea surface temperature (SST) and seawater carbonate chemistry in shaping inter-population differences in the thermal ecology and the physiological tolerances to OA of an ecosystem engineer, the limpet *Scurria araucana* (Gutiérrez et al., 2003). This species is a common aragonite calcifier (Vinogradov, 1953) that inhabits high-to-mid intertidal levels along the transitional biogeographic break situated at the South East Pacific coast (30–32°S) (Espoz, 2004). Along its distribution, populations of the limpet *S. araucana* are exposed to the abrupt discontinuity in upwelling regimes with clear temporal and geographic differences in SST and pH/pCO₂ variability. Consequently, our study explores the following questions: a) To what extent the interplay between biogeographic breaks and variability of SST and seawater carbonate system influences the thermal ecology and the tolerances to OA in natural populations? And b) do higher variability of SST and seawater carbonate conditions correlate with higher phenotypic plasticity in natural populations? We hypothesize that spatial and temporal differences in upwelling dynamics along the SEP have influenced geographic differences in thermal physiology and tolerance to OA because of local adaptation. Thus, we expect that limpets from more seasonal and fluctuating upwelling areas will be more plastic in response to temperature and pH/pCO₂ changes, while limpets from the strong and continuous upwelling will be more tolerant (less sensitive) to these environmental changes.

2 | METHODS

2.1 | Study area, phenotypic characterization and animal collection

In this work, we studied populations of the marine mollusc *S. araucana* along the biogeographic break zone in the Chilean coast (Figure 1): Huasco (27° 59' S, 71°18' W), Talcaruca (30° 29' S, 71°41' W) and Los Molles (32° 24' S, 71° 50' W). These sites exhibit different dynamics of upwelling regimes that create patterns of spatial and temporal variability in physical-chemical characteristics of coastal waters (Table 1). Talcaruca (30°S) demarcates the southern border of the Peruvian Province (Rivadeneira & Fernandez, 2005), and it is characterized by a strong, continuous upwelling, with cold waters supersaturated with dissolved CO₂ and low pH levels in near-shore areas (Torres et al., 2011). Huasco (27°S) and Los Molles (32°S), on the other hand, are exposed to seasonal, fluctuating upwelling events (SST, pH/pCO₂; see Broitman et al., 2011; Broitman et al. 2018) at both sides of break, in the Peruvian Province and in the transitional zone, respectively. Preliminary genetic information using restriction

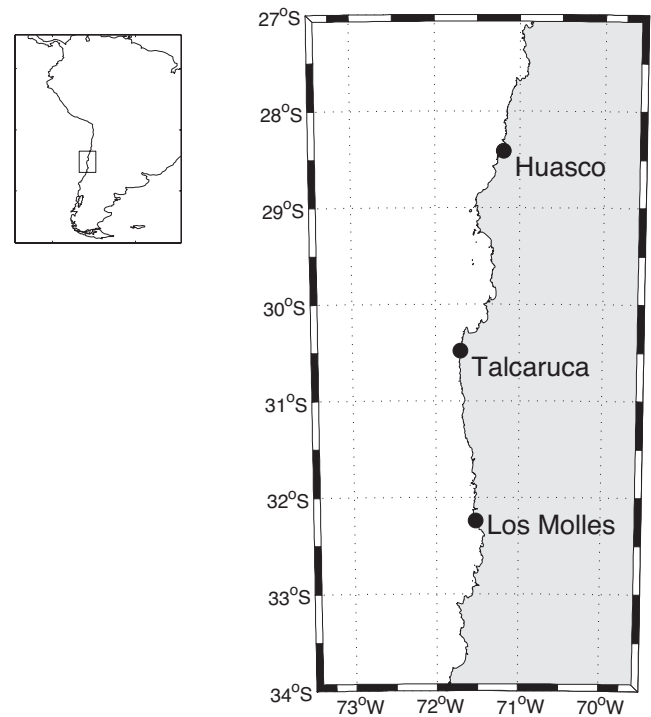


FIGURE 1 Location of the study sites along the biogeographic break localized around 30°S on the coast of Chile

site-associated DNA sequencing (RADseq) suggests the existence of genetically distinct populations in these sites with marked differences in genetic diversity (Lardies et al., in prep), findings that are consistent with other sympatric *Scurria* species (Haye et al., 2014).

In order to characterize and compare biometric effects of natural variability of SST and pH/pCO₂ on limpets from the three populations of *S. araucana* across the biogeographic break, we sampled each study site Huasco, Talcaruca and Los Molles every two months during a period of two years (5–8 limpets in each locality). Then, the following parameters from each of these animals were obtained in Huasco (n = 60), Talcaruca (n = 55) and Los Molles (n = 58): shell length (mm), total buoyancy weight (g), dry tissue weight and shell weight (g). For dry tissue and shell weights measurements, the soft body was removed from the shell of the limpet and then oven-dried at 60°C for 24 h. For Buoyant weight, each limpet was weighed under the water and verified with the dry weight measurements of the shells (Palmer, 1982).

In addition to the phenotypic characterization for each population, we conducted laboratory experiments. For this, individual limpets were collected and transported in coolers to the laboratory of the Universidad Adolfo Ibáñez at Santiago (Chile). Limpets were collected from rocky substrata and were randomly collected, simultaneously in all sites, during spring season at low tide by hand from the mid-upper intertidal zone in the lower limit of the mussels (i.e. *Perumytilus purpuratus*) band.

Animals were maintained in acclimation tanks at constant temperature (14°C) and salinity (33 ppt), in well-aerated, UV-filtered natural seawater for two weeks. Carbonate system parameters

Parameter	Locality		
	27° 59' S Huasco	30° 29' S Talcaruca	32° 24' S Los Molles
Salinity	34.50 ± 0.10	32.70 ± 0.34	32.06 ± 0.32
pH _{NBS}	8.15 ± 0.12	7.90 ± 0.41	8.11 ± 0.14
TA	2235.2 ± 20.0	2279.6 ± 64.3	2225.9 ± 30.2
CO ₃ ⁻²	155.3 ± 60.2	103.2 ± 39.6	144.3 ± 34.8
pCO ₂	420.1 ± 122.1	779.9 ± 332.3	454.1 ± 176.0
Ω _{calcite}	3.96 ± 1.02	2.51 ± 0.95	3.51 ± 1.59
Ω _{aragonite}	2.55 ± 0.82	1.60 ± 0.59	2.24 ± 1.01
SST (°C)	14.70 ± 1.93 (15.6, 20.1)	13.80 ± 3.74 (8.4, 16.8)	14.24 ± 2.16 (10.8, 16.4)
CV SST (%)	9.55	24.00	17.33
AT (°C)	16.4 (13.3, 22.9)	13.4 (9.8, 17.7)	13.2 (8.2, 17.6)

Note: pH_{NBS} is expressed in moles. TA and CO₃⁻² are expressed in micromoles per kilogram seawater. pCO₂ is expressed in micro-atmospheres. Parameters are as follows: total alkalinity (TA), carbonate (CO₃⁻²), the partial pressure of CO₂ (pCO₂), saturation states for aragonite Ω_{Ara} and saturation states for calcite Ω_{Cal}.

during acclimation period were 400 μatm pCO₂ and pH 8.02. This selected temperature and pCO₂ reflect the mean SST and pH during the animal collection period (spring-summer) and is associated to the upwelling dynamics, specifically to the relaxation phase, in the region (see Ramajo et al., 2020). During this period, animals were fed by placing rocks covered with microalgae from the sampling areas into the acclimation tanks. Shell epibiota were removed to reduce epibiont respiration and avoid interferences during the subsequent oxygen consumption and thermal performance measurements (Sections 2.2-2.4). After the acclimation period, physiological (metabolic rates) and morphological (e.g. body size, shell length) characteristics were assessed. Then, individuals from the three populations were subsampled (n = 40 per population) for experimental exposure to ocean acidification conditions (Section 2.3).

As part of this work, monthly seawater samples from the studied areas were taken for determination of carbonate system parameters. For pH measurements (total scale), two water samples were collected and analysed within 60 min of collection, using a Metrohm 826 pHMobile Meter© connected to a combined electrode (double juncture), calibrated using Tris buffers (pH = 8.089) at 25°C using a thermo-regulable water bath. For total alkalinity (AT) analyses, discrete water samples were collected using borosilicate glass bottles (Corning 500-mL), poisoned using mercuric chloride (0.2 cm³ of a 50% saturated solution) and sealed with Apiezon® L grease for transportation to the Universidad Adolfo Ibáñez laboratory. Water samples were stored for no more than 3 months in cool, dark conditions until alkalinity analysis. Three seawater subsamples of each bottle were used to estimate measurement of AT using automated potentiometric titration (Haraldsson et al., 1997). Partial pressure of CO₂ (pCO₂) and saturation states (Ω) for calcite and aragonite were estimated from the averaged values of pH_T, A_T and SST using the CO2SYS software (Pierrot et al., 2006).

TABLE 1 Summary of salinity and seawater carbonate chemistry variables measured in the three study locations. (Mean ± SD) during 2014 and 2016. Temperature information includes long-term daily averages of the sea surface temperature (SST) and the air temperature (AT) during the period 2014–2019. The minimum and maximum temperatures are shown between parentheses. CV indicates coefficient of variation. Ω_{calcite} = saturation states for calcite; Ω_{aragonite} = saturation states for aragonite

A continuous sea surface temperature (SST) monitoring programme was also established along central-northern Chile using submersible temperature data loggers (HOBO®, Onset Computer Corp., MA, USA) housed inside PVC pipes. These pipes were embedded in concrete blocks, attached to chains and deployed ~1 m below the surface. Details about data loggers recording and management can be found in Tapia et al. (2014) and Broitman et al. (2021).

2.2 | Cardiac activity and thermal performance curve (TPC)

Thermal fluctuating and low pH waters along the studied biogeographic break can be physiologically challenging for marine benthic organisms inhabiting this region. These environmental characteristics may modulate their thermal ecology and their physiological tolerances to environmental stress. Here, we used heart rate (cardiac activity) to evaluate potential signals of environmental/biogeographic influence on thermal physiological performance of *S. Araucana* population. Cardiac activity was measured using the method described by Gaitán-Espitia et al. (2014), Gaitán-Espitia, Bacigalupe, et al. (2017). After two weeks of acclimation, 12 individuals per population were used for analysis of thermal sensitivities. The parameters selected were as follows: a) the optimal temperature (T_{opt}), b) the maximal performance (μ_{max}) and c) the upper and lower limits of temperature at which the heartbeat frequency falls (CT_{min} and CT_{max}). Organisms were placed separately in plastic chambers with 6 subdivisions, installed in a thermo-regulated bath at constant seawater temperature (±0.5°C, LWB-122D, LAB TECH), for 30 min. Experimental temperatures for thermal performance curves were chosen between 0 and 37°C. The performance traits were measured at every 2°C in the extremes of each population's tolerance range and every 3°C units inside the range (12–25°C). First, we used a decreasing temperature

ramp from 14 to 0°C to determine CT_{min} . Second, after a two-day recovery period for all individuals, we used an increasing thermal ramp from 14 to 37°C to determine CT_{max} . Heart rate was determined by a heartbeat amplifier AMP 03 (Newshift Lda[®]) connected to an oscilloscope. The results were expressed in beats min^{-1} . The mean heart rate for each limpet at each experimental temperature was calculated with the aim of estimating the thermal performance curve (TPC) for each population. Here, several non-linear functions (e.g. Gaussian, Exponential Modified Gaussian, Quadratic Lorentzian, Weibull) were tested using the *lsfit* function implemented in the *easyns* R package v.5.0 m (Arnhold, 2017) and the *TableCurve2D* curve-fitting software (version 5.01; Systat Software, Inc.). TPC parameters (μ_{max} , T_{opt} , CT_{min} and CT_{max}) were extracted from the best fitted models (see Gaitán-Espitia et al., 2014 for further details).

2.3 | Exposure of *S. araucana* populations to OA

Geographic differences in local variability in SST and seawater carbonate parameters can potentially influence local adaptation and the capacity of the organisms to tolerate environmental and climate change (e.g. OA). Here, we assessed whether the environmental conditions along the biogeographic break have shaped divergent tolerances and phenotypic plasticity in populations of *S. araucana*. Subsampled individuals from each population (total $N = 120$; 40 per locality) were collected during spring 2019 and marked for identification using bee tags. Then, animals were independently exposed to contrasting CO_2 conditions (400 and 1300 μatm pCO_2) during a short-term period (20 days) in order to assess rapid acclimatory responses and plasticity. The selected pCO_2 concentrations were based on current globally averaged atmospheric levels and predicted levels by the year 2100 according to the RCP 8.5 scenario (see Moss et al., 2010 and IPCC, 2014). Forty animals from each population were individually kept in 1 L glass aquariums and randomly assigned (merging populations) to one of the experimental CO_2 treatments (either current or high pCO_2) with two tanks/blocks (10 individual aquariums = 10 limpets per tank) per treatment. Each individual aquarium was filled with the appropriate preconditioned seawater (see Section 2.5), which was replaced every 3 days in order to remove faeces, ammonia and detritus. Oxygen consumption and buoyancy weights were measured in each marked limpet at day 10 and 20 of exposure. During the experimental period, $pH_{(NBS)}$, total alkalinity and salinity were monitored every 3 days. Seawater temperature, pH/CO_2 and dissolved oxygen were constantly controlled using external chillers and bubbling the corresponding treatment gas mixture (see Section 2.6). pH measurements were estimated in closed 25 mL cells, at 25.0°C using a Metrohm 713 pH meter (input resistance $> 10^{13} \times 0.1$ 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 at 25°C. Total alkalinity (TA) values were obtained from 50 mL of seawater aliquots treated with 50 μl of saturated $HgCl_2$ solution.

Samples were analysed following Haraldsson et al. (1997) in an automatic titrator (Mettler Toledo, T50) with a glass combined electrode (expressed as μM). Temperature (14°C) and salinity (33 psu) were measured using a portable salinometer (Salt6+, Oakton[®], accuracy: $\pm 1\%$ and $\pm 0.5^\circ C$, respectively). Animals were fed as described during the acclimation period.

2.4 | Oxygen consumption and growth rate

Oxygen consumption rate was estimated in limpets from the three selected localities before, during and after the acidification experiments, as described by Lardies et al., (2014). Before oxygen uptake measurements, all individuals were kept under starvation for 24 h in their specific experimental conditions (i.e. pCO_2 concentration, temperature, salinity and photoperiod). Then, limpets were placed individually into a closed respirometric chamber (113 mL), filled with filtered seawater from the corresponding pCO_2 treatment. Once sealed, the chambers were placed into a tank with seawater at 14°C controlled by a chiller. Oxygen consumption rates were measured using a fibre-optic oxygen optode connected to a PreSens Microx TX3 temperature compensated oxygen meter (Precision Sensing, GmbH, Regensburg, Germany) with a tip diameter of 140 μm . Oxygen partial pressure measurements ran for at least 60 min and were never allowed to decrease below 80% O_2 saturation to avoid animals experiencing hypoxia. The first 10 min and the last 5 min of determinations were eliminated to avoid the possible disturbances caused by stress of animal manipulation. Oxygen consumption values were expressed as O_2 $mg\ h^{-1}g^{-1}$. Finally, growth rates ($mg\ d^{-1}$) of *S. araucana* individuals from the three selected populations were estimated from changes in the total buoyancy weight recorded at days 0, 10 and 20 of the experimental CO_2 exposure. Buoyant weight is a proxy of growth because it is comparable to the calcification rate and it is not influenced by the amount of seawater and tissue weight (Palmer, 1982).

2.5 | pCO_2 seawater equilibration and carbonate parameters

The CO_2 manipulation system was built as that described by Torres et al. (2013) and Navarro et al. (2013). Briefly, the manipulation system was composed by 2 polyethylene header tanks (60 L, hereafter referred as CO_2 equilibration tanks) filled with UV-filtered seawater. The equilibration tanks were used to adjust CO_2 concentration levels (400 and 1300 μatm) by bubbling atmospheric air or an air- CO_2 mixture respectively in the tanks. For high pCO_2 treatment, dry air was blended with pure CO_2 to the predetermined concentration using mass flow controllers (MFCs AalborgTM) for air and CO_2 . Treatment conditions into the aquariums were maintained by constantly bubbling with the corresponding CO_2 concentration (see SM Table 2). For both, field and laboratory seawater samples, pH , total alkalinity, phosphate and dissolved silicate (Strickland & Parsons, 1968) were

TABLE 2 Parameters of thermal performance curves in populations of the limpet *Scurria araucana* along the biogeographic break of the South East Pacific coast. Thermal optimum (T_{opt}), maximum performance (μ_{max}) and critical temperatures (CT_{min} and CT_{max})

Population	Latitude	CT_{min} (°C)	CT_{max} (°C)	T_{opt} (°C)	μ_{max}
Huasco	27° 59' S	-3.45	40.09	20.76	58.91
Talcaruca	30° 29' S	-4.13	40.23	25.31	61.65
Los Molles	32° 24' S	-3.99	40.18	23.64	56.88

used to calculate the carbon system speciation using the CO2SYS software (Pierrot et al., 2006). For these parameters, we used the dissociation constant from Mehrbach et al. (1973), refit by Dickson and Millero (1987) and Dickson (1990).

2.6 | Data analysis

Results were expressed as means \pm standard errors. Data were tested for normality and homogeneity by using the Shapiro–Wilk and Levene tests, respectively. When normality and homogeneity assumptions were not met, data were logarithm-transformed by ln. A mixed model analysis of covariance (ANCOVA) was performed to determine the effects of the interaction between population and pCO_2 level (hereafter population $\times pCO_2$) on oxygen consumption rate, using buoyancy weight as covariate. The fit of non-linear functions (e.g. Gaussian, Exponential Modified Gaussian, Quadratic Lorentzian, Weibull) to describe the TPC's of the three selected populations were analysed using the Akaike information criterion (Angilletta, 2006). Curve shapes and parameters were evaluated through confidence intervals (CI) computed from the likelihood profile and inter-population comparisons were done using AIC and the Extra Sum-of-Squares F test. Analyses of physiological performance among the three populations were conducted using a linear mixed model (LMM), with experimental temperature and populations as categorical predictors for heart rate whereas pCO_2 treatment and populations for oxygen consumption rate. In these analyses, *experimental temperature* and pCO_2 were treated as fixed factor, whereas *populations* were considered as a random factor because they represent a random subsample of the real populations along the species distribution. Statistical analyses were performed R and Prism (GraphPad Software).

3 | RESULTS

3.1 | Carbonate system speciation

Seawater carbonate chemistry parameters in samples collected from the field, varied among study sites (Table 1). The highest levels of seawater temperature, pH and carbonate chemistry variables (CO_3^{2-} ; $\Omega_{calcite}$ and $\Omega_{aragonite}$; one-way ANOVA $F_{2,54} = 10.549$; $p < .001$, $F_{2,54} = 18.015$; $p < .001$, and $F_{2,54} = 13.020$; $p < .001$, respectively) were observed at the extremes of the biogeographic break (27°S,

Huasco and 32°S, Los Molles), while the lowest levels were found in the middle of this transition zone (30°S, Talcaruca). This site also exhibited lower pH values and high pCO_2 (one-way ANOVA $F_{2,54} = 4.026$; $p < .001$, and $F_{2,54} = 16.048$; $p < .001$, respectively) as well as higher temporal variability in carbonate parameters and SST compared to the other areas at both sides of the biogeographic break (see Table 1). Patterns of environmental variability across the latitudinal gradient covered by our study sites differed by their SST climatology (long-term daily means). SST climatologies are heterogeneous across the region as was recently showed by Broitman et al. (2018), Broitman et al. (2021) with significant highest values of SST variance at the biogeographic break zone (i.e. Talcaruca).

3.2 | Characterization of *S. araucana* populations

3.2.1 | Natural variation in morphometric and physiological traits

In order to understand potential morphometric effects of natural spatial variability of SST and pH/ pCO_2 on natural populations of *S. araucana*, we assessed the scaling relationship between shell length and buoyant weight across the biogeographic break. Limpets in the biogeographic break at 30°S (Talcaruca) showed a high variability in this scaling relationship in comparison with Los Molles and Huasco populations. However, no significant differences were found in scaling relationship between shell length and buoyant weight among populations across the biogeographic break ($F_{2,157} = 0.006$, $p = .994$) (Figure 2). In terms of oxygen consumption, acclimated individuals from natural populations showed different energetic demands. Limpets from the southern part of the transition zone (32°S) exhibited higher oxygen consumption rates than those from the biogeographic break (30°S) and the northern part of the transition zone (27°S). However, significant differences between populations were not found after controlling by body weight (ANCOVA, $F_{2,40} = 1.140$, $p = .331$) (Figure S1 and Supplementary Material).

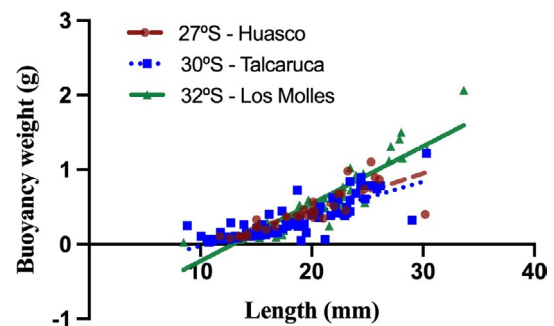


FIGURE 2 The length (L)–buoyant weight (BW) relationship for each selected population of the mollusc *Scurria araucana*. Dashed line, dotted line and solid line represent linear least square fit from Huasco (27°S), Talcaruca (30°S) and Los Molles (32°S) populations, respectively. Linear least square fit for Huasco: $BW = -0.72 + 0.06L$ ($R^2 = 0.84$); Talcaruca: $BW = -0.54 + 0.049L$ ($R^2 = 0.80$) and Los Molles: $BW = -0.99 + 0.077L$ ($R^2 = 0.86$)

3.2.2 | Cardiac activity, TPC and respiration

The interaction between the cardiac activity, as indicator of physiological performance among populations, and experimental temperatures followed the expected non-linear pattern (best fitted models in Supp. Table 1). This interaction was significant for all populations (LMM, $F_{26,496} = 12.38$, $p < .001$), evidencing differences in the shape of the TPCs (Extra Sum-of-Squares F test, $F_{8,796} = 2.17$, $p < .05$; Figure 3). These results were mostly explained by differences in T_{opt} between Talcaruca (95% CI_{Top} 23.67–26.95) and Huasco (95% CI_{Top} 18.08–23.44) (Table 2).

In terms of oxygen consumption, acclimated individuals from natural populations (transition zone and biogeographic break) did not show differences in energetic demands (controlling by body weight; ANCOVA, $F_{2,40} = 1.14$, $p = .331$) (SM Figure 1). Oxygen consumption rates ranged between 0.94 ± 0.20 and 1.39 ± 0.23 $mg\ O_2\ h^{-1}g^{-1}$.

3.3 | Physiological effects of OA

Metabolic rates were significantly influenced by the geographic context (LMM, $F_{2,62} = 4.48$, $p = .016$ and $F_{2,59} = 6.60$, $p = .003$ after 10 and 20 days of exposure respectively), but no significant interactions were found between populations and pCO_2 treatments (LMM, $F_{2,62} = 4.48$, $p = .301$ and $F_{2,59} = 0.45$, $p = .640$ after 10 and 20 days of exposure, respectively). Overall, CO_2 laboratory exposure did not influence differential oxygen consumption rates among populations (Figure 4). Under control conditions (400 μatm), populations showed differences in O_2 consumption rates that were consistent after 10 and 20 days (Figure 4). Higher rates were observed in limpets from populations in the southern area of the transition zone (32°S) and the biogeographic break (30°S), compared to animals from the northern part of the transition zone (Figure 4 and Figure S1).

In terms of growth rates, no significant differences were found between the three populations exposed to both pCO_2 treatments (LMM, population: $F_{2,59} = .476$, $p = 0.624$; pCO_2 treatment: $F_{1,59} = 0.320$, $p = .574$; population $\times pCO_2$ treatment: $F_{2,59} = 0.481$, $p = .621$). Although high pCO_2 did not have a significant effect on this trait across the biogeographic break, it induced higher variation in limpets from the middle and southern populations (Figure 5).

4 | DISCUSSION

Climatic and geographic breaks can have an important influence on eco-evolutionary patterns of phenotypic and genetic variation of natural populations, species diversification and distribution, as well as community structure across broad spatial scales (Cox & Moore, 2010; Keith et al., 2015). At the species level, these patterns have been associated with geographic differences in phenotypic plasticity and local adaptation among populations that result from the interplay between genetic discontinuities and gradients of natural environmental variation and selection (Barria et al., 2014;

Gaitán-Espitia et al., 2014; Gaitán-Espitia, Marshall, et al., 2017; Gaitán-Espitia, Villanueva, et al., 2017; Sivasundar & Palumbi, 2010). Many studies have explored this framework with a particular focus on the evolution of thermal tolerance of marine organisms across biogeographic breaks characterized by clines in temperature and the implications for species' responses (e.g. range shifts, local extinctions, adaptive evolution) to warming oceans (e.g. Donelson et al., 2019; Gaitán-Espitia et al., 2013, 2014; Gaitán-Espitia, Bacigalupe, et al., 2017; Lardies et al., 2010, 2011). However, there is less information and empirical studies documenting comparable ecological and evolutionary patterns shaped by natural variation in environmental factors related to other important global change stressors such as ocean acidification (OA) (Calosi et al., 2017; Gaitán-Espitia, Villanueva, et al., 2017). The South East Pacific (SEP) coast is an ideal natural laboratory to investigate this important gap of knowledge. This system exhibits extreme natural variability in seawater carbonate chemistry parameters related to temporal and spatial dynamics in upwelling events (Lardies et al., 2014; Navarro et al., 2013; Torres et al., 1999). Here, we investigated to what extent the spatial and temporal environmental variability (in SST and seawater carbonate conditions) that characterizes the biogeographic break in the SEP, influences intra-specific differences in the thermal ecology and the tolerances to OA of the limpet *Scurria araucana*. Our results indicate that the heterogeneous seascape of carbonate conditions and sea surface temperature across a biogeographic break in the SEP coast influence phenotypic differences in thermal physiology among populations of this ecosystem engineer. However, this natural variability does not influence divergent tolerances, sensitivities or responses of limpets to OA, at least in the short-term scale.

Morphometric and physiological traits such as metabolic rates (heart rate and oxygen consumption) can be assessed as proxies of organismal performance (Brown et al., 2004), as well as for inferring relative adaptation to different environments (e.g. Huey et al., 1991; Partridge et al., 1995). The analysis of these traits and their responses to changes in environmental factors (e.g. temperature; Thermal Performance Curves, TPCs) is considered an useful approach to predict the responses of species and populations to climate change (e.g. ocean acidification and global warming) (Broitman et al., 2021; Deutsch et al., 2008; Fernández et al., 2020; Gaitán-Espitia, Bacigalupe, et al., 2017; Huey & Kingsolver, 2011). This approach allows to identify the capacity for plastic phenotypic changes in natural populations (Gaitán-Espitia et al., 2013; Schulte et al., 2011). In our study, we found that natural spatio-temporal environmental variability in seawater carbonate parameters and temperature could be a key factor explaining phenotypic differences in morphometric and physiological traits for *S. araucana* populations along the SEP break (27°S–32°S). The scaling results between buoyant weight and length obtained from limpets in the studied populations showed that individuals from the central part of the break (30°S, Talcaruca) are characterized by a higher variability in buoyant weight per unit of shell length in comparison with individuals from the populations at both sides of the break (27°S–Los Molles and 32°S–Huasco). This can be attributed to the lower amount of calcium carbonate in the

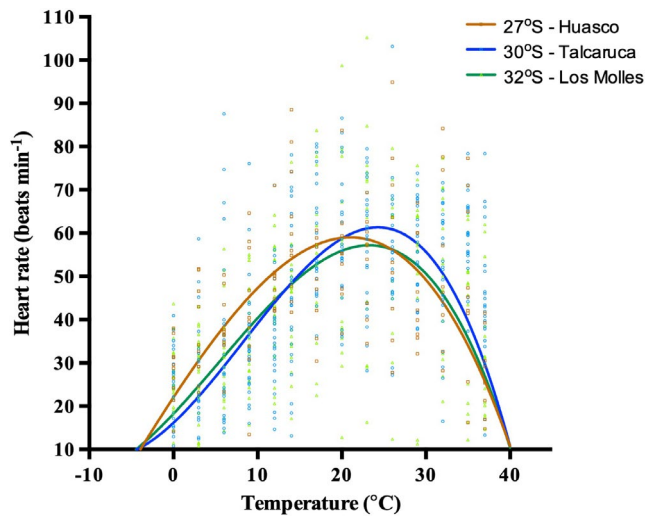


FIGURE 3 Thermal sensitivity curves (TPCs) for the three selected populations of the mollusc *S. araucana* along the main biogeographic break of Chilean coast

shells of animals from Talcaruca, which experience higher variability in the seawater carbonate system caused by the semi-permanent upwelling events occurring in this locality (see Broitman et al., 2018; Osores et al., 2017; Ramajo et al., 2015). For example, Talcaruca limpets with thinner shells could be beneficial from the energy budget point of view because calcification is an energetically costly process (Palmer, 1983, 1992; Waldbusser et al., 2013) and metabolic cost is higher in this population. Nevertheless, at the same time, thinner shells make limpets be more susceptible to predation and may alter predator–prey dynamics in zones with high temporal variability of carbonate system parameters and/or in projected scenarios of ocean acidification (Lord et al., 2019; Manríquez et al., 2014).

Similarly, the data provided by the analysis of the cardiac activity indicate that limpets from the central part of the break (30°S) exhibit higher thermal optimum (T_{opt}) compared to limpets from the northern part of the break, while maximum performance (μ_{max}) is marginally higher compared to animals from the southern part of the break. This could be related to the existing high environmental variability (both in temperature, pH and carbonate system parameters) in this locality that may favour resilience to environmental stress (see Broitman et al., 2018, 2021), local adaptation and/or the evolution of phenotypic plasticity (Chevin et al., 2010; Vargas et al., 2017). These characteristics could play an important role maximizing survival during rapid changes in environmental conditions associated with climate change (Thomsen et al., 2017). A recent study by Wang et al. (2018) showed that thermal tolerance of the limpet *Cellana toreuma* acclimated to CO_2 levels of $400 \mu atm$ was higher than that of limpets acclimated to $1000 \mu atm$. The authors concluded that the resilience of *C. toreuma* limpets to thermal stress will be constrained by future OA scenarios (Wang et al., 2018). Our results indicate, however, that these responses are context-dependent. Here, populations of *S. araucana* which are acclimated to high variability in seawater carbonate parameters and temperature, exhibit higher tolerance to thermal stress.

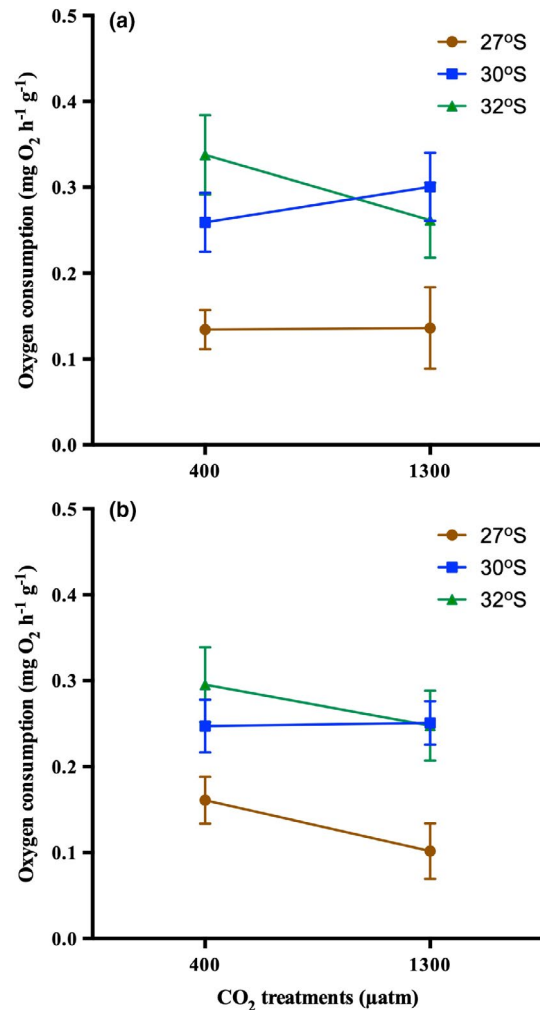


FIGURE 4 Reaction norms plots for metabolic rates (mean \pm SE) of populations of *Scurria araucana* collected along the biogeographic break recorded after 10 (a) and 20 (b) days of exposure at current pCO_2 ($400 \mu atm$) and at high levels of pCO_2 conditions ($1300 \mu atm$). Each line connects the average of population measured in both pCO_2 conditions

Global climate models predict major changes in coastal regions such as the SEP, driven by the rise of sea surface temperatures, the decrease of pH linked to OA, and the increase in the intensity and duration of upwelling events in the coming decades (Bakun et al., 2015; Turi et al., 2016; Xiu et al., 2018). Contrary to our expectations, the short-term (20 days) OA experimental exposure used in our study did not unveil differential capacity for plastic phenotypic responses and tolerance among populations of *S. araucana* along the SEP biogeographic break. All the three populations across showed similar capacities to tolerate short-term exposure to high pCO_2 . Traits such as buoyant weight, and thus growth, showed substantial intrapopulation variability between experimental pCO_2 treatments, but no significant differences among populations. These findings contrast with the negative growth rates under OA reported in other species such as blue mussel *Mytilus edulis* (Berge et al., 2006; Melzner et al., 2011). In terms of metabolic rates (O_2 consumptions), only biogeographic effects, but not pCO_2 effects were evidenced in our study.

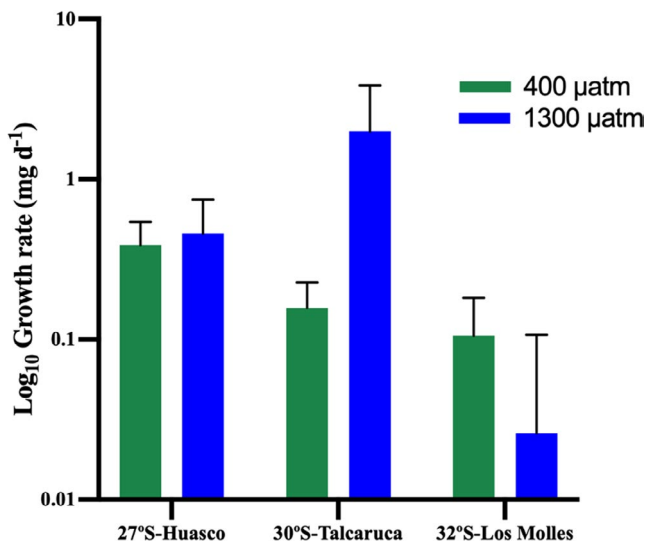


FIGURE 5 Growth rates measured as total buoyancy body weight change (mean \pm SE) of *S. araucana* individuals from the three selected populations after 20 days of exposure at current $p\text{CO}_2$ (400 μatm) and at high levels of $p\text{CO}_2$ conditions (1300 μatm)

This may imply that the mosaic of carbon chemistry conditions experienced by limpets across the biogeographic break is not driving physiological divergence nor differences in local adaptation among populations, at least when exposed to short-term OA. These results contrast with findings reported by Thomsen and Melzner (2010) in the bivalve *Mytilus edulis* in which the degree of phenotypic plasticity was related to the environmental variability faced by this bivalve. There is an inherent limitation in scope between the time-scale of the incubation period regarded in our study (i.e. 20 days) with the scales at which natural variation occur and projected event of OA will take place in nature. Nevertheless, ecosystem influenced by upwelling system, specifically on Punta Lengua de Vaca on northern-central coast of Chile, shows that natural scales of variation in environmental variables (i.e. pH, oxygen and temperature) have an average duration of 13 days during upwelling activation (see Ramajo et al., 2019, 2020). Thus, our incubation period is in agreement with natural scales of variation carbonate system parameters originated by upwelling activation. This simplistic view is common in short-term studies about OA effects and tend to disregard relevant responses that organism may express in the incoming decades like local adaptation that may also modulate the ecological relevance of the organisms upon the ecosystem functioning (but see Dupont et al., 2012).

Previous studies have suggested that geographic differences in phenotypic responses to OA are species-specific (Vargas et al., 2017) and are the result of local adaptation shaped by gradients of natural selection and variation in environmental conditions (Gaitán-Espitia, Marshall, et al., 2017; Gaitán-Espitia, Villanueva, et al., 2017). For instance, in the mussel *Mytilus chilensis*, populations across environmental gradients have shown differences in growth rate after 20 days of exposure to different $p\text{CO}_2$ levels (380, 700 and 1000 μatm) (Duarte et al., 2015). Moreover, in the sea urchin *Loxechinus albus*, larval morphology, physiology,

development/growth and survival showed geographic differences among populations exposed to elevated $p\text{CO}_2$ (1200 μatm), which were correlated to the activity of upwellings and fjords (Gaitán-Espitia, Villanueva, et al., 2017). In the clam *Ruditapes decussatus*, the growth rate response to OA also differed at local scales (Range et al., 2011), especially among organisms inhabiting coastal, estuarine and transitional waters due to the natural, high variability seawater carbonate chemistry properties (Lardies et al., 2017; Vargas et al., 2017). Although our findings in the present study do not provide new support to these general trends, it is important to highlight that natural environmental variation across temporal and spatial scale might be the underpinning driver of the overall high tolerance to OA evidenced by populations of the limpet *Scurria araucana* along the SEP.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ddi.13478>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at <https://doi.org/10.5061/dryad.cnp5hqc59>.

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BIOSKETCH

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SUPPORTING INFORMATION

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