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A contribution to the special feature 'Ocean acidification'.

Electronic supplementary material is available online at [rs.figshare.com](http://rs.figshare.com).**Global change biology****Spatio-temporal environmental variation mediates geographical differences in phenotypic responses to ocean acidification**Juan Diego Gaitán-Espitia<sup>1,2</sup>, Paola A. Villanueva<sup>3</sup>, Jorge Lopez<sup>3</sup>, Rodrigo Torres<sup>4</sup>, Jorge M. Navarro<sup>3,4</sup> and Leonardo Bacigalupe<sup>2</sup><sup>1</sup>CSIRO Oceans and Atmosphere, Hobart 7001 Tasmania, Australia<sup>2</sup>Instituto de Ciencias Ambientales y Evolutivas, and <sup>3</sup>Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile<sup>4</sup>Centro FONDAF de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes, Valdivia, Chile

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Phenotypic plasticity is expected to play a major adaptive role in the response of species to ocean acidification (OA), by providing broader tolerances to changes in  $p\text{CO}_2$  conditions. However, tolerances and sensitivities to future OA may differ among populations within a species because their particular environmental context and genetic backgrounds. Here, using the climatic variability hypothesis (CVH), we explored this conceptual framework in populations of the sea urchin *Loxechinus albus* across natural fluctuating  $p\text{CO}_2$ /pH environments. Although elevated  $p\text{CO}_2$  affected the morphology, physiology, development and survival of sea urchin larvae, the magnitude of these effects differed among populations. These differences were consistent with the predictions of the CVH showing greater tolerance to OA in populations experiencing greater local variation in seawater  $p\text{CO}_2$ /pH. Considering geographical differences in plasticity, tolerances and sensitivities to increased  $p\text{CO}_2$  will provide more accurate predictions for species responses to future OA.

**1. Introduction**

In an era of rapid environmental changes such as ocean acidification (OA), there is a pressing need to understand how organisms will respond to greater and less predictable variations in environmental conditions [1]. Intuitively, phenotypic plasticity seems a suitable strategy to cope with these changes by means of behavioural, physiological, life-history and morphological adjustments [2]. These plastic responses have long been recognized as important mechanisms by which organisms maximize fitness in heterogeneous environments [3], facilitating the persistence of natural populations by providing broader tolerances to environmental conditions [4]. Despite this central role of phenotypic plasticity, standard models aimed to predict the effect of climatic change on species persistence and distribution (i.e. the climate envelope models) do not incorporate differences in plastic responses among populations [5]. Geographical differences in plasticity may reflect contrasting selective pressures resulting from habitats with different environmental heterogeneity [6]. Under this context, the climatic variability hypothesis (CVH) offers a powerful conceptual framework with which to view the impact of future climate change (e.g. OA) on species persistence, by linking physiology, climate and biogeographic distributions [5]. The CVH states that in more variable environments, organisms should have broader

64 ranges of environmental tolerance and/or greater physiological  
65 flexibility that enable them to cope with fluctuating  
66 environmental conditions [7].

67 Environmental variability is an intrinsic characteristic of  
68 coastal ecosystems along the Southern Pacific coast of South  
69 America, where spatio-temporal changes in CO<sub>2</sub>, pH, temper-  
70 ature, nutrients and other factors, are the result of  
71 dynamic processes such as upwellings, riverine discharges  
72 and biological activity [8]. In this region, populations of  
73 marine organisms are exposed to natural variability in pH/  
74 pCO<sub>2</sub> with fluctuating CO<sub>2</sub>-supersaturated surface waters  
75 almost year-round in the northern section (18°S–30°S),  
76 seasonal CO<sub>2</sub>-supersaturated waters southward (30°S–39°S)  
77 and CO<sub>2</sub>-undersaturated surface waters in the southernmost  
78 section (more than 42°S) [9]. Like in other coastal regions,  
79 this natural variability can be far greater in magnitude than  
80 the predicted change due to OA [10], and may have prompted  
81 the evolution of a broad range of mechanisms by which coastal  
82 organisms can maintain the homeostasis for biological pro-  
83 cesses (e.g. calcification) [11]. Homeostatic capacity and  
84 tolerances to CO<sub>2</sub>/pH changes are known to differ across geo-  
85 graphical regions [12], highlighting the importance of this  
86 component in the understanding of species susceptibilities to  
87 future OA. In order to test the CVH, we explored morpho-  
88 physiological and developmental responses to elevated  
89 pCO<sub>2</sub>, in natural populations of a keystone species in the  
90 Pacific coast of Chile, the sea urchin *Loxechinus albus*. We  
91 predict that populations experiencing greater local variation  
92 in CO<sub>2</sub>/pH (i.e. continuous and seasonal upwelling subre-  
93 gions) will be less susceptible to OA than populations from  
94 less variable CO<sub>2</sub>/pH environments.

## 97 2. Material and methods

98 Adult sea urchins were collected from nine localities along  
99 the upwelling system and fjords of the Pacific coast of Chile,  
100 spanning approximately 4500 km (electronic supplementary  
101 material, figure S1). Animals were maintained in flowing  
102 seawater aquaria (13–14°C) and fed *ad libitum* with kelp until  
103 experiments. Gametes were obtained by standard methods [13]  
104 within 2–3 days after collection. From each locality, 20 indepen-  
105 dent crosses of a single male with pooled eggs of three females  
106 were developed to avoid male–female incompatibility. Eggs  
107 were fertilized using filtered (0.1 µm) seawater at ambient  
108 conditions (13°C and 390 µatm pCO<sub>2</sub>) and distributed in the  
109 experimental pCO<sub>2</sub> treatments (current global: 390 µatm and  
110 projected OA:1200 µatm [10,14]) with controlled temperature  
111 (13–14°C) at a concentration of approximately 0.7 embryos  
112 ml<sup>-1</sup>. Each cross was cultured separately in two replicate buckets  
113 for each treatment, in which the seawater carbonate chemistry  
114 was maintained using a semi-automatic flow-through CO<sub>2</sub>-  
115 mixing system [15], modified following [16] (electronic  
116 supplementary material, table S1). Embryos were sampled daily  
117 to record developmental progression (DP) and survival until  
118 early pluteus (4 arms, 80–84 h) when physiological (metabolic  
119 rate (MR) by oxygen uptake-VO<sub>2</sub>) and morphometric (total  
120 larval length and postoral arm length) analyses were performed  
121 (see the electronic supplementary material). Traits were analysed  
122 using linear mixed models in the ‘lme4’ package of R v.3.3 (R  
123 Core Team, 2016), with CO<sub>2</sub> treatment and subregion as fixed fac-  
124 tors and locality as random factor. Significance tests were  
125 performed with the ‘lmerTest’ package. Post hoc comparisons  
126 for mixed effects models were done with the ‘multcomp’ and  
‘lsmeans’ packages. DP curves were fitted and analysed with  
GraphPad Prism software (GraphPad, San Diego, CA, USA).

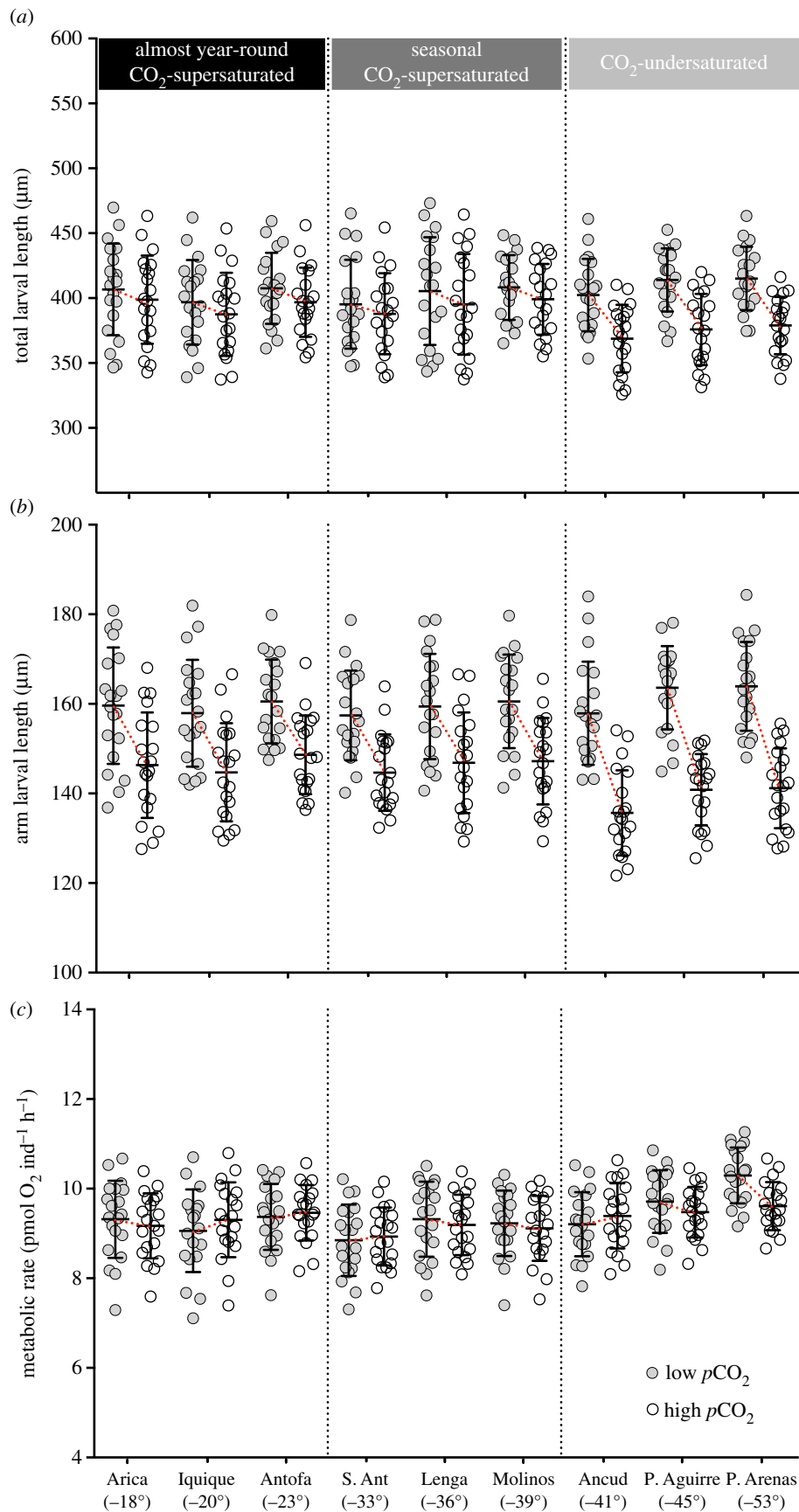
## 3. Results

There were significant effects of high pCO<sub>2</sub> on total larval  
length (TL:  $F_{1,348} = 32.4$ ,  $p < 0.05$ ), postoral arm length  
(POL:  $F_{1,348} = 221.7$ ,  $p < 0.05$ ), size-corrected MR ( $F_{1,348} =$   
 $35.8$ ,  $p < 0.05$ ), DP ( $F_{1,1792} = 11.35$ ,  $p < 0.05$ ) and survival  
( $F_{1,348} = 494.9$ ,  $p < 0.05$ ) of *L. albus*. These effects varied  
among subregions (significant interaction) for all of the  
morpho-physiological ( $X^2_{LRT} > 15$ ,  $p < 0.05$ ) and develop-  
mental traits (electronic supplementary material, table S2).  
Although POL, survival and DP were significantly affected  
( $F_{2,348} = 9.03$ ,  $F_{2,348} = 33.1$  and  $F_{20,1776} = 66.2$ ;  $p < 0.05$ ) by  
elevated pCO<sub>2</sub> in populations from the three subregions  
(figures 1 and 2), the major negative effects were detected  
in those within the CO<sub>2</sub>-undersaturated subregion (Post-hoc  
 $t_{ratio} < -12$ ,  $p < 0.05$ ). These three populations were the  
only ones with negative effects of high pCO<sub>2</sub> on TL and  
MR (post hoc  $t_{ratio} = -6.5$  and  $-4.4$ , respectively,  $p < 0.05$ ;  
figure 1a). Under low pCO<sub>2</sub> conditions, most of the pheno-  
typic traits did not differ among populations and subregions  
(TL:  $F_{2,177} = 1.08$ ,  $p = 0.34$ ; POL:  $F_{2,177} = 1.12$ ,  $p = 0.32$ ; MR:  
 $F_{2,177} = 1.92$ ,  $p = 0.15$ ; survival:  $F_{2,177} = 2.23$ ,  $p = 0.11$ ; figures 1  
and 2). However, DP differed geographically ( $F_{8,864} = 6.634$ ,  
 $p < 0.05$ ; figure 2), showing a faster rate in the southernmost  
population (figures 1 and 2a).

## 4. Discussion

This study highlights the role of naturally fluctuating pCO<sub>2</sub>/  
pH environments in determining geographical differences in  
phenotypic responses to projected OA. Although elevated  
pCO<sub>2</sub> affected larval morphology, physiology, development  
and survival, the magnitude of these effects differed among  
the three main subregions. The lack of clinal trends in pheno-  
typic responses to simulated OA suggests that geographical  
differences in average pCO<sub>2</sub> are not driving differences in  
tolerances among sea urchin populations. Instead, phenotypic  
differences in *L. albus* were consistent with the predictions of  
the CVH showing greater tolerance to OA in populations  
experiencing greater local variation in seawater CO<sub>2</sub>/pH  
[7,17]. Total larval length, postoral arm length (a proxy of  
larval calcification [18]), DP and survival were less affected  
(approx. 3% change) in populations within the CO<sub>2</sub>-supersatu-  
rated subregions (i.e. continuous and seasonal; 18–39°S) than  
in populations from the CO<sub>2</sub>-undersaturated subregion  
(approx. 9% change; more than 40°S). This geographical  
pattern of phenotypic responses may result from spatial differ-  
ences in OA-induced energetic costs for maintenance, growth  
and survival under elevated pCO<sub>2</sub> conditions [19]. In fact,  
larval MR was only affected by acidified seawater in popu-  
lations within the CO<sub>2</sub>-undersaturated subregion, supporting  
the idea that changes in energy allocation can be the main dri-  
vers of the negative effects of OA in *L. albus* [18,19]. Under  
present-day pCO<sub>2</sub> conditions, larvae of *L. albus* showed similar  
morpho-physiological characteristics among populations.  
Nonetheless, the faster growth rate observed in the southern-  
most population is likely due to the rearing temperature that  
was slightly higher (approx. 2°C) than the local conditions  
during its spawning/growing season (11°C) [13].

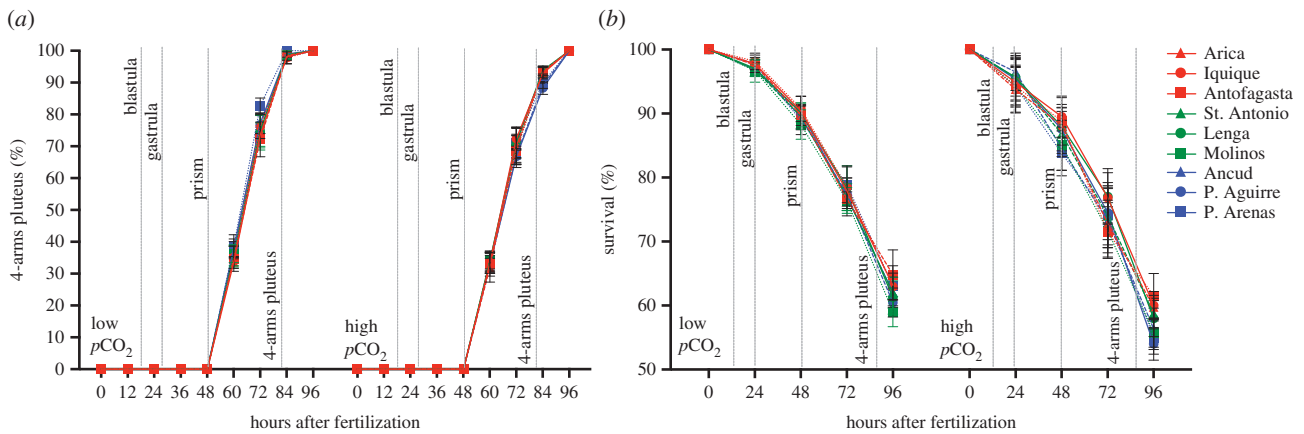
From a theoretical perspective, geographical differences  
in tolerances and sensitivities to high pCO<sub>2</sub> may reflect  
contrasting selective pressures along the spatial distribution



**Figure 1.** Geographical responses in (a) total larval length, (b) postoral arm length and (c) size-corrected metabolic rate under  $p\text{CO}_2$  treatments. Mean  $\pm$  s.d.

of *L. albus* [6]. Within the  $\text{CO}_2$ -supersaturated subregions, local selection may have promoted highly plastic phenotypes with broad tolerance to pH variation [20], and greater fitness (e.g. survival) under high  $p\text{CO}_2$  conditions [21] in comparison with phenotypes from the  $\text{CO}_2$ -undersaturated subregion. Similar geographical differences in performance and fitness

have been documented in other marine species along upwelling systems (e.g. [12,22]), suggesting that regional differences in carbonate chemistry may have acted as selective pressures maintaining phenotypic and genetic variation necessary for adaptive responses to changing pH [21,22]. Although gene flow can impede the adaptive divergence in populations of



**Figure 2.** Effect of ocean acidification on (a) developmental progression and (b) survival of sea urchin larvae. Mean  $\pm$  s.d.

*L. albus* [20], local adaptation may still occur if phenotype-specific mortality occurs after larval dispersal [23].

In a projected OA scenario, the stronger negative effects on larval growth and developmental dynamics in populations from the CO<sub>2</sub>-undersaturated region can lead to prolonged pelagic larval duration, increasing the susceptibility to predation and reducing the number of settlers due to the high mortality in the plankton [18]. For these populations, greater shifts in skeletal morphology caused by OA may influence larval feeding and their ability to disperse [24], affecting the energy transfer across trophic levels and potentially influencing population dynamics and predator–prey interactions in marine food webs [14]. In conclusion, our study reinforces the importance of considering geographical differences in plasticity, tolerances and

sensitivities to increased pCO<sub>2</sub> for predicting species responses to future OA.

**Ethics.** This study was conducted under approval of the UACH animal ethics committee no. 056/12.

**Data accessibility.** Data are available as the electronic supplementary material.

**Authors' contributions.** J.D.G.-E. and L.B. conceived the study, carried out analyses and drafted the manuscript. J.D.G.-E., P.A.V. and J.N.M. developed experiments. J.L. and R.T. analysed water chemistry. All authors agree to be held accountable for the content and approve the final version of the manuscript.

**Competing interests.** We have no competing interests.

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