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Effect of low pH on growth and shell mechanical properties of the Peruvian scallop *Argopecten purpuratus* (Lamarck, 1819)

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Abstract :

Dissolution of anthropogenic CO2 modifies seawater pH, leading to ocean acidification, which might affect calcifying organisms such as bivalve mollusks. Along the Peruvian coast, however, natural conditions of low pH (7.6–8.0) are encountered in the habitat of the Peruvian scallop (Argopecten purpuratus), as a consequence of the nearby coastal upwelling influence. To understand the effects of low pH in a species adapted to these environmental conditions, an experiment was performed to test its consequences on growth, calcification, dissolution, and shell mechanical properties in juvenile Peruvian scallops. During 28 days, scallops (initial mean height = 14 mm) were exposed to two contrasted pH conditions: a control with unmanipulated seawater presenting pH conditions similar to those found in situ (pHT = 7.8) and a treatment, in which CO2 was injected to reduce pH to 7.4. At the end of the experiment, shell height and weight, and growth and calcification rates were reduced about 6%, 20%, 9%, and 10% respectively in the low pH treatment. Mechanical properties, such as microhardness were positively affected in the low pH condition and crushing force did not show differences between pH treatments. Final soft tissue weights were not significantly affected by low pH. This study provides evidence of low pH change shell properties increasing the shell microhardness in Peruvian scallops, which implies protective functions. However, the mechanisms behind this response need to be studied in a global change context.

Highlights

► *Argopecten purpuratus* shell growth was reduced by 9% in low pH exposure. ► *A. purpuratus* net calcification was reduced about 10% in low pH exposure. ► *S*hell microhardness of *A. purpuratus* was positively affected by low pH.

Keywords : Ocean acidification, Low pH, Pectinid, Growth, Shell, Microhardness, Crushing force, Calcification, Upwelling systems

1. Introduction

Atmospheric carbon dioxide (CO_2) levels have increased since 1750 as a result of anthropogenic emissions (IPCC, 2019). Since then, the global ocean has absorbed about 30% of the emitted CO_2 through dissolution, which induced a decrease in seawater pH (Bindoff et al., 2019) and reduced availability of carbon ions, thus leading to the increasing instability of the mineral forms of calcium carbonate ($CaCO_3$). These changes in seawater properties may affect the organisms that produce exoskeletons from calcium carbonate biodeposition, such as marine bivalves. During the past 20 years, the vulnerability of calcifying organisms has been assessed by experimental exposure to reduced pH or enhanced pCO_2, and a wide variety of organism responses have been pointed out (see e.g. Findlay et al., 2009; Ries et al., 2009). Globally, bivalves showed negative responses (Bressan et al., 2014; Duarte et al., 2013; Gazeau et al., 2013; Lagos et al., 2016), as well as, positive responses (Findlay et al., 2009; Gutowska et al., 2010; Kroeker et al., 2013; Ries et al., 2009; Wood et al., 2008) in terms of growth and calcification. Such negative effects on growth might reflect an underlying energetic impairment (Sokolova et al., 2012; Stumpp et al., 2011), whose mechanism is still to be pinpointed (Brown et al., 2018). In addition, experimental low pH studies have highlighted a $CaCO_3$ chaotic crystal deposition that weakens the skeleton as Cnidaria, Annelida, Bivalvia, and Cephalopoda (Byrne and Fitzer, 2019). Nevertheless, under ocean acidification, mechanical characterization has only been applied to 9 species from 6 families of bivalve (Byrne and Fitzer, 2019), and for Pectinidae, just one specie was studied (Dell'Acqua et al., 2019). Mineral structure and mechanical integrity characterization are needed for a complete understanding of the impact of low pH on calcifying organisms (Byrne and Fitzer, 2019).

Argopecten purpuratus (Peruvian scallop) is the most important shellfish resource in Peru, and its exploitation supports an important part of the economy of the coastal populations of this country. *A. purpuratus* is found in the coastal bays of the Humboldt current upwelling system, which extends from Peru to northern Chile. The Chilean upwelling system, located between 30 and 41°S, is seasonally active (Aravena et al., 2014) and displays great variability in pH, specifically close to Tongoy bay (30°S) (Lagos et al., 2016). On the other hand, the Peruvian upwelling system (6°S-16°S) is active all year round (Chavez and Messié, 2009; Zuta and Guillén, 1970). In this area, the upwelled seawater is relatively cold, rich in nutrients, and shows low O_2 and high CO_2 concentrations (Friederich et al., 2008), causing the natural occurrence of low pH waters (~7.6) (Hernandez-Ayon et al., 2019; León et al., 2011). These waters can reach shallow coastal zones, like the bays in the Peruvian central coast, where natural and cultivated Peruvian scallop populations are found (Aguirre-Velarde et al., 2019a). However, pH variability is still not completely understood in this habitat. Additionally, in these shallow areas, local biogeochemical processes (such as respiration) may induce both dynamic and strong variations of pH, and thereby contribute to an additional reduction of environmental pH (Breitburg et al., 2015). On the other hand, natural variation in pH and local oceanographic conditions could lead to local adaptations and species-specific responses to low pH.

Previous laboratory experiments in *A. purpuratus* showed contradictory responses related to shell growth under low pH conditions. Lagos et al. (2016), reported reduced growth at pH 7.7, while Ramajo et al. (2016) reported increased growth at pH 7.6, and the response of *A. purpuratus* to ocean acidification is therefore unclear. Additionally, in Peru, where constant upwelling occurs, the response of this species to low pH has not yet been assessed, despite being of capital importance for the understanding of Peruvian scallop response mechanisms in this particular environment. The present study was designed to study the effect of a reduced pH (7.4), representing the environmental conditions experienced by *A. purpuratus* in Peru, on its growth and mechanical properties, in order to understand the effect of seawater acidification on this species of economic importance, as well as identify possible adaptations or vulnerabilities, crucial for this species in this actual context of climate change. We also discuss the importance of the methodology, the experimental conditions (suitable food), and of pH level selection according to naturally acidified water, in order to perform coherent studies of the effects of low pH on calcifying organisms.

2. Materials and Methods

2.1. Biological material and acclimation

Juvenile scallops belonging to the same cohort were obtained from the hatchery “La Arena" (Casma, Peru), and were transferred to the Laboratory of Marine Ecophysiology at the Instituto del Mar del Perú on the 27th of October 2015 (IMARPE, Callao, Peru). The scallops were acclimated for 19 days before the beginning of the experiment. During acclimation, scallops were placed in 300-L tanks continuously supplied with 1µm-filtered seawater, with a renewal rate equivalent to one tank volume per day. Scallops were continuously fed *ad libitum* with a mixture of microalgae *Chaetoceros calcitrans* and *Isochrysis galbana* (9:10 in terms of cell numbers). Water within the tanks was constantly homogenized by air bubbling. The temperature during acclimation was maintained at 17°C. Total pH (pH_T) was monitored daily (measurement details in 2.3 section) and was maintained on average at 7.83 (±SD 0.08). Tanks were cleaned from biodeposits daily.

2.2. Experimental setting

During the experiment, suitable oxygenation and stable pH of the water of each tank was achieved by using a 1000-L header tank supplied with 1-µm filtered seawater and with constant aeration (Fig. 1). The two 200-L experimental tanks were continuously supplied by seawater (open flow-through) from the header tank, with a flowrate equivalent to one tank volume per day. Experimental tanks were constantly homogenized using 1200 L min^{-1} aquaria pumps. Peristaltic pumps allowed to continuously supply them with a 2:5 mixture of *C. calcitrans* and *I. galbana* from the feeding tank, maintaining an algal concentration close to 5600 cells mL^{-1}. Water in each experimental tank was kept at 16 ± 1°C. Experimental tanks were daily cleaned from biodeposits and refilled with seawater from the header tank. A 12L:12D photoperiod was kept constant during the experiment period.

One tank was used as the control, with unmanipulated carbonate chemistry, while the other tank was used for the low pH treatment. In order to lower the pH of the water, gaseous CO2 was injected in a reactor placed inside the low pH tank. The CO_2 flow rate to the reactor was controlled using a bubble counter and allowed to maintain a pH at 7.4 (SD = 0.07). This value was chosen based on (1) the IPCC (2013) projection which estimates a reduction of surface pH of about -0.4 units in the ocean average for 2100 and (2) taking into account the naturally low pH found in the natural habitat of *A. purpuratus* in Peru (Paracas Bay: average 7.77, min 7.34 and max 8.4; Merma, 2016).

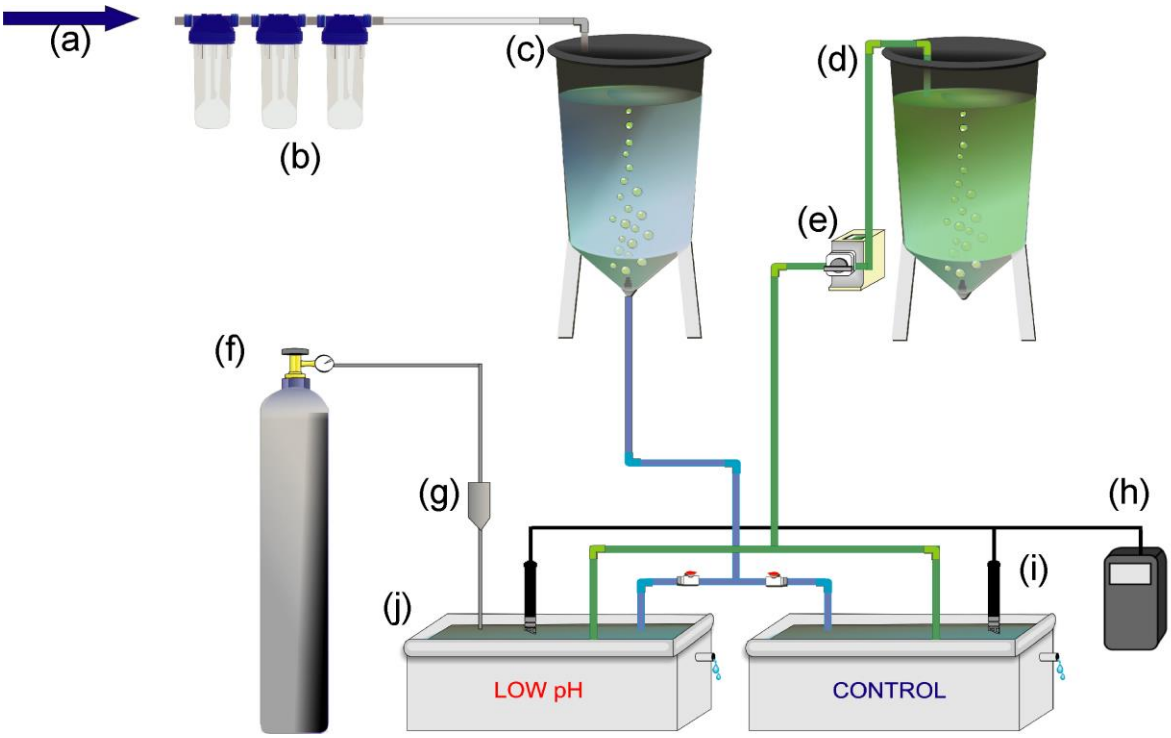

Figure 1: Schematic representation of the experimental setup. a) seawater inlet, b) cartridge filter (10, 5 and 1µm), c) head tank, d) feeding tank, e) peristaltic pump, f) CO_2 tank, g) counter of CO_2 bubbles, h) monitoring system of pH, O_2, and salinity, i) electrodes, j) experimental tanks.

Both control and low pH tanks received 238 juvenile scallops (height average = 14.0, SD = 1.3 mm, min = 11.6 and max = 16.0 mm), no gonadal maturation was observed. In each tank 83 individuals were labeled; 43 were used for nondestructive measurements of net calcification, and 40 individuals were used to estimate scallop growth rates. The remaining 155 individuals of each tank were used for measurements of growth in height and destructive measurements, such as ash free dry weight (AFDW) and shell weight. A third group composed of 30 empty valves, of a height similar to the scallop previously used, were put in each experimental tank to estimate the dissolution rates. Both tanks were monitored daily for mortality and at the end of the experiment, the remaining individuals were used for the shell mechanical properties tests (see section 2.5). The experiment was performed over a 28 days period.

2.3. Monitoring of seawater variables

Water temperature in each tank was recorded every 30 min using autonomous temperature loggers (TidbiT v2Temp, OnSet HOBO). Oxygen saturation and pH were recorded by using a multiparameter (WTW multi 3430) equipped with an optical oxygen sensor (WTW FDO 925) and a pH sensor (WTW Sentix 940). Daily, the pH sensor was calibrated against Tris seawater buffers and WTW Technical buffer solutions of pH 4.01, 7.00 and 10.01. Oxygen saturation and pH were measured every 5 min in the low pH treatment, and every hour in the control. The pH was expressed as total pH (pH_T), which was measured on the total hydrogen ion concentration scale (Dickson et al., 2007). Salinity was measured daily in the head tank using a salinometer (Portasal Wildine 8410A). Twice a day in each tank, water was sampled for counting phytoplankton cell concentration, using a Neubauer chamber and a microscope (Leica DM LS).

Water from both tanks was sampled daily for total alkalinity (A_T) determination. A_T was measured by open-cell titration (Dickson et al., 2007), using an automatic alkalinity titrator (Model AS-ALK2 Apollo SciTech) coupled to a pH meter (Orion Star Thermo Scientific A211 pH meter, Orion 8102BN pH electrode). All samples were analyzed at the experimental temperature (16°C). Measurement accuracy was checked against a certified reference seawater (CRM, batch no. 140 supplied by A. Dickson, University of California, San Diego CA).

Temperature, pH_T, A_T and salinity data were used to calculate other parameters of the carbonate system: partial pressure of CO_2 (pCO_2), aragonite saturation state (Ω_{arag}) and calcite saturation state (Ω_{calc}), using CO2SYS software for MS Excel (Pierrot et al., 2006) set with solubility constants by Millero (2010) and $KHSO_4$ (Dickson, 1990).

2.4. Growth, calcification, and dissolution measurements

2.4.1 Shell and soft tissues growth

Measurements of shell height and total wet weight were performed at days 14 and 28 of the experiment, by sampling 30 individuals in each tank. Flesh was removed from the shells, both soft tissues and valves were dry weighed after 48h at 60°C (until the weight remained constant), and AFDW was determined after 4h calcination at 450°C.

2.4.2 Net calcification

A group of 43 individuals was used for non-destructive estimation of net calcification. Shell weight was indirectly estimated from buoyant weight; this non-destructive method allows estimating the shell weight of a whole animal immersed in seawater (Palmer, 1982). For this purpose, the 43 labelled specimens were weighed in a basket full of seawater, which was suspended to a Sartorius balance (0.0001g). To establish the relationship between buoyant weight (BW) and dry shell weight (DSW), we used the scallops that remained at the end of experiment. BW of these scallops was assessed, and then a destructive measurement was performed on the same individuals for the measurement of DSW. Estimates for each pH condition allowed the estimation of DSW from BW, using the following relationships:

$$DSW(pH = 7.4) = 1.5483BW(R^2 = 1) \quad (1)$$
$$DSW(pH = 7.8) = 1.5696BW(R^2 = 1) \quad (2)$$

The same valves that were used to obtain DSW were calcined for 3h at 900°C, and allowed to establish the following relationship:

$$W_{CaCO_3} = 0.982DSW(R^2 = 1) \quad (3)$$

Net calcification rate (NCR), was calculated as the variation in shell weight reported to the initial shell weight (mg $CaCO_3$ d^{-1} g^{-1}) using the following equation (Palmer, 1982):

$$NCR = (((W_{CaCO_3 i} - W_{CaCO_3 f})/t)/W_{CaCO_3 i}) * 1000 \quad (4)$$

Growth rates were calculated as the difference between final and initial height, divided by the duration of the experiment (28 days).

2.4.3. Dissolution rate

To estimate the dissolution rates by using shells from the group which were put in each experimental tank, composed of 30 empty valves 14.0 ± 1.3 mm length. It was estimated using the difference between the weights of the empty shell at the end of the experiment, compared to the initial weight and divided by 28 days (experiment duration). All weights were measured with a Sartorius balance (0.0001g).

Finally, the measured variables (shell height, AFDW, shell weight, growth, calcification, and dissolution rates) were analyzed by using non-parametric Mann-Whitney test and T-test when normality and homoscedasticity were met (which was determined by performing the Shapiro and Levene tests, respectively), to test for differences between the two different pH groups.

2.5. Shell material properties

Shell mechanical compression assays were performed on dried empty shells from the end of the experiment between 18 to 24 mm (n = 29 for Control and n = 23 for Low pH), which were used for the previous morphometric measurements. For each sampled individual, and after removing the resilium, essays of maximum load and extensibility were performed on both right and left valves, using a material testing machine

(Zwick Roell Z0.5, with a crosshead speed of 0.25 mm min^{-1}). Compression was made first on the right valve, then on the left valve separately. Valves were identically positioned on the surface of the machine (see Fig S1 in supplementary information): shell length along the horizontal axis, outer shell surface facing upwards. Maximum load (crushing force) was identified as the highest point on the load-time curve before fracture, while the extensibility refers to the distance a shell will bend/flex before failure (see curves in Fig. 2S and 3S in supplementary information). As the crushing force depends on the height, a linear model type II was fitted for each of the pH groups and for the two valves (right and left) separately. Residuals of the fitted linear models were then compared by using the Mann-Whitney U test, to test for differences between the two pH levels.

Microhardness assays were performed on labeled shells, which were used for non-destructive measurement of growth rate (see Section 2.2),. Both valves of each scallop were embedded in Epoxy resin. After the resin hardened, valves were sectioned along the dorsoventral axis using a diamond saw. Sections were polished down to 1µm. Microhardness was determined using a standard Vickers hardness tester (Zwick Roell ZHV30 diamond indenter). By using a 200g load and 11s dwelling time, 3 to 6 indentations were performed in the region was formed during the experiment in both valves. Furthermore, during the test was difficult to establish exactly the part (inner or outer shell layer) in which were made indentation because boundaries were not clear. The median was used to obtain the microhardness value of each valve. Twelve samples from each treatment (control and low pH) were tested. An ANOVA was performed to test for differences between the microhardness of the valves (right and left separately). To compare microhardness between two pH levels the Mann-Whitney U test was used, because homoscedasticity and normality were not reached.

2.6. Shell surface observation and mineral composition

For shell surface observation, Scanning Electron Microscope (SEM) images were generated from randomly selected shells, from control and low pH. Two shells from each treatment (n=2) were randomly selected, they were gently brushed to clean the surface, and the valves were cut on an average area of 1x1 cm, from the edge of the shell by using a diamond saw in order to put in a pedestal stub and show the outer shell surface. The pieces of shell were subsequently mounted on SEM pedestal stubs, and examined under the SEM (Quanta 650 FEI equipped with Octane Pro AMETEK) without coating.

In order to determine the mineral composition of the shells, approximately 900 mg of the whole shell material was gently ground into a fine powder using an agate mortar and pestle. Powder samples of five shells per treatment were analyzed using an X-ray diffractometer (Shimadzu XRD-6000, Cu X-ray tube).

3. Results

3.1. Experimental conditions

During the experiment, the pH showed a decreasing trend (Fig. 2a), and the experimental setting allowed us to efficiently maintain two pH conditions significantly distinct (Mann-Whitney U test, $p < 0.05$). Oxygen saturation was kept close to normoxia (> 85%) in both treatments (Table1). Total alkalinity ranged between 1694 and 2045 µmol kg^{-1} in the control tank and between 1792 and 2116 µmol kg^{-1} in the low pH tank (Fig. 2b), with a significant difference between the two conditions (Mann-Whitney U test, $p < 0.05$). In both tanks, total alkalinity exhibited a decreasing trend according to the negative slope ($p < 0.01$). The pCO_2 in the control was lower compared to the low pH condition (Mann-Whitney U test, $p < 0.01$). Ω_{Calc} and Ω_{Arag} also showed significant differences between the two treatments (*t* test, $p < 0.05$). Salinity remained relatively constant (35.07 ± 0.004) recorded at the head tank. Average phytoplankton concentrations in the tanks ranged from 5414 to 5795 cells ml^{-1} in the control and the low pH, with no difference between the two treatments (Mann-Whitney U-test, $p > 0.05$).

Table 1: Physicochemical variables of seawater for the two treatments: temperature (T), dissolved oxygen saturation (DO), total pH (pH_T), total alkalinity (A_T), partial pressure of CO_2 in seawater (pCO_2), and saturation state for calcite (Ω_{Calc}) and aragonite (Ω_{Arag}). Significant differences are expressed with letters a and b, being a>b (Mann-Whitney U-test). Values in parenthesis represent the standard error (SE)

pH level	T(°C)	DO (%)	pH_T	A_T (µmol kg^{-1})	pCO_2 (µatm)	Ω_{Calc}	Ω_{Arag}
Low pH	16.14[a] (0.16)	86[b] (7.8)	7.415[b] (0.14)	1928[a] (173.7)	1729[a] (481.4)	0.88[b] (0.33)	0.56[b] (0.22)
Control	16.09[b] (0.31)	89[a] (5.1)	7.779[a] (0.20)	1858[b] (216.8)	678.5[b] (348.1)	1.86[a] (0.82)	1.20[a] (0.53)

Temperature (n = 1344 per pH treatment); dissolved oxygen and pH (low pH n = 8064 and control n = 672); total alkalinity, pCO_2, Ω_{Calc} and Ω_{Arag} (n = 28 per pH treatment)

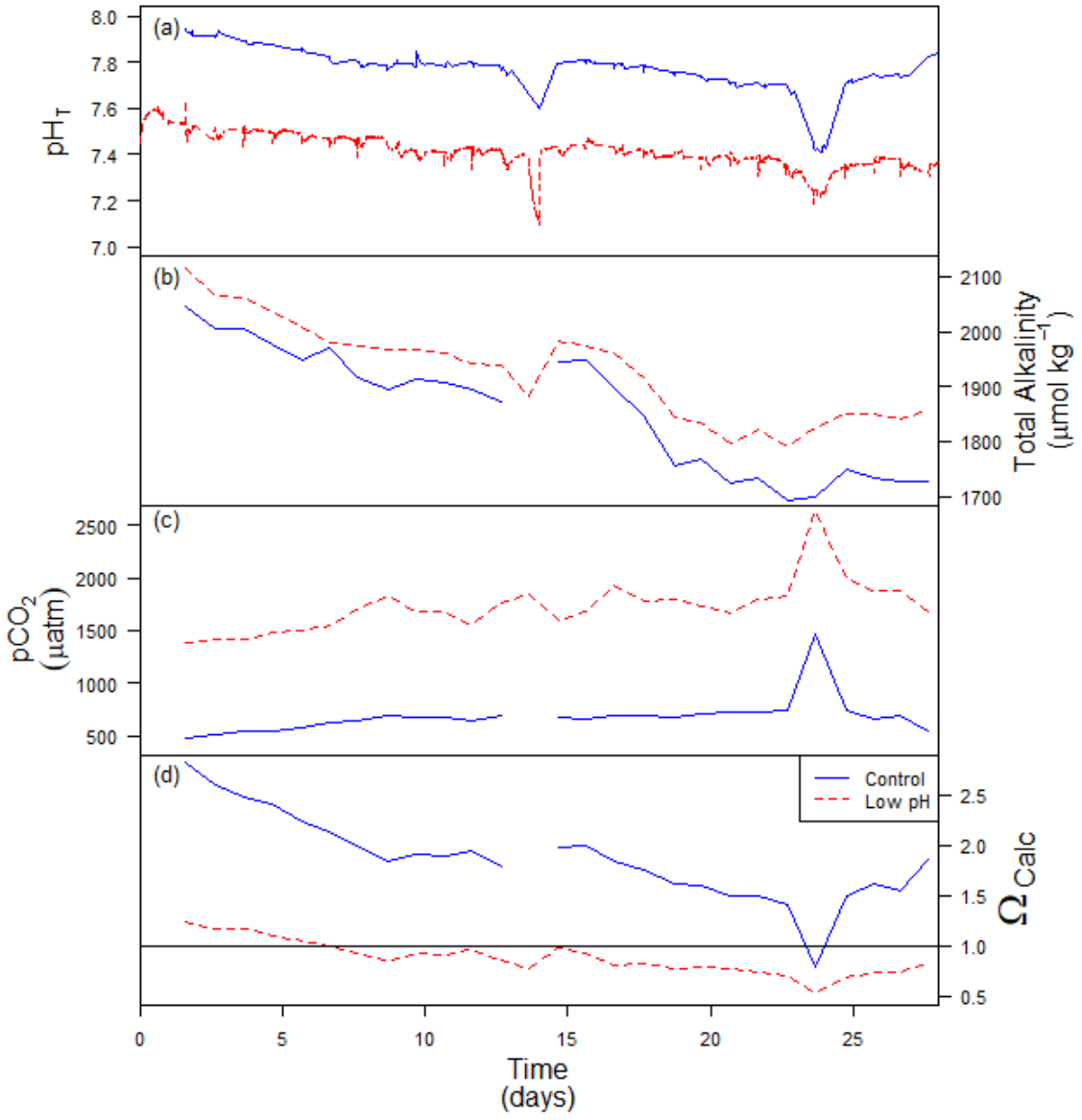

Figure 2: Trends in pH (a), total alkalinity (b), partial pressure of CO_2 (c), and the saturation state of calcite (d) of seawater during the experiment in the two treatments. Ω_{Calc} values situated above the solid black line represent supersaturated waters, while Ω_{Arag} values situated below the line represent undersaturated waters. pH (low pH n = 8064 and control n = 672); total alkalinity, pCO_2, Ω_{Calc} and Ω_{Arag} (n = 28 per pH treatment)

3.2. Growth

At the end of the experiment, shell height of scallops growing in control condition was significantly higher (Mann-Whitney U test, $p < 0.01$) than shell height of scallops from the low pH condition (Fig 3). The corresponding growth rates (mean ± CI) were 0.33 ± 0.017 mm day^{-1} in the control and 0.30 ± 0.018 mm day^{-1} in the low pH treatment. Shell weight was also significantly lower in the low pH treatment than in the control (t test, $p < 0.05$). Interestingly, values of AFDW at the end of the experiment were close in both conditions and were not significantly different ($p > 0.05$). However, at the end of the experiment, AFDW values showed a tendency to decrease in the low pH treatment, when compared to the control.

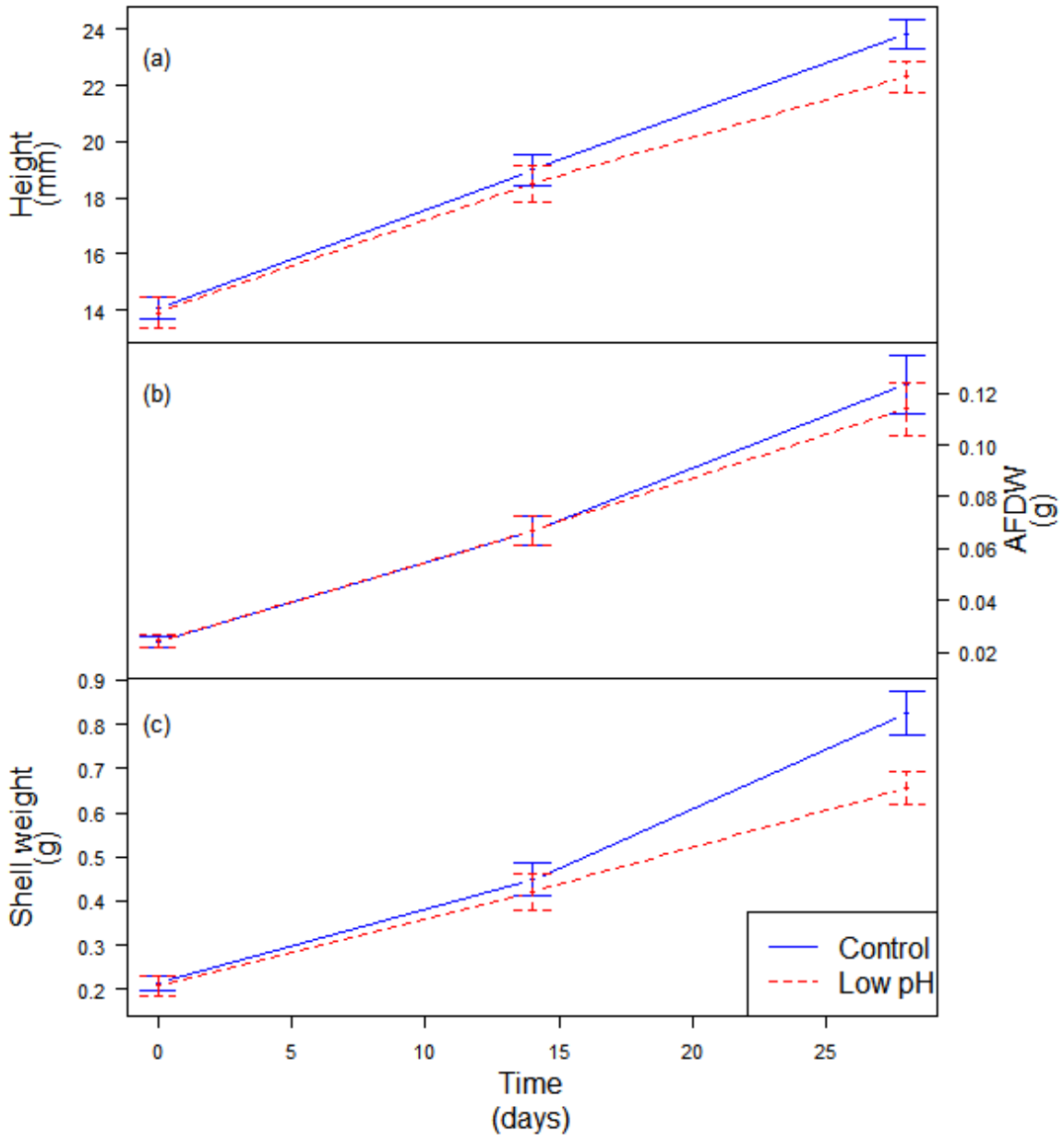

Figure 3: Morphometric changes of Peruvian scallop (*A. purpuratus*) during the experiment, in control (solid line) and low pH (dashed line) treatments: (a) shell height, (b) ash free dry weight (AFDW) y (c) shell dry weight. Vertical bars indicate the 95% confidence interval. Day 0 n = 30 individuals, day 14 n = 30 individuals, day 28 control n = 65 individuals and for low pH n = 58 individuals.

3.3. Net calcification and dissolution rate

The mean rate of net calcification was 73.5 mg $CaCO_3$ g^{-1} day^{-1} in the control, and 66.2 mg $CaCO_3$ g^{-1} day^{-1} in the low pH treatment, showing significant differences between the two conditions (Mann-Whitney U test, $p < 0.05$). Furthermore, the dissolution rate of empty shells was 6 times higher in the low pH treatment than in the control (Fig 4), with values of -0.37 and -0.06 mg day^{-1}, respectively (significant differences Mann-Whitney U test, $p < 0.05$).

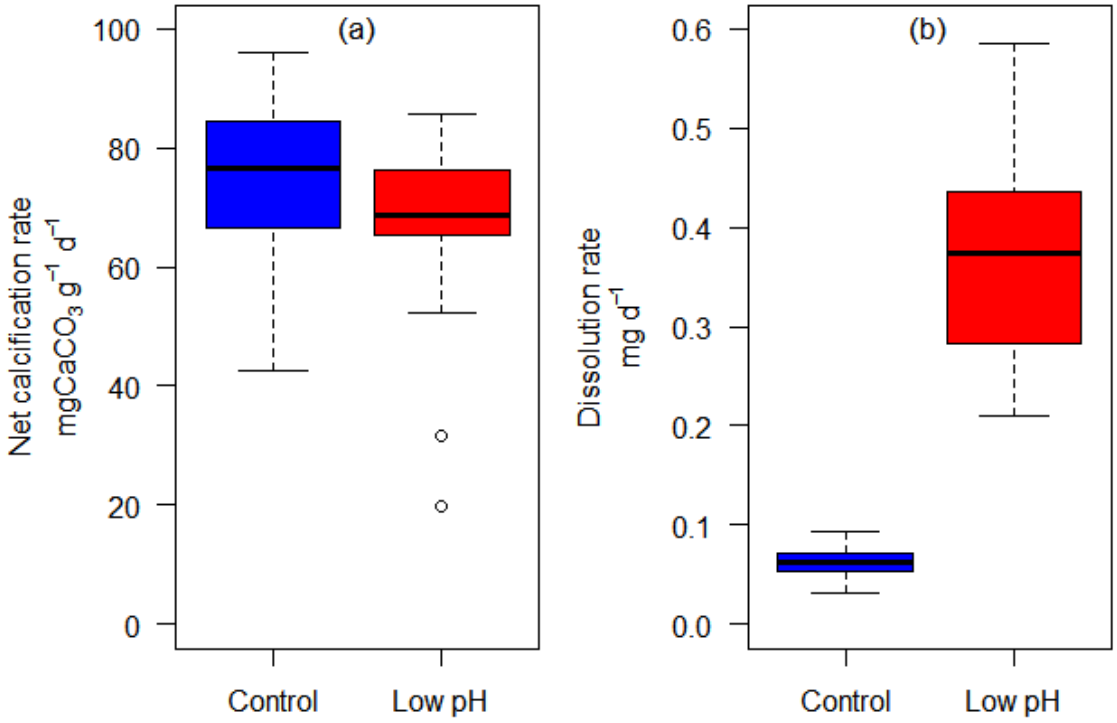

Figure 4: Box-plots of (a) net calcification rate (control n = 37 individuals and low pH n = 25 individuals) and (b) dissolution rate (n = 30 individuals per pH treatment) of Peruvian scallop (*A. purpuratus*) in the two pH treatments.

3.4. Shell material properties

The crushing force for both valves separately was compared using the linear model residuals in order to quit the height influence on crushing force. The linear models for left valves and right ones are shown (Fig. 5) separately because right valves are stronger than left ones (Mann-Whitney U test, $p < 0.05$). No significant difference between the two treatments was found for crushing force and extensibility in right and left valves (Mann-Whitney U test, $p > 0.05$).

No significant difference in microhardness between right and left valves was found, but it differed significantly between the two pH conditions, the microhardness of the valves being higher in the low pH treatment (Mann-Whitney U test, $p < 0.05$) (Fig 6) and no interaction between them was found (ANOVA, $p > 0.05$).

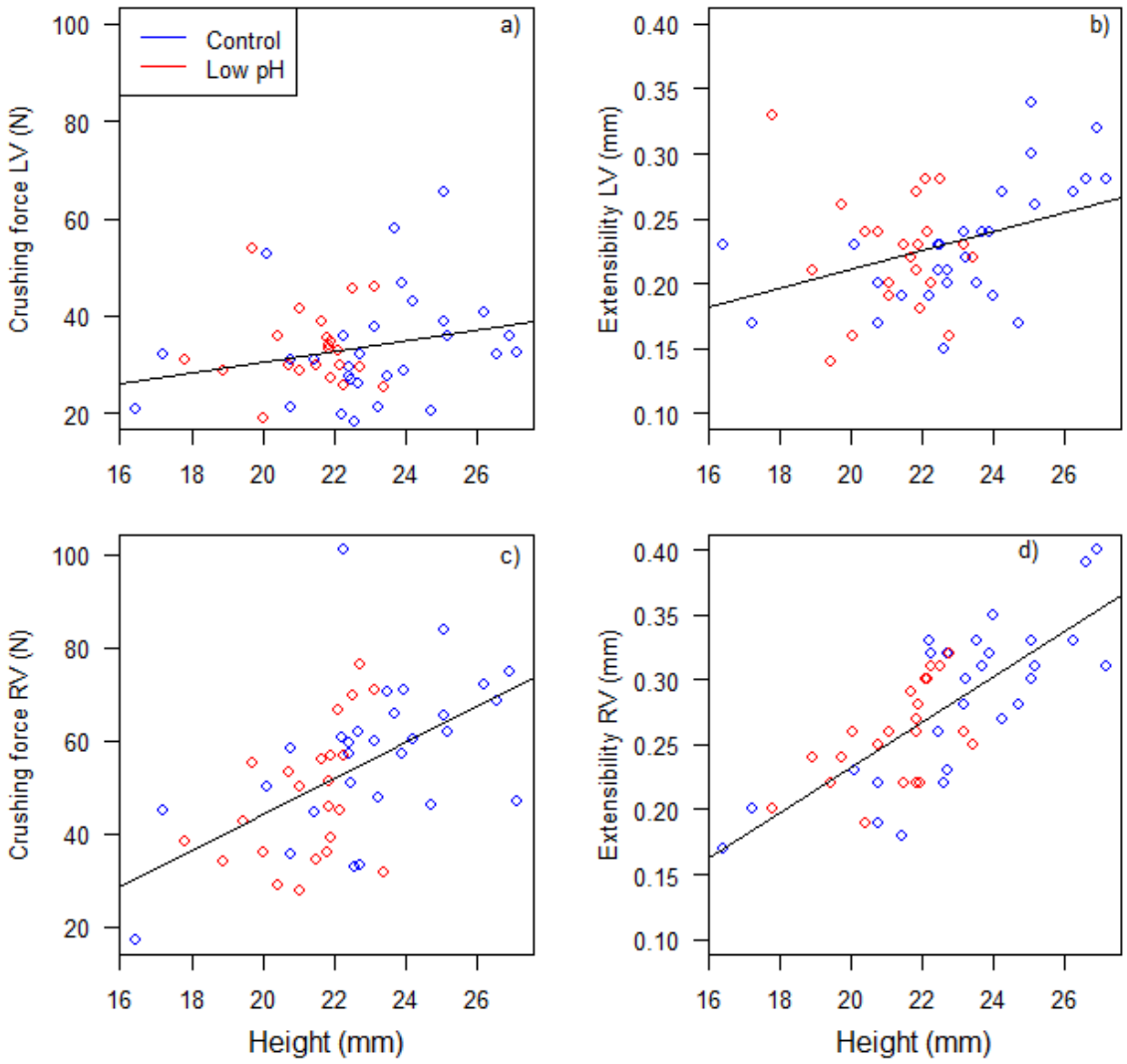

Figure 5: Linear regression of a) crushing force against left valve (LV) height, b) extensibility against left valve height, c) crushing force against right valve (RV) height, and d) extensibility against right valve height. Compression tests were performed in shells of Peruvian scallop (*A. purpuratus*) from the end of the experiment. Control n = 29 individuals and low pH n = 23 individuals.

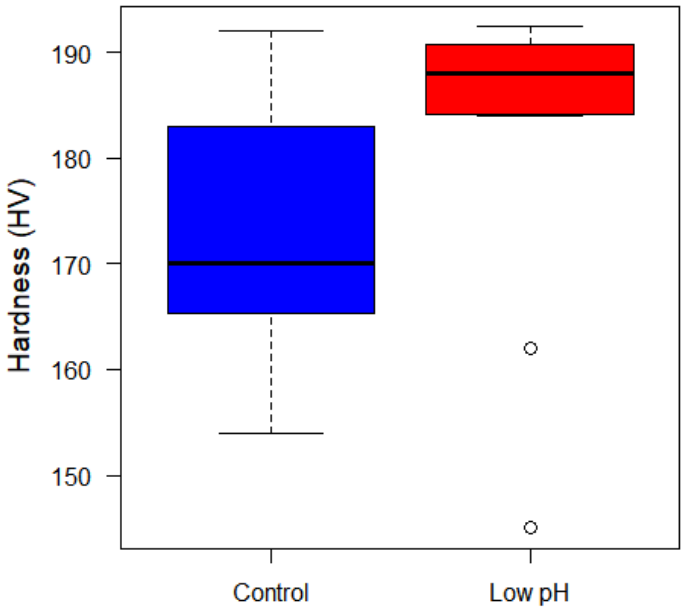

Figure 6: Microhardness of the shell of Peruvian scallop (*A. purpuratus*) for the two groups of pH treatment (n = 12 individuals per pH treatment).

3.6. Shell mineral composition

The analysis of the mineral composition of the shell revealed that the Peruvian scallop is a "monomineralic calcifier" that produces > 97 % low-Mg Calcite (Table 2). This species continued producing the same polymorph of $CaCO_3$ under the low pH treatment. Only in the control, a specimen with 8% high-Mg calcite was found.

Table 2. Mineral composition in % (SD) of Peruvian scallop (*A. purpuratus*) shells from control and low pH treatment.

Response	n	Control	Low pH	p value
Calcite %	5	96.9 (3.1)	98.4 (0.5)	$p > 0.05$
Aragonite %	5	1.4 (0.7)	1.4 (0.6)	$p > 0.05$

4. Discussion

Experimental conditions: pH of control and food conditions

The present experiment was carried out taking into consideration the natural conditions of the Peruvian scallop habitat in Peru. The pH of the control (7,8) was chosen because it represents the average conditions in shallow waters along the Peruvian coast (León et al., 2011), additionally, it was naturally supplied to the laboratory by the uptake of seawater from Callao Bay. However, the pCO_2 level of the control (~678 µatm pCO_2) was higher than the control level of ~385 µatm pCO_2 recommended by Riebesell et al. (2010). Nevertheless, the pCO_2 level of the present study was representative of the natural conditions found in the Peruvian upwelling system (Friederich et al., 2008). Furthermore, the experimental conditions (pH, temperature, and food) established in this study allowed us to achieve a growth rate in the control (0.33 mm d^{-1}) that was close to those found in natural conditions, ranging range from 0.20 to 0.39 mm d^{-1} for individuals with a height of 10 to 23 mm (Aguirre, 2011; Alcazar and Mendo, 2008; Cisneros et al., 2008). Similarly, in a study carried out with *A. purpuratus* in Chile, Lagos et al. (2016) achieved similar growth rates between the control and the natural growth rates of 0.08 to 0.17 mm d^{-1} that are found naturally in Chilean bays (Avendaño and Cantillanez, 2008; Ramajo et al., 2020). In contrast, two published experiments performed by Ramajo et al. (2016, 2019) showed that growth rates from the control ranged from 0.016 to 0.04 mm d^{-1}, which is 3 to 20 times lower than those observed in natural conditions. Such a huge discrepancy between natural and the control growth rates might be related to unsuitable, or poor nutritional conditions in the experimental design. Indeed, Ramajo et al. (2016, 2019) supplied lyophilized phytoplankton suspension of two flagellated microalgae (*Isochrysis sp*. and *Pavlova sp*.), instead of living algae diet as it was used in Lagos et al. (2016) and in the present work. Since growth is a function of food quantity and quality, poor food conditions might reduce the effects of experimental treatments, as growth rates should be more strongly affected by the food condition than by the pH. In the present study, the growth rate measured in the control was similar to the one found in natural conditions in central Peru, thus allowing us to extrapolate our results to the natural environment and local Peruvian scallop populations. About the alkalinity's decreasing trend in this experiment, we propose it depends on natural variations in the Callao Bay (12°S) because seawater was pumped into the head tank, furthermore, the seawater in the experimental tanks was overturned daily. The average total alkalinity in this study (1858-1928 µmol kg^{-1}) was lower compared to a similar experiment carried out by Cueto-Vega (2021) in which the TA mean was 1992-2021 µmol kg^{-1}. Additionally, an experiment in the Callao Bay had a TA between 2336-2338 µmol kg^{-1} (Chen et al., 2021). However, natural variation in the total alkalinity in Callao Bay remains poorly studied.

Growth, calcification, and dissolution at low pH

The exposure to reduced pH (7.4) for 28 days induced a reduction of shell growth and calcification in *A. purpuratus*, although this effect was not significant after 14 days. This negative effect is consistent with the work of Lagos et al. (2016), which showed a negative impact of a low pH of 7.7 on scallop growth after 18 days. However, these results are contradictory to those of Ramajo et al. (2016) who found a positive effect of a reduced pH (7.6) on growth after only 11 days. The duration of the exposure might be a key factor (Kroeker et al., 2013), as different effects were found for the same species, depending on the exposure time: continuous or diurnal variations (Gobler et al., 2017). Taking into account the generally low impact of reduced pH and the inter-individual variability in growth rate, an experiment long enough is needed to allow results from different experimental conditions to diverge from each other, and thus highlight significant differences. The lower the growth rate, the longer the experiment should last. These differences in growth rates, exposition times, and pH levels might explain the discrepancy between the results from Ramajo et al. (2016) on one side, and from Lagos et al. (2016) and the present study on the other side.

Similar results of reduced shell growth under low pH conditions were obtained in a wide range of mollusk species (Bressan et al., 2014; Kroeker et al., 2013; Melzner et al., 2011). In other bivalves, such as the mussel *Mytilus edulis*, the reduction of growth rates resulting from undersaturated seawater with respect to calcite ($\Omega_{Calc} < 1$) was shown to cause an increased calcification and shell maintenance costs under high CO_2 pressure (Melzner et al., 2011). In juvenile *A. purpuratus*, Lagos et al. (2016) hypothesized that the reduction in shell increments under low pH conditions could be due to the energy allocation being remobilized to other key physiological processes, such as acid-base balance, reproduction, and immune function. However, no

measurements of metabolic variables were performed to confirm this hypothesis. Considering an energetic approach, low pH could increase the maintenance cost of the scallop, resulting in less energy available for growth (Klok et al., 2014). Similarly, the intake or assimilation of energy by the organism could be affected by low pH (Leung et al., 2020), with less energy remaining to pay prioritized maintenance costs, resulting in even less energy allocated to growth. Therefore, reduced shell length for a given age may be related to the indirect effect of pH on the energetic cascade, in addition to its direct effect of dissolution of the shell.

The dissolution rate had a reduced effect on the calcification rate because it represented 0.4% (control pH 7.8) and 2.5% (low pH 7.4) at 16°C. In contrast, Lagos et al. (2016) found dissolution rates of 9.2% for pH 7.7 at 18°C and of 15% for pH 7.7 at 14°C relatives to net calcification in *A. purpuratus*. In order to make the comparison possible, results from Lagos et al. (2016) were converted from 14 to 16°C and the treatments of pH 7.7 and 7.8 were considered, resulting in dissolution rates 5 times higher for Lagos et al. (2016) compared to the present study. In absence of biological forcing (empty shells), dissolution rates may be affected by the organic composition and crystal size of the shells. According to Harper (2000), those factors could lead to differences in dissolution rates and could be more important than the mineral composition (calcite or aragonite) of the shell. Furthermore, variations in shell composition could be different between different populations of the same species (Clark et al., 2020).

Previous studies showed that the periostracum could protect shells from dissolution (Melzner et al., 2011). Nevertheless, the absence of periostracum was reported for many pectinids (Freitas et al., 2009), and the periostracum of scallops was described as "thin" (Beninger and Le Pennec, 2006; Clark, 1976;). Clark (1976) determined the periostracum of *Pecten diegensis* to be 0.2 µm thick, qualifying the periostracum of scallops and oysters as extremely thin, additionally, its main function is to assist in the marginal calcification, rather than being a protective layer. For *A. purpuratus*, in the oldest parts of the shell, situated close to the umbo, a structure surface loss was reported, due to natural abrasion (Gosselin et al., 2013), which probably leads to periostracum loss in these oldest parts. On the other hand, Ramajo et al. (2016) hypothesized that a thicker periostracum could explain the positive effect on growth, more than the organic composition of the periostracum, in the low pH (7.6) treatment. However, no photos nor measurements were available to confirm this statement. In the present study, the microscope resolution used did not allow us to observe the periostracum. Additionally, shell surface observations in the control (n = 2) and low pH (n = 2) showed a non-homogeneous surface, which is best visible in SEM images (Fig S4). Therefore, natural pH 7.8 could result in some degree of dissolution as well as a low pH, this observation is consistent with the dissolution rates estimated for both control and low pH. Further studies on periostracum structure and surface observation in this species are required to fully understand its role and response in a low pH environment.

Overall, under low pH conditions, juveniles of *A. purpuratus* seem to prioritize the maintenance of soft tissues, as no difference in weight was found between the two pH treatments. According to Aguirre-Velarde et al. (2019b), high energy cost is invested by this species in soft tissue growth. Considering that the dissolution rate only reached 2 % of calcification rate, this suggests that the reduction in calcification rate would be mainly due to an indirect effect of low pH (Klok et al., 2014) and that growth of soft tissues seems to be prioritized, resulting in reduced shell growth. Furthermore, other unknown processes driven by hypercapnic stress at the cellular level might increase energy demand (Pan et al., 2015). This process might include the regulation of intracellular acidosis (Stumpp et al., 2011) and thus might increase maintenance costs under low pH, as hypothesized by Jager et al. (2016). In this sense, some studies pointed out that calcification is a low energy consuming process (Palmer, 1992; Clark et al., 2020), and, under stress conditions like reduced salinity (Clark et al., 2020; and authors therein) and low pH, it is frequently affected (Findlay et al., 2009; Parker et al., 2013; and authors therein), probably due to indirect effects of low pH on metabolism (Klok et al., 2014).

Shell mechanical properties in low pH

In the present experiment, valves of the Peruvian scallop of the same size were lighter in the low pH condition than in the control, suggesting that valves could be more fragile in a low pH environment. However, it was not possible to establish if the lower weight was due to the lower density or reduced thickness of the shell, as these parameters were not measured. The difference in crushing force between the left and right valve of *A. purpuratus*, independently of the pH level, could be explained by differences in microstructure, composition, and shape between left (upper) and right (lower) valves (Checa et al., 2005, 2009). Furthermore, crushing force and extensibility are dependent on the shell height, when the influence of the height is removed there is no effect of low pH on the crushing force. A study in which shell biometrics had a relationship with crushing force was made by Auzoux-Bordenave et al (2019), in which the shell weight in abalone explained the reduced crushing force in low pH. Additionally, an interesting result was that the microhardness of the valves was higher under low pH. Similarly, increased hardness was reported in *Mytilus edulis* (Fitzer et al., 2015) and in *Austrocochlea constricta* (Leung et al., 2017, 2020) in low pH conditions, however, the mechanisms that explain this remain unknown. Nevertheless, a wide range of responses to low pH was described in a review by Byrne and Fitzer (2019), in which crystals and structure alteration, as well as weaker shells, were mostly found. Despite its diversity, the effect of low pH on bivalve shells seems to be species-specific, and scallops are still to be investigated. We suggest that the organic and inorganic composition and the microstructure of the valve, which play a key role in the nucleation, polymorph, growth, orientation, and morphology of the calcification process (Addadi et al., 2006; Kamat et al., 2000; Okumura et al., 2011; Suzuki and Nagasawa, 2013), could be altered by low pH, modifying the mechanical properties of the valves, such as strength and hardness. Therefore, a change in crystallographic orientation could increase the microhardness of new calcite formed under low pH and lead, as was found by Fitzer et al., (2015, 2014). Furthermore, a change in organic composition, previously reported for this species by Ramajo et al. (2016), could lead to some mechanical property change. However, the effect of biotic and abiotic factors on the proportions of mineral and organic matrix composition remains unknown (Clark et al., 2020). According to our results, low pH did not affect the proportion of calcite/aragonite in the whole shell. However, a detailed study in the newest parts of the shell, which would have grown in low pH conditions, is recommended, as well as the study of the ultrastructure in natural populations of Peruvian scallop.

Environmental implication for *A. purpuratus*

Highly variable and multi-stressor (i.e. oxygen, pH, temperature) environments, in which inhabits the Peruvian scallop, can affect this species (Aguirre-Velarde et al., 2019a; Merma, 2016). A highly variable habitat could give the species some plastic responses (Leung et al., 2017). Our results suggest that the Peruvian scallop shows some plasticity, because the growth rate recorded in the low pH condition is found in natural populations of Peru, and therefore our low pH condition of 7.4 should not be the worst scenario experienced by *A. purpuratus*. Furthermore, the increased microhardness in low pH could make the shell more brittle and susceptible to predators, however, mechanical properties have not been studied in natural populations yet and mechanisms of change are poorly understood. Therefore, a multi-stressor and high variability experimental approach (McElhany and Busch, 2012) are needed to improve the understanding of the responses of species living in coastal habitats, influenced by upwellings.

Conclusions

The exposure to low pH (7.4), considering the average pH in the habitat of Peruvian scallop *A. purpuratus*, resulted in negative responses on shell growth and calcification, neutral responses on soft tissue growth, and positive responses on microhardness at the end of the experimental period (28 days).

In the low pH treatment, dissolution did not seem to play an important role in reducing calcification rates. However, despite the adaptations of *A. purpuratus* to cope with the naturally acidic conditions found in the Peruvian bays, our results suggest that low pH should have a negative effect on the scallop metabolic processes, with consequences on growth.

Furthermore, this study emphasizes the importance of suitable control conditions for the experiment (eg. pH and food) which should represent natural variations or average conditions found in the natural environment. As a result, growth rates could be considered the quality control of laboratory experiments, when similar to those found in the natural environment.

Finally, to assess the vulnerability of *A. purpuratus* to ocean acidification and more generally to climate change, further multi-stressor experiments, focusing on the energetics of this species, would help us to better understand the processes that are affected, as well as determine the effect of each stressor, and their possible interactions.

Declarations of interest
None.

Author contributions
The authors substantially contributed to the study's conception and data acquisition. All authors have given approval to the final version of the manuscript. In particular, K. Córdova-Rodríguez carried out the experiment, performed the data collection and all the analyses, and wrote the manuscript; Mr. Rozas strongly contributed to the mechanical analyses; Mr. Fernandez planned the experimental design and revised the manuscript; Dra. Graco and Dr. Fly-Sainte-Marie discuss, revised the manuscript and funding acquisition; and Dr. Aguirre-Velarde conceptualization supervised all the analyses, revised the manuscript, approved the final version and funding acquisition

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Supplementary information

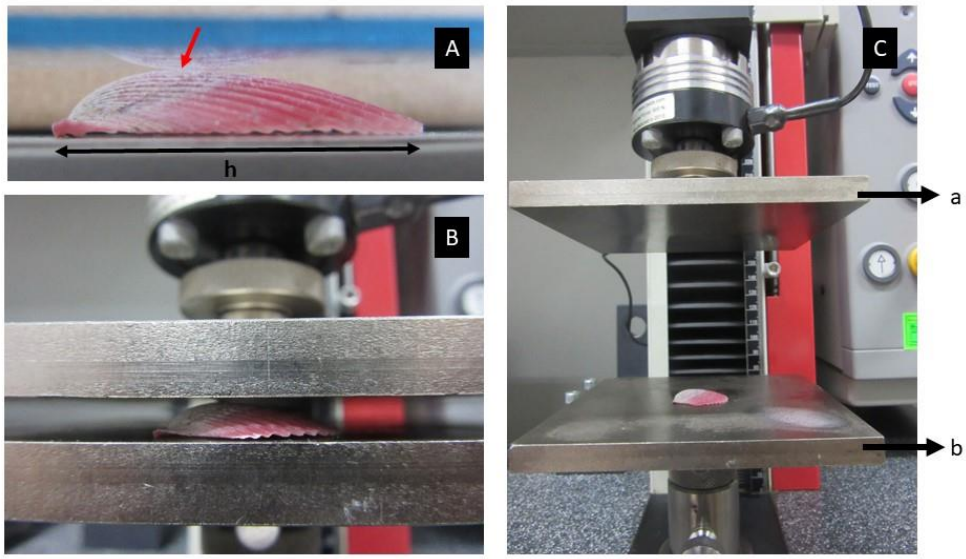

Fig S1. Compression test scheme. A) shell Orientation in which “h” means shell height and the red arrow points to the upper part of the valve that will hit the compression plate. B) Didactic representation of the compression test just before the mobile top plate hits the valve. C) Parts of compression tester in which “a” corresponds to the mobile top plate and “b” fixed bottom plate. Valve is laid in the middle of the bottom plate in every test.

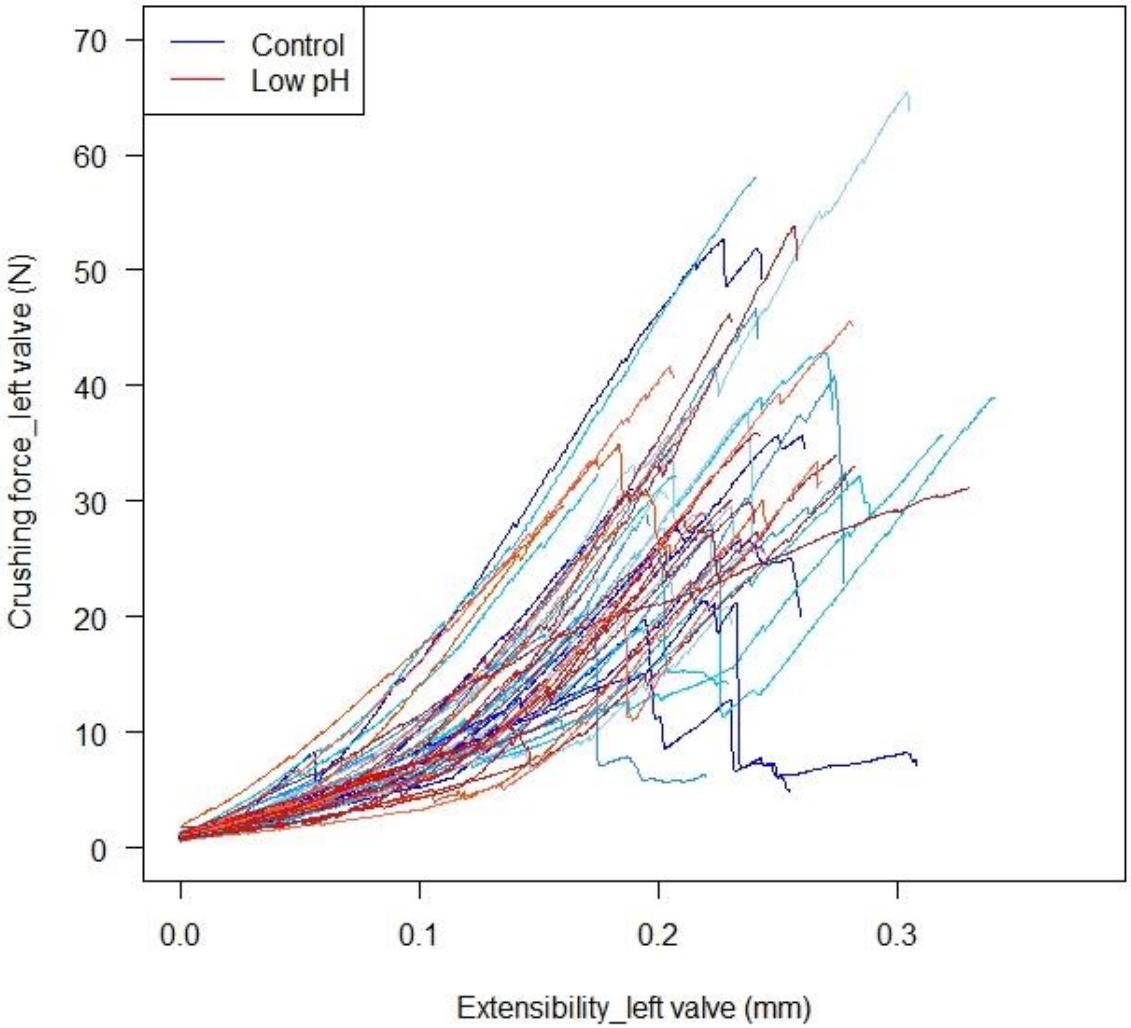

Fig S2. Compression test results crushing force against extensibility of the left valve showing the behavior of the complete shells. Control n = 29 individuals and low pH n = 23 individuals.

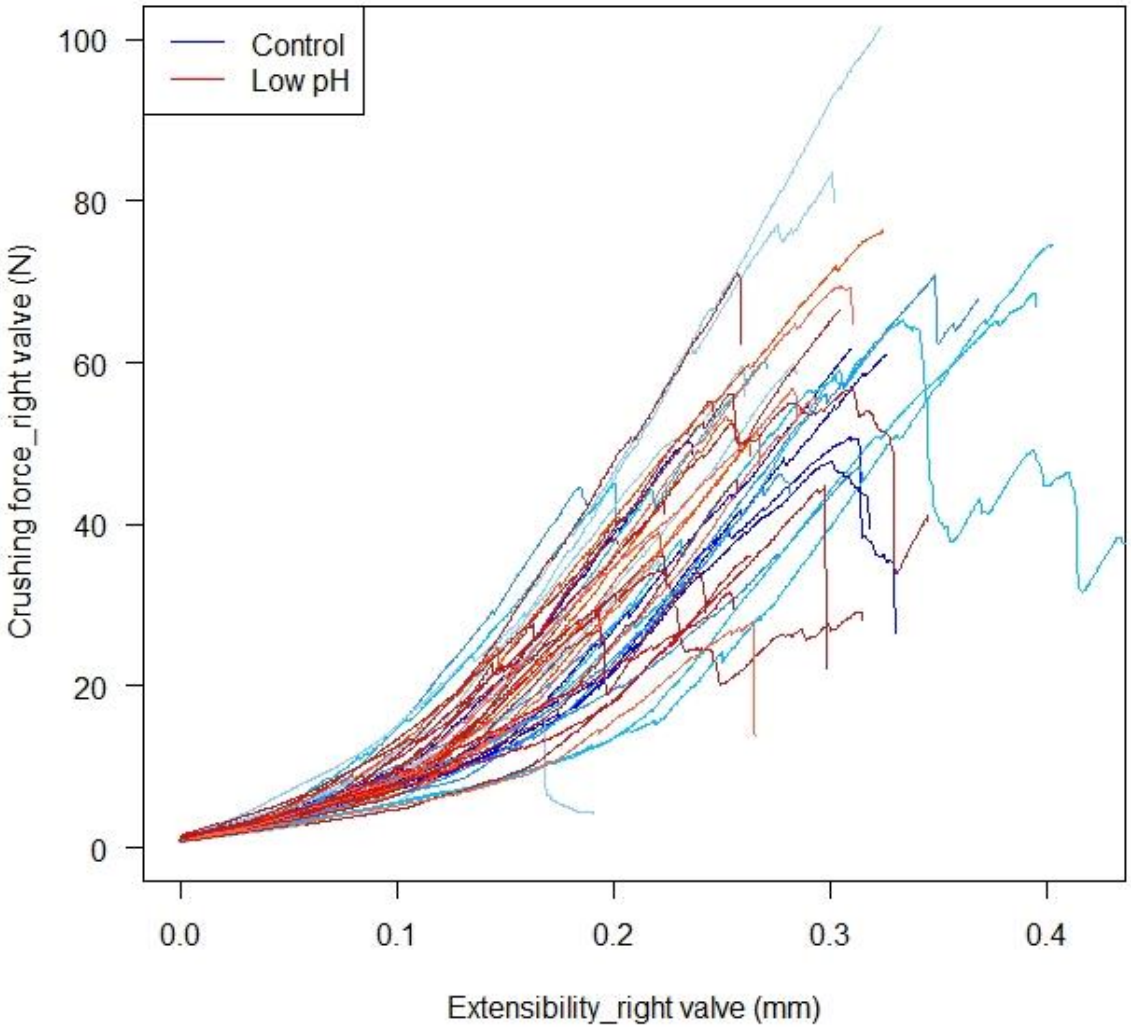

Fig S3. Compression test results crushing force against extensibility of the right valve showing the behavior of the complete shells. Control n = 29 individuals and low pH n = 23 individuals.

Shell surface observation

Shells from the control showed a regular surface (Fig. S4a), the ribs and intercostal channels presented no signs of damage (Fig. S4b), SEM images of the rib surface (Fig. S4c), and the structure of the intercostal channel (Fig. S4d). On the contrary, in shells from the low pH treatment, the shell surface (Fig. e), with the ribs and intercostal channels showing corroded ornaments, presented a white color on the surface (Fig. S4f). Furthermore, SEM images showed that the ribs of the shells from the low pH treatment were corroded (Fig. S4g), as well as the intercostal channel (Fig. S4h).

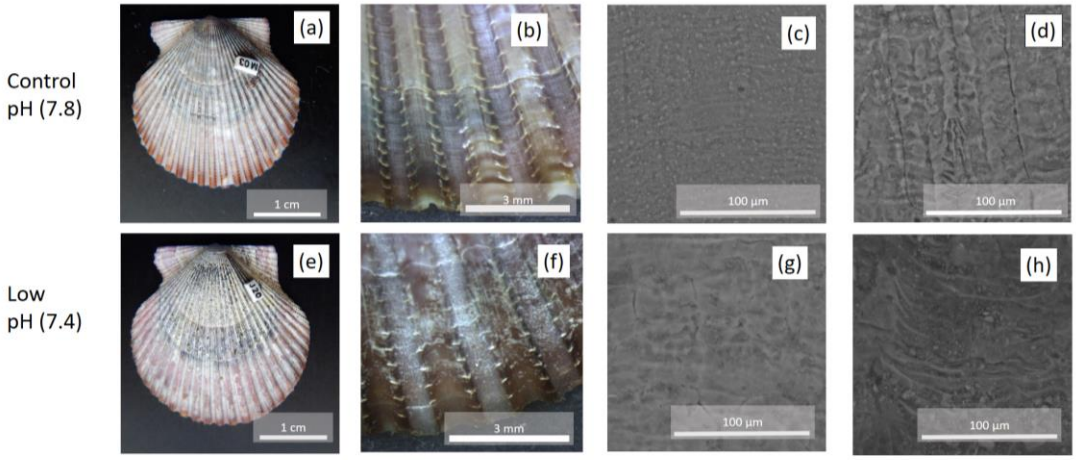

Fig S4: Overall view, stereoscopic photograph and Scanning Electron Micrograph (SEM) of outer shell surface of *Argopecten purpuratus* from normal (7.8) and low pH (7.4) treatments. a) Overall view of the outer left valve from the control (pH 7.8), (b) stereoscopic micrograph of the edge showing ribs and intercostal channels, (c) SEM of the rib, and (d) SEM of the intercoastal channel (pH 7.8). (e) Overall view of the outer left valve from the low pH (7.4), (f) Stereoscopic micrograph of the edge showing bleaching and corroded ornaments, (g) SEM of the rib showing a corroded surface, and (h) SEM of the intercoastal channel showing corrosion.