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Title: Biomineralization changes with food supply confer juvenile scallops (*Argopecten purpuratus*) resistance to ocean acidification

Running Head: Food supply and ocean acidification

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Abstract

Future ocean acidification (OA) will affect physiological traits of marine species, with calcifying species being particularly vulnerable. As OA entails high energy demands, particularly during the rapid juvenile growth phase, food supply may play a key role in the response of marine organisms to OA. We experimentally evaluated the role of food supply in modulating physiological responses and biomineralization processes in juveniles of the Chilean scallop, *Argopecten purpuratus,* that were exposed to control (pH ~ 8.0) and low pH (pH ~ 7.6) conditions using three food supply treatments (high, intermediate, and low). We found that pH and food levels had additive effects on the physiological response of the juvenile scallops. Metabolic rates, shell growth, net calcification, and ingestion rates increased significantly at low pH conditions, independent of food. These physiological

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responses increased significantly in organisms exposed to intermediate and high levels of food supply. Hence, food supply seems to play a major role modulating organismal response by providing the energetic means to bolster the physiological response of OA stress. On the contrary, the relative expression of chitin synthase, a functional molecule for biomineralization, increased significantly in scallops exposed to low food supply and low pH, which resulted in a thicker periostracum enriched with chitin polysaccharides. Under reduced food and low pH conditions, the adaptive organismal response was to trade-off growth for the expression of biomineralization molecules and altering of the organic composition of shell periostracum, suggesting that the future performance of these calcifiers will depend on the trajectories of both OA and food supply. Thus, incorporating a suite of traits and multiple stressors in future studies of the adaptive organismal response may provide key insights on OA impacts on marine calcifiers.

Introduction

About one third of anthropogenic CO_2 emissions are stored in ocean surface waters (Sabine *et al.,* 2004), altering seawater pH and carbonate ion concentration. By the end of this century, open-ocean seawater pH is predicted to decline from the present 8.0 to 7.8 (Wolf–Gladrow *et al.,* 1999), continuing to decline by 0.5 units by 2200 (Wigley *et al.,* 1996). These changes in seawater pH and carbonate ion concentration with ocean acidification (OA) may induce significant impacts on marine organisms, with calcifiers being particularly vulnerable (Hendriks *et al.,* 2010a; Kroeker *et al.,* 2013).

A recent meta-analysis of experimental assessments of the biological responses of organisms exposed to conditions expected with projected OA revealed effects across multiple physiological processes (e.g. calcification, growth, and metabolism), and confirmed that calcifiers are notably sensitive (Kroeker *et al.,* 2013). However, marine biota, including calcifiers, have evolved a range of mechanisms to cope with the broad variability in pH and carbonate ion concentration that characterizes many coastal ecosystems (Duarte *et al.,* 2013; Hendriks *et al.,* 2015). These mechanisms

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are, however, energy–demanding, suggesting that the resistance of marine calcifiers to OA may depend on food supply (Pan *et al.,* 2015).

The role of food availability in the capacity of bivalves to cope with stressful conditions, such as extreme temperature (i.e. Hoogenboom *et al.,* 2012), low salinity (i.e. Fernández-Reiriz *et al.,* 2005), or hypoxia (i.e. Iranon & Miller, 2012), has been amply documented. However, the role of food supply in possibly mitigating the negative effects of OA on marine calcifiers has received limited attention to–date. Experiments conducted with corals (i.e. Comeau *et al.,* 2013; Crook *et al.,* 2013), crustaceans (i.e. Pansch *et al.,* 2014), mollusc bivalves (i.e. Melzner *et al.,* 2011; Hettinger *et al.,* 2013), and echinoderms (Pan *et al.,* 2015) provide evidence that the negative effects of OA on calcification and growth disappear when the organisms are supplied adequate and high food levels.

Coastal regions affected by upwelling processes range among the most productive in the world and support very high yields of bivalves and other calcifiers (Lachkar, 2014). The high production of calcifier animals in upwelling ecosystems is in contrast with their expected vulnerability to the low saturation states for carbonate minerals that characterize the cold, nutrient–rich, and CO_2–rich deep-waters fertilizing these ecosystems (Feely *et al.,* 2008). This apparent paradox could be partially explained by the abundant food supply characteristic of upwelling systems, especially during upwelling relaxation periods (e.g. Peterson *et al.,* 1998; Figueiras *et al.,* 2002). This abundant food supply could provide calcifiers with the energy required to display the several biological mechanisms conferring resistance to OA (Hendriks *et al.,* 2015). However, many of the physiological mechanisms involved (e.g. mechanisms to up-regulate internal pH, Hendriks *et al.,* 2015) provide resistance while they are active and, therefore, depend on food availability during the periods when adverse and upwelled conditions occur. Hence, these mechanisms may not offer protection during the early phases of upwelling, when recently-upwelled cold, hypoxic, and CO_2–rich deep–waters have not yet developed high phytoplankton biomass. Indeed, reports of mass mortality of oysters at the Oregon coast have been attributed to the upwelling of corrosive waters (Barton *et al.,* 2012). Thus, the maintenance of highly productive populations of calcifiers in upwelling-influenced coastal regions requires that these organisms use periods of high-food supply to deploy structural

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mechanisms to resist the low pH and saturation states for carbonate minerals characteristic of these habitats during the adverse period of corrosive waters and limited food supply immediately following upwelling. A primary structural adaptation mechanism for bivalves is the development of protective organic layers, such as the shell periostracum, which can be deposited in periods of high food abundance to protect the shell carbonate minerals from corrosive waters in the adverse periods (Rodolfo–Metalpa *et al.,* 2010a; Cumming *et al.,* 2011; Gazeau *et al.,* 2013; Ramajo *et al.,* in revision). However, there is a lack of studies designed to understand the role of shell periostracum and the energetic costs of the biomineralization process in molluscs growing under these productive, but chemically challenging, upwelling conditions.

We experimentally tested the hypothesis that food supply enhances resistance to ocean acidification of calcifiers from populations occurring in upwelling-influenced coastal regions. In particular, we tested how different food supply affects the response of juvenile Chilean scallops, *Argopecten purpuratus*, a major resource in the Chilean region, to the pH projected by the end of the twenty–first century. We examined, in detail, the role of food supply in the deployment of biological and biomineralogical mechanisms confering resistance to OA, such as the deposition and structure of the periostracum. *A. purpuratus* responses were assessed in terms of growth, calcification, and metabolic and ingestion rates, as well as the expression of chitin synthase and the secretion of shell periostracum.

Material and Methods

The scallop, Argopecten purpuratus, in the Chilean upwelling region

The Chilean coast is influenced by wind-driven upwelling events of different intensities and frequencies (Strub *et al.,* 1998; Atkinson *et al.,* 2002), leading to major fisheries of molluscs, crustaceans, echinoderms, fishes, and seaweeds (Montecino & Lange, 2009) that account for 40% of the annual landings of the Humboldt Current System (Thiel *et al.,* 2007). The most intense upwelling centers in Chile are associated with headlands and embayment areas (Fonseca & Farias, 1987). The

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organisms tested were collected in Tongoy Bay, which extends between Choros Point (29°14′S, 71°27W) and Lengua de Vaca Point (30º18'S, 71º37'W), and represents one of the most important upwelling areas in Chile (Figueroa & Moffat, 2000). Upwelling events deliver low O_2 and low pH waters to the surface, resulting in CO_2 super-saturation in the bay (Torres *et al.,* 1999; Torres & Ampuero, 2009; Moraga–Opazo *et al.,* 2011).

The Chilean scallop, *Argopecten purpuratus,* a filter–feeding bivalve distributed from Panama (10ºN) to north–central Chile (30ºS) (Bore & Martinez, 1980) with an annual production of 16,000 tons in Chile (Sernapesca, 2012), is one of the most important resources in Tongoy Bay. Hence, the response of *A. purpuratus* to stresses, such as changes in temperature, salinity, and the quantify/quality of suspended particulate organic matter, have been examined experimentally (i.e. Martínez *et al.,* 2000; Fernández–Reiriz *et al.,* 2005), establishing the important role of diet on the digestive capacity (Navarro *et al.,* 1994), absorption processes (Labarta *et al.,* 1997), growth and energy budget (Martínez *et al.,* 1995, 2000), reproductive conditions (Uriarte *et al.,* 2004), and behavior (Fernández-Reiriz *et al.,* 2005). In contrast, the vulnerability of the juvenile stages of *A. purpuratus* to projected ocean acidification, and the role of food supply in conferring resistance, have not been assessed, despite the potential implications for the aquaculture and fishery industries supported by this species.

Animal Collection

A. purpuratus juveniles, collected from wild populations in Tongoy Bay, were obtained in January 2014 from the INVERTEC S.A. aquaculture company, located in Tongoy Bay (30º16'S; 71º35'W). Individuals were transferred to Estación Costera de Investigaciones Marinas (ECIM-Pontificia Universidad Católica de Chile) at the central coast of Chile (32º43'S, 71º37'W), where the experiment was conducted. At the laboratory, size (maximum length), and wet and buoyant weight, were determined. Dry weight (DW) was estimated from maximum length using the allometric equation described by Uribe *et al,* (2008). A total of 90 healthy juvenile scallops of similar size (18.14

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± 1.31 mm) were selected and labelled with numbered bee tags glued onto the shells to identify each individual during the experiment.

Experimental setup and carbon system parameter measurements

We evaluated the simultaneous effects of pH variation and food availability using a factorial design. Scallops were exposed to treated seawater pumped from Las Cruces with two levels of pH (control and low, see below) and three levels of food availability (5%, 2%, and 0.1% DW individual day^{-1}). Feeding treatments were chosen based on Martinez *et al.* (2000), who reported that a food supply of 5 to 6% of individual dry weight day^{-1} allowed for optimal juvenile scallop growth. pH treatments (present and future) were achieved by aerating seawater with a mix of air and pure CO_2 gases using Mass Flow Controllers (Aalborg, USA). Scallops were fed daily with a concentrated phytoplankton suspension (Phytogold-S, Brightwell Aquatics, Catawiss, PA) of *Isochrysis* sp. and *Pavlova* sp., as required by the experimental feeding treatments. The experiment contained three independent replicated 9 L aquaria for each combination of pH and food supply, thus involving a total of 18 aquaria. Aquaria were cleaned and re-filled with filtered water (10 µm plus UV filter) and CO_2 pre-treated water every two days to maintain water quality and stable salinity levels at 34‰. The aquaria received a natural light cycle of austral summer, and water temperature was maintained at 18 ± 1ºC using a chiller (BOYU, Model L075). A total of five individuals were randomly allocated to each of the 18 experimental aquaria where they were exposed to experimental conditions of pH and food supply for a total of 30 days (n = 6 treatments).

Discrete pH (NBS scale) measurements were taken once a week using a combined electrode (double juncture) connected to a Metrohm 826 pH Mobile-meter and calibrated with commercial buffers (Metrohm®) maintained at 25ºC using a temperature-controlled water bath. Two replicate water samples were collected for Total Alkalinity (A_T) analysis once a week, and fixed with supersaturated $HgCl_2$. A_T was measured using automatic titration (open-cell method) with HCl (Fixanal®) and double endpoint titration to pH 4.45 and 4.41 (NBS scale) following Dickson Sop 3b

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(version 3.01) with a Tritando 808 and Aquatrode plus (Metrohm®). The accuracy of measurements was checked against certified reference seawater supplied by the Scripps Institution of Oceanography in San Diego, CA (CRM, Batch 101). Temperature in each aquarium was recorded continuously using HOBO temperature loggers (Onset®). Salinity was determined daily using an optical refractrometer. Carbonate system parameters were estimated from the average values of salinity, pH_{NBS}, A_T, and SST using CO2SYS software (Pierrot *et al.,* 2006) and applying dissociation constants from Mehrbach *et al.,* (1973) refitted by Dickson & Millero (1987) and $KHSO_4$ (Dickson, 1990). The experimental water conditions in the experimental treatments are reported in Table 1.

Metabolic rate

Metabolic rate, measured as oxygen consumption, was determined using three or four individual scallops per replicate aquarium for each treatment. The animals were incubated individually in 113 ml respirometric chambers for two hours at a constant temperature of 18ºC controlled by an automated temperature chiller (BOYU, Model L075) in water equilibrated to the corresponding pH treatment. Before measurements, individuals were exposed to inanition during 48 hours in UV-treated filtered, CO_2 pre-treated seawater at the same temperature as used throughout the experiment. The oxygen consumption rate was determined using an optical fiber system (Presens Mini Oxy-4 Respirometer, PreSens, Regensburg, Germany), which quantified dissolved oxygen every 15 seconds for 120 minutes. Before measurements, sensors were calibrated with a solution of Na_2O_3S at 5% and aerated water for the values 0% and 100% air saturation, respectively (Storch *et al.,* 2010). Oxygen consumption rates were standardized to individual dry weight (mg O_2 h^{-1} g^{-1}).

Mortality, growth, and net calcification rates

Mortality was monitored daily. After 30 days, growth, net calcification, and metabolic rates were determined. Growth rates correspond to the difference in maximum length between two consecutive experimental sampling events, divided by the number of days elapsed (mm d^{-1}). The net calcification

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rate was calculated using the buoyant weight technique (Davies, 1984), where buoyant weight was converted into dry weight according to the equation:

$$\text{Dry weight} = \text{Buoyant weight} / (1 - \rho_{water} / \rho_{skeleton})$$

In this equation, ρ_{water} is the seawater density in which the scallops were weighed (calculated using salinity = 34‰, temperature = 14ºC) and $\rho_{skeleton}$ is the density of calcite (2.71 g cm^{-3}) (see methods Rodolfo-Metalpa *et al.,* 2010a). Net calcification rates were calculated as the change in dry weight between 0 and 30 days, normalized to the initial dry weight of the individuals (µg$CaCO_3$ g^{-1} d^{-1}). Net calcification represents the balance between gross calcification and dissolution of shells yielding the net accumulation of calcium carbonate, organic matrix, and inorganic carbon by the animal during the experiment (Rodolfo–Metalpa *et al.,* 2010a,b).

Ingestion rate

Scallops were removed from the respirometric chambers following metabolic rate measurements, returned to their respective aquaria, and fed according to their experimental treatment conditions for 24 hours before determining ingestion rates. Three of the *A. purpuratus* individuals from each aquarium used for metabolic measurements were individually sorted into 1000 mL acid-washed borosilicate bottles filled with filtered seawater (1µm and UV-filter) and equilibrated to the respective pH treatment. Constant food concentration was added to the bottles by pipetting 5 mL of an algal stock with a mean cell concentration of about $75x10^6$ cell mL^{-1} equivalent to 10 to18 µg Chl *a* L^{-1}, which represents the mean Chl *a* concentration in Tongoy Bay during spring/summer upwelling relaxation periods (Torres & Ampuero, 2009). A 100 ml subsample in an initial bottle of 1000 mL was immediately filtered to represent for Chl *a* analysis at T_0. Individuals for each experimental treatment were incubated for approximately 4 hours, and periodically cleaned (i.e. removing faeces or pseudo-faeces) to avoid sediment accumulation. Bottles were submersed in a container with a flow-through seawater system used to maintain experimental temperature (18º±1ºC), and mixed gently and

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periodically (every 30 minutes) to minimize cell sedimentation during incubation. After 4 hours, two sub-samples of 100 mL from each bottle were filtered through a GF/F glass fiber filter (0.7 µm), which was extracted of Chl *a* in 95% acetone in the dark before measurement on a Trilogy Turner fluorometer (Model 37200-040) (Strickland & Parsons, 1972). Clearance rate (CR), measured as the volume (mL) cleared of phytoplankton (as Chl *a*) per hour, was calculated following Coughlan (1969). The ingestion rate (IR) was estimated as the product of CR and the natural food concentration level used in the study (10 to 18 µg Chl *a* L^{-1}) (Vargas *et al.,* 2013), and standardized to dry weight (mg Chl *a* g DW^{-1} h^{-1}) to allow comparison among individuals and treatments.

Chitin synthase (CHS) expression

Total RNA extraction was performed using the adductor muscle of two to three scallops per aquarium and the Trizol® method (Invitrogen™), following the manufacturer instructions. Then, RNA was DNAse treated with DNAse I (RQ1, promega). cDNA was synthesized using 1 µg of total RNA using random hexamers and the ImProm II™ Reverse Transcription System (Promega), following the manufacturer instructions. Real Time PCR was performed using the Brilliant® SYBR® Green QPCR Master Reagent Kit (Agilent Technologies) and the Eco Real Time-PCR detection system (Illumina®), as described by Arias *et al.* (2011) on three replicates of each sample. The PCR mixture (10 µL) contained 2 µL of template cDNA (diluted 1/10) and 140 nM of each primer. Amplification was performed under the following conditions: 95 ºC, 30 seconds; melting temperature for 30 seconds; and 72 ºC, 40 seconds. At the end of PCR amplification, all products were subjected to a melt cycle from 55º C to 95º C. The primers used for the CHS were described by Cumming *et al.* (2011) (*Laternula eliptica* CHS F: 5' TGTCCGCTCCTATCAAAACC 3', CHS R: 5' GGCCTTATCTCCTTCCTTGG 3') and by Zapata *et al.* (2009) (*Argopecten purpuratus,* ActinF: 5' AGGCTCCATCTTGGCATCTCT3', Actin R: 5' AGATTCGTCGTATTCCTGTTTGC 3'). The melting temperature was 57 ºC and 60 ºC for CHS and Actin, respectively. Relative gene expression of *CHS* (GOI) was normalized to the level of the housekeeping Actin gene (HK), according to

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equation: $2^{(\Delta ctGOI-ctHK)}$. Gene expression levels were then normalized to the average value of the treatment with the lowest expression. HK expression was similar across samples, and the reaction specificities were tested with melt gradient dissociation curves and electrophoresis gels (agarose 2% of each PCR product).

Periostracum morphology and organic composition

We assessed the structure of the periostracum by analyzing the outer shell surface with scanning electron microscopy (SEM) using a Zeiss Auriga SEM (Germany). Prior to observation, samples were carbon-coated in a Hitachi UHS evaporator (Japan). The chemical composition of the periostracum was analyzed by Fourier Transform Infrared spectroscopy (FTIR). For FTIR analyses, the outer surface of intact shell samples (near the margin) of two animals per aquarium were pressed against an ATR (attenuated total reflection) diamond crystal window and the FTIR spectra recorded at a 2 cm^{-1} resolution over 100 scans using a FTIR spectrometer (model 6200, JASCO Analytical Instruments, Japan). The amount of water, protein, sulfate, carbonate, polysaccharides, and lipids were estimated from the absorption peak areas associated with the characteristic molecular group of each component (e.g. O-H:water; C–H:lipids or fatty acids; amide:proteins; C–O:carbonates; S-O:sulfates; COC:sugars/polysaccharides; Rodriguez–Navarro *et al.,* 2013).

Data analysis

We analyzed the variance in growth rate, net calcification rate, oxygen consumption, ingestion rate, and relative CHS expression using two–way ANOVA with seawater pH and food availability as factors, followed by the Tukey post hoc HSD test to assess differences between experimental treatments. Prior to the statistical analyses, we log–transformed data to satisfy the assumptions of normality and homogeneity of variance, and verified it using the Shapiro–Wilk and Levene tests. Two–way MANOVA was performed to determine if the

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organic structure of the periostracum was affected by pH and food availability treatments. We used Pearson correlation analysis to test for relationships between carbonate and the organic constituents forming the periostracum. A two–way ANOVA was used to evaluate the role of pH level and food availability treatments in accounting for differences in carbonate and organic constituent of the periostracum among individuals. All of the analyses were performed using JMP software for OS X (Version 9.0.1).

Results

Metabolic rate

Food supply and pH level affected the oxygen consumption rates of *A. purpuratus* individuals (Table 2). Juvenile scallops exposed to an increase in food supply showed a corresponding rise in metabolic rates for both pH treatments (Fig. 1a), although differences between the 2% and 5% food supply treatments were not significant (Tukey HSD test, $P > 0.05$, Table 2). Independent of food supply, scallops exposed to acidified seawater showed significantly elevated metabolic rates, measuring more than 1.5 times higher than the rates of animals exposed to control pH conditions (Fig. 1a, Table 2).

Mortality, growth, and net calcification rates

All animals survived the experiment in all treatments, but food supply and pH levels affected growth and net calcification rates of *A. purpuratus* (Table 2). Scallops grown in control pH at low food supply treatment (0.1%) presented the lowest growth rate (0.009 ± 0.002 mm d^{-1}), significantly lower than that of scallops that received higher food supply (0.020 ± 0.002 mm d^{-1} and 0.016 ± 0.003 mm d^{-1}, for treatments 2% and 5% d^{-1} foot supply, respectively). However, no significant differences were found between individuals exposed to 2% and 5% d^{-1} food treatments (Table 2, Fig. 1b). Scallops exposed to low pH levels showed significantly higher growth rates compared with those under control pH experimental conditions, independent of food supply treatment. Individuals exposed to low pH

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levels and receiving high food supply (2% and 5% d^{-1}) exhibited the fastest growth rates (0.1% diet: 0.017 ± 0.004 mm d^{-1}, 2% diet: 0.023 ± 0.008 mm d^{-1} and 5% diet: 0.033 ± 0.004 mm d^{-1}), but no significant differences were observed between these food supply treatments (Fig. 1b, Table 2). A similar pattern was observed for net calcification rate (Table 2). Individuals exposed to control pH and low food supply showed the lowest net calcification rate (3.59 ± 2.19 µg $CaCO_3$ g^{-1} d^{-1}). However, the net calcification rate doubled when food supply was increased (2% diet: 8.27 ± 1.94 µg $CaCO_3$ g^{-1} d^{-1}; 5% diet: 9.26 ± 2.17 µg $CaCO_3$ $g^{-1}d^{-1}$) (Fig. 1c). Organisms exposed to low pH showed significantly higher net calcification rates compared to those under control pH (Table 2). In fact, the calcification rate of *A. purpuratus* maintained at low pH increased more than two fold with increasing food supply (0.1% d^{-1}: 7.77 ± 3.73 µg$CaCO_3$ $g^{-1}d^{-1}$; 2% d^{-1}: 13.74 ± 2.54 µg$CaCO_3g^{-1}d^{-1}$; and 5 % d^{-1}: 17.87 ± 4.56 µg$CaCO_3g^{-1}d^{-1}$, Fig. 1c). No significant differences were found between 2% and 5% food supply treatments for both pH treatments (Table 2).

Ingestion rate

Food supply and pH levels affected ingestion rates of *A. purpuratus* individuals (Table 2). *A. purpuratus* individuals exposed to low pH values showed significantly higher ingestion rate values than organisms exposed to control pH values in all food supply treatments (Fig. 1d; Table 2). No significant differences were found between low and intermediate food supply (0.1% and 2% d^{-1}) (Table 2). However, we observed significant differences between the lowest and highest food supply (0.1% and 5% d^{-1}, Fig. 1d, Table 2). Individuals fed with 0.1% of their DW d^{-1} showed a five–fold greater ingestion rate under low pH relative to those in control pH conditions (control pH: 5.3 ± 2.3 mg Chl *a* g^{-1} h^{-1}; low pH: 29.3 ± 5.8 mg Chl *a* g^{-1} h^{-1}), compared to two–fold increase in ingestion rate at low pH relative to control pH for intermediate (control pH: 14.7 ± 5.9 mg Chl *a* g^{-1} h^{-1}; low pH: 29.9 ± 6.9 mg Chl *a* g^{-1} h^{-1}) and high (control pH: 22.3 ± 6.4 mgChl*a* $g^{-1}h^{-1}$; low pH: 46.9 ± 6.8 mgChl*a* $g^{-1}h^{-1}$) food supply (Fig. 1d).

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Chitin synthase (CHS) expression

CHS expression was significantly up-regulated for *A. purpuratus* individuals exposed to low pH, compared to those in control pH. However, food supply had no significant effect on CHS expression (Table 2, Fig. 2), regardless of interaction with pH (two–way ANOVA: $P = 0.105$) (Table 2). Scallops fed with the lowest food supply (0.1% DW d^{-1}) and exposed to low pH showed a 4.5 times higher up-regulation of CHS expression than those exposed to control pH conditions and equal food supply (Tukey HSD test: $P < 0.05$). For high food supply treatment (5% DW d^{-1}), CHS expression was 1.5 times up-regulated in scallops exposed to low pH compared to those under control pH, although the difference was not statistically significant (Tukey HSD test: $P > 0.05$). The CHS expression for scallops exposed to intermediate food supply (2% DW d^{-1}) was similar in control and low pH treatments (Tukey HSD test: $P > 0.05$) (Fig. 2).

Periostracum morphology and organic composition

IR spectra showed the presence of a periostracum layer in all specimens, but with a variable composition across treatments (Fig. 3). The IR spectra showed amide/protein bands at 1640 and 1520 cm^{-1}, sulfates at 1235 cm^{-1}, and polysaccharides centred at around 1065 cm^{-1} from periostracum proteins and chitin polysaccharides. Additionally, the underlying shell carbonate mineral contributes with a broad band centered at around 1400 cm^{-1} and two sharp peaks at 874 cm^{-1} and at 713 cm^{-1}, characteristic of calcite, the main mineral component of the shell. In all specimens, the most intense bands were those associated with carbonates. However, a more intense polysaccharide band appeared in specimens exposed to low pH compared with those exposed to control pH levels (Fig. 3). Pearson correlation analyses showed that the intensity of the carbonate absorption bands was negatively and significantly correlated with that of polysaccharide (Pearson coefficient = –0.937, P = 0.000), protein (Pearson coefficient = –0.723, $P < 0.001$), and lipid (Pearson coefficient = –0.681, $P < 0.001$) peaks. These negative correlations result from the fact that the shell carbonates underlying the periostracum become more exposed and contribute more to the IR spectra as the periostracum (composed of

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proteins and polysaccharides) becomes thinner. Because the ATR signal has very low penetration (about 2 μm), the contribution of the shell carbonate to the IR spectra is indicative of a very thin periostracum (Rodríguez–Navarro *et al.,* 2013).

Comparative analyses among specimens from different experimental treatments revealed significant differences in the periostracum composition depending on pH levels (Wilks' $\lambda = 0.512$, $P = 0.002$) and food availability (Wilks' $\lambda = 0.491$, $P = 0.016$). However, no interactive effects were observed between experimental treatments (Wilks' $\lambda = 0.664$, $P = 0.213$). More specifically, the FTIR spectra of specimens from the low pH treatments generally had more intense polysaccharide bands and less intense carbonate bands than specimens from control pH experiments (ANOVA, $P < 0.05$, Fig. 3, Tables 3 and 4). However, the intensity of protein and lipid bands did not differ significantly among treatments (Table 4). These observations reveal that specimens exposed to low pH conditions secreted a thicker periostracum, which was substantially enriched in polysaccharides. On the other hand, food supply affected the polysaccharide level of the periostracum (ANOVA, $P = 0.015$), being higher at intermediate and low food supply levels. Other chemical constituents (proteins, carbonate, lipids) were not significantly affected by food supply (see Table 4). Again, no interactive effects between pH and food supply were detected on the chemical composition of the periostracum (Table 4).

Discussion

The results presented demonstrate that (1) the juvenile *Argopecten purpuratus* scallop is highly tolerant to low pH representing ocean acidification conditions, and (2) that its physiological performance under experimental OA conditions can even exceed those observed under control pH when food is supplied. Juvenile scallops exposed to a decrease of 0.4 units in seawater pH for 30 days supported elevated metabolic, growth, calcification, and ingestion rates when receiving high and intermediate food supply compared to those under low food supply. Although no scallop mortality was observed, juvenile scallops exposed to low pH and low food supply conditions over–expressed CHS and experienced changes in the composition of their periostracum layer (higher polysaccharides

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concentration), suggesting these to be important biological mechanisms to respond to OA conditions in this species. While these results clearly show that food supply confers juvenile scallops' resistance to ocean acidification, extrapolation of the results obtained in such controlled, short term experiments in the field involves uncertainties, due to the limitations of the experimental conditions (cf. Hendriks *et al.,* 2010b; Cornwall & Hurd, 2015). In addition, the experiment conducted cannot possibly capture the complexity of upwelling systems. Upwelling of high–CO_2 waters is associated with the exposure of the organisms to low temperatures, which would depress the saturation state for aragonite below the level derived from high CO_2 alone (e.g. Feely *et al.,* 2008), and the impacts of low oxygen that may affect the metabolic status of the organisms (e.g. Mayol *et al.,* 2012). An alternative to the experimental design used here, where pH and food levels were used as treatment and all other factors were fixed, would have been to associate low pH with low temperature and low oxygen, relative to conditions under control pH, thereby attempting to reproduce the combination of environmental conditions associated with upwelling. However, such an experimental design would have been inconclusive in terms of assigning observed responses to ocean acidification. Indeed, experiments, such as those conducted here, provide worst-case scenarios in evaluating the impacts of OA on marine organisms, as these experiments isolate the organisms from environmental interactions that may also buffer the impacts and do not allow for the adaptive processes the populations may develop in the field (Hendriks *et al.,* 2010b).

Metabolic depression has been described as an intrinsic and adaptive strategy to extend survival time in response to short-term events of hypercapnia, hypoxia, or food deprivation (Guppy & Withers, 1999). However, metabolic up–regulation in low pH environments, as observed for the juvenile *A. purpuratus* scallop here, is a less frequently observed response. Yet, this response has been reported for some marine species exposed to stressful pH conditions as a requirement to maintain intracellular pH and cellular homeostasis (e.g. Lannig *et al.,* 2010; Cumming *et al.,* 2011; Lardies *et al.,* 2014), which is consistent with the metabolic costs of mechanisms developed by calcifiers to cope with acidic conditions (Hendriks *et al.,* 2015). Metabolic up–regulation to face stressful environments is energetically costly. This limits the energy available to sustain other

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physiological activities, which generates trade–offs (Wood *et al.,* 2008, Deigweiher *et al.,* 2010). For instance, the blue mussel, *Mytilus edulis,* exposed to acidic conditions responded with higher metabolic rates at the expense of decreasing calcification and growth rates (Thomsen & Melzner, 2010). The cost of increased metabolism at low pH conditions in the echinoderm, *Amphiura filiformis,* was evidenced by muscle wastage, although growth and calcification were not reduced (Wood *et al.,* 2008).

We observed enhanced growth and calcification rates in *A. purpuratus* juveniles under OA conditions. Although a large body of experimental studies reveals that, generally, growth and calcification rates are negatively affected under OA conditions across a wide range of taxas (see Kroeker *et al.,* 2013), a range of studies show positive effects on growth (e.g. Iglesias-Rodriguez *et al.,* 2008; Findlay *et al.,* 2009; Wood *et al.,* 2008; Gutowska *et al.,* 2010) and calcification (e.g. Wood *et al.,* 2008; Ries *et al.,* 2009). This suggests that the effects of OA on growth and shell precipitation are species-specific (Ries *et al.,* 2009; Kroeker *et al.,* 2013), and that carbonate shells can develop at low pH and carbonate under-saturated conditions well below the threshold of $\Omega_{arag} = 1$ proposed in the past (i.e. Wood *et al.,* 2008). For instance, there is evidence that some species have the ability to generate suitable conditions at the site of calcification, thereby buffering the impacts of OA (Gazeau *et al.,* 2013; Ries, 2011; Wittmann & Pörtner, 2013; Hendriks *et al.,* 2015). OA affects the pH in body fluids, such as the extrapallial fluid, affecting calcification and shell structures (see Cohen & Holcomb, 2009; Ries, 2011). In fishes, crustacean, and molluscs, HCO^{3-} accumulation has been described as an efficient mechanism to buffer pH in body fluids (hemolymp or extrapallial fluid) and to avoid a decrease of pH at calcification sites (Pane & Barry, 2007; Gutowska *et al.,* 2010). In the blue mussel, *M. edulis,* metabolic changes associated with protein degradation and an increase in ammonia excretion (De Zwaan *et al.,* 1976; Pörtner *et al.,* 2004; Michaelidis *et al.,* 2005) have been interpreted as an intracellular pH regulatory mechanism to cope with OA conditions (Fernández–Reiriz *et al.,* 2012). In other cases, an enhanced activity of carbonic anhydrase (CA) as was observed for the oyster, *Crassostrea virginica,* and the clam, *Mercenaria mercenaria,* acts as a compensatory response to higher pCO_2 and temperature conditions (Ivanina *et al.,* 2013).

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Previous studies on the *Argopecten purpuratus* reported important effects of food quantity, quality, and feeding regimes on growth, reproduction, and feeding behaviour (see Martinez *et al.,* 1995, 2000; Navarro *et al.,* 2004; Uriarte *et al.,* 2004; Fernández–Rieriz *et al.,* 2005). Studies analyzing the effect of OA on bivalves have shown negative effects on feeding activities, such as clearance and ingestion rates, possibly due to deficiencies in the functioning of the digestive and filtering systems (Bamber, 1987, 1990; Fernández–Reiriz *et al.,* 2011; Navarro *et al.,* 2013; Vargas *et al.,* 2013), with reduced feeding under OA conditions resulting in lower survival, growth rate, and calcification (Dupont & Thorndyke, 2008).

Our finding of significantly enhanced ingestion rates in juvenile scallops maintained under low pH conditions when exposed to natural food concentrations is consistent with similar results reported for other scallop species, *Chlamys nobilis* and *Pecten maximus,* when exposed to pH conditions comparable to those in our study (Wenguang & Maoxian, 2012; Sanders *et al.,* 2013). We acknowledge that increasing food supply to natural food concentration levels of 10 to 18 µg Chl*a* L^{-1} could have enhanced the ingestion rate of individuals from the low (0.1% DW) and intermediate (2% DW) food concentration treatment. However, we did not observe significant differences between low and intermediate food treatments for both pH treatments.

Although calcification rates were enhanced under OA conditions, the shell formation process was affected by low pH. In particular, we observed up-regulation of the expression of chitin synthase (CHS) for *A. purpuratus* juveniles under experimental OA conditions, especially for individuals receiving low food supply. Chitin is a key component of the organic matrix of mollusc shells (Weiner & Traub, 1984; Zentz *et al.,* 2001; Falini & Fermani, 2004), with a role in mineralization processes and shell formation (Falini *et al.,* 1996; Weiss *et al.,* 2006). CHS is responsible for the enzymatic synthesis of chitin (Glaser & Brown, 1957), and its expression plays an important role in the formation and mechanical strength of the shell (Schönitzer & Weiss, 2007). Thus, CHS expression has been proposed as a proxy of calcification (Cumming *et al.,* 2011). Important alterations in structure and shell functionality (i.e. CHS inhibition), with corresponding impacts over fitness, have been observed for mussel *M. galloprovinciallis* larvae under OA conditions (Weiss & Schönitzer,

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2006; Schönitzer & Weiss, 2007). Our results point at up–regulation of CHS expression in the experimental scallops exposed to OA as a possible acclimation and/or adaptive structural mechanisms to cope with projected low pH and low carbonate saturation state (Ω) scenarios (Cumming *et al.,* 2011).

Calcifying organisms growing under low pH conditions typically experience a reduction in the thickness of the periostracum and changes in its chemical composition, with important consequences for shell dissolution (Rodolfo-Metalpa *et al.,* 2010a; Gazeau *et al.,* 2013; Ramajo *et al.,* submitted). In our study, the carbonate signal was reduced under OA conditions, independent of food supply, while polysaccharides signals were strengthened under OA conditions, increasing as food supply decreased. The concentration of other organic components, such as lipids and proteins, were not affected by pH or experimental food treatments. These results are indicative of the secretion of a thicker periostracum involving higher levels of polysaccharides under OA conditions, particularly when food supply was at intermediate and low levels. However, an opposite pattern (lower polysaccharides and higher carbonate signals) was observed on the intertidal mussel, *Perumytilus purpuratus,* when it was exposed to low–pH freshwater inputs (Ramajo *et al.,* submitted), indicating that some biomineralogical responses to low pH conditions are also specie-specific. Hence, our results suggest that polysaccharides' synthesis during periostracum formation would be the main process affected by low pH, which is consistent with previous studies that found over–expression of tyrosinase, the gene involved in the formation of the organic matrix and periostracum for mussel, *Mytilus edulis,* when exposed to OA conditions (Hünning *et al.,* 2013).

Recent studies suggest that abundant food availability helps calcifiying species resist OA by supplying the energy required to support mechanisms involved in maintaining homeostasis (e.g. Melzner *et al.,* 2011; Thomsen *et al.,* 2013; Pan *et al.,* 2015). Although Martinez *et al.* (2000) determined that an *Argopecten purpuratus* individual filters 5 to 6% of its dry weight per day, we did not observe any differences in growth, calcification, metabolic rates, and CHS expression between organisms supplied with intermediate (2% DW d^{-1}) and

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high (5% DW d^{-1}) food levels. However, these physiological rates were considerably suppressed under the lowest food supply tested (0.1% DW d^{-1}) for both control and low pH treatments, indicating that limited food availability weakens the capacity of the organisms to cope with projected future acidified waters. Interestingly, juveniles of the other commercially exploited pectinid species, such as the king scallop, *Pecten maximus,* have also been reported to display tolerance to OA, as indicated by enhanced metabolic and clearance rates, when growing under abundant food supply (Sanders *et al.,* 2013).

In addition to the possible co-variation of temperature and oxygen with low-pH in upwelling areas, food quality may also co-vary with pH in these systems. Recent analyses have reported effects of OA on the growth (i.e. Wu *et al.,* 2010) and the nutritional status (i.e. low fatty acid concentration and composition, Rossoll *et al.,* 2012) of phytoplankton grown under low pH conditions, especially when also affected by light limitation (Gao *et al.,* 2012). However, a recent meta–analysis concluded that these effects are not consistent across phytoplankton species (Dutkiewicz *et al.,* 2015).

The variability in environmental conditions experienced by calcifying species in their native habitats affects their sensitivity to OA, conferring them with potential to adapt to future changes (Duarte *et al.,* 2013; Lardies *et al.,* 2014; Vargas *et al.,* 2014; Pansch *et al.,* 2014). Furthermore, high natural variation in seawater chemistry in coastal ecosystems implies that resident organisms often experience pH regimes at present comparable to those projected for the year 2100 (Hofmann *et al.*, 2011; Duarte *et al.,* 2013). Our results suggest that juvenile scallops of *Argopecten purpuratus* from Tongoy Bay are tolerant to these projected low pH scenarios. In Chile, *A. purpuratus* cultures are located in zones continuously affected by upwelling processes that transport cold and hypoxic waters under-saturated in carbonate to nearshore habitats (Torres *et al.,* 2011, see also Feely *et al.,* 2008). This is the case for Tongoy Bay, where the donor scallop population in our experiment grows. Tongoy Bay is exposed to the semi–permanent upwelling of Lengua de Vaca Point (see Torres & Ampuero, 2009; Torres *et al.,* 2011).

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Research on the effects of OA on calcifier species in upwelling systems has demonstrated the ability of some species to maintain physiological processes under the high pCO_2 conditions and ample food supply (Thomsen *et al.,* 2010) characteristic of these highly productive ecosystems. It has been suggested that organisms with intrinsic high metabolic rates are less sensitive and more resistant to OA in such productive environments (Melzner *et al.,* 2009; Parker *et al.,* 2013; Gazeau *et al.,* 2013). Indeed, the performance of the scallops tested here improved under low pH conditions and medium and high food supply, the conditions prevailing in their habitat, as compared with the elevated pH representing the average pH in the open ocean typically used as a "control" in OA experiments (Hendriks *et al.,* 2010a, Kroeker *et al.,* 2013).

The results presented suggest that phenotypic plasticity in physiological, biochemical, and morphological traits of *Argopecten purpuratus* contribute to the resistance of this commercial species under OA conditions. Hence, the production of this important resource should be maintained in a high–CO_2 ocean, provided that upwelling dynamics continue to supply abundant food to the Chilean coast. However, under reduced food and low pH, the organismal adaptive response was to trade-off growth towards the expression of biomineralization molecules and altering the organic composition of shell periostracum, indicating that the future performance of these calcifiers will depend on the trajectories of both OA and food supply.

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References

Arias MB, Poupin MB, Lardies MA (2011) Plasticity of life-cycle, physiological thermal traits and Hsp70 gene expression in an insect along the ontogeny: effect of temperature variability. *Journal of Thermal Biology*, **36,** 355-362.

Atkinson LP, Valle–Levinson A, Figueroa D, De Pol-Holz R, Gallardo VA, Schneider W, Blanco JL, Schmidt M (2002) Oceanographic observations in Chilean coastal waters between Valdivia and Concepción. *Journal of Geophysical Research-Oceans*, **107**, C7. DOI:10.1029/2001JC000991.

Bamber NR (1987) The effects of acidic sea water on young carpet-shell clams *Venerupis decussata* (L.) (Mollusca: Veneracea). *Journal of Experimental Marine Biology and Ecology*, **108,** 241–260.

Bamber NR (1990) The effects of acidic seawater on three species of lamellibranch molluscs. *Journal of Experimental Marine Biology and Ecology*, **143,** 181–191.

Barton A, Hales B, Waldbusser GG, Langdon C, Feely RA (2012) The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. *Limnology and Oceanography*, **57,** 698–710.

Bore, D., Martinez C (1980) Catálogo de recursos pesqueros de Chile-Santiago. Chile. IFOP CORFO, 83 pp.

Cohen AL, Holcomb M (2009) Why corals care about ocean acidification: uncovering the mechanism. *Oceanography*, **22,** 118–127.

This article is protected by copyright. All rights reserved.

Accepted Article

Comeau S, Carpenter RC, Edmunds PJ (2013) Effects of feeding and light intensity on the response of the coral *Porites rus* to ocean acidification. *Marine Biology*, **160,** 1127–1134.

Cornwall CE, Hurd CL (2015) Experimental design in ocean acidification research: problems and solutions. *ICES Journal of Marine Science*, DOI: 10.1093/icesjms/fsv118.

Coughlan J (1969) The estimation of filtering rate from the clearance of suspensions. *Marine Biology*, **2,** 356–358.

Crook ED, Cooper H, Potts DC, Lambert T, Paytan A (2013) Impacts of food availability and pCO_2 on planulation, juvenile survival, and calcification of the azooxanthellate scleractinian coral *Balanophyllia elegans*. *Biogeosciences*, **10,** 7599-7608

Cummings V, Hewitt J, Van Rooyen A *et al.,* (2011) Ocean acidification at high latitudes: potential effects on functioning of the Antarctic bivalve *Laternula elliptica*. *PLoSOne*, **6,** e16069.

Davies PS (1989) Short–term growth measurements of corals using an accurate buoyant weighing technique. *Marine Biology*, **101,** 389–395.

Deigweiher K, Hirse T, Bock C, Lucassen M, Pörtner HO (2010) Hypercapnia induced shifts in gill energy budgets of Antarctic notothenioids. *Journal of Comparative Physiology B*, **180,** 347–359.

de Zwaan A, Wijsman TCM (1976) Anaerobic metabolism in bivalvia (Mollusca) Characteristics of anaerobic metabolism. *Comparative Biochemistry Physiology*, **54B,** 313-323.

Dickson AG (1990) Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 298.15K. *Deep–Sea Research I*, **37,** 755–766.

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep–Sea Research I*, **34,** 1733–1743.

Duarte CM, Hendriks IE, Moore TS *et al.* (2013) Is Ocean Acidification an Open–Ocean Syndrome? Understanding Anthropogenic Impacts on Marine pH. *Estuaries and Coasts*, **36,** 221–236.

This article is protected by copyright. All rights reserved.

Accepted Article

Dutkiewicz S, Morris JJ, Follows MJ, Scott JR, Levitan O, Dyhrman S, Berman-Frank I (2015) Impact of ocean acidification on future phytoplankton communities. *Nature Climate Change*. DOI:10.1038/nclimate2722

Dupont S, Thorndyke MC (2010) Impact of CO_2–driven ocean acidification on invertebrates early life-history – What we know, what we need now and what we can do. *Bigeoscicences Discussions*, **6,** 3109–3131.

Falini G, Albeck S, Weiner S, Addadi L (1996) Control of aragonite or calcite polymorphism by mollusk shell macromolecules. *Science,* **271,** 67–69.

Falini G, Fermani S (2004) Chitin mineralization. *Tissue Engineering*, **10,** 1–6.

Feely RA, Sabine CL, Hernandez–Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of corrosive 'acidified' water onto the continental shelf. *Science*, **320,** 1490–1492.

Fernández-Reiriz MJ, Navarro JM, Labarta U (2005) Enzymatic and feeding behavior of *Argopecten purpuratus* under variation in salinity and food supply. *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology*, **141,** 153–63.

Fernández-Reiriz MJ, Range P, Alvarez-Salgado XA, Labarta U (2011) Physiological energetics of juvenile clams *Ruditapes decussatus* in a high CO_2 coastal ocean. *Marine Ecology Progress Series*, **433,** 97–105.

Fernandez-Reiriz MJ, Range P, Alvarez-Saldago XA, Espinosa J, Labarta U (2012) Tolerance of juvenile *Mytilus galloprovincialis* to experimental seawater acidification. *Marine Ecology Progress Series*, **454**, 65-74.

Figueiras FG, Labarta U, Fernánedez–Reiriz MJ (2002) Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. *Hydrobiologia*, **484,** 121-131.

Figueroa D, Moffat C (2000) On the influence of topography in the induction of coastal upwelling along the Chilean Coast. *Geophysical Research Letters*, **27.** DOI: 10.1029/1999GL011302.

This article is protected by copyright. All rights reserved.

Accepted Article

Findlay HS, Kendall MA, Spicer JI, Widdicombe S (2009) Future high CO_2 in the intertidal may compromise adult barnacle (*Semibalanus balanoides*) survival and embryon development rate. *Marine Ecology Progress Series*, **398,** 193–202.

Fonseca T, Farías M (1987) Estudio del proceso de surgencia en la costa chilena utilizando percepción remota. *Investigaciones Pesqueras*, **34,** 33-46.

Gao, KS, Xu JT, Gao G *et al.* (2012) Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. *Nature Climate Change,* **2,** 519–523.

Gazeau FL, Parker LM, Comeau S *et al.* (2013) Impacts of ocean acidification on marine shelled molluscs. *Marine Biology,* **160,** 2207–2245.

Glaser L, Brown DH (1957) The synthesis of chitin in cell-free extracts of *Neurospora crassa. The Journal of Biological Chemistry,* **228,** 729–742.

Guppy M, Withers PC (1999) Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biological Review,* **74,** 1–40.

Gutowska MA, Melzner F, Langenbuch M, Bock C, Claireaux G, Pörtner HO (2010) Acid–base regulatory ability of the cephalopod (*Sepia officinalis*) in response to environmental hypercapnia. *Journal of Comparative Physiology B,* **180,** 323–335.

Hendriks IE, Duarte CM, Álvarez M (2010a). Vulnerability of marine biodiversity to ocean acidification: a meta–analysis. *Estuarine, Coastal and Shelf Science,* **86,** 157–164.

Hendriks IE, Duarte CM (2010b). Ocean acidification: Separating evidence from judgment: a reply to Dupont et al. *Estuarine, Coastal and Shelf Science,* **89,** 186-190.

Hendriks IE, Duarte CM, Olsen YO *et al.* (2015) Biological mechanisms supporting adaptation to ocean acidification in coastal ecosystems. *Estuarine, Coastal and Shelf Science,* **152,** A1 – A8.

Hettinger A, Sanford E, Hill TM, Hosfelt JD, Russell AD, Gaylord B (2013) The influence of food supply on the response of Olympia oyster larvae to ocean acidification. *Biogeosciences,* **10,** 6629–6638.

This article is protected by copyright. All rights reserved.

Accepted Article

Hofmann GE, Smith JE, Johnson KS *et al.* (2011) High–frequency dynamics of ocean pH: a multi–ecosystem comparison. *PLoSOne,* **6,** e28983.

Holcomb M, McCorkle DC, Cohen AL (2010) Long-term effects of nutrient and CO_2 enrichment on the temperate coral *Astrangia poculata* (Ellis and Solander, 1786). *Journal of Experimental Marine Biology and Ecology,* **386,** 27–33.

Hoogenboom MO, Campbell DA, Beraud E, DeZeeuw K, Ferrier–Pagès C (2012) Effects of light, food availability and temperature stress on the function of photosystem II and photosystem I of coral symbionts. *PLoSOne,* **7,** e30167.

Hüning AK, Melzner F, Thomsen J *et al.* (2013) Impacts of seawater acidification on mantle gene expression patterns of the Baltic Sea blue mussel: implications for shell formation and energy metabolism. *Marine Biology,* **160,** 1845–1861.

Iglesias-Rodríguez MD, Halloran PR, Rickaby REM *et al.* (2008) Phytoplankton calcification in a high-CO_2 world. *Science,* **320,** 336–340.

Iranon NN, Miller DL (2012) Interactions between oxygen homeostasis, food availability, and hydrogen sulfide signaling. *Frontiers in Genetics of Aging*, **3,** 257. DOI: 10.3389/fgene.2012.00257

Ivannia AV, Beniash E, Etzkorn M, Meyers TB, Ringwood AH, Sokolova IM (2013) Short-term acute hypercapnia affects cellular responses to trace metals in the hard clams *Mercenaria mercenaria*. *Aquatic Toxicology,* **140/141**, 123-133.

Kroeker KJ, Kordas RL, Crim R *et al.* (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology,* **19,** 1884–1896.

Labarta U, Fernández–Reiríz MJ, Babarro JMF (1997) Differences in physiological energetics between intertidal and raft cultivated mussels *Mytilus galloprovincialis. Marine Ecology Progress Series,* **52,** 167-173.

This article is protected by copyright. All rights reserved.

Accepted Article

Lachkar Z (2014) Effects of upwelling increase on ocean acidification in the California and Canary Current systems. *Geophysical Research Letters.* DOI:10.1002/2013GL058726.

Lannig G, Eilers S, Pörtner HO, Sokolova IA, Bock C (2010) Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas*. Changes in metabolic pathways and thermal response. *Marine Drugs,* **8,** 2318–2339.

Lardies MA, Arias MB, Poupin MJ *et al.* (2014) Differential response to ocean acidification in physiological traits of *Concholepas concholepas* populations. *Journal of Sea Research,* **90,** 27–134.

Martinez G, Caceres LA, Uribe E, Diaz MA (1995) Effects of different feeding regimens on larval growth and the energy budget of juvenile Chilean scallops, *Argopecten purpuratus* Lamarck. *Aquaculture,* **132,** 313–323.

Martínez G, Brokordt K, Aguilera C, Soto V, Guderley H (2000) Effect of diet and temperature upon muscle metabolic capacities and biochemical composition of gonad and muscle in *Argopecten purpuratus* (Lamarck 1819). *Journal of Experimental Marine Biology and Ecology,* **247,** 29–49.

Mayol E, Ruiz–Halpern S, Duarte CM, Castilla JC, Pelegrí JL (2012) Coupled CO_2 and O_2–driven compromises to marine life in summer along the Chilean sector of the Humboldt Current System. *Biogeosciences,* **9,** 1183–1194.

Merhbach C, Culberso CH, Hawley G, Pytkowic RM (1973) Measurement of apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography,* **18,** 897–907.

Melzner F, Gutowska MA, Langenbuch M *et al.* (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences*, **6,** 2313–2331.

This article is protected by copyright. All rights reserved.

Accepted Article

Melzner F, Stange P, Trübenbach K *et al.* (2011) Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoSOne,* e24223.

Michaelidis B, Ouzounis C, Paleras A, Pörtner H-O (2005) Effects of long–term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Marine Ecology Progress Series,* **293,** 109–118.

Montecino V, Lange CB (2009) The Humboldt Current System: Ecosystem components and processes, fisheries, and sediment studies. *Progress in Oceanography,* **83,** 65-79.

Moraga–Opazo J, Valle–Levinson A, Ramos M, Pizarro-Koch M (1991) Upwelling-triggered near-geostrophic recirculation in an equatorward facing embayment. *Continental Shelf Research,* **31,** 19-20.

Navarro JM, Fernández–Reiriz MJ, Labarta U (2004) Short-term feeding response of the scallop *Argopecten purpuratus* exposed to two different diets. *Journal of the Marine Biological Association of the UK,* **84,** 775 – 779.

Navarro JM, Torres R, Acuña K *et al.* (2013) Impact of medium-term exposure to elevated pCO_2 levels on the physiological energetics of the mussel *Mytilus chilensis*. *Chemosphere,* **90,** 1242-1248.

Pan TCF, Applebaum SL, Manajan DT (2015). Experimental ocean acidification alters the allocation of metabolic energy. *Proceedings of the National Academy of Science USA.* **112,** 4696–4701.

Pane EE, Barry JP (2007) Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. *Marine Ecology Progress Series,* **334,** 1-9.

Pansch C, Schaub I, Havenkand J, Wahl M (2014) Habitats traits and food availability determine the response of marine invertebrates to ocean acidification. *Global Change Biology,* **20,** 765–777.

Parker L, Ross PM, O'Connor WA, Pörtner HO, Scannes E, Wright JN (2013) Predicting the response of molluscs to the impact of ocean acidification. *Biology,* **2,** 651–692.

This article is protected by copyright. All rights reserved.

Accepted Article

Peterson WT, Arcos DF, McManus GB, Dam H, Bellantoni D, Johnson T, Tiselius P (1988) The nearshore zone during coastal upwelling: daily variability and coupling between primary and secondary production off central Chile. *Progress in Oceanography,* **20,** 1-40.

Pierrot D, Lewis E, Wallace DWR (2006) MS Excel Program Developed for CO_2 System Calculations. ORNL/CDIAC–105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.

Pörtner HO, Langenbuch M, Reipschläger A (2004) Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. *Journal of Oceanography,* **60,** 705–718.

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2–induced ocean acidification. *Geology,* **37,** 1131–1134.

Ries JB (2011) Skeletal mineralogy in a high–CO_2 world. *Journal of Experimental Marine Biology and Ecology,* **403,** 54–64.

Rodolfo–Metalpa R, Lombardi C, Cocito S, Hall–Spencer JM, Gambi MC (2010a) Effects of ocean acidification and high temperatures on the bryozoan *Myriapora truncata* at natural CO_2 vents. *Marine Ecology,* **31,** 447–456.

Rodolfo-Metalpa, R, Martin S, Ferrier-Pagés C, Gattuso J-P (2010b) Response of the temperate coral *Cladocora caespitosa* to mid- and long-term exposure to pCO_2 and temperature levels projected for the year 2100 AD. *Biogeosciences,* **7,** 289–300.

Rodríguez-Navarro AB, Dominguez-Gasca, N, Muñoz A, Ortega-Huertas M (2013) Change in the chicken eggshell cuticle with hen age and egg freshness. *Poultry Science,* **92,** 3026-3035.

Rossoll D, Bermúdez R, Hauss H *et al.* (2012) Ocean Acidification-Induced Food Quality Deterioration Constrains Trophic Transfer. *PLoS ONE,* **7,** e34737.

Sabine CL, Feely RA, Gruber N *et al.* (2004) The oceanic sink for anthropogenic CO_2. *Science,* **305,** 367–371.

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Accepted Article

Sanders MB, Bean TP, Hutchinson TH, Le Quesne WJF (2013) Juvenile king scallop, *Pecten maximus*, is potentially tolerant to low levels of ocean acidification when food is unrestricted. *PLOSOne*, **8**, e74118.

Schönitzer V, Weiss IM (2007) The structure of mollusc larval shells formed in the presence of the chitin synthase inhibitor Nikkomycin Z. *BMC Structural Biology,* **7,** 71.

Sernapesca (2012) Anuario Anual de Pesca. http.://www.sernaspesca.cl

Storch D, Fernández M, Navarette S, Pörtner HO (2011) Thermal tolerance of larval stages of the Chilean kelp crab *Taliepus dentatus*. *Marine Ecology Progress Series,* **429,** 157–167.

Strickland JDH, Parsons TR (1968) A practical handbook of seawater analysis. Ottawa: Fisheries Research Board of Canada, Bulletin 167.

Strub T, Mesías J, Montecino V, Rutllant J, Salinas S (1998) Coastal ocean circulation off western South America. Coastal segment. In: *The Sea* (eds Robinson AR, Brink KH), pp. 273–313, Wiley and Sons, USA.

Thiel M, Macaya E, Acuña, E *et al.* (2007) The Humboldt Current System of northern and central Chile: oceanographic processes, ecological interactions and socioeconomic feedback. *Oceanography and Marine Biology: An Annual Review,* **45,** 195–344.

Thomsen J, Melzner F (2010) Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis. Marine Biology,* **157,** 2667–2676.

Thomsen J, Gutowska MA, Saphöerster J *et al.,* (2010) Calcifying invertebrates succeed in a naturally CO2–rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences,* **7,** 3879–3891.

Thomsen J, Casties I, Pansch C, Körtzinger A, Melzner F (2013) Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis:* laboratory and field experiments. *Global Change Biology,* **19,** 1017–1027.

Torres R, Ampuero P (2009) Strong CO_2 outgassing from high nutrient low chlorophyll coastal waters off central Chile (30ºS): The role of dissolved iron. *Estuarine, Coastal and Shelf*

This article is protected by copyright. All rights reserved.

Accepted Article

Science, **83,** 126–132.

Torres R, Turner D, Silva N, Rutllant J (1999) High short-term variability of CO_2 fluxes during an upwelling event off the Chilean coast at 30ºS. *Deep-Sea Research I,* **46,** 1161–1179.

Torres R, Pantoja S, Harada N *et al.* (2011) Air–sea CO_2 fluxes along the coast of Chile: From CO_2 outgassing in central–northern upwelling waters to CO_2 sequestering in southern Patagonian fjords. *Journal of Geophysical Research,* **116**. DOI:10.1029/2010JC006344.

Uriarte I, Farías A, Hernández J, Schäfer C, Sorgeloos P (2004) Reproductive conditioning of Chilean scallop (*Argopecten purpuratus*) and the Pacific oyster (*Crassostrea gigas*): effects of enriched diets. *Aquaculture,* **230,** 349–357.

Uribe E, Moraga J, Zuíga S, Rosales S, Alvarez G, Avalos P, Chirino S (2008) Proyecto FIP No 2006-37 'Establecimiento de un protocolo de seguimiento ambiental para la determinación de la capacidad de carga para el cultivo del ostión del Norte. Informe Final.

Vargas CA, de la Hoz M, Aguilera V *et al.* (2013) CO_2–driven ocean acidification reduces larval feeding efficiency and change food selectivity in the mollusk *Concholepas concholepas*. *Journal of Plankton Research.* DOI:10.1093/plankt/fbt045.

Vargas CA, Aguilera VM, San Martín V *et al.* (2014) CO_2-driven ocean acidification disrupts the filter feeding behavior in Chilean gastropod and bivalve species from different geographic localities. *Estuaries and Coasts,* **38,** 1163-1177.

Weiner S, Traub W (1984) Macromolecules in mollusc shells and their functions in biomineralization. *Philosophical Transactions B,* **304,** 421–438.

Weiss IM, Schönitzer V, Eichner N, Sumper M (2006) The chitin synthase involved in marine bivalve mollusk shell formation contains a myosin domain. *FEBS Letters,* **580,** 1846–1852.

Weiss IM, Schönitzer V (2006) The distribution of chitin in larval shells of the bivalve mollusk *Mytilus galloprovincialis. Journal of Structural Biology,* **153,** 264–277.

This article is protected by copyright. All rights reserved.

Accepted Article

Wenguang L, Maoxian He (2012) Effects of ocean acidification on the metabolic rates of three species of bivalve from southern coast of China. *Chinese Journal of Oceanology and Limnology,* **30,** 206-211.

Wigley TML, Richels R, Edmonds JA (1996) Economic and environmental choices in the stabilization of atmospheric CO_2 concentrations. *Nature,* **379,** 240–243.

Wittmann AC, Pörtner HO (2013) Sensitivities of extant animal taxa to ocean acidification. *Nature Climate Change,* **3,** 995–1001.

Wolf–Gladrow DA, Riebesell U, Burkhardt S, Bijma J (1999) Direct effects of CO_2 concentration on growth and isotopic composition of marine plankton. *Tellus B. Chemical and Physical Meteorology,* **51,** 461–476.

Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. *Proceedings of the Royal Society B,* **275,** 1767–1773.

Wu Y, Gao K, Riebesell U (2010) CO_2-induced seawater acid- ification affects physiological performance of the marine diatom *Phaeodactylum tricornutum*. *Biogeosciences,* **7,** 2915– 2923.

Zapata M, Tanguy A, David E, Moraga D, Riquelme C (2009) Transcriptomic response of *Argopecten purpuratus* post-larvae to copper exposure under experimental conditions. *Gene,* **442,** 37–46.

Zentz F, Bedouet L, Almeida MJ, Milet C, Lopez E, Girau M (2001) Characterization and quantification of chitosan extracted from nacre of the abalone *Haliotis tuberculata* and the oyster *Pinctada maxima. Marine Biotechnology,* **3,** 36–44.

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TABLES

Table 1. Average (± SE) water conditions and carbonate system parameters of each experimental treatment during incubations of juvenile scallops exposed to control and low pH and three levels of food supply for 30 days. pH (NBS scale), A_T (mmol/kgSW), partial pressure of CO_2 (µatm), and saturation states of the water with respect to calcite and aragonite. Salinity was stable at 34 (psu).

Exp. Treatments		T (ºC)	pH	A_T	pCO_2	Ω_{calc}	Ω_{arag}
pH	Food			(mmol/kgSW)	(µatm)		
Control	5%	18.32 (0.3)	8.02 (0.02)	2204 (17)	552 (23)	3.2 (0.1)	2.1 (0.1)
	2%	18.32 (0.3)	8.09 (0.02)	2234 (20)	459 (15)	3.7 (0.1)	2.4 (0.0)
	0.10%	18.34 (0.2)	8.14 (0.01)	2251 (6)	405 (17)	4.0 (0.1)	2.6 (0.1)
Low	5%	18.29 (0.3)	7.62 (0.01)	2206 (9)	1519 (28)	1.40 (0.0)	0.9 (0.0)
	2%	18.33 (0.3)	7.65 (0.00)	2233 (12)	1426 (5)	1.51 (0.0)	1.0 (0.0)
	0.10%	18.37 (0.3)	7.69 (0.01)	2238 (8)	1304 (9)	1.64 (0.0)	1.1 (0.0)

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Table 2. *Argopecten purpuratus.* Effect of pH (two levels: 7.6 – 8.0) and food availability (three levels: 0.1%, 2%, and 5% of the scallops dry body mass) on growth, net calcification, metabolic, ingestion rates, and CHS expression of juvenile scallops. Bold numbers indicate significant p-values at $\alpha = 0.05$. Results for Tukey pairwise comparison among level of the main factors are also shown.

Biological response	Source	DF	MS	F	*P*-value	Tukey post hoc comparisons
Metabolic rate	pH	1	0.27	26.18	**<0.001**	7.6 > 8.0
($mgO_2\ h^{-1}\ g^{-1}$)	Food	2	0.064	6.22	**0.014**	0.1 % < 2 % = 5 %
	pH × Food	2	0.001	0.11	0.900	
	Error	12	0.01			
	Total	17				
Growth Rate	pH	1	0.256	5.72	**0.034**	7.6 > 8.0
($mm\ day^{-1}$)	Food	2	0.279	6.23	**0.014**	0.1 % < 2 % = 5 %
	pH × Food	2	0.042	0.93	0.420	
	Error	12	0.045			
	Total	17				
Calcification Rate	pH	1	0.45	5.17	**0.042**	7.6 > 8.0
($\mu gCaCO_3\ g^{-1}\ d^{-1}$)	Food	2	0.458	5.26	**0.023**	0.1 % < 2 % = 5 %
	pH × Food	2	0.017	0.2	0.821	
	Error	12	0.087			

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	Total	17				
Ingestion Rate	pH	1	0.941	30.27	**0.000**	7.6 > 8.0
(mg Chla g^{-1} h^{-1})	Food	2	0.216	6.95	**0.010**	0.1 % = 2% < 5 %
	pH × Food	2	0.095	3.06	0.084	
	Error	12	0.031			
	Total	17				
CHS expression	pH	1		5.86	**0.032**	7.6 > 8.0
	Food	2		0.17	0.846	
	pH × Food	2		2.73	0.105	
	Error	12				
	Total	17				

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Table 3. Normalized intensity (mean ± SE) of main absorption bands of ATR-FTIR spectra of the outer shell surface of *Argopecten purpuratus* from different experimental treatments. These parameters were used to characterize the composition of the shell periostracum.

Periostracum compounds	Control pH			Low pH		
	0.1%	2%	5%	0.1%	2%	5%
CO_3	0.264 ± 0.011	0.246 ± 0.014	0.266 ± 0.003	0.225 ± 0.011	0.209 ± 0.019	0.261 ± 0.016
Polysaccharides	0.236 ± 0.011	0.246 ± 0.018	0.201 ± 0.006	0.294 ± 0.021	0.329 ± 0.013	0.250 ± 0.029
Proteins	0.100 ± 0.007	0.118 ± 0.008	0.131 ± 0.004	0.120 ± 0.008	0.158 ± 0.003	0.129 ± 0.009
Lipids	0.008 ± 0.001	0.009 ± 0.001	0.011 ± 0.001	0.011 ± 0.001	0.009 ± 0.003	0.009 ± 0.000

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Table 4. *Argopecten purpuratus.* Effect of pH (two levels: 7.6 – 8.0) and food availability (three levels: 0.1%, 2%, and 5% of the scallops dry body mass) on periostracum organic composition of juvenile scallops. Bold numbers indicate significant p-values at $\alpha = 0.05$.

	pH		Food		pH × Food	
Periostracum compounds	$F_{(1, 12)}$	*P*	$F_{(2, 12)}$	*P*	$F_{(1, 12)}$	*P*
CO_3	5.81	**0.033**	3.63	0.058	0.98	0.405
Polysaccharides	18.60	**0.001**	6.15	**0.015**	0.51	0.615
Proteins	2.60	0.132	1.89	0.192	0.98	0.404
Lipids	0.03	0.868	0.28	0.762	0.97	0.407

FIGURE CAPTIONS

Figure 1. *Argopecten purpuratus*. Metabolic (a), growth (b), net calcification (c), and ingestion rate (d) in the different pH and food availability treatments in juvenile scallops. Data presented are mean ± SE.

Figure 2. Expression of chitin synthase (CHS) of *Argopecten purpuratus* juveniles' muscle tissue after 30 days at experimental pH and food availability treatments. Data shown are means ± SE.

Figure 3. ATR-FTIR spectra of the outer shell surface of scallops from different experimental groups (food availability treatments) subjected at low and high pH seawater. The spectra are offset along the Y-axis. Main IR bands are highlighted in the graph.

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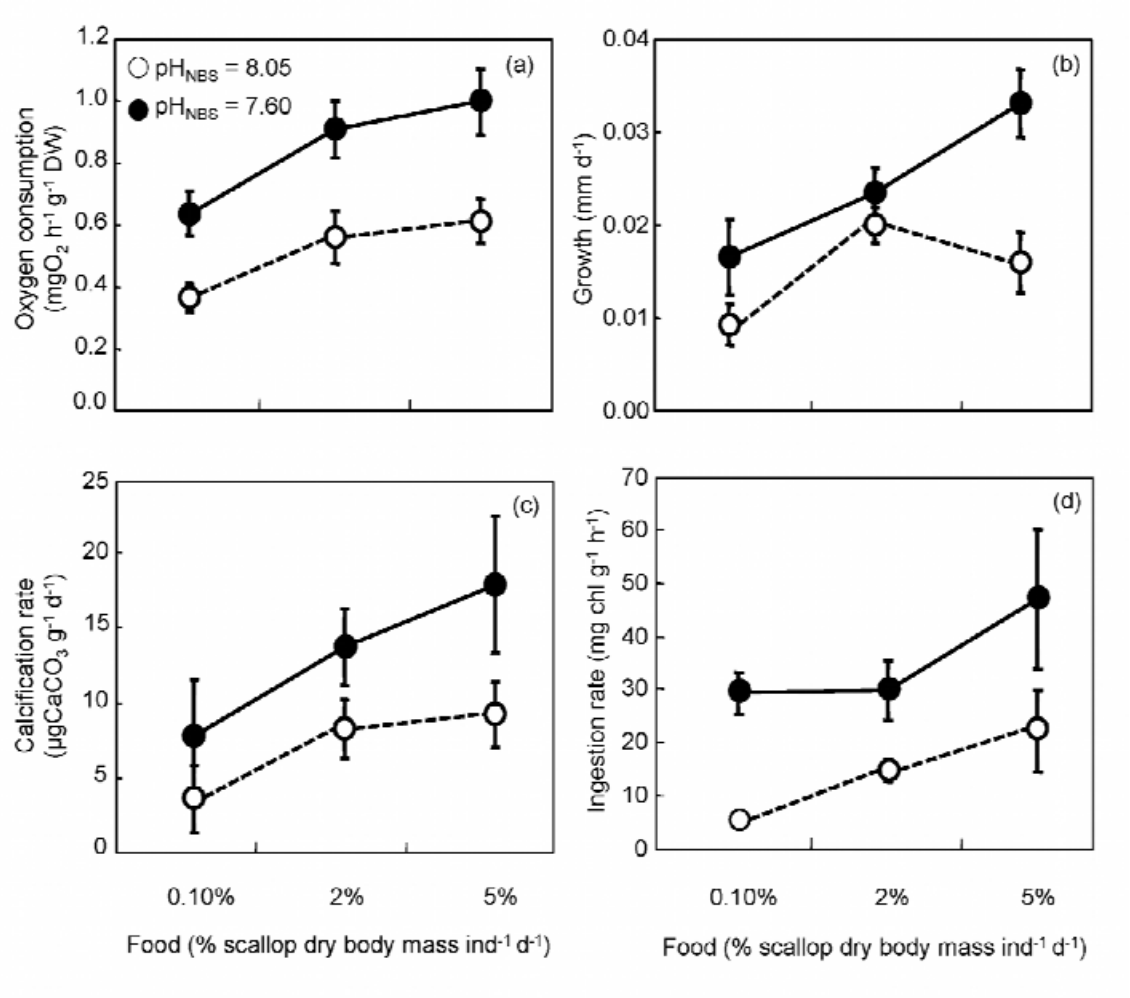

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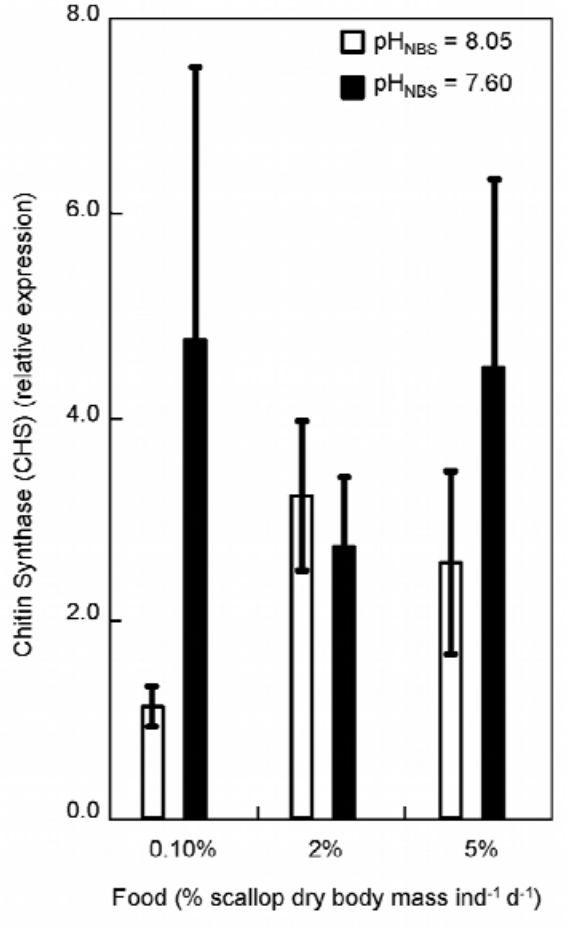

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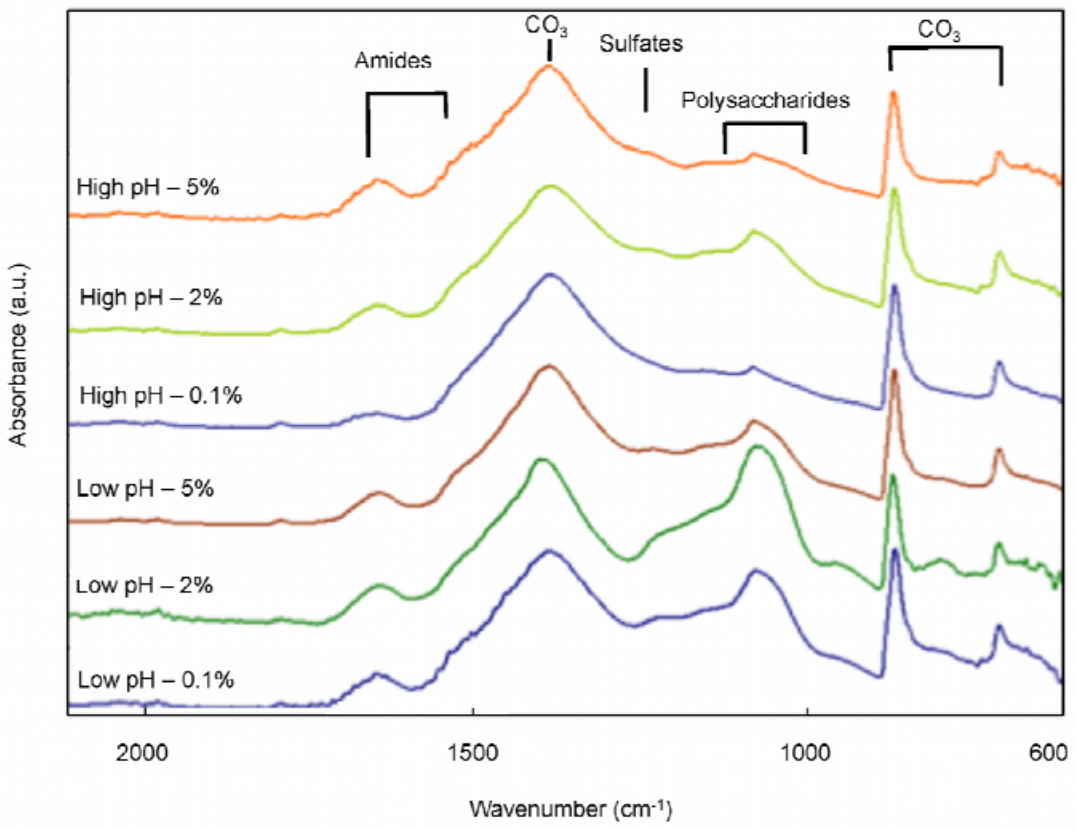

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