Effects of temperature and ocean acidification on shell characteristics of *Argopecten purpuratus*: implications for scallop aquaculture in an upwelling-influenced area

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ABSTRACT: Coastal upwelling regions already constitute hot spots of ocean acidification as naturally acidified waters are brought to the surface. This effect could be exacerbated by ocean acidification and warming, both caused by rising concentrations of atmospheric CO_2. Along the Chilean coast, upwelling supports highly productive fisheries and aquaculture activities. However, during recent years, there has been a documented decline in the national production of the native scallop *Argopecten purpuratus*. We assessed the combined effects of temperature and pCO_2-driven ocean acidification on the growth rates and shell characteristics of this species farmed under the natural influence of upwelling waters occurring in northern Chile (30°S, Tongoy Bay). The experimental scenario representing current conditions (14°C, pH ~8.0) were typical of natural values recorded in Tongoy Bay, whilst conditions representing the low pH scenario were typical of an adjacent upwelling area (pH ~7.6). Shell thickness, weight, and biomass were reduced under low pH (pH ~7.7) and increased temperature (18°C) conditions. At ambient temperature (14°C) and low pH, scallops showed increased shell dissolution and low growth rates. However, elevated temperatures ameliorated the impacts of low pH, as evidenced by growth rates in both pH treatments at the higher temperature treatment that were not significantly different from the control treatment. The impact of low pH at current temperature on scallop growth suggests that the upwelling could increase the time required for scallops to reach marketable size. Mortality of farmed scallops is discussed in relation to our observations of multiple environmental stressors in this upwelling-influenced area.

KEY WORDS: Calcification · Shell growth · Scallop farming · Upwelling · Chile

INTRODUCTION

The increasing concentration of carbon dioxide (CO_2) in the atmosphere due to anthropogenic activities has triggered major changes in the global climate system, leading to increased global mean temperatures (i.e. global warming) of approximately 0.7°C, a trend that will continue and fluctuate over a broad range (1–4°C) towards the end of the century (IPCC 2014). These changes in the thermal environ-

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ment may influence the distribution and abundances of the marine biota through challenges to the eco-physiological capabilities of different organisms (Helmuth et al. 2006). However, environmental changes due to warming alone are only one of a set of possible interacting climatic variables that will drive ecological and evolutionary responses in marine ecosystems (Harley et al. 2006). Rising atmospheric CO_2 levels are also driving an increased uptake of CO_2 into the ocean, which in turn produces a series of changes to the carbonate chemistry of seawater, a process now well established as ocean acidification (Caldeira & Wickett 2003, Orr et al. 2005, Gattuso et al. 2015). Ocean acidification (OA) negatively affects a variety of marine calcifying organisms by reducing calcification rates or by increasing the dissolution of calcareous structures (Fabry et al. 2008, Doney et al. 2009, Ries et al. 2009, Gazeau et al. 2013, Kroeker et al. 2013). Studies of mollusks specifically have suggested that the negative impacts of OA include reduced shell mass along with compromised shell structural integrity and strength (Buschbaum et al. 2007, McClintock et al. 2009, Welladsen et al. 2010, Bressan et al. 2014, Duarte et al. 2014, Fitzer et al. 2014, Mackenzie et al. 2014). However, the re-sponses of different species and populations are highly variable when the impacts of OA are assessed in combination with warming and other stressors such as salinity and oxygen (e.g. Duarte et al. 2014, 2015, Ko et al. 2014). Thus, the combined effects of OA and warming on commercial shellfish species are of growing concern given that mollusks represent an important source of global seafood production (Branch et al. 2013). In addition, as global warming and OA are occurring concomitantly, affecting many physiological processes of marine organisms, their combined effects must be evaluated (Byrne & Przeslawski 2013).

Shellfish aquaculture is reliant on growing individ-uals at high densities, meaning that shell robustness and size are important individual attributes with important implications for levels of production. Shell integrity is particularly important during the thinning process in which bivalves are physically handled as their densities are manipulated to reduce the inter-ference in feeding so the scallops can grow faster to market size (e.g. Fréchette et al. 2010, Cubillo et al. 2012). OA and warming could also have implications for decision-making processes associated with the adequate timing of release of hatchery-reared mol-lusks into nature (Jory & Iversen 1988, Grefsrud & Strand 2006). Thus, in addition to knowing how OA and warming may affect the ability of the shell to

provide physical support for soft internal organs and defense against predators and/or other environmen-tal stressors (Waldbusser et al. 2013), a knowledge of how shell characteristics may change under future environmental conditions is crucial information for underpinning the future management strategies employed by the aquaculture industry (e.g. Grefsrud & Strand 2006, Welladsen et al. 2010, Mackenzie et al. 2014).

The aquaculture industry relies on key ecosystem services provided by the coastal ocean, where most shellfish production takes place. Larval production in onshore hatcheries provides some control over the natural variability observed in coastal waters, such as food content and water temperature, but they are transferred as juveniles to the natural envi-ronment, where they are reared until harvest. How-ever, the carbon chemistry of seawater is also sub-ject to natural variability, especially in coastal upwelling areas, where it may negatively impact aquaculture production (Barton et al. 2012). In northern Chile, the fishery of the native scallop *Argopecten purpuratus* (Mollusca; Bivalvia; Pec-tinidae) collapsed during the mid-1980s following overexploitation of natural stocks. The fishery is still banned and was replaced by production from sus-pended culture activities, although current produc-tion only represents a fraction (10–15%) of the his-torical national landings (Stotz 2000). During the last decade, the Chilean landings of mollusks in general have ranged from 300 000 to 550 000 t. Over the same period, the production of scallops showed a decreasing trend, with the production in 2014 being equivalent to only 20% of the landings recorded 10 yr before (SERNAPESCA 2014; Fig. 1). This reduction in scallop production is challenging
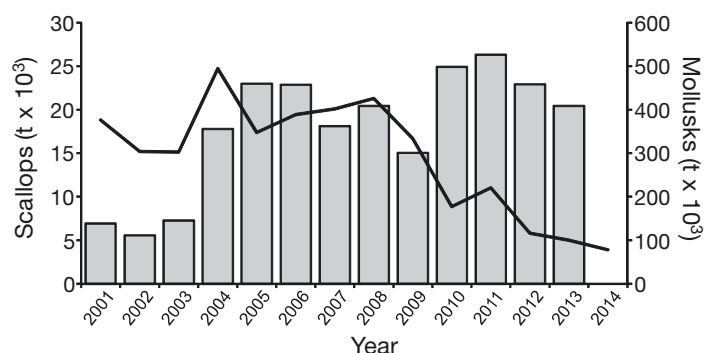

Fig. 1. Landings of *Argopecten purpuratus* (black line) and total mollusks harvested in Chile for the period 2001 to 2014 (source: Servicio Nacional de Pesca, www.sernapesca.cl; accessed 4 June 2015)

the sustainability of this socio-economic sector in northern Chile, thus highlighting the need to perform studies aimed at understanding causes of this reduction and to provide information to support mitigation and adaptation strategies. For example, studies carried out with the mussel *Mytilus chilensis*, the most widely cultivated species in Chile, showed that OA, but not temperature increase, will decrease the biomass production of this highly valuable bivalve by 30% (Navarro et al. 2013, Duarte et al. 2014, 2015).

The commercial production of *A. purpuratus* is currently restricted to aquaculture activities which use suspended cultures in natural embayments, with northern Chile and Tongoy Bay (30° 12′ S, 71° 34′ W, Fig. 2) being among the most important locations for scallop aquaculture along the Chilean coast (Thiel et al. 2007). Tongoy Bay is located ca. 10 km north of the Pt. Lengua de Vaca upwelling center (30° S, Fig. 2). The bay is part of the highly productive Coquimbo upwelling system, which is characterized by the presence of cold, CO_2-enriched, and poorly oxygenated waters, particularly during the spring and summer months (Thiel et al. 2007). More recent evidence suggests that this upwelling area is naturally acidified and characterized by low pH levels and a

low carbonate saturation state (Torres & Ampuero 2009). These conditions are similar to projections of progressive acidification in other eastern boundary coastal upwelling areas, such as off the coasts of Oregon and California, USA (Feely et al. 2008, Gruber et al. 2012). This environmental pattern implies that the sustainability of the shellfish aquaculture industry within Tongoy Bay could be vulnerable to the penetration of corrosive waters upwelled at Pt. Lengua de Vaca.

Upwelling is intensifying in eastern boundary current systems (Sydeman et al. 2014), particularly in the Oregon–California and Humboldt systems, which may further increase the intrusion of corrosive waters in the coastal domain. This ongoing global trend emphasizes the challenge of establishing a coherent scientific basis with which to predict the response of specific aquaculture species living in regions subject to upwelling disturbances (Barton et al. 2012, Ekstrom et al. 2015). The current study represents the first effort specifically aimed at experimentally evaluating the combined effects of temperature and OA upon the native scallop *A. purpuratus* cultured under the influence of upwelling waters. A range of biological responses were measured, including calcification rates, shell dissolution, shell thickness, dry shell weight, and tissue biomass. These results are discussed in relation to updated information on the physical–chemical variability inside Tongoy Bay and events of *in situ* mortality of *A. purpuratus* observed in the commercial aquaculture operations in the bay.

MATERIALS AND METHODS

Scallop collection

Juvenile individuals of *Argopecten purpuratus* (~41 mm ± 1.3 SD in shell length) were obtained on 20 August 2014, from suspended culture facilities (ca. 2 m depth) belonging to the at-sea commercial aquaculture production operations from Invertec–Ostimar Co. located in Tongoy Bay (Fig. 1). After collection, scallops were transported in a thermobox (ca. 14°C, emersed but wet conditions) to the Calfuco Coastal Laboratory (Valdivia, Chile) and aquaria (9 l, 15–20 scallops per aquarium). Before the experimental period, scallops were kept for 3 d in running seawater (ca. 13–14°C), with natural photoperiod, and fed daily with microalgae (*Tetraselmis* spp., ~65 × 10^6 cells ml^{-1}) to allow the scallops to acclimate to laboratory conditions.
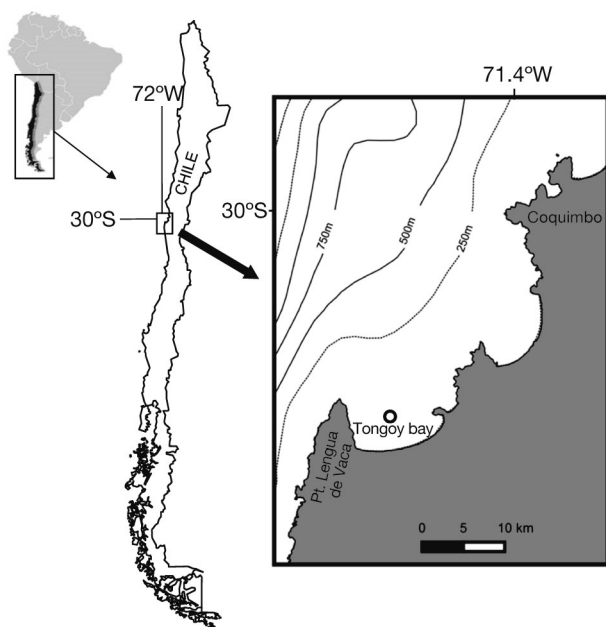

Fig. 2. Geographic location of Tongoy Bay and Pt. Lengua de Vaca upwelling center. The circle indicates the location of the CEAZAMET buoy, which is moored adjacent to the aquaculture facilities of the Invertec–Ostimar aquaculture company

Experimental setup

To examine the effects of pH and temperature, 4 experimental treatments were selected: (1) present-day conditions (control): 14°C and pH ~8.0; (2) control temperature (14°C) and low pH (~7.7); (3) high temperature (18°C) and control pH; (4) high temperature and low pH. Control conditions in temperature represent annual average sea surface temperature (14°C, a value currently recorded during the scallop collection, see 'Results'), whereas high temperature, in addition to the projected increase in 4°C (IPCC 2014), represents the maximum surface temperature (18°C) reported for Tongoy Bay (Aravena et al. 2014). The temperature of each treatment was stabilized using external chillers (±0.1°C). To obtain the different pH scenarios, the methodology previously described by Torres et al. (2013) (see Navarro et al. 2013, Duarte et al. 2014, 2015) was followed. Briefly, for present-day conditions (i.e. ~390 μatm pCO_2 in seawater), atmospheric air was bubbled into experimental aquaria and head tanks; for a low pH scenario, blended dry air was generated by compressing atmospheric air (117 psi) using an oil-free compressor with pure CO_2 using mass flow controllers (MFCs, Aalborg^{TM}); this blend was then bubbled into experimental aquaria and head tanks reaching ~900 μatm pCO_2 in seawater. The seawater of each aquarium was replaced every 2 d using the pre-equilibrated seawater from the head tank. In the current experimental system, this increase in pCO_2 in seawater resulted in a corresponding drop in pH (~0.3 units) yielding a target pH level of ~7.7 for the low pH scenario, while the present-day pH level remained at ~8.0 units (Table 1). These pCO_2 levels in seawater were selected taking into account the rate of change projected for

the atmospheric CO_2 by the year 2100, consistent with the IPCC A2 emission scenario (e.g. Meinshausen et al. 2011, IPCC 2014).

Four scallops were randomly assigned to each of 20 aquaria (9 l), and each aquarium was then randomly assigned to a pH/temperature treatment following a systematic design, with each treatment being replicated 5 times. All animals were labeled using bee tags glued to the shell. In addition, 1 empty shell of *A. purpuratus* of similar size to those in the experimental aquaria, previously tagged and weighed, was placed in each container to provide an estimate of net shell dissolution in each treatment. Beforehand, dead shells were cleaned with distilled water and then dried for 24 h (60°C) until constant dry weight. Before assigning individuals to experimental treatments, live animals were characterized in terms of their shell length, shell height, and buoyant weight. There were no statistical differences across treatments in these shell characteristics at the beginning of the experiments (1-way ANOVA, p > 0.05 in all cases). The exposure period lasted for 18 d, and scallops were fed daily with *Tetraselmis* spp. and maintained under natural photoperiod conditions, as in the previous acclimation period.

Carbonate system parameter monitoring

The seawater in each aquarium was gently replaced every day, with the corresponding seawater pre-equilibrated at the target pCO_2 levels in the head tank. Over the experimental period, pH and total alkalinity (A_T) were monitored on Days 2, 6, 10, 14, and 18 to estimate carbonate system parameters. Samples for pH were collected in 50 ml syringes, avoiding formation of bubbles during collection and handling of the sample, and immediately transferred

Table 1. Carbonate system parameters (mean ± SE) registered at each experimental treatment combining present-day (low pCO_2/pH ~8.0) and future acidification conditions (high pCO_2/pH ~7.7) with temperatures dominating in Tongoy Bay, Chile, during the experimental period (14°C) and under warmer conditions (18°C). NBS: National Bureau of Standards

Carbonate system parameter	14°C		18°C	
	pH ~8.0	pH ~7.7	pH ~8.0	pH ~7.7
Temperature (°C)	14.13 ± 0.19	14.08 ± 0.14	18.13 ± 0.11	18.14 ± 0.13
Salinity (PSU)	34.48 ± 1.78	35.36 ± 0.50	33.28 ± 1.97	34.00 ± 0.68
pH$_{NBS}$	8.058 ± 0.017	7.754 ± 0.027	8.032 ± 0.041	7.720 ± 0.021
Total alkalinity (μmol kg^{-1})	1580.41 ± 206.40	1778.14 ± 127.82	1666.75 ± 117.55	1695.96 ± 133.49
CO_3^{-2} (μmol kg^{-1})	86.54 ± 10.15	53.76 ± 5.58	94.76 ± 3.92	52.13 ± 5.07
pCO_2 (μatm)	367.77 ± 62.17	891.26 ± 77.76	435.96 ± 74.43	969.48 ± 74.27
$\Omega_{calcite}$	2.07 ± 0.24	1.28 ± 0.13	2.29 ± 0.09	1.25 ± 0.13
$\Omega_{aragonite}$	1.32 ± 015	0.82 ± 0.09	1.48 ± 0.06	0.81 ± 0.08

to a 25 ml thermostated closed cell at 25.0 ± 0.1°C for standardization (DOE 1994, Torres et al. 2013), using a Metrohm® pH–meter with a glass combined double junction Ag/AgCl electrode (Metrohm model 6.0258.600) calibrated with standard buffer of pH 4 (Metrohm® 6.2307.200), pH 7 (6.2307.210), and pH 9 (6.2307.220). pH values are reported on the National Bureau of Standards scale. Samples for A_T were poisoned with 50 µl of saturated $HgCl_2$ solution and stored in 500 ml borosilicate bottles (Pyrex, Corning®) with ground-glass stoppers lightly coated with Apiezon L® grease and stored in the dark at room temperature. Additionally, temperature and salinity were monitored during incubations by using a portable Salinometer (Salt6+, Oakton®, accuracy: ±1% and ±0.5°C, respectively). A_T was determined using the open-cell titration method (Dickson et al. 2007) using an automatic alkalinity titrator (Model AS-ALK2 Apollo SciTech) equipped with a combination pH electrode (8102BNUWP, Thermo Scientific) and temperature probe (Star ATC, Thermo Scientific) connected to a pH meter (Orion Star A211, Thermo Scientific). All samples were analyzed at 25°C (±0.1°C) with temperature regulated using a water bath (Lab Companion CW-05G). Accuracy was controlled against a certified reference material (CRM; batch no. 140 supplied by A. Dickson, University of California, San Diego, CA). Every sample was analyzed with 2 or 3 replicates, and an accuracy of 2 to 3 µmol kg^{-1} was observed with respect to CRM. Temperature and salinity data were used to calculate the rest of the carbonate system parameters (e.g. pCO_2, CO_3^{2-}) and the saturation stage of aragonite ($\Omega_{aragonite}$) and calcite ($\Omega_{calcite}$). Analyses were performed using CO2SYS software for MS Excel (Pierrot et al. 2006) set with Mehrbach solubility constants (Mehrbach et al. 1973) refitted by Dickson & Millero (1987). The $KHSO_4$ equilibrium constant determined by Dickson (1990) was used for all calculations (Table 1).

Biological responses

At the end of the experimental period, shell thickness (mm) was measured and averaged over 3 measurements taken between the ribs located at the posterior region of scallop shells (i.e. the edge of the newly deposited shell; Bibby et al. 2007, Thomsen et al. 2010, Bressan et al. 2014). The tissue was removed and the final dry shell weight (mg) was measured after drying the shell at 60°C overnight (Binder) and then weighed in an analytical balance (to the nearest 0.01 mg, Mettler Toledo). Because potential problems of water held in the shell and soft tissues, the final wet biomass was described using a relative index estimated as the difference between total and buoyant weight (see below), and expressed as a percentage of the total weight. Relative indexes are traditionally used to describe bivalve quality under aquaculture conditions (e.g. Maguire et al. 1999, Filgueira et al. 2013). Individual growth rates based on shell height (length perpendicular to the hinge) and length were estimated based on measurements performed (to the nearest 0.01 m using a Mitutoyo® caliper) on Days 1 and 18 of the experiment. Measurements of both shell height and length are used routinely during the thinning process of scallops in commercial aquaculture operations (Hennen & Hart 2012). In addition, calcification and dissolution rates (see also Gazeau et al. 2015) were estimated from changes in the buoyant weight of individual scallops and changes in empty shell weight recorded on Days 1 and 18 of the experiment using an analytical balance. Briefly, the buoyant weight is a non-destructive technique useful to estimate the shell weight in gastropods, where the whole animal is immersed in seawater (Palmer 1982). Due to differences in specific gravity of shell and tissue, when immersed in seawater the mass of the animal is mainly accounted for by the shell. In addition, buoyant weight increment is an important proxy for growth because it is equivalent to the calcification rate and is not affected by the amount of seawater and tissue weight (Palmer 1982). However, the changes in empty shell weight used as a proxy of shell dissolution must be interpreted with caution (Nienhuis et al. 2010), given the role of organic layers such as the shell periostracum in protecting live animals from dissolution (Tunnicliffe et al. 2009). To avoid errors from air trapped inside the animals during the measurements of buoyant weight, each specimen was gently moved from the rearing container to the analytical balance. Following this procedure, only seawater remained within the valves and yielded consistent buoyant weights for the experimental specimens. The relationship between dry shell weight (SW) and buoyant weight (BW) was verified by constructing the scaling relationship between both variables using an additional batch of scallops encompassing all sizes available from the Invertec-Ostimar farm (Log_{10} SW = 0.1995 + 0.9950 Log_{10} BW; n = 71; p < 0.001; r^2 = 0.99). This methodology was successfully validated previously for the mussel *Mytilus chilensis* (Duarte et al. 2014). No mortality was recorded in any treatment during the experimental period.

In situ biological and environmental monitoring

During July 2014, the Center for Advanced Research in Arid Zones (CEAZA) in collaboration with the Invertec−Ostimar Co. monitored water quality parameters in Tongoy Bay in close proximity to the area of scallop farming. This monitoring involved the deployment (10 m depth) of a CTD SBE 16plus v2 RS232 (Sea-Bird Electronics) measuring conductivity (S m^{-1}), salinity (PSU), temperature (°C), and dissolved oxygen (mg l^{-1}, ml l^{-1}, and % saturation) every 15 min (www.ceazamet.cl/index.php? pag=mod_estacion&e_cod=BTG). In this study, the environmental data from July to December 2014 are summarized, jointly with estimates of the cumulative mortality of scallops for several size ranges recorded during the thinning process performed by the Invertec−Ostimar Company. The thinning process consists of a mechanical separation of scallops, reducing density to make room for those remaining in order to ensure better growth and productivity of the individuals (Maguire et al. 1999, Filgueira et al. 2013). During the thinning process, individuals that have died during the growing period are also removed. In this study, we report the cumulative mortalities recorded after 4 to 5 mo of growing period for 4 shell range sizes (i.e. 7−15 to 45−50 mm in shell height), and their corresponding densities (i.e. from 200 to 25 scallops per lantern level). The adult fraction of the scallops size range corresponds to individuals between 45 and 50 mm, which are cultivated at densities of 25 individuals on each level of the lanterns. The harvest is performed when the scallop shell sizes reach 65 to 70 mm. During December 2014, the first-ever survey of carbonate system parameters (pH$_T$, A$_T$, and estimates of pCO$_2$ and Ω) within Tongoy Bay was conducted, using similar methodology as described above. However, in contrast to the monitoring techniques employed within the laboratory, the field monitoring campaign used seawater buffer supplied by CRM, and pH was computed on the 'total' hydrogen scale (pH$_T$). This study only presents the pH$_T$ and temperature data, and more environmental field data will be published elsewhere.

Statistical analyses

In each analysis, the replicate was the average response of the 4 scallops in each tank. Differences between treatments in scallop shell thickness and dry shell weight recorded at the end of the experi-ment were tested using ANCOVA models, including the final measurement of shell length and buoyant weight as covariates, respectively, in order to control for the inherent positive scaling relationship between shell length and both shell weight and thickness. ANCOVA results evidenced no significant influence of the shell length upon scallop shell thickness (covariate, $F_{1,15} = 1.74$; p = 0.207), but a significant influence of buoyant weight upon dry shell weight (covariate, $F_{1,15} = 82.69$; p < 0.001). In this case, further comparisons among treatments were done estimating the predicted value (least square mean) of the regression between dry shell weight with buoyant weight, and comparing them at the mean value of this covariate (3.681 g ± 0.37 SD; see also Watson et al. 2012). Two-way ANOVAs were used to evaluate whether temperature, pH levels, or the interaction between factors affected the measured biological responses (biomass, shell growth, calcification, and shell dissolution rates) during the experiment. Tukey's HSD was used as an *a posteriori* test when the main factors indicated significant differences between levels of the corresponding factor (Underwood 1997). These ANOVAs were implemented in a generalized linear model and the coefficient of the interaction term was estimated. Positive or negative coefficients for significant interaction terms were interpreted as synergistic or antagonistic effects between temperature and pH upon the corresponding biological response (Kroeker et al. 2013). Only growth rates based on shell length and height required log$_{10}$ transformation to meet ANOVA assumptions. In both ANOVA and ANCOVA, normality was assessed over model residuals using the Kolmogorov−Smirnov test, and homoscedasticity was evaluated using Bartlett tests (Sokal & Rohlf 1995). Finally, a χ^2 test on scallop cumulative mortality data recorded in the sea farm was used to test whether, over time (month), mortalities were dependent on the shell range sizes used in the scallop thinning process. All analyses were carried out using Minitab v14.

RESULTS

The experimental setup and average environmental and carbonate chemistry parameters recorded during the experiments with *Argopecten purpuratus* are shown in Table 1. Low A$_T$ values were observed for all treatments (<2000 µmol kg^{-1}), due to the influence of riverine discharges (Valdivia River Estuary) close to the coastal laboratory (Torres et al. 2013).

pCO_2 values ranged from 367 to 435 µatm for the low pCO_2 treatment at 14 and 18°C, respectively, and from 891 to 969 µatm for the high pCO_2 at 14 and 18°C, respectively. $\Omega_{aragonite}$ varied less between temperatures, but undersaturation was reached under the high pCO_2 treatment ($\Omega_{aragonite}$ from 0.81 to 0.82; Table 1).

At the end of the experimental period, shell thickness, dry shell weight, and wet biomass of the scallops were significantly lower in the treatments representing combinations of low pH levels at increased temperature when compared with current environmental conditions (i.e. 14°C and pH ~8.0; Fig. 3). These patterns of variability were dominated by differences in temperature among treatments and to a lesser extent to pH variability (Table 2). Thus, thicker and heavier shells and increased biomass were observed on scallops growing under current environmental conditions when compared with all other combinations of temperatures and pH level (Tukey HSD, p < 0.05).

Control or current temperature (~14°C) and low pH (~7.7) significantly restricted the shell growth in length and height (Table 2, Tukey HSD, p < 0.05, Fig. 4), but elevated temperatures appeared to ame-

Table 2. Summary results comparing *Argopecten purpuratus* shell thickness and shell weight (ANCOVA), biomass and growth rates in shell height and length, and calcification and dissolution rates (2-way ANOVA) between combinations of pH (7.7 and 8.0) and temperature (14 and 18°C) used in experimental treatments. Significant p-values (p < 0.05) are shown in **bold**

Biological response	Environmental variable	df (source, error)	F	p
Shell thickness (mm)	Temperature	1, 15	18.07	**0.001**
	pH	1, 15	1.24	0.283
	Temperature × pH	1, 15	0.50	0.488
Shell dry weight (g)	Temperature	1, 15	6.08	**0.026**
	pH	1, 15	3.89	0.067
	Temperature × pH	1, 15	2.19	0.159
Wet biomass (%)	Temperature	1, 16	8.36	**0.011**
	pH	1, 16	2.52	0.132
	Temperature × pH	1, 16	2.61	0.126
Growth in height (mm d^{-1})	Temperature	1, 16	7.4	**0.015**
	pH	1, 16	6.11	**0.025**
	Temperature × pH	1, 16	6.52	**0.021**[a]
Growth in length (mm d^{-1})	Temperature	1, 16	14.1	**0.002**
	pH	1, 16	9.00	**0.008**
	Temperature × pH	1, 16	4.28	0.055
Calcification rate (g d^{-1})	Temperature	1, 16	24.39	**<0.001**
	pH	1, 16	0.97	0.340
	Temperature × pH	1, 16	3.39	0.066
Dissolution rate (mg d^{-1})	Temperature	1, 16	2.21	**0.015**
	pH	1, 16	12.90	**0.002**
	Temperature × pH	1, 16	0.72	0.408

[a]Significant synergistic effects of both variables

liorate the negative impacts of reduced pH (i.e. combination pH ~7.7/18°C; Fig. 4). Temperature and pH showed a significant and positive interaction term (coefficient = 0.0104 ± 0.0041 SE; Table 2), thus indi-
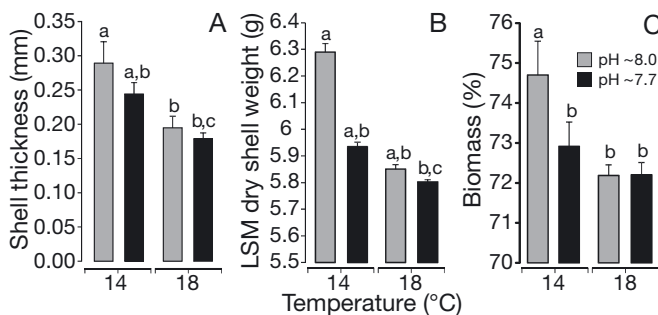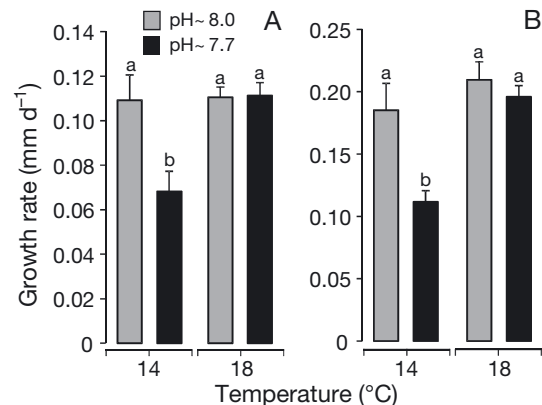

Fig. 3. Mean ± SE (A) shell thickness, (B) dry shell weight, and (C) wet biomass of *Argopecten purpuratus* reared at nominal pH levels in seawater and at 2 temperatures. All measurements were recorded at the end of the study period (18 d). LSM: least square mean, which corresponds to the predicted value of dry shell weight at the mean value of buoyant weight, used as covariate in an ANCOVA model (see 'Materials and methods'). Different letters indicate significant differences among treatments (n = 5) using a post hoc Tukey HSD test

Fig. 4. Growth rates measured as increments in the shell height and length (mean ± SE) of *Argopecten purpuratus* individuals reared at 2 temperatures and 2 nominal pH levels in seawater. These rates were estimated based on the difference between initial and final measurements after the 18 d experimental period. Different letters indicate significant differences among treatments (n = 5) using a post hoc Tukey HSD test

cating a synergistic effect of temperature and pH on the growth rate in shell height of the scallops. For the remaining biological responses, we found non-significant interactive effects of pH and temperature (Table 2).

The net calcification rate over the study period was not affected by low pH, but temperature had a significant positive effect (Table 2, Fig. 5A). Shell dissolution, estimated from changes in shell weight of dead scallops, was significantly higher at the combination of low pH and at control temperature (i.e. pH ~7.7/14°C; Fig. 5B).

The monitoring program that started during 2014 provided updated information on the seawater properties of Tongoy Bay. In particular, during the experimental period (mid-August to mid-September), the seawater temperature measured at 10 m depth inside the bay ranged between 12 and 15°C, with low levels of oxygen concentration (3–6 ml l^{-1}) and low variability in salinity (ca. 34.4 psu; Fig. 6A–C). On 2 September, cooling events were observed that coincided with periods of oxygen depletion, with the second event displaying oxygen levels below 2 ml l^{-1}, values that are consistent with a hypoxia event (Fig. 6B). During the rest of the year (October to December), the temperatures regularly fluctuated below 15°C, with drops in temperature being concurrent with decreased levels of dissolved oxygen. From July to December 2014, the cumulative mortalities (integrating the last 4–5 mo) of scallops were dependent on shell size (χ^2 = 142, df = 15, p < 0.001, Fig. 6D). In particular, scallop mortality was reduced during September, but an important

Fig. 6. Temporal fluctuations in (A) temperature, (B) oxygen concentration, and (C) salinity measured from June to December 2014 by the CEAZAMET buoy at 10 m depth in Tongoy Bay. (D) Cumulative mortalities in 4 shell range sizes of *Argopecten purpuratus* recorded during the scallop thinning procedure performed from July to December 2014 at the Invertec–Ostimar Co. scallop farm; each growing period lasted for ca. 4 to 5 mo. The initial number of scallops at each level of the lantern nets and in each size range (see 'Materials and methods') is shown in brackets. Inset in (A) shows the histogram for temperature observations recorded during the study period; the horizontal dashed line in (B) indicates hypoxia levels, and the stippled area represents the timing of the experimental period
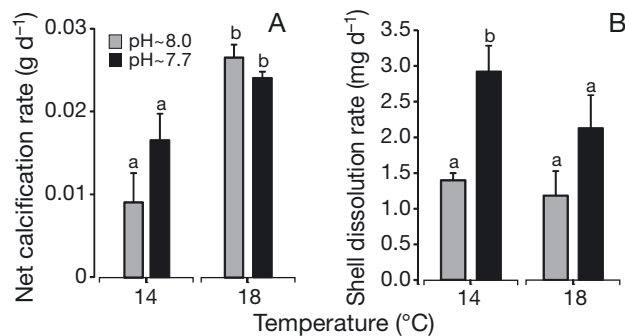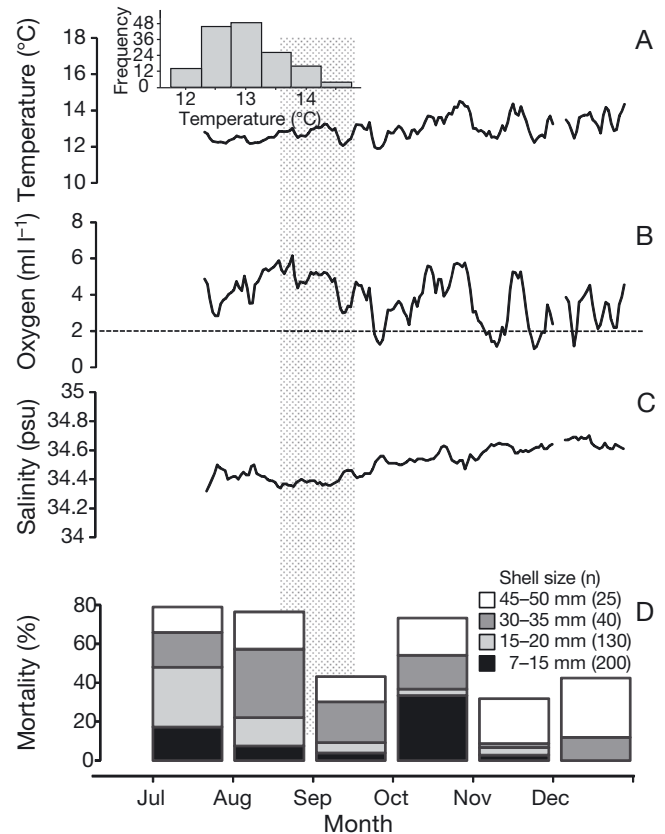

Fig. 5. (A) Net calcification rate (mean ± SE) of *Argopecten purpuratus* reared at 2 temperatures and 2 nominal pH levels in seawater. (B) Dissolution rate (mean ± SE) of dead shells of the scallops exposed to the same experimental conditions. These rates were estimated based on the difference between initial and final measurement after the 18 d experimental period. Different letters indicate significant differences among treatments (n = 5) using a post hoc Tukey HSD test

increase in mortality was observed in October mostly in small scallops (7–15 mm). The mortality of scallops with larger shell sizes (>45 mm) remained fairly constant (ca. 20–30%) throughout the monitoring period (Fig. 6D).

DISCUSSION

Despite the short period of exposure used in our study (18 d), we detected significant effects of OA and temperature on juvenile individuals of *Argopecten purpuratus*; our results were similar to a previous study, in which a short exposure time was suf-

ficient to detect OA and warming effects on this marine mollusk (Nienhuis et al. 2010, Duarte et al. 2014). These results suggest that OA and warming might have significant effects on the fitness of these individuals in a short period of time. This information is relevant to understand the effects of upwelling conditions on these animals, because this phenomenon is of short duration and is not constant. In this study, no mortality effects were found on juveniles in any of the experimental treatments, which indicates that the projected increase of CO_2 levels and temperature had chronic, but not lethal, effects on these animals. This lack of mortality may result from an underlying natural tolerance of *A. purpuratus* to the changes in temperature and pH used in the current study, all of which fall within the extremes of environmental variability observed inside Tongoy Bay (see discussion below and Fig. 7).

Despite the lack of lethal effects, significant physiological responses to both temperature and pH changes were observed. Shell thickness, shell weight, and live biomass of *A. purpuratus* individuals were significantly reduced at low pH (pH ~7.7) and increased temperature (18°C) compared to control individuals (pH = 8.0 at 14°C). Reduced shell growth and increased dissolution rates were observed at
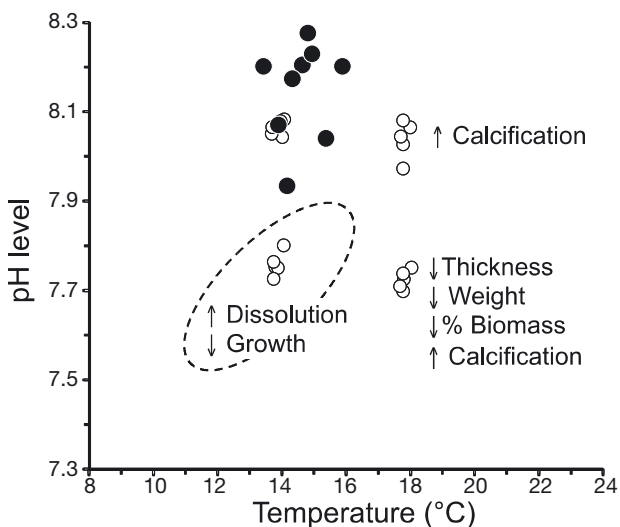

Fig. 7. Graphical summary for the variability of each temperature/pH combination in the experimental treatments (open circles); the natural variability in temperature/pH recorded inside Tongoy Bay in December 2014 (black dots) during a field survey, and the environmental range of pH and temperature reported for Pt. Lengua de Vaca upwelling area (dashed ellipse, see 'Discussion'). The significant effects recorded in each shell characteristic of *Argopecten purpuratus* are indicated by up or down arrows and presented beside each pH/temperature combination

ambient temperature (14°C) in low pH treatments. These dissolution rates fluctuated between 3 and 10% of net calcification, but did not preclude animal growth, as illustrated by increased shell length and weight across all treatments. Impacts of lowered pH were ameliorated at warmer temperatures (18°C), through a significant increase in calcification rates. Thus, the results of this study agree with previous studies where shell characteristics, such as shell dissolution, shell size, and calcification rates, were used to examine the impacts of temperature and OA on marine calcifying organisms, and that warming could reverse the negative effects of OA (e.g. Beniash et al. 2010, Welladsen et al. 2010, Byrne 2012, Chan et al. 2013, Thomsen et al. 2013, Bressan et al. 2014, Fitzer et al. 2014). Although we did not assess additional biological sources of stress in the present study, they may also affect shell formation and structure. For instance, food limitation could be crucial if feeding rates increased beyond the food supply provided in this experiment (Mackenzie et al. 2014).

Significant reductions of shell thickness and weight (ca. −36% and −7%, respectively) were observed under experimental conditions of low pH at 18°C with respect to control scallops (i.e. present-day conditions, 14°C/pH ~8.0). Such a response could have implications for the long-term survival of individuals, as shell strength in scallops is a function of shell height, thickness, corrugation, and convexity (Pennington & Currey 1984). Lafrance et al. (2003) demonstrated that the shells of wild scallops were significantly heavier and stronger than those of cultured scallops, suggesting that cultured juvenile scallops have an increased vulnerability to shell-cracking predators. This reduction in shell strength was associated with factors related to the suspended culture (e.g. density, suspension depth, fouling, handling), leading to the study of mechanisms enhancing shell strength and strategies involving the timing of release of scallops into bottom culture (Grefsrud & Strand 2006). Our study suggests that, in addition to factors related to suspended culture conditions, other stressors such as low seawater pH also influence the shell strength of *A. purpuratus*. Similar reductions in shell thickness and associated robustness have been described by several studies in other mollusks exposed to low pH conditions (Bibby et al. 2007, Gazeau et al. 2013). In particular, the larval shell of the scallop *A. irradians* decreased in thickness when exposed to the low pH (high pCO_2) seawater conditions projected for the end of this century (Talmage & Gobler 2010). However, other studies reported that mussels grew thicker shells at warmer temperatures

independent of pH conditions (*Mytilus galloprovincialis*, Kroeker et al. 2013), or showed no reduction in shell thickness (*M. edulis*, Thomsen et al. 2013). More recently, Bressan et al. (2014) pointed out that a reduction in shell thickness could be species specific and could take place after 3 and 6 mo of incubation under acidification conditions in clams *Chamelea gallina* and mussels *M. galloprovincialis*, respectively. Since low pH conditions can reduce the shell thickness in scallops, it is realistic to expect effects in areas, such as Tongoy Bay, that are under the influence of low pH upwelling waters (Torres & Ampuero 2009, see below and Fig. 6). Thus, studies that aim to improve our understanding of the mechanisms that govern scallop shell strength and related mineralogical properties are urgently needed.

Reduced shell growth under acidified or low pH conditions have been reported specifically for scallops (Talmage & Gobler 2009, White et al. 2013) and other mollusks (Melzner et al. 2011, Hiebenthal et al. 2013, Kroeker et al. 2013, Bressan et al. 2014). As in those studies, we found that a significant reduction in shell length and height of *A. purpuratus* occurs even though seawater remained above the saturation state for carbonate precipitation ($\Omega_{calcite} > 1$). In addition, our experimental scenarios are within the environmental conditions described as setting a threshold for negative effects of pH (7.5–7.4; Berge et al. 2006, Bressan et al. 2014) and temperature (25°C) on shell growth of bivalves (Hiebenthal et al. 2013, Kroeker et al. 2013). Thus, to a lesser extent but also important, the reduction in shell growth of *A. purpuratus* may be ascribed to dissolution as recorded in dead shells exposed to low pH and low temperature conditions (Nienhuis et al. 2010, Duarte et al. 2014). In contrast to other materials, the kinetic dissolution/solubility of carbonate shells increases at low temperatures (Morse et al. 2007). This property of carbonates may help to explain the increase in shell calcification of *A. purpuratus* at warmer temperatures. However, saturation states were roughly similar across temperature treatments at respective pH levels. This suggests a major role for warmer temperatures, which could potentially offset the reduction in calcification caused by OA (Byrne & Przeslawski 2013, Kroeker et al. 2013, Duarte et al. 2014). However, calcification must be regarded as part of the whole biology of the individuals and thus the reduction in shell increments under low pH could be because energy allocation is being diverted away to other key physiological processes such as acid–base balance, reproduction, and immune function (Wood et al. 2008, Findlay et al. 2009). Finally, the increment in the calcification rates

at 18°C registered in our study (see Fig. 7) reinforces the notion that *A. purpuratus* can benefit from warmer temperatures and agrees with previous observations relating increased developmental rates and population abundances of *A. purpuratus* during El Niño – Southern Oscillation (ENSO) events which regularly impact upwelling ecosystems in northern Chile (Thiel et al. 2007). This finding indicates that scallop aquaculture in northern Chile will remain exclusively associated with embayment areas, where increased temperatures may provide a 'refuge' for scallop production.

Currently, there is no consensus of how the effects of warming and acidification will interact (e.g. additive, synergistic, or antagonistic) to influence the fitness of mollusks (e.g. Berge et al. 2006, Findlay et al. 2009, Gooding et al. 2009, Comeau et al. 2010, Byrne 2011, Kroeker et al. 2013, Duarte et al. 2014). These discrepancies have led to generalizations that temperature may have a stronger effect on the overall survival of invertebrates (Findlay et al. 2009, Lischka et al. 2011), while OA will have its greatest influence primarily on calcification and growth rates (Byrne & Przeslawski 2013). In our study, temperature increase and OA operated synergistically only on growth rates of *A. purpuratus,* estimated through changes in shell length and height, a result which is in agreement with previous evidence for synergistic effects of temperature and acidification on mollusks (Rodolfo-Metalpa et al. 2011). Thus, our study suggests that changes in shell growth can result from increased dissolution at low pH and temperature conditions and from increased calcification rates at warmer temperatures, which may actually overcome the negative impact of acidification. However, we also record a moderate but significant reduction in biomass (−2%) under low pH, regardless of seawater temperature. This would imply that low pH was increasing the biological cost of living for the scallops, which could have additional implications for the commercial production of *A. purpuratus* under the influence of upwelling waters (e.g. Talmage & Gobler 2010, Barton et al. 2012).

The increased calcification rates of *A. purpuratus* observed at warmer temperatures appeared to be occurring at the expense of reducing the shell thickness, weight, and live biomass. For instance, at the end of the experiment, the mean shell length was positively correlated with mean dry weight (Pearson's r = 0.99; p = 0.003, n = 4). That is, across treatments, larger shells imply heavier shells. However, calcification rate was negatively associated with shell thickness (Pearson's r = −0.97; p = 0.033, n = 4), which

suggests that those shell size/weight increments promoted by increased temperature at both pH conditions could occur at the cost of reducing shell thickness. This potential trade-off highlights some strategies that may be implemented by this cultured species to cope with variability in both climate stressors. In particular, our study suggests that scallops may increase their calcification rate and reach larger shell size during warmer periods (e.g. ENSO) but would be thinner and frailer, and thus more vulnerable to predators and parasites (e.g. perforating polychaetes), as well as increasing the risks of shell breakage and mortality during the manipulation and mechanical sorting symptomatic of the commercial thinning process. In addition, under projected global acidification scenarios (which are already occurring in our study area due to upwelling events, see below and Fig. 7), the observed reduction in growth rates would suggest that it will take longer for scallops to reach marketable size and will require a modification to the timing of the thinning process performed by the aquaculture industry. Future studies should examine strategies that facilitate the survival of scallops when subjected to the thinning and sorting process.

Coastal upwelling modulates the physical and chemical properties of seawater over large areas of the coastal ocean. In relation to the current study, the Pt. Lengua de Vaca upwelling increases CO_2-fluxes (fCO_2 ~ 1000 µatm) and promotes strong reductions in pH (pH ~7.6) in Tongoy Bay and the surrounding area (Torres & Ampuero 2009). These pH values have previously been proposed as a threshold for negative effects of OA on shell growth in bivalves (e.g. Berge et al. 2006, Bressan et al. 2014). Thus, the significant net shell dissolution and reduced growth rates of *A. purpuratus* registered at acidified (pH ~7.7) and current temperature (14°C) conditions suggest that the shell characteristics of the farmed scallops in Tongoy Bay are already compromised during upwelling periods. More generally, it has been suggested that changes in ocean chemistry, which have been driven by rising anthropogenic CO_2 emissions, may be inhibiting the development and survival of larval shellfish, and are probably contributing to a global decline in bivalve populations of particular scallop species (Talmage & Gobler 2010). Some upwelling areas of the Humboldt Current system are showing cooling trends (Gutierrez et al. 2011), which may represent an additional stressor due to the increased kinetic dissolution of carbonate at lower temperatures (Morse et al. 2007). Thus, further environmental monitoring is required to better understand the extent, timing, and persistence of upwelling on the physical-chemical properties of the waters masses circulating inside Tongoy Bay, and their role in reducing shell growth and biomass production of *A. purpuratus* farming operations.

Initial physical-chemical monitoring and water sampling related to the scallop aquaculture was started during 2014. This monitoring has already indicated that during the austral spring (December), the seawater inside Tongoy Bay had pH values between 7.9 and 8.3, and temperatures between 14 and 16°C. This variability compares well with the present-day experimental scenario used in the current study (Fig. 7). This implies that, in absence of upwelling influences, Tongoy Bay provides suitable conditions to support scallops with appropriate shell thickness, shell weight, and live tissue biomass as required by scallop aquaculture. In addition, previous studies in the area have suggested the role of upwelling influences in providing an adequate food supply into Tongoy Bay (Gonzalez et al. 1999). However, Tongoy Bay also showed low levels of oxygenation at 10 m depth (Fig. 6B), which adds a third climatic stressor associated with upwelling ecosystems (e.g. Gruber 2011). Occasional occurrences of hypoxic conditions may also affect the development of *A. purpuratus* and decrease their physiological capacity to withstand other stressors often encountered by cultured scallops (Brokordt et al. 2013). The mortality data gathered at Invertec-Ostimar farms suggest that, under current temperatures, drops in oxygen concentration below 2 mg l^{-1} (i.e. hypoxia events, Díaz & Rosenberg 2008) may be related to the increased mortality of *A. purpuratus* juveniles (7−15 mm) in September and October, while the mortality of the adult fraction (>45 mm) remains almost stable from July to October. However, hypoxic events are more common from November to January and correspond with an important reduction in mortality of juveniles. This suggests that, compared to the adult fraction, juveniles are more resilient to sources of mortality potentially induced by the underlying environmental variability occurring during the Nov−Jan monitoring period inside Tongoy Bay. Ramajo et al. (2016) recently reported that juvenile *A. purpuratus* are tolerant to OA conditions and showed increased metabolism, shell growth, net calcification, and ingestion rates under OA conditions (pH ~7.6). However, as in our study, these positive responses to OA also occurred at increased temperatures (i.e. at 18°C). These observations reflect the occurrence of multiple stressors that may interfere with temperature and acidification

upwelling-influenced areas with potential impacts on growth and production of the native scallop. Overall, the results of the current study indicate that a review of existing data for scallop production and environmental variability in the area would be valuable, as would be additional experimentation using a multiple-stressor approach.

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