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Ocean acidification alters anti-predator responses in a competitive dominant intertidal mussel

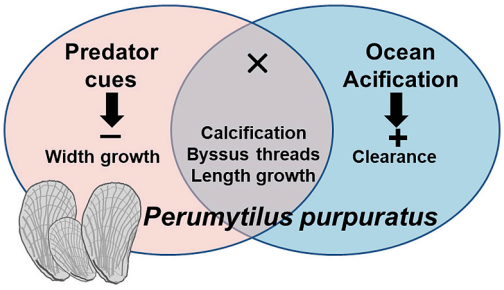
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HIGHLIGHTS

- Combined effects of predation cues (PC) and ocean acidification (OA) were evaluated.
- Combined effects of PC and OA reduced growth in mussels.
- Calcification rates were driven by the interaction between PC and OA.
- Byssus production in mussels increased with OA, in conditions without PC.
- OA alters the natural response of mussels to predation risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Widespread intertidal mussels are exposed to a variety of natural and anthropogenic stressors. Even so, our understanding of the combined influence of stressors such as predation risk and ocean acidification (OA) on these species remains limited. This study examined the response of the purple mussel (*Perumytilus purpuratus*), a species distributed along Pacific southeastern rocky shores, to the effects of predation risk and OA. Using a laboratory 2 × 2 cross design, purple mussels were either devoid or exposed to predator cues from the muricid snail *Acanthina monodon*, while simultaneously exposing them to current (500 ppm) or projected OA conditions (1500 ppm). The response of purple mussels to these factors was assessed using growth, calcification, clearance, and metabolic rates, in addition to byssus production. After 60 d, the presence of predator cues reduced mussel growth in width and length, and in the latter case, OA enhanced this response making the effects of predator cues more severe. Calcification rates were driven by the interaction between the two stressors, whereas clearance rates increased

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only in response to OA, likely explaining some of the growth results. Mussel byssus production also increased with pCO_2 but interacted with predation risk: in the absence of predator cues, byssus production increased with OA. These results suggest that projected levels of OA may alter and in some cases prevail over the natural response of purple mussels to predation risk. Considering the role played by this mussel as a dominant competitor and ecosystem engineer in rocky shores, these results have community-wide implications.

1. Introduction

Human activities are altering the natural conditions in which coastal and oceanic organisms have evolved (e.g., Caldeira and Wickett, 2003; Orr et al., 2005). One well-established example is the increment in atmospheric CO_2, which has risen 100 ppm since the industrial revolution and today stands at ~420 ppm (IPCC et al., 2019). Such anomaly is rising the CO_2 uptake into the ocean, reducing seawater pH, the concentration of CO_3^{-2}, and the saturation state of Calcium carbonate minerals, a process generically known as Ocean Acidification (hereafter OA) (Orr et al., 2005; Feely et al., 2009; Gattuso et al., 2015). OA has been linked with changes in marine resources (Doney et al., 2020), species survival (Busch and McElhany, 2016) and various aspect of species' life history (Fabry et al., 2008; Kroeker et al., 2013; Watson et al., 2013). The latter changes include negative and positive effects on various behavioral (see review by Wang and Wang, 2020), physiological and morphological traits (Findlay et al., 2010; Navarro et al., 2013; Duarte et al., 2016; Manríquez et al., 2021), including changes in growth (Duarte et al., 2015), calcification (Fabry et al., 2008), and metabolic rates (Benítez et al., 2018). OA may also alter predator-prey interactions, in an interplay of natural and anthropogenic stressors. For instance, in simple trophic chains associated with tidal pools, reduced water pH has been shown to have negative effects on the response of herbivore snails to their predators (sea stars), while weakening indirect interactions with macroalgae (Jellison and Gaylord, 2019). OA is therefore expected to be relevant at the community level, but its ultimate effects are still far from being fully understood (Fabry et al., 2008; Kroeker et al., 2014; Gaylord et al., 2015).

The ability of a predator to find its prey is often matched by the prey's ability to detect such predator through chemical cues (Cheung et al., 2009; Manríquez et al., 2014; Kong et al., 2019) or to reduce predation effects by growing thorns, thicker shells or strengthening the attachment to substrates by byssus threads (Smith and Jennings, 2000; Smee and Weissburg, 2006; Li et al., 2015; Sui et al., 2015). On rocky intertidal communities, these types of responses have been well documented among mollusks (Kroeker et al., 2014; Wang and Wang, 2020), particularly in mussels, which as juveniles and adults lack the ability to escape predators by moving or migrating. For instance, under perceived risk of predation species such as the blue mussel (*Mytilus edulis*) is known to thicken its shells (Sherker et al., 2017), whereas the Asian green mussel (*Perna viridis*) increases calcification and byssus thread production (Cheung et al., 2004; Fässler and Kaiser, 2008), and the thick shell mussel (*Mytilus coruscus*) enhances byssus production and shell closing strength (Li et al., 2015; Sui et al., 2015). OA may indirectly also alter the outcome of interactions between mussels and a variety of their predators, including seabirds, crustaceans, echinoderms, and mollusks (e.g., Li et al., 2015; Kong et al., 2019). An illustrative example is the production of byssus threads by the mussel *Mytilus coruscus*, a well-known antipredator response that is triggered and enhanced in the presence of predator cues, but is partially inhibited in the presence of OA (Li et al., 2015) and other stressors (e.g. Sui et al., 2017).

Mussels are ubiquitous in rocky shore ecosystems and as such represent suitable models for the study of natural and anthropogenic stressors (e.g., OA and predation). When mussels are also key ecological players, such as competitive dominant species (Suchanek, 1978, 1986, 1986; Reusch and Chapman, 1997; Prado and Castilla, 2006; Duarte et al., 2006), their response to stressors likely have community-level effects: any impacts of OA on these species could have direct or indirect consequences for substrate (space) availability for potential competitors and for trophic interactions with the predators relying on these species for food (Seed, 1996; Firth and Crowe, 2010; Wang and Wang, 2020). Some autoecological studies have shown that OA can alter mussel growth and shell calcification rates (Duarte et al., 2014, 2015), in addition to their production of byssus threads (O'Donnell et al., 2013; Dickey et al., 2018; Sui et al., 2015, 2017, 2017). OA may also reduce the capacity of these organisms to respond to predation risk, making them more vulnerable to predators (Manríquez et al., 2014). An interesting model species for the study of OA and mussel responses to predators is *Perumytilus purpuratus* (hereafter the purple mussel). This species forms dense intertidal beds along the southeastern Pacific and is both a competitive dominant species and an ecosystem engineer (Prado and Castilla, 2006; Valdivia et al., 2021). Given its wide distribution along the Pacific coast, the purple mussel is also exposed to considerable local-scale variation in e.g., temperatures and food availability, associated with scattered upwelling centers along the Chilean littoral (Ramajo et al., 2019; Weidberg et al., 2020). Such variation is a valuable aspect in the choice of a model species, considering its importance for studies attempting to forecast changes in systems exposed to OA.

Purple mussels are vulnerable to various predators. In response to highly mobile predators such as the crab *Acanthocyclus hassleri*, this species has been shown to modify the thickness of their shell and ramp-up the production of byssus threads (Caro et al., 2008). Some evidence suggests that such antipredator responses may be modified in the presence of environmental stressors. Purple mussel populations from northern Chile, also exhibit a complex repertoire of responses to crabs and large keystone predators (*Concholepas concholepas*), that interact with chemical and physical variables such as OA and elevated water temperatures (20 °C; Manríquez et al., 2021). One important aspect that has not been studied in the context of OA is the purple mussel interaction with one of its most common predators, the small muricid snail *Acanthina monodon* (Poblete et al., 1987; Reid and Osorio, 2000), which feeds on this species and surrounding barnacles (López et al., 1995; Moreno, 1995; Soto et al., 2004). Our study focused on this predator-prey system at a distinct geographic setting, the rocky littoral linked to an upwelling system in central Chile. In this system, we assessed the combined effects of *A. monodon* predator cues and OA upon morphological, physiological, and behavioral traits of the purple mussel. Although the evidence this far on these factors is not fully consistent, and do not include an abundance of examples from upwelling-linked habitats, our working hypothesis was that the ability of purple mussels to respond to *A. monodon*'s predation risk is halted by OA.

2. Material and methods

2.1. Collection, acclimation and feeding of experimental animals

Juvenile purple mussels (8.0 mm in shell length, SL; N = 160 individuals) and predatory snails (*A. monodon*; 18.5 mm in peristomal length; n = 32 individuals) were collected by hand during September 2019. Both species were collected in the intertidal zone of Quintay (33°11′00″ S; 71°41′10″ W), a rocky shore system associated with regular upwelling waters occurring in the central Chile coast (Aravena et al., 2014). The animals were transported in coolers to the laboratory (Centro de Investigaciones Marinas de Quintay, CIMARQ, Universidad Andrés Bello), where they were kept in 1 μm filtered seawater at~ 12 °C and 33 psμ for one month of acclimation. During that period (and

subsequently, during the experimental period), purple mussels were fed daily with 20 mL of a microalgae culture (*Isochrysis galbana*; ~25 × 10^6 cells ml^{-1}), while the predatory snails were kept in separate tanks and fed with additional purple mussels obtained in the same collection site (five ~8 mm SL purple mussels per week).

2.2. System of CO_2 mixing and temperature

To adjust the water conditions to the two pCO_2 levels required (500 and 1500 ppm), we used the mixing system described by Torres et al. (2013). In brief, to obtain the target CO_2 concentrations, we blended dry air with pure CO_2 using mass flow controllers (MFCs, www.aalborg.com) for air and CO_2. This blend was then bubbled into two equilibration tanks (60 L) filled with filtered seawater. Such seawater was delivered to two 20 L tank immersed in baths with controlled temperature (15 °C). Then, seawater with the corresponding pCO_2 was transferred to the experimental tanks using an electric pump. Seawater parameters, including pH, total alkalinity, temperature, and salinity were measured once a week in four randomly chosen tanks per treatment (Table 1). pH measurements were taken in closed 60 mL cells thermo-stated at 25 °C using a Metrohm 914 pH meter and an electrode (NTC (6.0228.010) calibrated with a buffer Metrohm® 4 (6.2307.200), 7 (6.2307.210), 9 (6.2307.220) at 25 °C. Hence, values of pH are reported on a NBS scale (DOE et al., 1994). We acknowledge that in seawater, the use of a NBS scale is less accurate than an absolute pH scale. However, our choice is in line with other ecophysiological studies and, from that perspective, the use of a free hydrogen ion concentration scale (NBS) seemed more suitable than the total scale as it does not include sulfate protonation in its definition (see Riebesell et al., 2011; Ramajo et al., 2020; Lardies et al., 2021; Schubert et al., 2021).

Temperature and salinity were measured with a digital thermometer (Hanna®, model HI 98509) and an Eco Sense EC 300, respectively. Measures of Total alkalinity (AT) were done using potentiometric titration (Metrohm® Titrando 888 model; Haraldsson et al., 1997), equipped with a pH electrode (Aquatrode Plus with Pt1000, Metrohm®). Certified Reference Material (CMR, provided by Andrew Dickson, Scripps Institution of Oceanography, San Diego, USA) was used to control analysis accuracy. A_T samples were analyzed at 25 °C (±0.1 °C) regulating the temperature using a water-bath (Lab Companion CW-05G). Values of pH, temperature, salinity, and AT were used to estimate the remaining parameters of the carbonate system and the saturation stage (Ω) of aragonite and calcite using CO_2SYS software for MS EXCEL (Pierrot et al., 2006) and Mehrbach solubility constants (Mehrbach et al., 1973) refitted by Dickson and Millero (1987) (Table 1).

2.3. Experimental setup

We used a 2 × 2 cross-design to compare the performance of purple mussels (n = 8 replicates per treatment) when exposed to (i) the presence or absence of *A. monodon*'s cues, and to (ii) current (500 ppm; Vargas et al., 2017; Broitman et al., 2018) or projected OA levels (1500 ppm; Meinshausen et al., 2011 IPCC, 2014). After acclimation, the experimental animals, i. e., 5 purple mussels per replicate (amount based on prior experience with similar experiments; C. Duarte, Unpublished Data), were randomly assigned to tanks filled with 0.6 L of filtered seawater characterizing one of these four treatments: current (500 ppm) or projected (2200) pCO_2 conditions (1500 ppm; IPCC, 2014), with or without *A. monodon* cues (Table 1). To maintain the two pCO_2 levels, each experimental tank was constantly bubbled with the corresponding pCO_2 concentration. To create fresh predator cues, one *A. monodon* snail was confined into the first of three compartments, which fed cues into a second compartment, which was connected to a third compartment containing the five purple mussels (see Electronic Appendix). This setup exposed purple mussels to the input of *A. monodon* cues, while preventing any physical contact between predators and prey. Each experimental tank (with its three compartments) had a snap on lid and four perforations; one for the feeding system (drip-feed, in the large compartment), a second one to deliver the treated seawater, a third to input the corresponding gas-mix, and a fourth one to collect water samples for monitoring pH and alkalinity. The walls dividing the three compartments of each tank were perforated in decreasing heights to allow for a one-direction water flow (i.e., a flow direction that completely prevented the mix of water from different treatment conditions). All the tanks were semi-immersed in the water bath to maintain the target temperature. A perforation made in the external wall of the last compartment and placed 1 cm above the water level in the water bath was used as a water outlet. The water pumping system delivered the corresponding mix of water at a rate of 0.078 L h^{-1}. As indicated above, each treatment included eight replicated tanks which were linked to a recirculation system with chillers to maintain a consistent temperature (15 °C). Experiments lasted 60 days.

Table 1
Seawater carbonate system parameters (Mean ± SD) used to maintain *Perumytilus purpuratus* mussels and the predator snail *Acanthina monodon* during the exposure period. See Material and Methods for details on the pCO_2 used in this study.

Parameter	500 µatm	1500 µatm
pH_{NBS} at 25 °C	7.876 ± 0.020	7.615 ± 0.019
pH_{NBS} in situ	8.017 ± 0.021	7.750 ± 0.020
Salinity (psu)	30.82 ± 0.23	30.38 ± 0.31
Temperature (°C)	15.55 ± 0.12	15.36 ± 0.18
TA (mmol kg^{-1})	2140 ± 18	2123 ± 20
pCO_2 in situ (ppm)	620 ± 32	1211 ± 55
$[CO_3^{2-}]$ in situ (µmol kg^{-1})	134 ± 5	76 ± 3
Ωca	2.42 ± 0.14	1.38 ± 0.08
Ωar	1.59 ± 0.09	0.81 ± 0.05

2.4. Growth, calcification and clearance rates

Three estimates of purple mussel growth were calculated from changes in shell length and width (to the nearest 0.01 mm) and total body weight (to the nearest 0.1 mg) between the beginning and end of the experimental period (0 and 60 d).

Mussel calcification rates were estimated from changes in individual buoyant weight (i.e., underwater weight, see Palmer, 1992), based on measurements done at the beginning and end of the experimental period (0 and 60 d).

Clearance rates were measured in individuals from each replicated treatment (5 purple mussels per tank) taken at the end of the experimental period and transferred to incubation glass containers for 1 h. These containers were filled with 200 mL of seawater with similar pCO_2 conditions as to the one used in their corresponding experimental treatment. At the start of the incubation, food availability was ~4 × 10^5 cells mL^{-1} of microalgae (*I. galbana*), and the content of the containers was constantly mixed to avoid sedimentation. At the end of the incubation, the algal concentration was recalculated, and the equation by Sui et al. (2016), based on the original equation by Coughlan (1969), was applied to estimate clearance rate (CR; mL $ind^{-1}h^{-1}$):

$$CR = V \times (LnCi - LnCf) / T \times N$$

where V is the volume (mL), Ci and Cf are initial and final algal concentrations (cells mL^{-1}), T is the filtration time (h) and N stands for the number of mussels. For phytoplankton cell counts, 1 mL of each container was taken, fixed with a Lugol solution and counting was done in triplicate using a Neubauer chamber.

2.5. Metabolic and byssus thread production rates

At the end of the experiment, mass-specific oxygen consumption

rates (mg O_2 $L^{-1}g^{-1}h^{-1}$) were estimated separately for each individual mussel in each replicate and treatment. The measurements were carried out in filtered seawater with the same conditions to which the animals were exposed in the experiment. The mussels were starved for 24 h for determining standard metabolic rates (SMR) and then placed in individual glass chambers (~9 mL) equipped with a sensor spot, filled with 1 μm filtered seawater, and oxygen concentrations were measured every 15 s for 1 h using a Presens Oxy-4 SMA reader. In parallel, identical glass chambers without mussels and with the corresponding seawater conditions, were reared and used as controls. Oxygen concentration declines in the controls never reached above 3%. To avoid potential biases associated with handling, the first 5 min of measurements were not considered, so oxygen consumption estimations included measurements conducted along 55 min. Standard O_2 concentration slopes were estimated from the decline in oxygen concentrations in the chambers, normalizing with the buoyant weight of the mussels (mg O_2 $L^{-1}g^{-1}h^{-1}$).

To quantify byssus thread production, at the end of the experiment the five mussels from each replicate and treatment were placed in petri dishes. The mussels were placed at equidistant distances from each other, on their right valve, and were semi-immersed in seawater with the same conditions they were exposed to during the experiment. After 24 h, the petri dishes were placed under a stereomicroscope and the number of byssus threads was counted. For each Petri dish (i.e., each replicate with five mussels), an average of the number of byssus threads was calculated). Only attached byssus threads were considered in the count and were standardized by the number of mussels per dish and time. Although alternative methodologies have more throughoutly examined byssus thread production (i.e., dissecting length, diameter or strength; see for instance Li et al., 2015; Sui et al., 2017), our rather simple methodology has been used successfully before to measure thread production in this species (Manríquez et al., 2021).

2.6. Statistical analyses

The seven response variables measured in purple mussels (growth in length, width, and weight, in addition to calcification, clearance, metabolic and byssus thread production rates), were analyzed using separate two-way ANOVAs examining the influence of two explanatory variables: pCO_2 (500 versus 1500 ppm), predator cues (absence versus presence), and their interaction. Assumptions of normality and equal variance were examined in each analysis using the Anderson-Darling and the Levene's tests, respectively (no significant violations were detected). The analysis of mussel mortality rates was deemed unnecessary given the complete lack of mortality across treatments and replicates.

3. Results

3.1. Water characteristics and survival of purple mussels

Seawater parameters are summarized in Table 1. In average (±SD), pH values were 7.898 (0.03) and 7.620 (0.03) for pCO_2 equivalent to 500 and 1500 ppm, respectively. The seawater was under-saturated only with respect to aragonite in the high CO_2 treatment (1500 ppm, Table 1). Across the four treatments, survival of purple mussels was not affected by neither explanatory variable (no mussel mortality was recorded).

3.2. Growth rate

Mussel length growth was significantly associated with the presence of predator cues and pCO_2 conditions (two-way ANOVA $p < 0.01$ and $p = 0.022$, respectively), without a significant interaction between these factors (two-way ANOVA $p = 0.122$) (Table 2). In the absence of predator cues (noPC), growth rates nearly doubled the ones recorded when those cues were present (PC; Fig. 1A). Those growth rates increased in high pCO_2 conditions, particularly in the absence of predator cues (~60% increase in growth with respect to current pCO_2) (Fig. 1A). Growth in mussel width was only related with the presence of predator cues (two-way ANOVA $p = 0.04$; Table 2). As with shell length, width growth rates were twice higher in the absence of predator cues (noPC) than in their presence (PC), irrespective of pCO_2 conditions (Fig. 1B). Lastly, growth in mussel weight was less variable and was not significantly related to either predator cues or pCO_2, or the interaction of these factors (two-way ANOVAs $p \geq 0.448$; Table 2; Fig. 1C).

Table 2
Summary of two-way ANOVAs, showing the influence of pCO_2, predator cuesand their interaction on biological responses measured in *Perumytilus purpuratus*. DF: Degrees of freedom, MS: Mean squares. Significant p-values are presented in bold.

Response variable	Source of Variation	DF	MS	F	p
Length growth	pCO_2	1	0.0254	5.93	**0.022**
	Predator cues - PC	1	0.0600	14.001	**< 0.001**
	pCO_2 × PC	1	0.0109	2.537	0.122
	Error	28	0.0043		
Width growth	pCO_2	1	0.000164	0.114	0.738
	Predator cues - PC	1	0.006670	4.645	**0.040**
	pCO_2 × PC	1	0.000006	0.00429	0.948
	Error	27	0.001440		
Weight growth	pCO_2	1	0.0000517	0.592	0.448
	Predator cues - PC	1	0.0000092	0.106	0.748
	pCO_2 × PC	1	0.0000074	0.0842	0.774
	Error	27	0.0000873		
Calcification rate	pCO_2	1	0.0000031	1.12	0.300
	Predator cues - PC	1	0.0000058	2.135	0.156
	pCO_2 × PC	1	0.0000192	7.028	**0.013**
	Error	26	0.0000027		
Clearance rate	pCO_2	1	6.43E-07	5.404	**0.029**
	Predator cues - PC	1	2.76E-08	0.232	0.634
	pCO_2 × PC	1	4.87E-08	0.409	0.529
	Error	24	1.19E-07		
Metabolic rate	pCO_2	1	0.00314	0.239	0.629
	Predator cues - PC	1	0.02670	2.034	0.167
	pCO_2 × PC	1	0.00211	0.161	0.692
	Error	23	0.01310		
Byssal threads	pCO_2	1	10.580	8.442	**0.007**
	Predator cues - PC	1	0.405	0.323	0.574
	pCO_2 × PC	1	8.405	6.707	**0.015**
	Error	28	1.253		

3.3. Calcification and clearance rates

Mussel net calcification rates were neither affected by pCO_2 or predator cues (two-way ANOVA $p = 0.300$ and 0.155, respectively), but they were significantly associated with the interaction between these two factors (two-way ANOVA $p = 0.013$) (Table 2; Fig. 2A). Calcification rates measured at current pCO_2 conditions (~0.4–0.5 mg d^{-1} in average), either increased or declined at projected (1500 ppm) pCO_2 conditions, depending on the absence (no PC) or presence of predator cues (PC) in the water, respectively (Fig. 2A). In fact, at 1500 ppm, the difference between calcification rates measured in the absence and presence of predator cues increased considerably.

Purple mussel clearance rates were affected exclusively by pCO_2 conditions (two-way ANOVA $p = 0.029$; Table 2). A significant increase in those clearance rates (>40%) was recorded at 1500 ppm, regardless of the presence of predator cues (Fig. 2B).

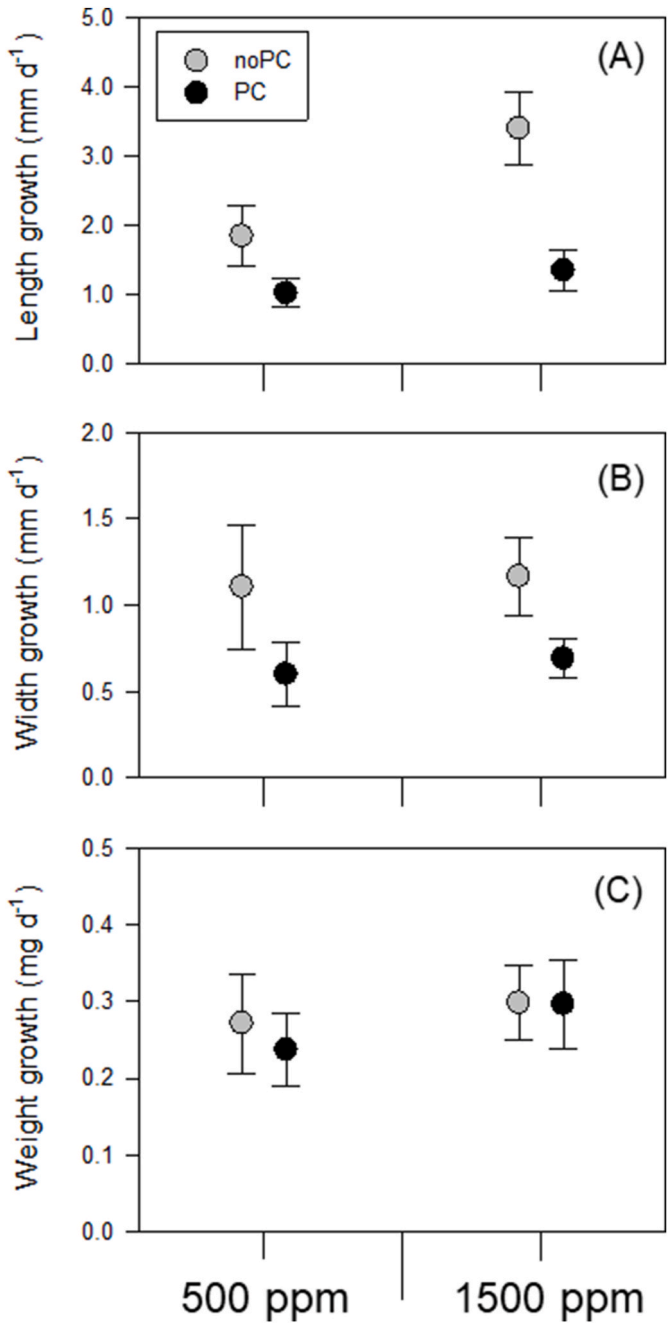


Fig. 1. Mean (+/− SE) purple mussel growth rates in (A) shell length, (B) shell width, and (C) body weight, in the absence (noPC) and presence of predator cues (PC), under current (500 ppm) or projected levels of pCO_2 (1500 ppm). Two-way ANOVAs detected a significant ($p < 0.05$) effect of pCO_2 and predator cues in (A), and predator cues alone in (B).

3.4. Metabolic and byssus thread production rates

Metabolic rates were variable (in average ~0.3–0.65 mg O_2 $g^{-1}h^{-1}$), but were not significantly related to pCO_2, the presence of predator cues or the interaction of these factors (two-way ANOVAS $p \geq 0.167$; Table 2; Fig. 3A). By contrast, the rate of production of byssus threads was affected by pCO_2 (two-way ANOVA $p = 0.007$) and by the interaction between pCO_2 and predator cues (two-way ANOVA $p = 0.015$) (Table 2). In the absence of predator cues (noPC), byssus threads production more than double at 1500 ppm with respect to 500 ppm; meanwhile, in the presence of predator cues (PC), thread production rates remained nearly unchanged between the two pCO_2 conditions (Fig. 3B).

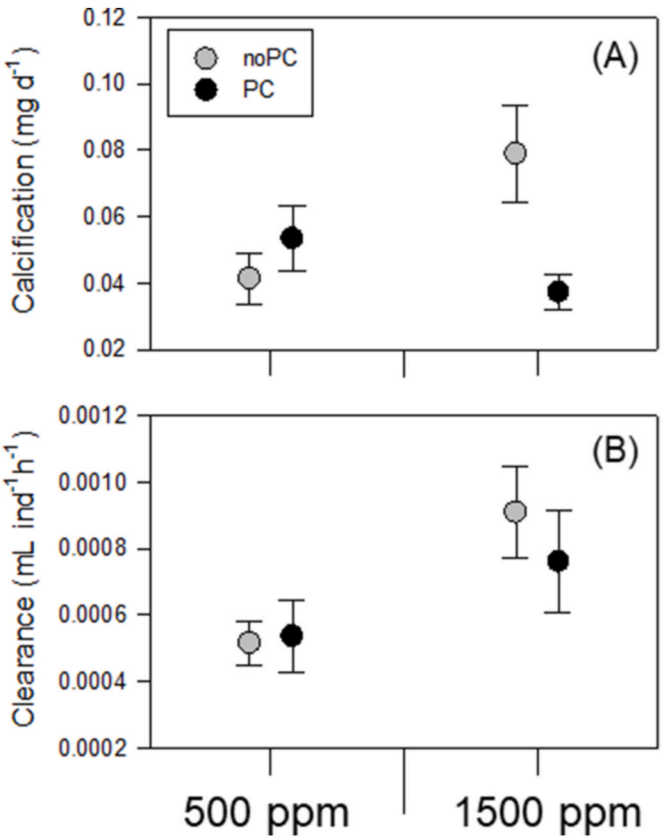


Fig. 2. Mean (+/− SE) purple mussel calcification (A) and clearance rates (B), in the absence (noPC) and presence of predator cues (PC), under current (500 ppm) and projected levels of pCO_2 (1500 ppm). Two-way ANOVAs detected a significant ($p < 0.05$) effect of the interaction between factors in (A), and pCO_2 alone in (B).

4. Discussion

There is a pressing need for studies assessing the effects of both natural and anthropogenic stressors on marine organisms (Carrier-Belleau et al., 2021), including but not restricted to OA, and for research disentangling their individual and combined influences (Poore et al., 2013; Duarte et al., 2016; Manríquez et al., 2017, 2020). This study focused on the purple mussel response to the risk of predation by one of its main consumers (*A. monodon*; Moreno, 1995; Soto et al., 2004) and to a global stressor, OA (Gazeau et al., 2013). Over the course of 60 days, the survival of purple mussels was not affected, suggesting that in the conditions recreated in our mesocosm, natural (predator cues) and anthropogenic (OA) stressors only had chronic, in some cases severe, but never lethal effects on this species. However, at a regional scale, it is undeniable that OA may have an impact on the survival of many species (e.g., Busch and McElhany, 2016). The absence of mortality is likely related to the plasticity of purple mussel populations, most of which are intertidal, and as such, naturally exposed to a wide range of variation in seawater carbonate parameters (see Duarte et al., 2015; Vargas et al., 2017). Predation risk influenced some morphological traits, whereas pCO_2 levels and the interaction between both factors accounted for the variation observed in length growth, clearance rates and byssal production.

4.1. Purple mussel variation in growth

The presence of predator cues, used here as a proxy of mortality risk due to predation (Cheung et al., 2009; Johnston et al., 2012), had a significant influence on mussel size (growth in width and length) but not

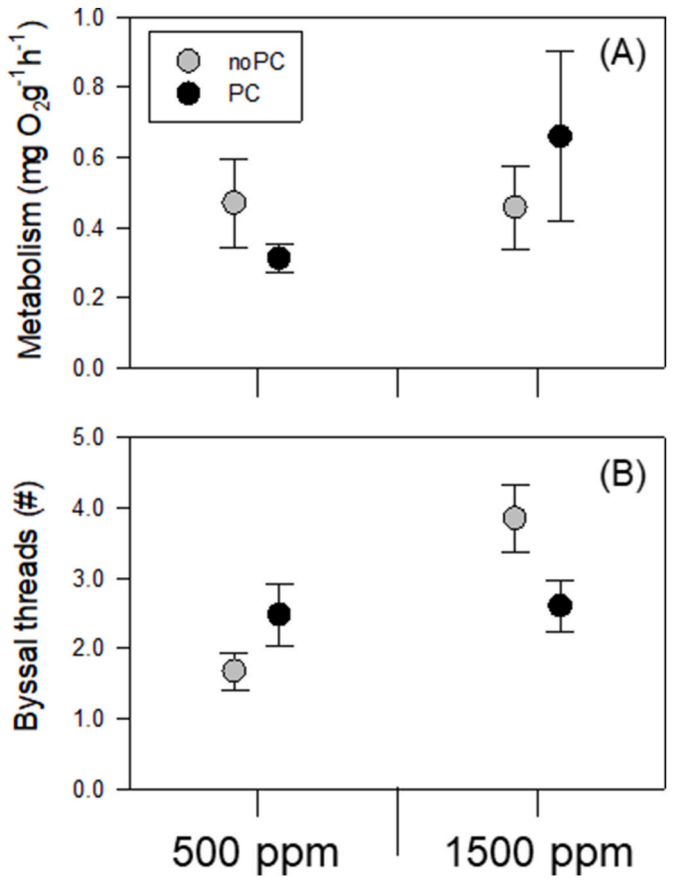


Fig. 3. Mean (+/− SE) purple mussel metabolic (A) and byssus thread production rates (B), in the absence (noPC) and presence of predator cues (PC), under current (500 ppm) and projected levels of pCO_2 (1500 ppm). The two-way ANOVAs detected significant ($p < 0.05$) effects of pCO_2 and the interaction between factors in (B).

weight. In comparison to control conditions, growth in size was significantly lower when *A. monodon* cues were present in the water. This was anticipated considering that prey species face a tradeoff between the use of energy and resources towards growth versus defense from predators (e.g., Harter and Heck, 2006). In this case, it is clear that the perceived risk of predation was detrimental to the increase in mussel size, which did not occur in the absence of that risk. Predator cues did not significantly interact with pCO_2 levels in any of the measurements of growth. However, in the particular case of shell length, pCO_2 levels were also influential and caused an increase in length at high levels of OA. These results were unexpected, as the decrease in carbonates that is typically associated with OA, most often reduces rather than increases, growth rates (Michaelidis et al., 2005; Gazeau et al., 2013; Duarte et al., 2015; Grenier et al., 2020). Hence, these results also broaden up the range of changes reported in the literature as a result of exposure to OA.

It is unclear why purple mussels would grow longer when exposed to OA than in current (natural) conditions. However, this might be related to an increase in their feeding rates, which we could speculate, might be partially reflected in the higher clearance rates measured at 1500 ppm (see below). We did not measure feeding rates, but if we venture to correlate feeding and clearance rates, our results are in line with those reported for other populations of purple mussels from coastal systems unaffected by estuarine runoffs (Saavedra et al., 2017), and other species of bivalves such as the Akoya pearl oyster (*Pinctada fucata*) from southern China (Liu and He, 2012). In both cases, those authors found that feeding rates or clearance rates increased at higher $pCO2$ levels. That is also in line with our own measurements of other mollusks from the same collection site, for which we detected higher feeding and growth rates when exposed to OA conditions (Duarte et al., Unpublished Data). Another intriguing result was that OA only influenced the purple mussel's length, without significantly affecting its width and weight; in other words, OA made purple mussels longer but not wider or heavier. Although purple mussels exhibit considerable phenotypic plasticity (Briones et al., 2014; Manríquez et al., 2021), our experiments only lasted 60 d, so we remain cautious and only suggest that further studies should explore such potential short-term effects of OA on this and other species.

4.2. Calcification and clearance rates

At current pCO_2 conditions (500 ppm), calcification rates showed the exact opposite pattern than growth. This is consistent with the general tradeoff identified above and suggests that while growth in size is lower in the presence of predator cues, calcification rates should be expected to be higher. In the light of these results, we can link higher calcification rates with potential shell thickening. This is a well-known direct response of prey to prevent or reduce predator's effects (e.g., Amaral et al., 2013; Briones et al., 2014). Examples showing shell thickening in response to predators include among others the mussels *Mytilus edulis* and *M. trossulus* in the presence of the carnivorous snail *Nucella lapillus* (Sherker et al., 2017), and the common periwinkle (*Littorina littorea*) in the presence of blue crabs (*Callinectes sapidus*) (Bibby et al., 2007). Higher calcification rates in response to predator cues were also found in a prior study conducted with the northernmost populations of purple mussels (Manríquez et al., 2021). Ultimately, mussel calcification rates measured in our experiment were driven by a significant interaction between predator cues and pCO_2 levels. In fact, at high pCO_2 levels, the apparent influence of predation risk switched, and calcification levels were higher in the absence of predator cues. Such increase in calcification at high pCO_2 levels was unexpected, as in general severe acidification is often detrimental to the thickening or strengthening of shells (Bibby et al., 2007; Amaral et al., 2013; Kroeker et al., 2014). However, it might be related to one of the various mechanisms used by marine organisms to cope with acidification (Rodolfo-Metalpa et al., 2011; Hendriks et al., 2015; Ramajo et al., 2016). For example, some species regulate the acid-base condition in their extra-pallial fluid in order to cope with a decrease in pH, avoiding the negative effects of ocean acidification on shell production (Gutowska et al., 2010; Hendriks et al., 2015). Further studies may shed light into the specific mechanisms involved in the response documented here.

Our results show that purple mussel clearance rates were driven solely by pCO_2 levels, without an apparent influence by predator cues. We interpret the higher clearance rates measured in the mussels at high projected acidification levels (1500 ppm) as a response to cope with the stress associated with OA. As indicated above, clearance rates have often been associated with food consumption rates and consequently, with the gathering of energy reserves (and in our experiment, with mussel growth in length). Such energy may confer marine organisms the ability to cope and resist the stress caused by OA (Melzner et al., 2011; Thomsen et al., 2013; Ramajo et al., 2016). However, it is important to mention that clearance rates are highly variable in response to pCO_2 levels (Navarro et al., 2013; Range et al., 2014; Wang et al., 2015; Saavedra et al., 2017). For example, unlike the results reported here, OA caused a reduction in clearance rates in Chilean mussels (*Mytilus chilensis*) (Navarro et al., 2013, 2016, 2016). Distinct responses have been also reported for mollusks from the southern coast of China (Liu and He, 2012). Among other variables, these authors measured clearance rates in three different species in response to various levels of acidification. Similar to our results, they found an increase in clearance rates in response to acidification in an oyster (*Pinctada fucata*), but opposite responses in the scallop and the mussel they studied (Liu and He, 2012). We mirror their interpretation and suggest wide variability in the ability of different species to cope or adapt to OA. Based on several other studies measuring the influence of predators on prey's clearance rates (e.g., Kulakovskii

and Lezin, 2002; Naddafi et al., 2007; Naddafi and Rudstam, 2013), *A. monodon* cues were expected to have an influence on purple mussels. However, in our study the influence of pCO_2 clearly prevailed over *A. monodon*'s cues.

4.3. Metabolic rates and byssus production

Oxygen consumption rates have been traditionally used as indicators of metabolic rates (Pörtner, 2002) and, surprisingly, they were affected by neither predator cues nor ocean acidification. Most literature so far shows that a rise in metabolic rates in prey species, is part of the "fight or flight response" used to escape predators (see Slos and Stoks, 2008; Beckerman et al., 2007). Although purple mussels are a well-known prey of *A. monodon* (Moreno, 1995; Soto et al., 2004), cues from this predator did not elicit a strong response at the metabolic level. We can only argue that such a response might have been more evident from the exposure to larger and more mobile predatory species, including upper-trophic level snails (*Concholepas concholepas*) or shell-crushing crabs (*Acanthocyclus hassleri*) (Manríquez et al., 2021). Similarly, changes in metabolic rates have been linked before to ocean acidification (Navarro et al., 2016; Manríquez et al., 2020), although the direction of such changes is less clear, even in our own prior research. Some studies have found that a rise in pCO_2 reduces metabolic rates in *Mytilus coruscus* (Wang et al., 2015) and *M. chilensis* (Navarro et al., 2016). Meanwhile, others have found that it increases metabolic rates in other purple mussel populations (Manríquez et al., 2021), or influences metabolic rates but only when combined with substantial salinity changes (Duarte et al., 2018). We stand on the side of caution and, given that the response of purple mussels at the metabolic level is rather complex, we call for further research addressing thoroughly metabolic changes.

Byssus threads enable mussels to attach to any hard surface available, including conspecific neighbors' shells, normally reducing potential predation risks (Brown et al., 2011; de Jager et al., 2017). In current (natural) conditions, byssus thread production seemed to reflect that, with a higher average of threads being measured in the presence of predator cues. This is in line with previous studies on other populations of purple mussel as well as populations of the dwarf mussel (*Semimytilus algosus*), which in the presence of predation risk by the crab *Acanthocylus gayi,* enhanced their byssus production (Caro et al., 2008). However, the significant interaction between predator cues and pCO_2 dictated a reversed pattern at high pCO_2 levels: considerably higher byssus thread production was only evident in the absence of predator cues. A significant influence of pCO_2 on the biogenesis of byssus has been reported before (Seguin-Heine et al., 2014; Babarro et al., 2018; Zhao et al., 2017). However, all those studies concluded that OA had a negative effect on byssus production, i.e., a reduction in the amount, length or strength of the byssus produced, which entails a decline in the ability of these species to firmly anchor themselves to the substrate. Again, the interaction of predator cues and pCO_2 levels did have a significant influence on the production of byssus threads in purple mussels. However, their effects were not additive or synergic, and the overall outcome with regards to pCO_2 effects was counterintuitive.

5. Conclusions

This study shows that the response of purple mussels to predator cues and projected levels of ocean acidification vary considerably. As expected, predator cues were detrimental to the mussels' growth in size, and add to the growing body of literature highlighting the importance of trait mediated interactions (e.g., Sherker et al., 2017; Jellison and Gaylord, 2019; Catalán et al., 2021). Meanwhile, projected levels of ocean acidification unexpectedly enhanced both mussel growth in length and clearance rates. Although we didn't measure mussel feeding rates, a potential link between clearance rates and food consumption as described above, is a plausible explanation for the unexpected increase in growth rates under OA exposure. Similar responses have been found in a few prior studies (e.g., Wang et al., 2015), but there is no doubt that most research continues to associate OA with negative effects on the behaviour of physiology of mollusks (see review by Wang and Wang, 2020; Doney et al., 2020). Exposure to OA also modulated (interacted with) the effects of predator cues on the outcome of two other traits: shell calcification and byssus thread production rates. The interaction between the cues of a fairly small predator (*A. monodon*) and OA were complex and counterintuitive at times, likely reflecting the outcome of most interactions between natural and anthropogenic stressors.

Considering the importance of purple mussels in rocky shore community assembly, i.e., their role as one of the competitive dominant species and as an ecosystem engineer (Prado and Castilla, 2006; Firth and Crowe, 2010; Valdivia et al., 2021), the finding of expected and unexpected responses warrants further studies. Similarly, research on populations from locations unexposed to upwelling conditions, or habitats with narrower ranges of natural variation than the intertidal (e.g., subtidal populations) may shed additional light onto the changes to be expected for the full distributional scope of purple mussels. Additional traits, potentially more sensitive than metabolic rates, may also be explored, including valve-gaping (Smee and Weissburg, 2006; Robson et al., 2007) and heart beats frequency (Zittier et al., 2015), among others. The complex response of purple mussels to natural and anthropogenic stressors such as ocean acidification (Widdicombe and Spicer, 2008; Sunday et al., 2011; Duarte et al., 2015; Vargas et al., 2017), suggest further research is necessary to narrow down the most likely future scenarios.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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