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Inducible defenses in an estuarine bivalve do not alter predator handling times and are not affected by climate change

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ABSTRACT: Mussels use their shells for protection which they can thicken or grow in response to predator cues, commonly referred to as an inducible defense. Oceans are experiencing elevated $p\mathrm{CO}_2$ due to climate change. Elevated $p\mathrm{CO}_2$ can have negative effects on bivalve morphology and physiology, but we are still learning about the consequences of these effects on predator–prey interactions, a key motivation of this study. Using a 4 wk (short-term) laboratory experiment, we orthogonally manipulated 2 levels of $p\mathrm{CO}_2$ (ambient or elevated to predicted future conditions that mimicked diel variability) and 2 levels of predator presence (absent or present) of blue crabs *Callinectes sapidus* to determine their effects on the morphology and predator handling times on southern ribbed mussels *Geukensia granosissima*. Experimental results indicated that shell length and width increased in mussels in response to the predator cues, and these inducible defenses were not affected by elevated $p\mathrm{CO}_2$. Unexpectedly, mussels exposed to elevated $p\mathrm{CO}_2$ exhibited greater growth in shell depth independent of the predator treatment, resulting in shells with rounder shapes. These effects on mussel morphometrics did not affect average crab handling times, but mussels exposed to the presence of a predator under elevated $p\mathrm{CO}_2$ conditions had highly variable handling times. This work highlights the complexity of animal physiology, morphology, and interspecific interactions on predator–prey relationships in a changing ocean.

KEY WORDS: Ocean acidification · Predator–prey relationships · Predation cues · Environmental stressors · Abiotic stressors · Stress response

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1. INTRODUCTION

Predator–prey interactions are complex and can be affected by both biotic and abiotic conditions (Connell 1961, Hughes & Seed 1981, Blundon & Kennedy 1982, Kishida et al. 2010, Gestoso et al. 2015). In addition to behavioral responses to predators such as hiding, migration, and habitat changes (Bollens & Frost 1991, Zamzow et al. 2010, Parsons et al. 2018), prey can exhibit morphological plasticity to reduce predation risk. In bivalves, this inducible defense can result in thickened shells (Caro & Castilla 2004) that may increase handling times of their predators (Sherker et al. 2017), further allowing them to outgrow vulnerable sizes of predation (Elner & Hughes 1978). Thus, inducible defenses are an important component of predator–prey interactions (Kishida et al. 2010). However, abiotic stressors in the physical environment can have negative effects on an organism's ability

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to respond to predation threats and form their inducible defenses. For example, elevated pCO_2 and associated changes in water chemistry caused by ocean acidification (e.g. pH, saturation state, dissolved inorganic carbon [DIC]; IPCC 2014) can be detrimental to calcareous organisms since it causes dissolution of the current calcareous structure (e.g. shell) and disrupts the formation of new calcified material needed for inducible defenses.

Ocean acidification is having complicated and variable effects on calcareous prey. Elevated pCO_2 and changes in water chemistry have been shown to decrease calcification, dissolve calcareous structures, and affect energy expenditure (Guinotte & Fabry 2008, Mostofa et al. 2016). For example, corals invested more energy for calcification to prevent decreased growth and skeletal density when exposed to elevated pCO_2 (Hoegh-Guldberg et al. 2007). As a consequence, they had less energy to heal wounds inflicted by corallivorous fishes, potentially leading to further population declines (Rice et al. 2019). Similarly, bivalves exposed to elevated pCO_2 experienced decreased growth (Berge et al. 2006), thickness (Berge et al. 2006, Melzner et al. 2011), and calcification rates (Gazeau et al. 2007) of their shells as well as reductions in byssal strength and attachment (Sui et al. 2015, Kong et al. 2019). Consequently, oysters exposed to elevated pCO_2 were more susceptible to predation by invasive snails due to a reduction in shell size (Sanford et al. 2014). Despite the examples of negative effects of elevated pCO_2 on some calcareous organisms, responses are equivocal, with evidence of both null effects and the ability to adapt to future ocean acidification conditions. For example, shell strength of *Mytilus edulis* was not affected by lowered pH (Mackenzie et al. 2014), suggesting shell-building processes were not compromised due to ocean acidification. Leung et al. (2020) found that snails had greater shell strength and integrity under future ocean acidification conditions due to more organic matter being incorporated into the shell. These results demonstrate how some organisms may be able to adapt to future ocean acidification conditions by modifying their shell structure (Fitzer et al. 2015a,b). The negative effects of elevated pCO_2 could ultimately disrupt an organism's ability to allocate energetic resources to developing inducible defenses (Trussell & Nicklin 2002, Kroeker et al. 2014a), thus making them more susceptible to predation by reducing predator handling times (Amaral et al. 2012). The consequences of the effects of elevated pCO_2 on predator–prey interactions remain unclear due to their complexity, limited and equivocal research, and theoretical predictions for interspecies relations (Gazeau et al. 2013, Kroeker et al. 2014b). Furthermore, theoretical predictions and even empirical evidence for how future ocean conditions may affect predator–prey interactions often come from studies that maintain static environmental conditions of elevated pCO_2, which may not fully capture the environmental and ecological reality for many organisms living in dynamic coastal habitats.

Estuarine environments are dynamic, with fluctuating abiotic conditions (e.g. temperature, salinity, pH, pCO_2) often requiring organisms to have a high tolerance for extreme conditions. For example, *Etheostoma spectabile* from habitats with highly fluctuating temperatures were found to have a higher thermal tolerance than individuals from stable habitats (Feminella & Matthews 1984). Conversely, estuarine organisms commonly exist at the edges of their abiotic tolerance thresholds and can be sensitive to extreme changes (Connell 1972, Somero 2002, Davenport & Davenport 2005) such as fluctuating pCO_2 conditions (Mangan et al. 2017). Therefore, some estuarine organisms are already living near or at their physiological tolerance limits (Somero 2010), which could hinder their ability to form inducible defenses. This potential sensitivity of estuarine organisms to extreme environmental conditions presents a challenge as ocean chemistry undergoes significant changes due to climate change-induced increased pCO_2 (Meehl et al. 2007). This effect is especially pronounced for sessile organisms, such as bivalves, due to their inability to move from unfavorable conditions (Nicholson 2002). The variability of estuarine habitats could make it challenging for some organisms to adapt to additional stressors such as elevated $pCO_{2.}$

The present study was designed to investigate the morphological responses of an estuarine bivalve to 2 simultaneous stressors: elevated pCO_2 and the presence of waterborne predation cues. Based on results from previous research (Bibby et al. 2007, Kroeker et al. 2014b), we predicted that the bivalve prey would develop inducible defenses in the form of shell growth in the presence of predation cues, but that this effect would be disrupted in elevated pCO_2 conditions. Specifically, we tested whether (1) bivalve growth and morphology was affected by elevated pCO_2 and the presence of waterborne predation cues, and (2) any effects of elevated pCO_2 and waterborne predation cues on growth and bivalve morphology led to differences in handling times by predatory crabs.

2. MATERIALS AND METHODS

2.1. Study organism and ecosystem setting

We focused on the southern ribbed mussel *Geukensia granosissima*, a common bivalve often associated with oyster-reef intertidal and estuarine habitats in the eastern Gulf of Mexico. The estuarine habitats where ribbed mussels are found are typically dynamic in abiotic conditions, including background pCO$_2$ levels (Maher et al. 2015), and the effects of predicted elevated pCO$_2$ on their inducible defenses remain unclear. Ribbed mussels in these habitats are consumed by many predators, including the shell-crushing blue crab *Callinectes sapidus* (Peterson et al. 2003). For our experimental trials, we collected southern ribbed mussels (mean ± SD shell length: 17.38 ± 1.46 mm, n = 140) from intertidal habitats in Tampa Bay, Florida, USA, and live blue crabs (n = 32; n = 8 for cues study, n = 24 for handling study, more details in Section 2.2) were obtained from local fishermen and transported to the lab <24 h after capture.

2.2. Laboratory setup

We maintained southern ribbed mussels in 1 of 4 orthogonal pCO$_2$ × predator treatments: 400 ppm (present-day pCO$_2$) or 600 ppm above ambient (i.e. target of 1000 ppm as the predicted pCO$_2$ for the year 2100; Meehl et al. 2007, Sokolov et al. 2009, IPCC 2014), and the presence (P$^+$) or absence (P$^-$) of waterborne chemical cues from predatory blue crabs (mean ± SD carapace width: 134 ± 5 mm, n = 8), for a 4 wk period. Experimental exposure time in ocean acidification research on marine invertebrates has been highly variable, ranging from hours to months (reviewed by Wang & Wang 2020). The 4 wk period we chose has been previously demonstrated to be an appropriate timeframe to observe the effects of ocean acidification and waterborne predation cues on mussel morphology (e.g. Berge et al. 2006, Kroeker et al. 2014a, Gestoso et al. 2016), with some studies observing effects in as little as 20 d (Duarte et al. 2015). Mussels were housed in an outdoor experimental pCO$_2$ system at the Climate Change Laboratory, University of Miami in Florida, USA. We followed the standard operating procedure for the design of the experimental system as described in Riebesell et al. (2011). The design of the system consisted of 1-way flow-through glass aquaria within a mesocosm (Fig. 1). From the sump, water was pumped to the primary tanks which held the crabs for the predator experiments and gravity-fed to the secondary tanks in the system which held the mussels. From the secondary tanks, water overflowed into a water bath that buffered temperature fluctuations (e.g. Freytes-Ortiz & Stallings 2018). We maintained the system at a targeted temperate of 30°C (mean ± SE: 29.8 ± 0.13°C), which did not differ among treatments (ANOVA, $F_{3,76}$ = 0.74, p = 0.53). The resulting system contained a total of 4 primary and 4 secondary tank replicates per treatment. The separation of the crabs and mussels allowed for the predator chemical cues to reach the mussels without direct interaction between crabs and mussels. Mussels exposed to experimental conditions were frozen directly after we took the last live growth measurements and subsequently oven-dried to determine

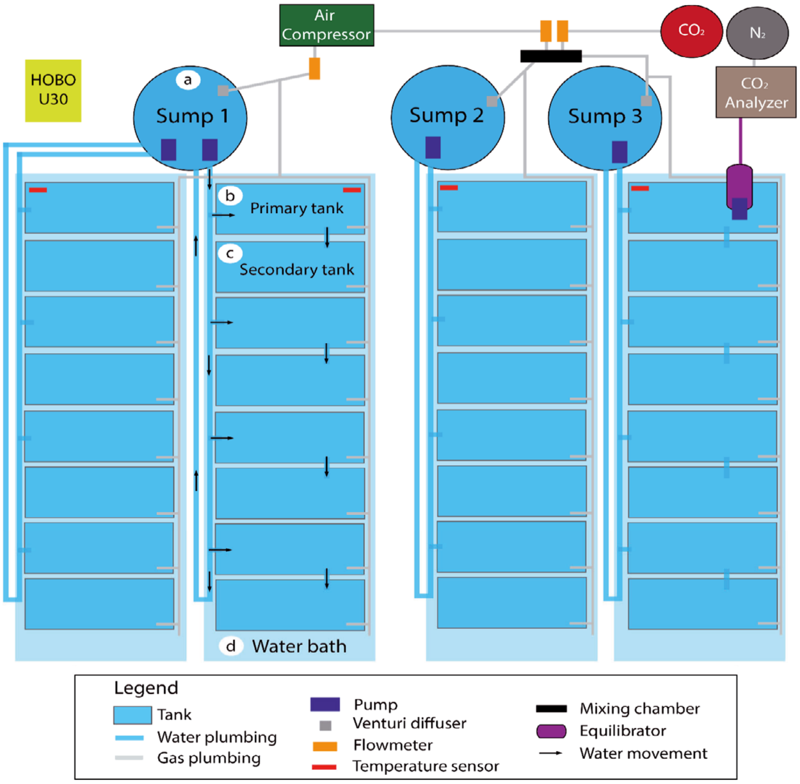

Fig. 1. Detailed schematic of ocean acidification experimental system. Letters represent the flow-through trajectory of water from the sump (a) to the water bath (d)

soft tissue and shell weight growth (see Section 2.4). We maintained a second group of mussels in the same experimental conditions for the predator handling time experiments (see Section 2.5). Each experimental tank housed a total of 24 mussels, 10 of which were monitored for the growth experiment, and 14 were included in the handling time experiment. No mussel or crab specimens were used for both experiments, to ensure independence.

Natural seawater for the experimental system was supplied and filtered at 10 µm from intakes near Bear Cut, Key Biscayne, Florida, USA. Blue crabs are rarely observed near the water intake pipe (i.e. fewer than 6 have been observed in the past 10 yr, P. Glynn & P. Gillette pers. comm.). Thus, any presence of these predator cues would have been extremely low in concentration and any uncontrolled external cues should have been consistent across experimental treatments. Due to the flow rate of the system, we did not anticipate the occasional presence of diluted predator cues to influence our results.

Control tanks were bubbled with ambient air directly from an oil-free air compressor. Sumps in the elevated $p\mathrm{CO}_2$ treatments were bubbled through Venturi diffusers with a mixture of ambient air and pure CO_2 gas, regulated by adjusting mass flow controllers (Sierra Instruments 810C). The experimental tanks were open to gas exchange with the atmosphere; therefore, they were also aerated with the appropriate gas mixture to maintain the targeted $p\mathrm{CO}_2$ levels. CO_2 concentrations were monitored in the experimental tanks in real time using a showerhead equilibrator/Licor (LI-820) CO_2 analyzer system, calibrated against a zero and a 700 ppm span gas at the beginning of the experimental period. The partial pressure of CO_2 in the equilibrator was logged every 5 min by a HOBO U30 data acquisition system. Experimental tanks were exposed to natural light conditions, attenuated by a neutral density shade cloth, and water flow was maintained at a constant rate of 0.3 l min^{-1}.

It is important to note that this experimental system did not actively control $p\mathrm{CO}_2$ like other systems. This system elevated normally fluctuating $p\mathrm{CO}_2$ levels due to diel cycles by a constant factor, allowing the system's $p\mathrm{CO}_2$ to fluctuate as expected in natural environments in the coming century. Our intention was to capture ecological reality by allowing the $p\mathrm{CO}_2$ levels to fluctuate as they naturally do. Monitored $p\mathrm{CO}_2$ data from this system had similar daily fluctuations in the elevated $p\mathrm{CO}_2$ as in the ambient experimental tanks (see Fig. 2). Therefore, this design successfully mimicked dynamic estuarine conditions which were more ecologically relevant than using an active and static $p\mathrm{CO}_2$-controlled system.

2.3. Chemical and physical parameter measurements

The water chemistry monitoring and reporting followed Riebesell et al. (2011). Temperatures in the experimental tanks were measured and logged every 5 min by the HOBO system. Salinity has low variation at the study site (~0.3 psu with tidal cycles) and was recorded weekly using a YSI Model 30 temperature/salinity meter that was calibrated prior to each use against a 50.0 mS standard solution. To monitor water chemistry, we collected 150 ml water samples each week from experimental tanks at 10:00 h (the time of day when the CO_2 level in the tanks crossed the midpoint between the early morning maximum and the late afternoon minimum), fixed them with 60 µl of saturated HgCl_2, and measured their total alkalinity (TA) and DIC (Table S1 in the Supplement at www.int-res.com/articles/suppl/m679p073_supp.pdf). TA was measured on an automated open-cell Gran titrator (precision: 0.2%) and has minimal diel variation at the study site. The HCl titrant was standardized against certified reference material (Andrew Dickson Lab, Scripps Institution of Oceanography). DIC was measured using a DIC analyzer (Apollo SciTech) and standardized to the same certified reference seawater. We computed the carbonate system parameters $p\mathrm{CO}_2$ and aragonite saturation state (Ω_{Ar}) from the measured temperature, salinity, DIC, and TA using the CO2Sys Macros in Excel (version 2.1, 18 September 2012).

2.4. Specimen collection and response parameters

Upon collection, we acclimated mussels at ambient $p\mathrm{CO}_2$ for 2 d, after which $p\mathrm{CO}_2$ was increased by 150 ppm every 24 h from ambient until reaching target levels. We fed the mussels 2.1×10^4 cells d^{-1} mussel^{-1} of refrigerated phytoplankton (Shellfish Diet 1800) to standardize food availability across treatments and through time (Kroeker et al. 2014a). The mussels did not receive significant nutrition from plankton in the seawater supply due to intake water filtration (Towle et al. 2015). Homogenate conspecific signals have been shown to induce anti-predator traits in prey (Yamada et al. 1998, Robson et al. 2010); therefore, crabs were fed southern ribbed mussel conspecifics once a day to mimic natural conditions and increase the probability of eliciting induced defenses on mussel morphology.

We calculated southern ribbed mussel growth rates from morphometric traits (i.e. shell length, shell width, and mussel wet weight in air and in water) measured weekly during the 4 wk experimental period. Mussels were removed from their holding tanks to measure their weight in water (buoyant weight) to the nearest 0.001 g, blot-dried for wet weight to the nearest 0.001 g, and shell morphometrics to the nearest 0.01 mm. Mortality was low (percent mortality per treatment: ambient $p\text{CO}_2 \times \text{P}^-$: 7.5%, ambient $p\text{CO}_2 \times \text{P}^+$: 5.0%, elevated $p\text{CO}_2 \times \text{P}^-$: 2.5%, and elevated $p\text{CO}_2 \times \text{P}^+$: 2.5%), and mussels that did not survive the 4 wk period were not included in the analyses. We considered a mussel to be dead when it did not close its valves after being mechanically stimulated.

We calculated mussel shell and soft tissue weight growth rates from changes in their buoyant and wet weights, respectively, verified with dry weight measurements of the shells after the end of the experiment (Palmer 1982). At the end of the growth experiment, we froze the mussels at −80°C and transported them to the College of Marine Science, University of South Florida, St. Petersburg, Florida, USA where they were dried at 60°C for 48 h, weighed, and dried again in a muffle furnace at 500°C for 6 h. We used a regression model to estimate shell and soft tissue weight growth rates using a separate subset of mussels not used for the growth experiment, which varied in shell length between 11.34 and 33.33 mm (mean ± SD shell length: 16.31 ± 3.87 mm, n = 30). Regressions for dry weight vs. wet weight were extremely precise (shell weight linear regression, $r^2 = 0.999$; soft tissue weight linear regression, $r^2 = 0.857$). We used these calculated values for soft tissue and shell weight for all further analyses, since they were significantly and strongly correlated to post-experiment measured values (soft tissue weight linear regression, $r^2 = 0.493$, $F_{2,136} = 134.2$, $p = 0.001$; shell weight linear regression, $r^2 = 0.999$, $F_{2,131} = 17.4$, $p = 0.001$).

2.5. Handling time experiment

After the 4 wk exposure period, we conducted an experiment to determine how any effects of elevated $p\text{CO}_2$ and predation cues on southern ribbed mussel morphology affected their susceptibility to predation. Specifically, we measured handling times of mussels by predatory crabs across the orthogonal experimental treatments. We acclimated blue crabs of appropriate size to consume the mussels (mean ± SD carapace width: 133 ± 8 mm, n = 24) to target $p\text{CO}_2$ levels (either ambient or elevated) for a period of 4 d prior to the feeding experiment. Note that the 24 crabs used in the handling time experiment were independent of the 8 used for the cue responses in the growth experiment. Crabs were fed southern ribbed mussel conspecifics ad libitum for 3 d and starved for 1 d prior to the feeding trial to normalize hunger among test crabs. We placed each crab in separate tanks and allowed them to feed uninterrupted for 1 h on mussels exposed to the experimental treatments. We measured handling times from video that recorded the trials to prevent altered feeding behaviors due to human presence. We measured handling time from the crab's first crushing behavior to when it abandoned the empty shell.

2.6. Statistical analyses

We used non-parametric permutation-based tests using the Fathom Toolbox for Matlab (Jones 2014) because the morphometrics data did not meet the assumptions for parametric analyses. Tests were performed using 5000 permutations of the data and a significance level of $\alpha = 0.05$. We used 3-way non-parametric ANOVA (npANOVA) to test whether individuals in different tanks exhibited different responses independently of the $p\text{CO}_2$ and predator treatments to which they were exposed. Since there was no significant effect of exposure tank on growth of any morphometric, nor were there synergistic effects of tank with any other variable (all p-values > 0.05), individuals within a tank were considered replicates for all further analyses. Two-way npANOVAs were also used to test effects of $p\text{CO}_2$ and predator treatments on the measured carbonate system parameters DIC and TA. We analyzed morphometric growth data using 1-way non-parametric ANCOVA (npANCOVA) in response to the $p\text{CO}_2$ and predator cue treatments, with the initial morphometric measurement as a covariate. Initial values of shell length, width, and depth were directly measured, while those for soft tissue and shell weights were estimated from buoyant and wet weight (see Section 2.4; Palmer 1982). We did not find any significant interactions between the covariate and the 2 levels of either $p\text{CO}_2$ or the predator cue treatments (i.e. the slopes were not different between treatment levels), so we report only the main effects on growth of each morphometric. Lastly, we tested the effects of $p\text{CO}_2$ and waterborne predator cues on crab handling times using a generalized linear mixed model (GLMM), with crab identity included as a random variable. The GLMM was performed using the *nlme* package (Pinheiro et al. 2021) in the R statistical language (R Core Team 2021, version 4.1.0).

We conducted a power analysis after the experiments to determine if the sample sizes were large enough for the growth morphometrics and the handling time experiments. Using an $\alpha = 0.05$, we found the power was 0.75 for the growth experiment and 0.89 for the handling experiment (R Core Team 2021, version 4.1.0), thus sample sizes were deemed sufficient to avoid Type II errors.

3. RESULTS

3.1. Experimental system

The experimental system successfully maintained target $p\text{CO}_2$ levels throughout the study (Table S2), with no significant differences across the control and predator-present treatments (Table S3). Saturation differed strongly between target $p\text{CO}_2$ levels for the duration of the study (Fig. S1). As expected, TA did not differ across treatments (Fig. S2), while DIC was significantly higher in the elevated $p\text{CO}_2$ than the ambient treatment (Fig. 2). No observed difference in TA across treatments provided evidence that observed differences in $p\text{CO}_2$ and Ω_{Ar} were driven by differences in DIC. Rather than static conditions, this experimental system was designed to expose mussels to more ecologically relevant variable $p\text{CO}_2$ conditions, which it did successfully (Fig. 2).

3.2. Mussel growth response

Southern ribbed mussels exposed to the presence of a predator exhibited significantly higher growth rates in shell length and shell width than those in the control treatment, independent of the $p\text{CO}_2$ treatment (Table 1; Figs. S3 & S4). Mussels had increased shell depth in elevated $p\text{CO}_2$ conditions compared to ambient $p\text{CO}_2$ conditions (Table 1; Fig. S5). Growth of soft tissue was slightly higher for mussels exposed to predators, and neither experimental treatment affected shell weight (Table 1; Figs. S6 & S7).

3.3. Handling time experiment

Average handling times for crabs on mussels exposed to the presence of predator cues did not differ from those exposed to control conditions, for either ambient or elevated $p\text{CO}_2$ treatments ($t_{21} = 0.44$, $p = 0.67$; Fig. 3). However, under elevated $p\text{CO}_2$ conditions, handling times were significantly more variable for mussels exposed to the presence of a predator than those in the control group (dispersion $F_{1,66} = 4.24$, dispersion $p = 0.042$). Under elevated $p\text{CO}_2$ conditions, handling times for mussels exposed to predators ranged from 32 to 334 s, compared to 38 to 213 s for the predator-free treatment (Fig. 3).

4. DISCUSSION

Our results indicate that inducible defenses of adult southern ribbed mussels *Geukensia granosissima* were not affected by exposure to elevated $p\text{CO}_2$. Both length and width of mussel shells increased when exposed to predators, regardless of $p\text{CO}_2$ level. Interestingly, growth in shell depth was higher for mussels exposed to elevated $p\text{CO}_2$ conditions. Although these effects on mussel morphometrics did not affect mean crab handling times, they were more variable for mussels exposed to the presence of a predator under elevated $p\text{CO}_2$ conditions.

Southern ribbed mussels exhibited greater growth rates in both shell length and width in the presence of predation cues compared to the control, independent of $p\text{CO}_2$ treatment. Increased shell growth was expected under at least the ambient $p\text{CO}_2$ conditions, as mussels use this as an inducible defense against the threat of predation (Leonard et al. 1999, Smith & Jennings 2000, Caro & Castilla 2004, Freeman 2007). In their studies, both Freeman (2007) and Smith & Jennings (2000) reported that mussels thickened their shells in response to cues from crab predators, but linear shell growth was not affected. We observed the opposite effect; increased shell length and width were not accompanied by increased shell weight. Although we did not measure shell thickness or shell crushing force in the present study, growth in shell weight is positively related to shell thickness (Reimer & Tedengren 1996, Frandsen & Dolmer 2002) and shell thickness is positively correlated to a mussel's ability to withstand crushing force (Leonard et al. 1999). This indicates thicker shells are less likely to be consumed by predatory crabs (Palmer 1985). Therefore, these mussels would have been just as vulnerable to predation as those not exposed to predator cues, consistent with our results from the handling time experiments.

Changes in both shape and structural integrity of bivalve shells can be affected by elevated $p\text{CO}_2$ lev-

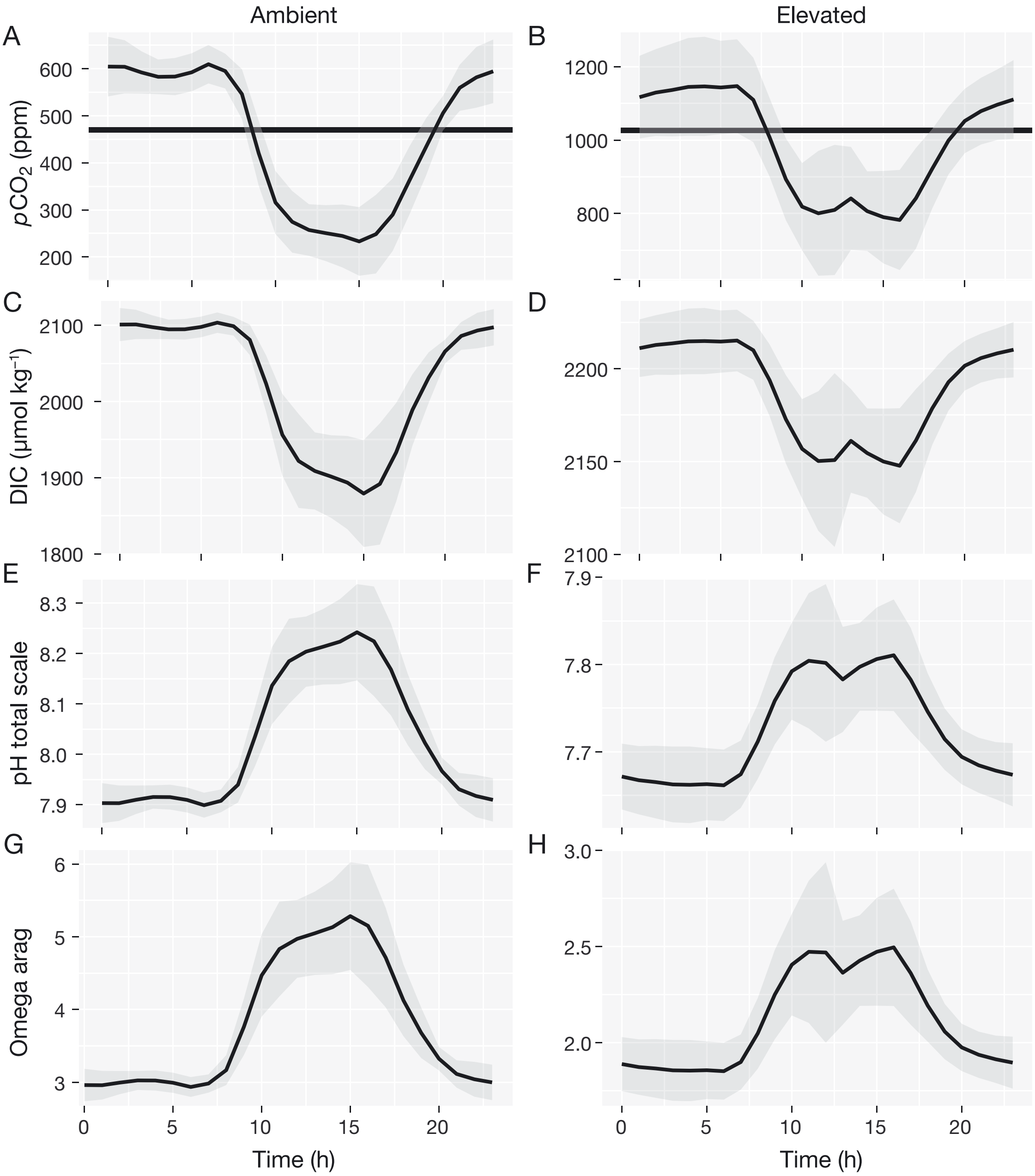

Fig. 2 Carbonate chemistry in experimental tanks showing the method of CO_2 control mimicked natural diel variability in (A,B) pCO_2, (C,D) dissolved inorganic carbon (DIC), (E,F) pH, and (G,H) aragonite saturation state (Omega arag) (note the different *y*-axis scales). Treatments with (A,C,E,G) ambient pCO_2, and (B,D,F,H) elevated pCO_2 levels. Data are aggregated across the 4 wk study, including ±SE (grey shading) and pCO_2 treatment means (horizontal lines in A and B)

els and alter their ability to protect themselves against predation (Amaral et al. 2012). The composition and structure of calcium carbonate layers can be compromised under elevated pCO_2 (Fitzer et al. 2014, 2015a, Byrne & Fitzer 2019), leading to shells with brittle, less stiff layers, with a decreased ability to flex before failing, and therefore, a higher vulnerability to fractures, like those that might occur during a predator attack (Mackenzie et al. 2014, Fitzer et al. 2015a,b). Although mussels in our study grew in shell depth and developed more globular shapes in the presence of predation cues under elevated pCO_2, these changes might not have been enough to protect them against predation. Despite no differences in average handling times across predator and pCO_2 treatments, they were more variable for mussels

Table 1. One-way non-parametric ANCOVA for the effects of $p\text{CO}_2$ and predator (blue crabs *Callinectes sapidus*) treatments on measured morphometrics of southern ribbed mussels *Geukensia granosissima*, with initial morphometric measurement as covariate. **Bold**: significant at $\alpha = 0.05$

Mussel morphometric	Treatment	df	*F*	p
Shell length	$p\text{CO}_2$	1	2.46	0.120
	Predator	**1**	**4.36**	**0.038**
Shell width	$p\text{CO}_2$	1	0.74	0.387
	Predator	**1**	**3.98**	**0.044**
Shell depth	$\boldsymbol{p\text{CO}_2}$	**1**	**5.61**	**0.021**
	Predator	1	0.72	0.395
Soft tissue weight	$p\text{CO}_2$	1	0.06	0.448
	Predator	**1**	**3.82**	**0.049**
Shell weight	$p\text{CO}_2$	1	3.80	0.052
	Predator	1	2.88	0.091

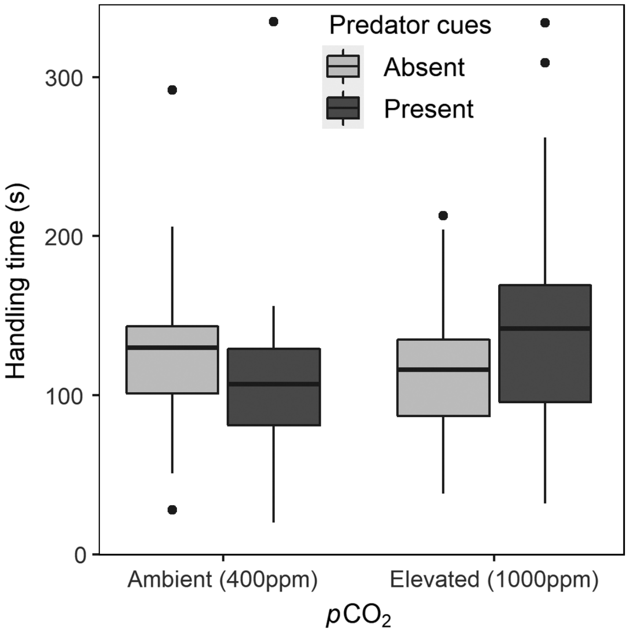

Fig. 3. Handling times of southern ribbed mussels *Geukensia granosissima* by blue crabs *Callinectes sapidus* in predator-absent and predator-present treatments at ambient and elevated $p\text{CO}_2$ treatment levels. The boxes reflect the interquartile range: (horizontal middle line: median, bottom and top of box: 25th and 75th percentiles, respectively); whiskers: 1.5 times the interquartile range below 25th and 75th percentiles; dots: values beyond the whiskers.

exposed to predation cues under elevated $p\text{CO}_2$. This indicates that some of those mussels experienced longer handling times than those not exposed to predator cues under elevated $p\text{CO}_2$. A similar effect of more variable predator handling times for southern ribbed mussels exposed to waterborne cues from blue crabs was found in experimental trials with current-day water temperatures compared to predicted higher temperatures (Freytes-Ortiz & Stallings 2018). In both Freytes-Ortiz & Stallings (2018) and the current study, it is possible that the changes in shell shape or structural integrity provided some protection against predation, but not enough to elicit differences in average handling times. However, future work may benefit from both longer exposure periods (Wang & Wang 2020) and a focus on among-individual variability instead of mean responses (Bolnick et al. 2011).

The question remains of why mussels would grow in shell length and width instead of shell weight, since this latter trait offers the most effective protection against predation. Adult blue crabs prefer to consume ribbed mussels with shells shorter than 25 mm to maximize energy obtained while minimizing energy expenditure and handling time (Elner & Hughes 1978, Hughes & Seed 1981). It is possible that mussels in the present study expended energy in growth in shell length and width, instead of shell weight, to move out of this preferred feeding size range, but did not do so in the 4 wk study period, again suggesting that a longer exposure period may have resulted in stronger differences between $p\text{CO}_2$ levels. Mussels from the predator-present treatment in our study, under both $p\text{CO}_2$ conditions, were similarly consumed in both timeframe and proportion as mussels exposed to the absence of a predator, indicating this response was unable to provide protection from predation.

Linear growth is commonly the only shell morphometric measured or reported in bivalve research, but has been deemed a poor indicator of $p\text{CO}_2$ effects on mussel growth and morphology (Gazeau et al. 2013). Our results indicate that mussel growth rates in shell depth increased under elevated $p\text{CO}_2$ conditions, independent of predator treatment. Thus, mussels exposed to the presence of a predator under elevated $p\text{CO}_2$ conditions were the only group that increased growth in all 3 morphometrics (shell length, width, and depth). These mussels developed rounder, more globular shapes, and experienced significantly more variable handling times compared to other treatments. Rounder, thicker shells have been shown to improve survival from shell-crushing predators, such as crabs (Brönmark et al. 2011). Therefore, the observed change in shell shape to round and globular could have been a form of inducible defense to improve protection, even when mussels were not able to develop thicker shells (Fitzer et al. 2015a).

Understanding how elevated $p\text{CO}_2$ affects predator–prey interactions in adult bivalves is an emerging field of study, and future research could consider other factors, such as study lengths. Although the length of our study was relevant to the temporal dynamics of $p\mathrm{CO}_2$ observed in estuarine systems (Chen et al. 2013), it was relatively short compared to the direction the field is heading to account for longer and cross-generational effects. Offspring of bivalves exposed to elevated $p\mathrm{CO}_2$ can be more resilient to this environmental stressor (Parker et al. 2012), and species with greater phenotypic variation have more options under natural selection (Sunday et al. 2011). However, bivalve sperm (Esposito et al. 2020) and larval development (Gazeau et al. 2010, Kapsenberg et al. 2018) are harmed when exposed to elevated $p\mathrm{CO}_2$. These traits could affect how mussels respond to $p\mathrm{CO}_2$-induced seawater acidification and predation cues under stressful environmental conditions. It is also important to note that the present study focused on adult southern ribbed mussels, not early life stages. More research is needed to elucidate how these changes in mussel morphology could affect crab predator handling times under $p\mathrm{CO}_2$-induced seawater acidification, perhaps through longer exposure periods and analysis of the structure of shells (including thickness), or a focus on cross-generational effects. Likewise, a focus on how elevated $p\mathrm{CO}_2$ affects the predator, rather than solely on the prey, is a fruitful direction for future research on predator–prey interactions (e.g. Wu et al. 2017, Wang et al. 2018). There is some evidence to suggest that under elevated $p\mathrm{CO}_2$ conditions, crustacean behavior and morphology could be altered (de la Haye et al. 2012, Landes & Zimmer 2012, Dodd et al. 2015). The effects of elevated $p\mathrm{CO}_2$ on crab predator morphology and behavior could have further consequences for their interactions with mussel prey.

As intertidal estuarine organisms, southern ribbed mussels are usually exposed to variable environmental conditions (Lent 1969). Although some intertidal organisms can be vulnerable to extreme environmental changes (Somero 2010), others have shown greater resilience when exposed to stressful physical conditions (reviewed by Wang & Wang 2020). Sensitivity to extreme environmental conditions is species-specific, and research has only recently examined how these sensitivities relate to the effects of $p\mathrm{CO}_2$-induced seawater acidification on bivalve morphology (Gazeau et al. 2013, Shaw et al. 2013, Guo et al. 2015). It is possible that southern ribbed mussels in our study were tolerant of elevated $p\mathrm{CO}_2$ levels due to their adaptation to these highly variable environments. Guo et al. (2015) similarly suggested that the tolerance of oysters to elevated $p\mathrm{CO}_2$ levels might be due to their adaptability to estuarine habitats with fluctuating environmental parameters.

In the growing field of experimental climate change research, most work has maintained static levels of ambient and predicted $p\mathrm{CO}_2$. However, $p\mathrm{CO}_2$ can be highly variable during diel cycles (Shaw et al. 2013), making static experimental conditions unrealistic. Recently, more work has studied the effects of fluctuating $p\mathrm{CO}_2$ on bivalve morphology (Shang et al. 2020), but the effect of these abiotic conditions on predator–prey relationships remains largely understudied. Through the present study, we investigated how predator–prey interactions were affected by $p\mathrm{CO}_2$-induced seawater acidification by including these diel dynamics, using an experimental system that allowed for diurnal $p\mathrm{CO}_2$ variability and including exposure to predation cues as a simultaneous stressor. As with Freytes-Ortiz & Stallings (2018), the results of the present study were complex, since elevated $p\mathrm{CO}_2$ led to more variable handling times by a crushing predator despite no effects on the inducible defenses of the prey. As scientists continue to study the complex effects of climate change and associated stressors such as ocean acidification, we will need to continue to incorporate such ecological reality into our study designs.

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