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Biological responses of the predatory blue crab and its hard clam prey to ocean acidification and low salinity

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ABSTRACT: How ocean acidification (OA) interacts with other stressors is understudied, particularly for predators and prey. We assessed long-term exposure to decreased pH and low salinity on (1) juvenile blue crab *Callinectes sapidus* claw pinch force, (2) juvenile hard clam *Mercenaria mercenaria* survival, growth, and shell structure, and (3) blue crab and hard clam interactions in filmed mesocosm trials. In 2018 and 2019, we held crabs and clams from the Chesapeake Bay, USA, in crossed pH (low: 7.0, high: 8.0) and salinity (low: 15, high: 30) treatments for 11 and 10 wk, respectively. Afterwards, we assessed crab claw pinch force and clam survival, growth, shell structure, and ridge rugosity. Claw pinch force increased with size in both years but weakened in low pH. Clam growth was negative, indicative of shell dissolution, in low pH in both years compared to the control. Growth was also negative in the 2019 high-pH/low-salinity treatment. Clam survival in both years was lowest in the low-pH/low-salinity treatment and highest in the high-pH/high-salinity treatment. Shell damage and ridge rugosity (indicative of deterioration) were intensified under low pH and negatively correlated with clam survival. Overall, clams were more severely affected by both stressors than crabs. In the filmed predator–prey interactions, pH did not substantially alter crab behavior, but crabs spent more time eating and burying in high-salinity treatments and more time moving in low-salinity treatments. Given the complex effects of pH and salinity on blue crabs and hard clams, projections about climate change on predator–prey interactions will be difficult and must consider multiple stressors.

KEY WORDS: Ocean acidification · Predator–prey dynamics · Salinity · Multiple stressors · Climate change · Blue crab · Hard clam

1. INTRODUCTION

1.1. Ocean acidification

Since the Industrial Revolution, the combustion of fossil fuels has increased CO_2 emissions into the atmosphere by an order of magnitude (Doney & Schimel 2007). This atmospheric CO_2 has been tempered by oceanic uptake, which accounts for almost one-third of anthropogenic CO_2 added to the atmosphere (Sabine et al. 2004, Sabine & Feely 2007). Consequences of oceanic uptake of CO_2 include alterations to the ocean carbonate system, causing reduced pH (termed 'ocean acidification', OA) (Caldeira & Wickett 2003). In the past few decades, OA has prompted a multitude of studies on its biological impacts (e.g. Raven et al. 2005, Fabricius et al. 2011, Kroeker et al. 2013). Most have focused on the effects on a single species (Bibby et al. 2007, Landes & Zimmer 2012). While these are valuable, they are not fully representative of the complex interactions between biotic and abiotic factors. Further, recent

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comparisons of open ocean, coastal, and estuarine systems revealed that estuaries will react differently than the open ocean, prompting the term 'estuarine acidification' (EA) (MdOA 2015). EA presents a new stress to organisms living in a highly variable environment, and combined stressors could alter the estuarine community in a complex manner.

1.2. Estuarine acidification

While OA with respect to open ocean systems has been well studied, the extent to which EA challenges estuarine communities remains understudied. Estuaries are more susceptible to acidification, since they have increased acidic inputs from terrestrial runoff and a reduced buffering capacity due to the lower salinity relative to open ocean systems (Cai & Wang 1998, Cai et al. 2017). Thus, estuaries may experience more drastic acidification sooner than the open ocean (Waldbusser et al. 2011), although the variability in estuaries makes it difficult to tease out the anthropogenic signal (Najjar et al. 2010). EA is characterized by substantial, rapid, and cyclic changes in pH (MdOA 2015, Klein et al. 2020). Diel cycles of pH in estuaries can range from 0.3 to more than 1 pH unit (Duarte et al. 2013, Carstensen & Duarte 2019). Estuaries are influenced on spatial and temporal scales much more dramatically than the open ocean, and future climate scenarios predict increased variability with increased CO_2 in estuaries (Miller et al. 2009). Besides CO_2 variability, estuaries experience regular shifts in nutrient concentrations, salinity, temperature, and dissolved oxygen (Paerl 2006). Future salinity trends in the Chesapeake Bay are particularly uncertain since the number of extreme rainfall events has been increasing over time (Jay et al. 2018), but sea level rise threatens to inundate estuaries with full-strength seawater (Hilton et al. 2008, Najjar et al. 2010, Carter et al. 2014). The pH variability and little information on the effects of multiple stressors (Denman et al. 2011, Baumann & Smith 2018, Baumann 2019) hamper our ability to predict results of stressors on food webs in coastal systems.

1.3. Multiple stressors and food-web interactions: responses to acidification and salinity

Decreased availability of CO_3^{2-} will likely impact bivalves' shell-building abilities, but the effects of this vary among life stages and species (Gazeau et al. 2007, Ries et al. 2009, Beniash et al. 2010). Larvae and juveniles are typically more sensitive to reduced pH (Van Colen et al. 2012). Decreased calcification rates under increased CO_2 levels (resulting in decreased availability of CO_3^{2-}) affect snails (Bibby et al. 2007, Ellis et al. 2009), juveniles of the hard clam *Mercenaria mercenaria* (Ries et al. 2009, Waldbusser et al. 2010), juveniles and adults of the eastern oyster *Crassostrea virginica* (Miller et al. 2009, Waldbusser et al. 2011), the blue mussel *Mytilus edulis* (Gazeau et al. 2007), and the sea scallop *Placopecten magellanicus* (Cameron et al. 2022). Live weight and shell length are reduced by increases in CO_2 alone in the striped venus clam *Chamelea gallina* (Bressan et al. 2014).

Many juvenile and adult crustaceans are more tolerant of OA than bivalves in the short-term (hours to days) due to their ability to physiologically regulate internal pH (Whiteley 2011), although this may not hold for larval crustaceans. Brachyuran crabs, including the blue crab *Callinectes sapidus* (Glandon & Miller 2017) and the Dungeness crab *Cancer magister* (Pane & Barry 2007), have mechanisms for effective acid–base regulation, allowing them to counteract short-term increases in CO_2. Shell hardening in juveniles post-molt, however, takes longer in low pH (Lane et al. 2013), and exoskeleton dissolution occurs in adults of the Tanner crab *Chionoecetes bairdi* (Dickinson et al. 2021). Exoskeleton dissolution could also expose underlying muscle leading to muscle damage (Dickinson et al. 2021). For larval crabs, low pH reduces growth and survival in blue crab (Giltz & Taylor 2017) and decapods in general (Bednaršek et al. 2021).

Combined effects of low pH and other stressors can have additive or interactive effects. For bivalves, shell structure is negatively impacted by simultaneous increases in CO_2 and decreases in salinity (Dickinson et al. 2012, 2013). In addition, OA and increased temperatures have negative effects on hard clam (Miller & Waldbusser 2016). Multiple stressors also impact crabs, although there are fewer studies relative to bivalves (Whiteley 2011, Breitburg et al. 2015). Blue crabs are strong osmoregulators, yet low salinity causes increased oxygen consumption, because crabs must use more energy to maintain homeostasis (King 1965, Findley et al. 1978). In *Callinectes danae*, reduced pH resulted in the loss of osmoregulatory capacity at low salinity (Ramaglia et al. 2018).

Predation is a key determinant of the abundance and size structure of prey populations, and the structure and functioning of communities (Paine 1966, Menge 1995, Bruno & O'Connor 2005). Few studies

have, however, focused on both predators and prey under low pH (Landes & Zimmer 2012, Parker et al. 2013, Dodd et al. 2015). Changes in physical and behavioral reception or response to cues with environmental stress can affect predator–prey interactions (Ockendon et al. 2014). When mud crabs are stressed under acidification, they reduce consumption rates (de la Haye et al. 2011, Dodd et al. 2015) and decrease prey handling time (Dodd et al. 2015). Acidification can also alter the number of prey consumed and decrease the responsiveness of prey to predator cues; clams typically avoid crab predation by reducing siphon pumping activity, and this response is altered under OA (Glaspie et al. 2017). Consumer stress theory predicts that predators will be more affected by stress than prey (Menge & Sutherland 1987), such that the effects of OA combined with other stressors on crabs are uncertain. Most studies of OA and predator–prey interactions have been short-term (hours to days) laboratory experiments (Bibby et al. 2007, Jellison et al. 2016) with few longer than 8 wk (e.g. Landes & Zimmer 2012, Dodd et al. 2015), and further tests employing long-term studies (weeks to months) are thus warranted.

1.4. Predator (blue crab) and prey (hard clam) experimental system

The blue crab *Callinectes sapidus* is a large portunid species abundant in the Chesapeake Bay (Hines et al. 1987, 1990, Lipcius & Van Engel 1990) that preys heavily upon bivalves (Hines 2007, Lipcius et al. 2007). Feeding efficiency (the number of prey caught over time) of blue crabs varies with prey availability, prey and predator density, and habitat complexity (Lipcius & Hines 1986, Mansour & Lipcius 1991, Eggleston et al. 1992, Micheli 1997). Blue crabs are the main predators of adult hard clams in the Bay (Hines et al. 1990), but their diet also includes other bivalves and juvenile blue crabs, along with fishes and invertebrates (Seitz et al. 2011). Blue crabs also support one of the most important fisheries in Chesapeake Bay, with over 55 million pounds landed in Virginia and Maryland, USA, in 2019, which is worth just over US$81 million to the economy (NOAA 2019).

The hard clam is a shallow-dwelling infaunal bivalve with short, retractable siphons that can be sealed tightly, indicative of an armor defense strategy (Vermeij 1987, Seitz et al. 2001). This species inhabits soft-sediment habitats throughout the lower Chesapeake Bay, where there is an important fishery for it (Mann et al. 2005). The hard clam is also an osmoconforming species and weak acid–base regulator, such that the external environment dictates its internal chemistry (DuPaul & Webb 1974, Melzner et al. 2009). Low salinity and acidification can, moreover, degrade the periostracum (i.e. the outermost layer of the shell) altering shell structure relative to high-salinity and high-pH conditions (Dickinson et al. 2013).

1.5. Research rationale, objectives, and hypotheses

Accurate predictions of the effects of OA and other stressors on bivalves and their predators are necessary, especially for food web models (Lipcius & Latour 2006), because ignoring predator–prey interactions likely underestimates the effects of multiple stressors (Parker et al. 2013). The objectives of our research were to assess the effects of long-term exposure (weeks to months) to decreased pH and salinity on: (1) juvenile blue crab pinch force, (2) hard clam survival, shell structure and growth, and (3) predator–prey interactions under low pH and low salinity via filmed mesocosm trials. We hypothesized that, whereas low salinity would not impact pinch force in blue crab given their euryhaline tolerances (Curtis & McGaw 2010), low pH would weaken pinch force indirectly due to exoskeleton dissolution in the claw (Dickinson et al. 2021). Longer exposure time to low pH would also weaken pinch force. We defined exposure time as the number of days a crab's carapace was exposed to a treatment. Regarding clams, we hypothesized that concurrent low pH and low salinity would reduce clam growth and weaken shell structure in general (Dickinson et al. 2013). Finally, we hypothesized that during predator–prey trials, clams would be easier to open and consume in low pH, regardless of salinity, but that crabs would be more stressed under low pH than high pH, therefore, predate upon fewer clams (Glaspie et al. 2017).

2. MATERIALS AND METHODS

2.1. Treatments

In 2018, we collected 24 juvenile blue crabs 50–90 mm carapace width (CW) from lower Chesapeake Bay tributaries off the York River, including Sarah's Creek, Allens Island, Perrin marsh, and Guinea marsh. We collected individual crabs via

Fig. 1. Photos of 2018 and 2019 tank configurations. (A) Wide-view of the 8 treatment tanks (2 replicate tanks per treatment) in 2018. (B) Overhead view of one of the 2018 treatment tanks. Each tank was separated into 4 chambers: 3 chambers held 1 blue crab each and the fourth chamber held 14 hard clams. (C) 2019 treatment tanks for clams (2 replicate tanks per treatment). (D) 2019 treatment tanks for crabs (2 replicate tanks per treatment). Individual crabs were separated by the same chambers used in 2018

beach seining and scraping from a boat. We obtained 112 juvenile hard clams 10–15 mm shell length (SL) from a private clam grower on the Eastern Shore of Virginia. Animals were kept in replicate 71 cm diameter cylindrical tanks with 5 cm of sand on the bottom (2 replicate tanks per treatment for both species with 3 blue crabs and 14 hard clams per tank; Fig. 1A); individual tanks were divided evenly into mesh compartments to keep crabs separate from each other and from clams (Fig. 1B). Treatment conditions were crossed high (30) or low (~15) salinity and low (7.0–7.2) or ambient (8.0–8.3) pH. We chose the low-pH values to represent those predicted to occur by the year 2100 (Donohue et al. 2012, Glandon & Miller 2017, Glaspie et al. 2017). An automated pH controller (model PHCN-37, Omega Engineering), electromagnetic solenoid valve (Grainger) and pH probes (Omega Engineering) controlled the pH of the acidified tanks by adjusting the flow of CO_2 bubbled into the tanks. We did not control the pH of the ambient water, which fluctuated naturally, but we did control salinity using Instant Ocean Sea Salt (Spectrum Brands) mixed with filtered river water and stored in non-treatment tanks. We manually checked temperature and salinity every other day using a datasonde (YSI) and verified the pH values measured by the automated pH controllers using a handheld probe (Omega Engineering). The experiment ran for 11 wk. We took water samples 3 times over the course of the experiment and analyzed each for dissolved inorganic carbon (DIC) using a DIC Analyzer (Apollo SciTech). We calculated carbonate alkalinity (CA) and partial pressure of CO_2 (pCO_2) (from pH and DIC measurements and water quality parameters) using CO2SYSv3 for MATLAB (MATLAB and Statistics Toolbox Release 2020, Sharp et al. 2020). Clams were fed Shellfish Diet 1800 (Instant Algae Marine Paste) every other day, and crabs were fed pieces of raw oyster.

In 2019, we collected 40 juvenile blue crabs 45–72 mm CW from the same lower Chesapeake Bay tributaries as in 2018 and held them in 96 cm diameter cylindrical tanks with 5 cm of sand on the bottom (Fig. 1C). Tanks of equivalent treatments (2 per treatment) were connected by Filstar XP external canister filters (Rena Aquatic Supply) so water would recirculate and minimize pH and salinity differences. Crab sample size was increased in 2019 due to access to new tanks, which also allowed us to create separate systems for crabs and clams so that predator and prey were not in the same tank (as in 2018). Predator cues could have been induced when crabs and clams were held together, hence the configuration change in 2019. We obtained 112 hard clams 10–15 mm SL from Cherrystone Aqua Farms (Cheriton, VA). Individuals were kept in rectangular tanks (43 cm length × 33 cm width) at 14 clams per tank (Fig. 1D). Clams and crabs were fed the same as in 2018, and we monitored pH and water quality with the same regularity and procedures as in 2018. We also took water samples for DIC 3 times over the course of the experiment, analyzed DIC, and calculated total alkalinity (TA) and pCO_2 with the same procedures. The experiment ran for 10 wk.

Crab and clam densities in the tanks were 1.4 and 19.4 m^{-2}, respectively, which are within the natural range of blue crabs (Hovel & Lipcius 2001) and hard clams (Dahl & Allam 2015). In addition, to further assess the potential for pseudoreplication, we used Tank as a random factor in the analysis, which was not significant (see Section 2.6). Given our use of realistic densities and that Tank was not a significant factor, we assume that pseudoreplication was not an issue in our experiments.

2.2. Crab claw pinch force

Following 10–11 wk of treatment exposure, we measured crab claw pinch force using a custom-designed instrument comprised of a load cell (Celtron model STC-10kgAL, Vishay Precision Group) and bridge amplifier (model DP25B-S-A, Omega). The analog output of the bridge amplifier was digitized using a data acquisition module (model USB-1208FS-Plus, Measurement and Computing Corporation) and recorded using software developed using Dasylab ver 13 (Measurement and Computing Corporation). We verified every crab to be in the inter-molt stage, or C_4, prior to measuring pinch force to standardize the likelihood of foraging among crabs. We used the right claw on each crab since most blue crabs have normal laterality, with the stronger crusher claw on the right-hand side (Hamilton et al. 1976, Blundon & Kennedy 1982).

2.3. Clam growth rate

We measured shell length on the longest side, as a proxy for growth, weekly in 2018 and every other week in 2019, using calipers accurate to 0.1 mm. After treatment exposure, growth rate was calculated as the final shell length minus initial length, divided by the total number of weeks.

2.4. Clam survival

We recorded clam survival weekly in 2018 and every other week in 2019. We discarded dead clams. Survivors were returned to the treatment tanks and allowed to re-bury.

2.5. Clam shell structure and ridge rugosity

Following the 11 wk treatment exposure in 2018, surviving clams were frozen for later processing. In fall 2019, we used a Phenom ProX Desktop Scanning Electron Microscopy (SEM, Thermo-Fisher Scientific) with integrated energy-dispersive X-ray diffraction to examine differences in exterior clam shell structure by treatment. Clam meat and shell were separated and dried at 60°C for 7 d. We then separated shells from each other and mounted them whole on specimen stubs. Finally, a location in the middle of each shell was photographed using the SEM at 260× magnification and 10 kV voltage (Fig. S1A in the Supplement; www.int-res.com/articles/suppl/m701p067_supp.pdf). Samples were not coated or cleaned prior to imaging. We used these images to quantify shell surface deterioration by treatment using a damage index (DI) scale as follows: 0 – no damage; 1 – periostracum discoloration and very minor breakage; 2 – further damage to the periostracum layer and prismatic layer visible; 3 – major area of periostracum layer flaking and prismatic layer dissolution (Bressan et al. 2014).

Images were further used to quantify shell ridge rugosity (indicative of shell deterioration; Dickinson et al. 2013). We virtually traced an exterior growth ridge on each clam and compared the length of this curve to the length of a continuous (nominally

undamaged) ridge curve (Fig. S1B). Greater rugosity indicated greater damage.

2.6. Statistical analyses

We analyzed response variables using an information theoretic approach (Burnham & Anderson 2002, Anderson 2008). We formed generalized linear models (g_i) based on multiple alternative hypotheses (Chamberlin 1890) regarding the influence of independent variables on response variables, and compared them using the Akaike information criterion (AIC) with R (glm, R Core Team 2021) and RStudio (RStudio Team 2021, version 2021.09.0) statistical software. The response variables of claw pinch force, clam growth rate, and clam rugosity were continuous, whereas clam survival was discrete (dead, alive). The independent continuous variables were crab size (mm CW) and exposure time (d), defined as the number of days a crab's carapace was exposed to a treatment since placement in the treatment or since its last molt. The factors were Salinity (low, high), pH (low, high), Gender, and Year (2018, 2019). Salinity and pH were kept in all models, even when the effect of either factor was non-significant. Doing so altered parameter estimates or their standard error of the mean (SEM) minimally.

The linear models, which did not involve 3-way interactions, had the form:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_j + \beta_{i+1} x_1 x_2 \ldots \quad (1)$$

where y = response variable, β_0 = parameter (constant) for the baseline condition, and β_i = parameters representing increases or decreases in the response variables due to independent variables x_j; 2-way interaction terms are represented as $\beta_i x_j x_{j'}$. Models were subsets of the global model and represented alternative hypotheses, which differed based on the specific response variable (Tables S1–S4 in the Supplement).

The generalized linear models used a gamma family, log link for claw pinch force and clam rugosity; Gaussian family for clam growth rate; and binomial family, logit link for clam survival. After selection of the best-fitting model, we added Tank as a random factor to the best-fitting model. Tank was non-significant and had a trivial effect in all analyses. Hence, we eliminated Tank as a factor from the best-fitting model for each analysis.

From the best-fitting models, which always included at least pH and Salinity, we generated means and standard errors for pH and Salinity, adjusted for the other independent variables using the emmeans package in R. We tested treatment means against each other using the pairs command in emmeans.

2.7. Filmed predator–prey interactions (2019)

We determined the interactive effects of pH and Salinity on predator–prey interactions via filmed mesocosm trials in 2019. Juvenile hard clams, which are susceptible to crab predation (Eggleston et al. 1992), and blue crabs were pre-treated for 10 wk with crossed treatments of low and high salinity and low and high pH (as in the single-species exposure). Subsequently, we transplanted clams into a 0.25 m^2 plot within a circular mesocosm of 96 cm diameter with 15 cm of sand at natural densities of 28 clams m^{-2} (Peterson 1983) and 35 cm of water overlying the sand. Water chemistry in the mesocosms mirrored the crossed treatments mentioned above with 1 mesocosm per treatment. Sample size was dependent on clam survival following the 10 wk pre-treatment. The low-pH, high-salinity treatment had n = 6, both low-salinity treatments had n = 2, and the control treatment had n = 4. Following pre-treatment, we starved each blue crab for 24 h, then introduced it into a mesocosm with clams under the same pre-treatment as the crab. All animals remained in the same treatment conditions in which they were pre-treated to prevent shock. For example, clams pre-treated in the low-pH, high-salinity treatment were transplanted to a mesocosm with the low-pH, high-salinity treatment. For these trials, we quantified crab behavior (i.e. time spent moving, buried, and eating) and predation on clams (clam mortality).

Due to low sample sizes per treatment, we were not able to perform statistical analyses on either crab behavior or predation. We originally allowed crabs to forage for 24 h, as in previous studies (Glaspie et al. 2017). Because of 100% clam predation in the first few trials, trial length was shortened to 12 h. Crabs in the 12 h trials did not eat, however. Thus, crab behavior and clam predation varied significantly depending on trial length, and crab sample size of each treatment was too small to analyze data from long trials and short trials separately. Consequently, long and short trials were combined to examine trends in the data. In addition, we combined both pH levels to examine trends by salinity alone, and both salinity levels to examine trends by pH alone. We visualized trends in data on crab behaviors between the 2 pH and 2 salinity levels using stacked bar graphs (see Fig. 7).

3. RESULTS

3.1. Seawater chemistry

High and low pH and salinity treatments were maintained in both 2018 and 2019 pre-treatments and in the 2019 predator–prey interactions (Table S5). Disssolved oxygen (DO) levels were above stressful levels (i.e. remained above 4 mg l^{-1}) in both years. Temperature was higher in 2019 than in 2018, likely due to variable tank heaters. This temperature difference, however, did not influence any of our response variables. pH in the 2019 ambient crab tanks was lower than in the 2018 ambient tanks (Table S5), but this was not caused by metabolically produced CO_2 because all the tanks were aerated. Moreover, the interaction between year and pH was not significant in model g_{16} for pinch force (Table S6). DIC, TA, and pCO_2 were highest in the 2018 low-pH/high-salinity treatment. In general, TA was lowest in the low-salinity treatments, and DIC and pCO_2 decreased with increasing pH.

3.2. Crab claw pinch force

Claw pinch force values were distributed as a gamma distribution with a heavy right tail, and were analyzed with a gamma family, log link. The independent variables included size, exposure time, salinity, pH, sex, and year (Table S6). The data were best fit by the additive model of crab size, year, and pH (g_{12}), with a weighted probability of 0.42 (Table S6), but we used model g_9, which also included salinity as a factor (Table 1). Parameter estimates for pH were virtually identical for the 2 models. Claw pinch force was positively related to crab size and was higher in 2019 (Table 1). Low pH significantly decreased claw pinch force by 26% (14.6 to 10.8 N) regardless of year, whereas low salinity had no significant effect (Fig. 2, Table 1).

Table 1. Estimate, SE, and statistical significance of the parameters from the generalized linear model with a gamma family log link (g_9) for crab claw pinch force in the 2018 and 2019 experiments. Of the 16 models, those including interaction effects did not fit the data as well as g_9, which explained 40.5% of the residual deviance. Model g_9 fit the data significantly better than the null model (likelihood ratio χ^2 test, p = 0.01), and the global model did not improve the fit significantly over model g_9 (likelihood ratio χ^2 test, p = 0.73). Base condition (Intercept) was high salinity, high pH and 2018

Parameter	Estimate	SE	p
Intercept	−611.50	267.25	0.03
Size	0.02	0.01	0.02
Salinity (low)	−0.09	0.12	0.44
pH (low)	−0.30	0.13	0.03
Year	0.30	0.13	0.03

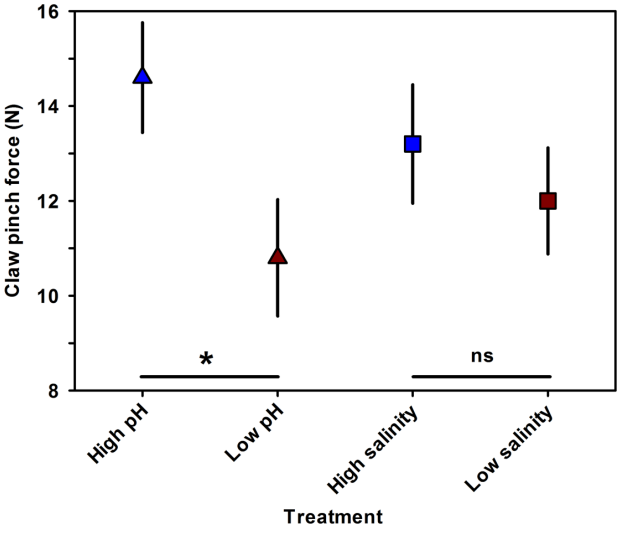

Fig. 2. Mean blue crab claw pinch force. Values are averaged over the levels of Size and Year. Further, values for pH and Salinity are averaged over Salinity and pH, respectively. Error bars = ±1 SEM. *: *t*-test, df = 29, p = 0.03; ns: not significant, *t*-test, df = 29, p = 0.44

3.3. Clam growth rate

Hard clam growth rate values were distributed and analyzed as a Gaussian distribution. The independent variables included salinity, pH, and year (Table S7). Growth rate data were best fit by the additive model of pH, salinity and year (g_6), with a weighted probability of 0.50 (Table S7). Clam growth rate was positive under high pH and high salinity (Fig. 3). Under low pH and low salinity, growth rate was significantly lower (Table 2) and negative (Fig. 3), indicative of shell dissolution.

3.4. Clam survival

Clam survival values were distributed as a binomial distribution (0, 1), and were analyzed with a binomial family, logit link. The independent variables included clam size (initial clam length), salinity, pH and year (Table S8). Clam survival probability was fit best by the additive model of clam size, pH and salinity (g_5), with a weighted probability of 0.23

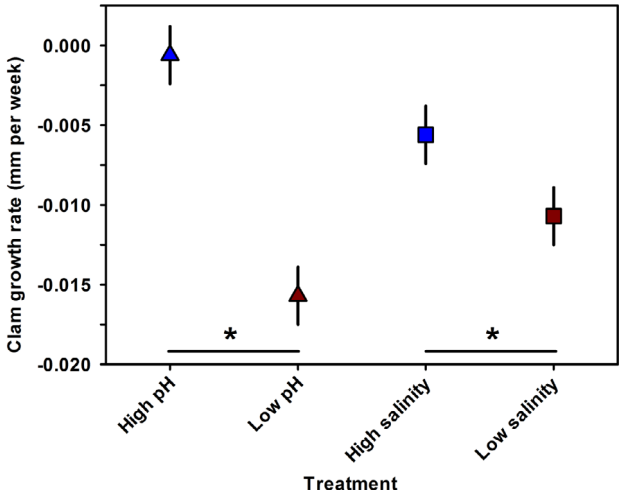

Fig. 3. Mean clam growth rate per week. n = 19 clams per treatment in both years. Values are averaged over the levels of Year, and values for pH and Salinity are averaged over Salinity and pH, respectively. Error bars = ±1 SEM. *: *t*-test, df = 138, p < 0.05001

Table 2. Estimate, SE, and statistical significance of the parameters from the best generalized linear model with a Gaussian family (g_6) for clam growth rate in the 2018 and 2019 experiments. Of the 7 models, those including interaction effects did not fit the data as well as g_6, which explained 25.0% of the residual deviance. Model g_6 fit the data significantly better than the null model (likelihood ratio χ^2 test, p < 0.001), and the global model did not improve the fit significantly over model g_6 (likelihood ratio χ^2 test, p = 0.88). Base condition (Intercept) was high salinity, high pH and 2018

Parameter	Estimate	SE	p
Intercept	−0.0006	0.0024	0.81
Salinity (low)	−0.0052	0.0025	0.04
pH (low)	−0.0152	0.0025	<0.001
Year	0.0052	0.0025	0.04

(Table S8). Survival probability was positively related to clam size and was significantly lower under low pH and low salinity (Table 3). Low pH decreased survival probability by 15% (0.79 to 0.67), while low salinity lowered survival probability by 26% (0.83 to 0.61) (Fig. 4, Table 3).

3.5. Clam shell structure and ridge rugosity

The most stressful treatment (low pH and low salinity) had the highest mean shellDI score of 2.0 (± 0.2 SE), while the control treatment (high salinity, high pH) had the lowest mean DI of 0.5 (± 0.2 SE). The other treatments were intermediate: low pH and high salinity (DI = 1.5 ± 0.2 SE) and high pH and low salinity (DI = 1.2 ± 0.3 SE). Shells in the low pH treatments had more severe damage than shells in the high pH treatments (Fig. 5).

Table 3. Estimate, SE, and statistical significance of the parameters from the best generalized linear model with a binomial family logit link (g_5) for clam survival in the 2018 and 2019 experiments. Of the 13 models, those including Year as a factor or interaction effects did not fit the data as well as g_5, which explained 7.4% of the residual deviance. Model g_5 fit the data significantly better than the null model (likelihood ratio χ^2 test, p < 0.001), and the global model did not improve the fit significantly over model g_5 (likelihood ratio χ^2 test, p = 0.39). Base condition (Intercept) was high salinity, high pH

Parameter	Estimate	SE	p
Intercept	−0.60	1.42	0.67
Size	0.21	0.12	0.07
Salinity (low)	−1.11	0.32	<0.01
pH (low)	−0.62	0.32	<0.01

Clam shell rugosity values were distributed as a gamma distribution with a heavy right tail and were analyzed with a gamma family log link. The independent variables included salinity and pH (Table S9). Rugosity was best fitted by the model of pH alone (g_3), with a weighted probability of 0.62 (Table S9), but we used model g_4, which also included salinity as a factor (Table S9). Parameter estimates of pH were virtually identical for the 2 models. Rugosity was significantly higher, which is indicative of shell damage

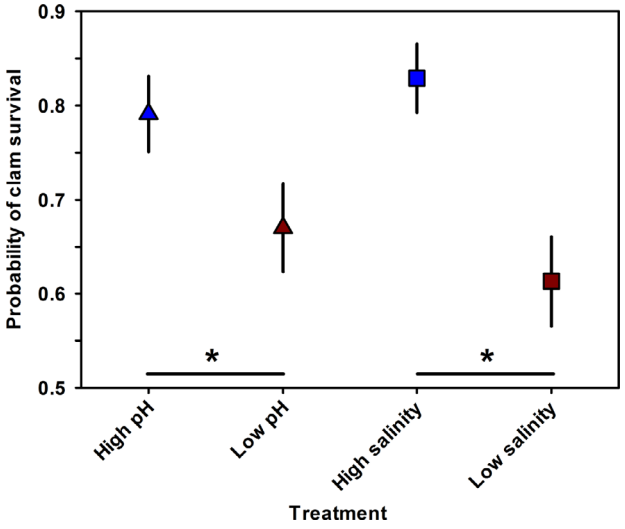

Fig. 4. Mean probability of clam survival. Values are averaged over the levels of Size. Values for pH and Salinity are averaged over Salinity and pH, respectively. Error bars = ±1 SEM. *: *t*-test, df = 220, p < 0.05

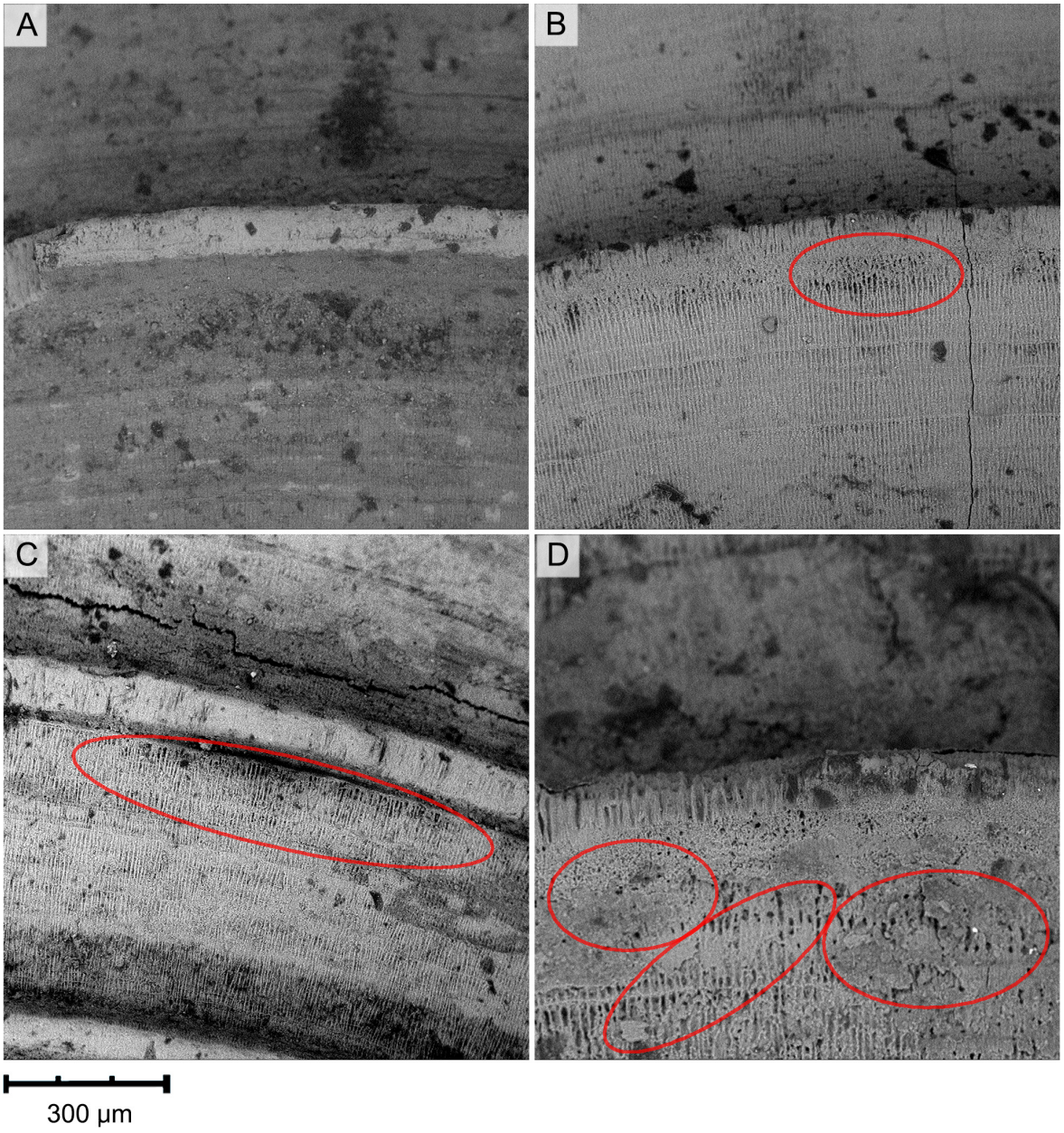

Fig. 5. Sample scanning electron microscope images of the exterior of clam shells from different treatments taken at 260×. (A) Shell from the control treatment (high-pH, high-salinity) with damage index (DI) score DI = 0. (B) Shell from the low-pH, high-salinity treatment with DI = 1. (C) Shell from the high-pH, low-salinity treatment with DI = 2. (D) Shell from the low-pH, low-salinity treatment with DI = 3. Shell deterioration circled in red

(Fig. 5), under low pH (Table 4). Low pH intensified rugosity by 57.5% (1.20 to 1.89) (Fig. 6), whereas low salinity did not have a significant effect (Table 4).

3.6. Filmed predator–prey trials (2019)

Salinity had a minor impact on crab behavior (Fig. 7), with crabs spending more time eating and burying in high-salinity treatments but more time moving in low-salinity treatments (Fig. 7A). This could be the result of higher sample size in high salinity. Because of differential survival and availability of clams after treatment exposure, the number of trials in high salinity was, however, more than double the number of trials in low salinity. pH did not affect crab behavior, and time spent eating was not different in high- and low-pH trials (Fig. 7B).

Table 4. Estimate, SE, and statistical significance of the parameters from the generalized linear model with a gamma family log link (g_4) for clam ridge rugosity in the 2018 experiment. Of the 5 models, the one including interaction effects did not fit the data as well as g_4, which explained 46.7% of the residual deviance. Model g_4 fit the data significantly better than the null model (likelihood ratio χ^2 test, $p < 0.001$), and the global model did not improve the fit significantly over model g_4 (likelihood ratio χ^2 test, $p = 0.70$). Base condition (Intercept) was high salinity, high pH

Parameter	Estimate	SE	p
Intercept	0.116	0.095	0.24
Salinity (low)	0.128	0.110	0.26
pH (low)	0.457	0.110	<0.001

Clam mortality during the trials was directly linked to trial length; clams in longer trials (24 h) were consumed much more than clams in shorter trials (12 h). Trials of different lengths were not evenly distributed among treatments. Furthermore, the trends toward increased crab eating behavior at high salinity were not reflected in clam mortality. When averaged by treatment, crabs consumed 3.3 (±1.5 SE) clams in the low-pH//high-salinity treatment (n = 6 trials), 3.5 (±3.5 SE) clams in the low-pH//low-salinity treatment (n = 2 trials), 7.0 (±0 SE) clams in the high-pH//low-salinity treatment (n = 2 trials), and 2.5 (±1.33 SE) clams in the control high-pH//high-salinity treatment (n = 4 trials).

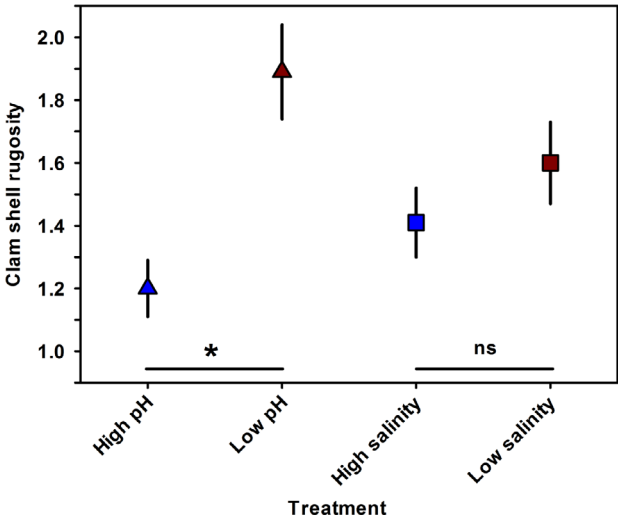

Fig. 6. Mean clam ridge rugosity. Values for pH and Salinity are averaged over Salinity and pH, respectively. Error bars = ±1 SEM. *: *t*-test, df = 21, $p < 0.001$; ns: not significant, *t*-test, df = 21, $p = 0.26$

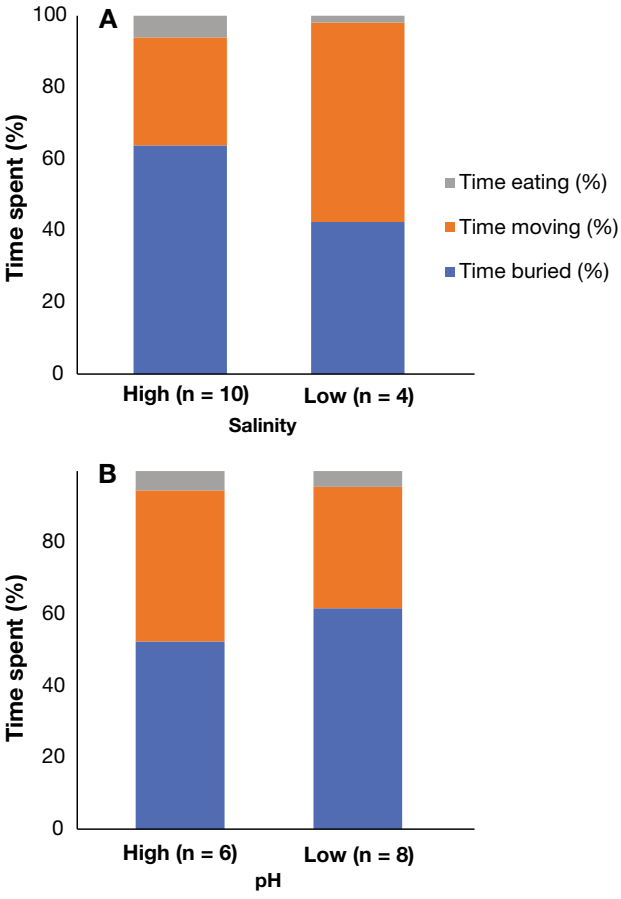

Fig. 7. Stacked bar graphs of crab behavior in percentages by (A) salinity and (B) pH. Number of trials for each parameter (high/low salinity, high/low pH) indicated in parentheses

4. DISCUSSION

In 2 consecutive years with multiple stressors, both juvenile crabs and clams were most impacted by pH, compared to salinity, and multiple stressors exacerbated pH effects. In both years, crab claw pinch force was weaker when individuals were exposed to acidified conditions. Clam growth, survival, exterior shell structure and ridge rugosity were negatively impacted by both pH and low salinity. Despite the impacts on each species separately, predator–prey trials suggested no alteration of the relationship due to salinity or low pH, though the results from the predator–prey trials are inconclusive due to low sample sizes.

4.1. Crab claw pinch force

Crab claw pinch force was significantly reduced by acidification in both years, regardless of salinity. This

trend is consistent with studies where low pH negatively impacted the muscle length and strength of green crab *Carcinus maenas* (Landes & Zimmer 2012), the endocuticle microhardness in juvenile red and blue king crabs, *Paralithodes camtschaticus* and *Paralithodes platypus*, respectively (Coffey et al. 2017), and an increase in Mg^{2+} in the velvet swimming crab *Necora puber* chelae (an indication of reduced chelae strength; Small et al. 2010). Ecologically, decreased claw strength could be detrimental to future blue crab populations under lower pH. Individuals could become inefficient at consuming prey, such as clams (Hines et al. 1990), and unable to defend themselves from predators, as suggested in other studies (Lane et al. 2013, Glandon & Miller 2017, Glandon et al. 2018).

4.2. Clam growth, survival, shell structure, and ridge rugosity

Low pH had the greatest negative impact on both growth and survival in 2018. Low salinity also reduced clam survival, but this effect was not as strong as that for pH. In 2019, the additive effects of pH and salinity lowered clam growth and survival more than pH alone. Salinity contributed more significantly in 2019 than in 2018, with survival being higher in both high-salinity treatments relative to low-salinity treatments. Because clams are osmoconformers (Anderson & Prosser 1953, DuPaul & Webb 1974) and weak acid–base regulators (Melzner et al. 2009), these results are not surprising.

Reduced pH also reduced juvenile hard clam survival in a previous 21 wk study involving pH levels 8.2, 8.1, and 7.7 and salinity levels 16 and 32 (Dickinson et al. 2013). To determine the effects of even lower pH, we replicated the salinity levels from Dickinson et al. (2013), but at a lower pH. This allowed us to determine both lethal and sublethal effects of lower pH. Early-life-stage hard clams also experienced reduced growth in combined low-pH and low-DO treatments (Gobler et al. 2014); thus, multiple stressors can exacerbate effects of OA. Finally, initial shell length of clams played a role in survival, with smaller clams having a lower survival rate. Shells of younger clams are not as developed as those of adult clams; thus, young clams have difficulty overcoming net dissolution (Waldbusser et al. 2010). OA has also proved detrimental in other marine calcifiers. Mediterranean mussels exposed to low pH experienced shell dissolution (Bressan et al. 2014), juvenile eastern oysters exposed to low pH and low salinity had significant mortality (Dickinson et al. 2012), and shells of pearl oysters were weakened under acidification (Welladsen et al. 2010).

As seen in SEM images depicting growth ridges, shells in acidified treatments were both qualitatively and quantitatively more damaged than shells in high-pH treatments. In addition, shells in low-salinity treatments were more damaged than shells in high-salinity treatments, and the most damaged shells were in the low-pH/low-salinity treatment. Shell dissolution under such stressful conditions could make clams more vulnerable to predation (Vermeij 1987, Seitz et al. 2001). If future salinity levels in the Chesapeake Bay decrease because of increased storm activity, as predicted (Jay et al. 2018), clams would be compromised.

4.3. Predator–prey interactions

Filmed predator–prey trials were inconclusive due to low sample sizes, but trends in the data suggested that pH had little impact on crab behavior after 10 wk of exposure. Given that crabs and clams were placed into mesocosm treatments that mirrored pre-treatment conditions, any resultant behaviors would not have been a result of shock. Other brachyuran crabs acclimated to low pH show similar responses (Whiteley et al. 2018), though some species, such as mud crabs *Panopeus herbstii* and *Pagurus bernhardus*, decrease prey handling time and consumption rates (de la Haye et al. 2011, Dodd et al. 2015). Because crab pinch force is negatively impacted, one must ask how long an individual can maintain homeostasis and normal behavior. Behavior may eventually change as the energetic trade-offs, such as prevention of muscle wastage (Landes & Zimmer 2012) become too great because of the high costs of increased calcification (Spicer et al. 2007, Wood et al. 2008). OA increased the foraging time and decreased consumption rates of the brown crab *Cancer pagurus* on blue mussels *Mytilus edulis* (Wang et al. 2018), as well as for the Asian paddle crab *Charybdis japonica* on various prey (Wu et al. 2017). Alternatively, if both predator and prey are altered (as in the present study), the result could be no net impact on their interaction (Landes & Zimmer 2012, Glaspie et al. 2017). In our experiments, clam shells were degraded and crab claw pinch force was weaker, suggesting that under future acidified conditions, there might be no net alteration in the crab–clam predator–prey interaction.

Behavior analyses also suggested that blue crabs spend more time eating in high-salinity treatments. If

storm activity increases and future salinity levels decrease in the Chesapeake Bay, blue crabs could be less efficient consumers, decreasing their nutrient and energy intake and potentially leading to lower growth rates (Stickle et al. 2007). Because maximal energy absorption and scope for growth in blue crabs are influenced by salinity (Guerin & Stickle 1992), fluctuations in salinity could strongly influence foraging efficiency of the blue crab in the future.

As the Chesapeake Bay undergoes OA and greater fluctuations in salinity due to climate change, such stressors could pose problems for marine calcifying species. Although the blue crab may be able to withstand future stressful conditions, the energetic tradeoffs (e.g. maintaining carapace calcification versus muscle strength) may determine whether they can maintain effective foraging and reasonable growth rates. For sedentary species like hard clams and other bivalves, however, the detrimental effects of OA and increased precipitation could enhance their susceptibility to predation. Climate change has already decreased the production of economically valuable mollusks (Doney et al. 2009), with a predicted loss of US$100 billion by 2100 (Narita et al. 2012). Understanding how OA interacts with other stressors to affect species responses is necessary for future management of exploited species, particularly under climate change.

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