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Ocean acidification impairs crab foraging behaviour

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Anthropogenic elevation of atmospheric CO_2 is driving global-scale ocean acidification, which consequently influences calcification rates of many marine invertebrates and potentially alters their susceptibility to predation. Ocean acidification may also impair an organism's ability to process environmental and biological cues. These counteracting impacts make it challenging to predict how acidification will alter species interactions and community structure. To examine effects of acidification on consumptive and behavioural interactions between mud crabs (*Panopeus herbstii*) and oysters (*Crassostrea virginica*), oysters were reared with and without caged crabs for 71 days at three pCO_2 levels. During subsequent predation trials, acidification reduced prey consumption, handling time and duration of unsuccessful predation attempt. These negative effects of ocean acidification on crab foraging behaviour more than offset any benefit to crabs resulting from a reduction in the net rate of oyster calcification. These findings reveal that efforts to evaluate how acidification will alter marine food webs should include quantifying impacts on both calcification rates and animal behaviour.

1. Introduction

Predation has been a central focus of community ecology over the past several decades due to its importance in mediating community structure [1–3]. Predator–prey interactions can directly or indirectly manifest in a broad range of lethal and sub-lethal effects, with far-reaching consequences for community dynamics and evolutionary processes. Predation risk has resulted in the evolution of physical (e.g. coloration and morphological structures, such as spines and calcified exoskeletons), chemical (e.g. production of toxins in seaweeds; [4]) and behavioural (e.g. refuge use and predator avoidance; [3,5]) defences among prey. Strong predator effects are often revealed when these 'evolutionary arms races' [6] are shifted by events such as removal of top predators from a system [7,8] or introduction of non-native predator species [9]. Although these more immediate disturbances to ecosystems have greatly informed our understanding of the importance of top-down forcing, forecasting how longer-term perturbations such as environmental forcing will impact predator–prey interactions and community structure more broadly requires incorporating their potential impacts into ecological experiments.

It is well established that global change can significantly alter predator–prey interactions [10]. For instance, rising carbon dioxide (CO_2) in the atmosphere is driving rapid, ubiquitous and increasing change in global ocean chemistry and ecosystems [11,12]. Ocean surface pH has already decreased by 0.1 since 1800 and is predicted to drop by an additional 0.1–0.4 units by end of century [13,14]. This pH prediction for 2100 will result in a nearly 50% reduction in the carbonate ion concentration of seawater and a corresponding decrease in its calcium carbonate saturation states [14]. The trend has raised concerns about the myriad of calcifying marine organisms that construct their shells and skeletons from calcite and/or aragonite polymorphs of $CaCO_3$, and has prompted

numerous studies investigating the potential effects of ocean acidification on rates of calcification. These studies have shown that marine calcifying species exhibit differing responses to CO_2-induced ocean acidification [15–18]. Variation in these responses is largely due to their differing abilities to regulate protons at the site of calcification, the relative solubility of their skeletal mineral polymorphs, the extent to which they cover their shells or skeletons with protective organic layers, and whether they use photosynthesis that is fertilized by elevated pCO_2 [17].

Ocean acidification is expected to have largely negative impacts on bivalve species [17]. For instance, calcification rates of *Crassostrea virginica* (eastern oyster)—along with shell hardness and fracture resistance—decrease linearly with CO_2-induced ocean acidification [17,19–21]. Crustaceans, in contrast, exhibit the potential for neutral to increasing calcification rates [17,22–24] with CO_2-induced ocean acidification. This pattern may include important estuarine predators, such as *Callinectes sapidus* (blue crab), *Panopeus herbstii* (mud crab) and *Menippe mercenaria* (stone crab). All of these crustacean species are important estuarine predators that prey upon various life stages of *C. virginica*; directly and indirectly affecting their population dynamics [25–27]. Additionally, oysters typically respond to predators by altering their calcification pattern to increase shell strength [28]. However, acidification has been shown to disrupt the induction of morphological defences in *Littorina littorea* [29], and may affect *C. virginica* similarly, further weakening its resistance to predation. Increased susceptibility of bivalves to predation, as suggested by opposing trends in calcification responses to acidification, could largely alter community dynamics throughout affected ecosystems.

Anthropogenically induced environmental changes throughout terrestrial and aquatic ecosystems often mediate changes in animal behaviour (e.g. [30,31]). Behaviour can become altered through three broad and often interacting pathways: information disruption, physical change and avoidance of altered environments [32,33]. For avoidance to occur, disturbances must be relatively local and organisms must be able to immigrate to a more favourable location [33]. The relatively slow and ubiquitous advance of ocean acidification may limit the ability of organisms to find more favourable locations and, therefore, the utility of avoidance as a coping strategy.

Physical changes in response to ocean acidification are much more common and can include changes to metabolism (e.g. [34]), calcification (e.g. [19]) and muscle strength [35,36]. The metabolic costs of survival in altered environments can result in reduced energy available for other behaviours, particularly for energetically expensive behaviours such as foraging and aggression [33,34]. For instance, reduced calcification caused by ocean acidification causes *L. littorea* to compensate by increasing predator avoidance behaviour [29].

Information disruption affects behaviour more directly, altering an organism's ability to perceive or process environmental information through a number of potential pathways [32]. For instance, information disruption has been shown to impair alarm responses in several fishes exposed to acidified waters [37,38]. Laboratory evidence suggests that an alarm pheromone of these fishes suffers an irreversible change in structure that renders it non-functional [39]. This particular effect occurs completely external to the impaired organism. Growing evidence exists for widespread internal information disruption via interference with neurotransmitter function [40]. In an effort to maintain internal acid–base homeostasis, some marine species alter intracellular concentrations of Cl^- and/or HCO_3^- [41,42], which can lead to changes in ion gradients at neuron synapses and improper activity of some γ-aminobutyric acid (GABA) receptors [40]. GABA receptors are widespread in both vertebrates and invertebrates, making many marine species potentially vulnerable to this effect [43]. GABA receptor disruption has been shown to cause abnormal olfactory preferences and changes in swimming patterns in two coral reef fishes [40], as well as impaired predator escape behaviour in a marine gastropod [44]. These studies suggest that ocean acidification can negatively impact animal behaviour, and in turn disrupt the transfer of energy to higher trophic levels.

To investigate how CO_2-induced ocean acidification will influence oyster reef communities, laboratory experiments were conducted to examine the impact of elevated pCO_2 on predator–prey interactions between *P. herbstii* and juvenile *C. virginica*. Experiments were designed to test the hypothesis that calcification rates of the oyster *C. virginica* are more negatively impacted by CO_2-induced ocean acidification than calcification rates of the crab *P. herbstii*, thereby increasing the oysters' susceptibility to mud crab predation. Alternatively, ocean acidification may disrupt the ability of mud crabs to locate or consume prey resources, thereby decreasing mud crab predation on oysters.

2. Material and methods

(a) Growth conditions

Juvenile wild-strain *C. virginica* (18.7 ± 3.8 mm shell height) were obtained from Jonny Oyster Seed of St. Leonard, Maryland. Spat were separated from cultch shell using a diamond-embedded lapidary saw and as much of the excess shell was removed as possible. Spat were then individually attached to plastic microscope slides with cyanoacrylate epoxy. Thirty oysters were suspended 40 cm from the bottom of each tank on 1.7 mm diameter plastic cord. Adult *P. herbstii* (23–28 mm carapace width) were collected from Middle Marsh near Beaufort, North Carolina in early May 2011. Crabs were maintained in half of the tanks containing oysters and chambered during the growth period to control individual feeding rates and inhibit cannibalism while allowing for water and cue circulation. Oysters were raised in an orthogonal 3 × 2 design with three acidification levels and two crab cue presence levels (present or absent), while crabs were raised in a 3 × 1 design with three acidification levels. All treatment combinations were replicated threefold.

Crabs and oysters were raised in isolated 34 l tanks for 71 days in seawater with calculated $pCO_{2(gas-e)}$ values (±SD) of 499 (±114), 785 (±154), and 9274 (±2243) µatm (table 1), corresponding to near-modern pCO_2, the predicted end-century pCO_2, and a level that exceeds the highest pCO_2 predicted to be experienced by these organisms. Although the high pCO_2 treatment is higher than is predicted to occur in the atmosphere and open-ocean for the foreseeable future, comparable conditions already can occur in both healthy and degraded estuaries as high dissolved inorganic carbon (DIC) from detrital organic matter, pollution and stratification combine to elevate local pCO_2 in estuarine waters inhabited by both species [45,46]. Furthermore, our high pCO_2 treatment was not formulated solely to target pCO_2 levels predicted for the foreseeable future, but rather to target pH levels and calcite saturation states that are predicted to occur over that timeframe. And because of the temporal and spatial variation in

Table 1. Mean (standard deviation) seawater parameters for pCO_2 treatments: calculated pCO_2 of mixed gases in equilibrium with experimental seawaters [$pCO_{2(gas\text{-}e)}$]; calcite saturation state (Ω_C); pH; and DIC. Full seawater parameters available as electronic supplementary material.

	control pCO_2	moderate pCO_2	high pCO_2
$pCO_{2(gas\text{-}e;\ ppm\text{-}v)}$	499 (114)	785 (154)	9274 (2243)
Ω_C	6.7 (2.0)	5.1 (1.3)	0.8 (0.2)
pH	8.20 (0.11)	8.04 (0.08)	7.05 (0.09)
DIC	2360 (303)	2549 (256)	3432 (207)

salinity ($5 < \text{salinity} < 35‰$) that occurs within estuarine waters that are inhabited by the investigated species, the pH (*ca* 7.0) and calcite saturation states (*ca* 0.8) that were maintained in the high pCO_2 treatment are realistic for low to moderate salinity and correspondingly low-alkalinity (TA < 1000 µmol) estuarine waters equilibrated with an atmospheric pCO_2 of *ca* 2400 µatm (predicted for year 2600, assuming a conservative annual increase of 3.5 µatm yr^{-1}). Furthermore, recent studies have revealed that estuaries such as the Chesapeake Bay [21], Elkhorn Slough, California [47], and Charleston Harbor Estuary, South Carolina [46] presently experience significant annual (8.2–7.6, Chesapeake Bay) and tidal (8.1–7.4, Elkhorn Slough; 7.6–6.9, Charleston Harbor Estuary) pH variation due to fluctuations in salinity (and resulting total alkalinity (TA)) and local enrichments in DIC (resulting from seasonal re-mineralization of benthic organic matter). Lastly, experimental seawaters were formulated to encompass a range of carbonate system parameters (pH < 7 and undersaturation with respect to calcite) that were similar to those that were employed in recent studies on related subjects (e.g. [29,34,48,49]).

Partial pressures of CO_2 were established by mixing pure CO_2 with compressed air using *Aalborg* digital mass flow controllers. Experimental seawater was bubbled with microporous ceramic airstones into triplicate glass tanks. The pCO_2 of the mixed gases was measured with a *Qubit S151* infrared pCO_2 analyser calibrated with certified air–CO_2 gas standards (precision = ±2.0%; accuracy= ±1.8%). Salinity (s.d.) was formulated at 31.72 (0.76) with *Instant Ocean Sea Salt* and deionized water and temperature (s.d.) was maintained at 25.97°C (1.15) with 50 W electric heaters. Although the trace elemental composition of *Instant Ocean Sea Salt* differs subtly from that of natural seawater, its major and minor elemental composition, as well as its carbonate chemistry, was the most similar to that of natural seawater when compared with eight other commercial sea salt mixes [50]. Every 2 days, oysters were fed 14 ml per tank of a commercial algal blend containing *Nannochloropsis oculata*, *Phaeodactylum tricornutum* and *Chlorella* sp. with a cell size of 2–20 µm (DT's Live Marine Phytoplankton, Sycamore, IL, USA). Each crab was provided 50 mg ± 7 mg dry weight of *Artemia* sp. (brine shrimp) on the same 2-day feeding schedule. Temperature, pH and salinity were measured every 2 days, while pCO_2 of mixed gases were measured weekly (*sensu* [17]) (table 1; see electronic supplementary material for all measured and calculated seawater parameters).

Seawater within each tank was continuously filtered (757 l h^{-1}) with a hanging power filter that contained a nylon-floss activated-carbon filter. Circulation and turbulence of seawater within each tank was enhanced with a 400 l h^{-1} powerhead. Each tank was covered with a transparent 3-mm Plexiglas sheet and both the tank and the attached filtration system were wrapped with cellophane to promote equilibration between the gas mixtures and the experimental seawaters and to minimize evaporative water loss. Tanks were illuminated for 12 h per day with standard white fluorescent lights (32 W, T8 6500 K) to simulate oysters' and crabs' natural light cycle.

Following the 71-day growth period, mud crabs and oysters were moved to tanks with a quartz sand substrate and with seawater chemistry that matched their respective experimental growth conditions. Twenty oysters, left attached to the plastic slides, were randomly selected then haphazardly arranged on the floor of the experimental tank. Two mud crabs were placed in each tank and allowed to prey upon these oysters for 48 h or until oyster mortality exceeded 75%, whichever occurred first. Two mud crabs were used in each assay to incorporate effects of acidification on conspecific aggression [51], which can be an important component of *P. herbstii* foraging ecology [52,53]. The number of oysters that was consumed was quantified every 2 h for the first 12 h and then sporadically for the remaining 36 h.

(b) Measurement and calculation of carbonate system parameters

The temperature within experimental tanks was measured every other day with a NIST-calibrated partial-immersion organic-filled glass thermometer (precision ± 0.3%, accuracy ± 0.4%). Salinity was measured every other day with a *YSI* 3200 conductivity meter with a *YSI* 3440 cell ($K = 10$) that was calibrated with seawater standards of known salinity provided by the laboratory of Prof. A. Dickson of Scripps Institute of Oceanography. Seawater pH was measured every other day with a *Thermo Scientific Orion 2 Star* benchtop pH meter with an *Orion* 9156BNWP pH probe, calibrated with 7.00 and 10.01 *Orion* NBS buffers traceable to NIST standard reference material (for slope of the calibration curve) and with seawater standards of known pH also provided by Prof. Dickson's laboratory (for *y*-intercept of the calibration curve). Seawater DIC was measured via coulometry (*UIC 5400*) and TA was measured via closed-cell potentiometric Gran titration calibrated with certified Dickson TA/DIC standards. Measurement of DIC and TA of the certified reference materials (CRMs) were consistently within 0.3% of certified values. Differences between the measured and certified TA and DIC values of the CRMs were used to correct measurements of experimental seawater solutions.

Seawater pCO_2, pH, carbonate ion concentration ($[CO_3^{2-}]$), bicarbonate ion concentration ($[HCO_3^-]$), aqueous CO_2 and calcite saturation state (Ω_C) were calculated from measured DIC, TA, temperature and salinity with the program CO2SYS [54], using Roy *et al.* [55] values for K_1 and K_2 carbonic acid constant [55], the Mucci [56] value for stoichiometric aragonite solubility product [56], and an atmospheric pressure of 1.015 atm.

(c) Quantification of calcification rates via buoyant weighing

Calcification rates of oysters and crabs were estimated using an empirically calibrated buoyant weight technique [17]. Specimens were weighed at the beginning of the experiment and at 71 days. Each specimen was suspended by aluminium wire from a *Cole-Parmer* bottom-loading scale (precision ± 0.001; accuracy ± 0.002) at a depth of 10 cm in a tank filled with experimental seawater maintained at a temperature of 25°C and salinity of 33. A plastic-coated zinc mass standard was intermittently weighed to ensure consistency of the buoyant weight method throughout the duration of the experiment.

Buoyant weight-dry $CaCO_3$ weight relationships for oysters and crabs were empirically derived by plotting final dry $CaCO_3$ weights (after removal of organic matter) against final buoyant weights of 49 oysters and 18 crabs randomly selected from the three pCO_2 (control—499 µatm, moderate—785 µatm,

high—9273 µatm) treatments used in experiments. Oyster dry $CaCO_3$ weight was the dry weight (70°C, 24 h) of the shell after mechanical removal of soft tissue. Crab dry $CaCO_3$ weight was the dry weight of the crab carapace after organic matter was removed via combustion in a muffle furnace at 500°C for 6 h. Buoyant weight–dry $CaCO_3$ weights for specimens from all treatments were highly correlated (linear regression of: oyster: $R^2 = 0.9976$, $p < 0.001$; crab: $R^2 = 0.9828$, $p < 0.001$) and similar among treatments, indicating that densities of crab and oyster shells do not vary appreciably among treatments [17]. Thus, a single linear equation for each species was used to convert buoyant weight to dry weight for purposes of estimating net calcification rates:

$$\text{Oyster: dry weight (mg)} = 1.5996 \times \text{buoyant weight (mg)} - 0.5013;$$
$$\text{Crab: dry weight (mg)} = 1.3411 \times \text{buoyant weight (mg)} - 0.0107.$$

(d) Video analysis of behaviour

Each feeding trial was recorded to explore the impacts of acidification on *P. herbstii* foraging behaviour. Tanks were continuously illuminated to improve video quality. Predation in control pCO_2 treatments corresponds well to published predation rates under natural light regimes in similar conditions, suggesting that the continuous lighting was not detrimental to crab consumption [57]. Two 30-min segments of video were analysed for each trial, with one starting point randomly selected from each of the following time intervals: 1.50–4.58 and 4.58–7.67 h after the start of the experimental trial. Each video segment includes only active experimental time (i.e. before 75% oyster mortality was observed in a trial). Analysis of crab behaviour included variables such as general activity (i.e. any movement of a claw), agonistic behaviour (i.e. physical confrontations and delayed movements when in close proximity to each other during those confrontations), prey handling time, number of predation attempts and average time spent in an unsuccessful predation attempt. Crabs often exhibited mild avoidance behaviour, typically maintaining a minimum separation of approximately 8–10 cm, which was not considered agonistic behaviour. Time spent handling prey included any use of the crabs' chela to grasp and manipulate an oyster. This definition encompasses all observed oyster manipulations except for brief pushing activity conducted with closed dactyls. An attempted predation event was defined as any generally continuous grasping contact regardless of periods of inactivity. Small periods of non-contact (less than 20 s) were not considered a new event, as these events occurred infrequently and appeared to be either disengagement to perform brief displays of dominance or the result of the crab accidentally dropping the oyster, rather than intentionally terminating a predation attempt. The mean duration of unsuccessful predation attempts was quantified to examine how perseverant crabs were in attempting to consume oysters. The first five unsuccessful attempts identified in each tank were averaged to get a tank mean. If a replicate treatment did not include five attempts during the initial hour of analysed video, additional segments were haphazardly selected from the two video analysis windows until a total of five unsuccessful attempts was reached. Several high pCO_2 replicates still failed to reach five attempts, and, as a result, this treatment was excluded from the analysis of mean duration of unsuccessful predation attempts.

(e) Statistical analyses

Cochran's *C* test for heteroscedasticity of variances was conducted on all main effects in each analysis [58]. Change in oyster buoyant weight was analysed with a two-way ANOVA with pCO_2 (control, moderate and high) and crab cue (present versus absent) as fixed factors. Change in crab buoyant weight was analysed with a one-way ANOVA with pCO_2 as a fixed factor. Proportion of oyster consumed after 12 h $tank^{-1}$ was arcsine transformed and a two-way ANOVA was conducted with pCO_2 and crab cue as fixed factors. However, two trials were terminated prior to 12 h due to prey depletion; therefore, the proportion of oysters consumed at the time of termination was used in the analysis. Use of the consumption count at the time of termination is a conservative estimate, as we assumed no additional predation following termination. Behavioural metrics of prey handling, general activity and agonistic behaviour were arcsine transformed, while prey encounters and mean duration of an unsuccessful predation attempt were Box Cox transformed. All behaviour metrics are values per tank and were analysed with separate two-way ANOVAs with acidification and crab presence as fixed factors. All post hoc tests were performed with Ryan's *Q* tests [59].

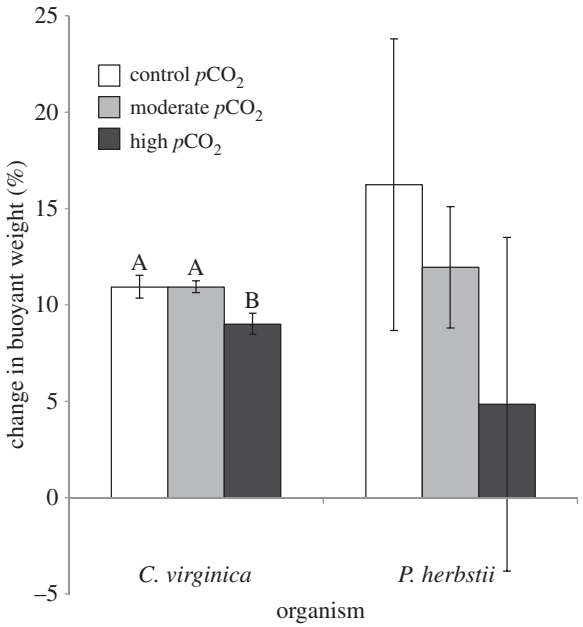

Figure 1. Mean (±s.e.) change in buoyant weight for *P. herbstii* and *C. virginica* after 71 days.

3. Results

(a) Calcification rates

Acidification negatively affected oyster calcification rates. The interaction between crab presence and pCO_2 ($F_{2,15} = 0.39$, $p = 0.687$) and the main effect of crab presence ($F_{1,15} = 1.12$, $p = 0.310$) were not significant, but pCO_2 did significantly affect calcification rates ($F_{2,15} = 4.68$, $p = 0.031$). Oyster calcification rates were significantly lower in the high pCO_2 treatment as compared with both the control and moderate treatments (Ryan's *Q* test, $p < 0.05$; figure 1), but the control and moderate treatments were not significantly different form each other. Crab calcification rates were not affected by pCO_2 treatment ($F_{2,6} = 0.70$, $p = 0.534$; figure 1).

(b) Crab consumption of oysters

In addition to impacting oyster calcification rates, acidification reduced crab consumption of juvenile oysters. The interaction between crab presence and acidification ($F_{2,12} = 2.88$, $p = 0.095$) was not significant, but acidification did significantly influence consumption rates ($F_{2,12} = 42.7$, $p \leq 0.001$). The percentage of oysters consumed per tank was greatest in the control pCO_2 treatment (67.5 ± 10%), intermediate in the

moderate pCO_2 treatment (41 ± 7.5%) and lowest in the high pCO_2 treatment (1 ± 1%; Ryan's Q test: $p < 0.05$ for all pairwise comparisons of acidification treatments; figure 2*a*). Meanwhile, crab presence during the growth period did not affect crab consumption of oysters ($F_{1,12} = 1.39$, $P = 0.26$).

(c) Crab behaviour

Acidification impacted crab foraging behaviour. Prey handling did not vary with the interaction of acidification or predator presence ($F_{2,12} = 1.97$, $p = 0.182$), nor with the main effect of crab presence ($F_{1,12} = 0.16$, $p = 0.695$). However, prey handling was significantly different across acidification treatments ($F_{2,12} = 6.08$, $p = 0.015$), with the control and high treatments significantly differing from each other (figure 2*b*). There was a similar pattern for mean duration of an unsuccessful predation attempt, with no effect of the interaction between acidification and crab presence ($F_{1,8} = 1.23$, $p = 0.300$) or the main effect of crab presence ($F_{1,8} = 1.13$, $p = 0.320$). However, there was a significant effect of acidification ($F_{1,8} = 10.63$, $p = 0.012$; figure 2*c*), with moderate acidification reducing the mean duration of unsuccessful predation attempts by 84.6% compared with the control acidification treatment.

There was no effect of acidification or crab presence on the number of predation attempts by crabs: acidification × crab presence interaction ($F_{2,12} = 0.53$, $p = 0.600$), crab presence ($F_{1,12} = 0.04$, $p = 0.842$) or acidification ($F_{2,12} = 2.87$, $p = 0.096$). Yet, there was a trend of decreasing prey encounters for the high pCO_2 treatment, with that treatment averaging approximately one-third of the encounters of the other pCO_2 treatments and 11 out of 15 observed high pCO_2 encounter events occurring in a single replicate.

General activity of crabs did not vary with the interaction of acidification and crab presence ($F_{2,12} = 0.14$, $p = 0.875$), nor with either the main effects of acidification ($F_{2,12} = 2.16$, $p = 0.158$) or crab presence ($F_{1,12} = 1.00$, $p = 0.336$). Similarly, agonistic behaviour did not vary significantly with the interaction term ($F_{2,12} = 2.59$, $p = 0.116$), the main effect of acidification ($F_{2,12} = 0.78$, $p = 0.482$) or the main effect of crab presence ($F_{1,12} = 0.42$, $p = 0.528$).

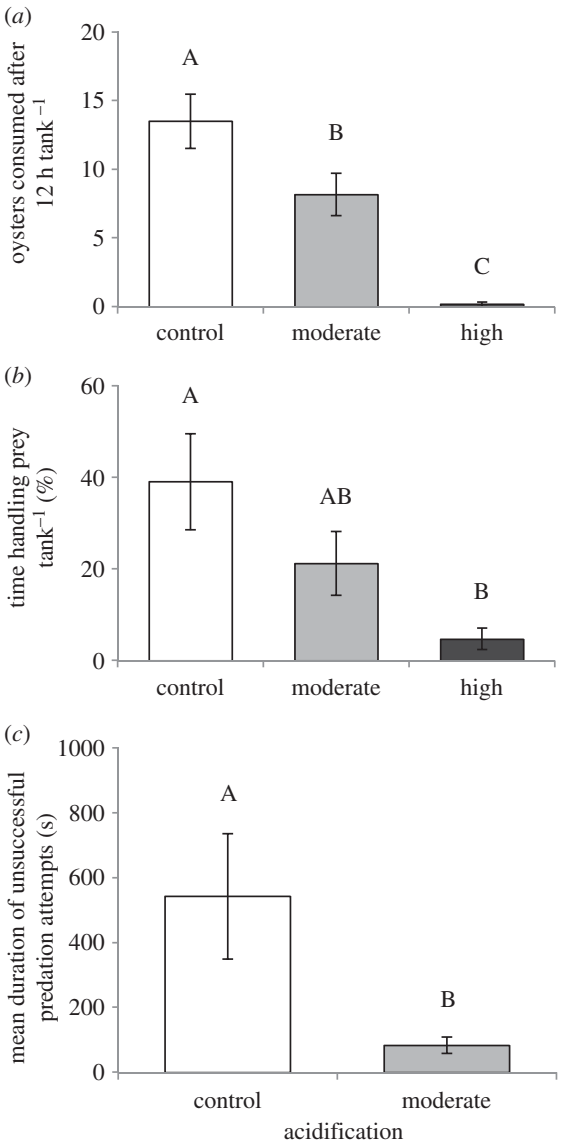

Figure 2. (*a*) Oyster consumption, (*b*) prey handling and (*c*) predator persistence at different acidification treatments. Data are untransformed means ± s.e.; letters denote significant differences from Ryan's Q post hoc tests. Several high pCO_2 replicates failed to reach five unsuccessful predation attempts; therefore, the high acidification treatment was excluded from (*c*).

4. Discussion

Ocean acidification reduced *P. herbstii* (mud crab) predation on juvenile *C. virginica* (eastern oyster). This finding was counter to our initial hypothesis that potentially differential effects of ocean acidification on crab and oyster calcification [17] would facilitate crab consumption of oysters. This counterintuitive result cannot be explained by negative effects of ocean acidification on crab shell mass because acidification did not significantly influence *P. herbstii* calcification rates. Furthermore, *C. virginica* calcification differed only between control and high pCO_2 treatments. Despite oyster calcification rates in the intermediate pCO_2 treatments being statistically indistinguishable from the control pCO_2 treatments, several metrics of crab behaviour differed significantly between these two treatments. Furthermore, the reduced calcification rates of oysters in the high treatment likely rendered them more vulnerable to predation by mud crabs, which is counter to what we observed in the behavioural assays. Thus, the negative effect of acidification on the ability of mud crabs to prey upon oysters more than offset any advantage conferred to the crabs from the decline in net rate of oyster calcification under the highest pCO_2 treatment.

Other experiments investigating the impact of CO_2-induced ocean acidification on predator–prey dynamics of oysters or crabs either support the hypothesis that acidification either increases the predation risk for oysters and other calcifying crab prey or has no effect. Amaral *et al.* [60] found that oysters exposed to low pH due to acidic run-off from sulfatic soils are more susceptible to drill predation than oysters from reference sites lacking acidic run-off [60]. Meanwhile, Landes & Zimmer [35] found no increase in predation on *L. littorea* by the green crab *C. maenas* under acidified conditions [35]. Differences between our experimental results and those of Amaral *et al.* [60] and Landes & Zimmer [35] may stem from the latter studies conducting predation trials at control pH conditions, whereas we conducted trials at the acidification levels under which the organisms were originally reared. Furthermore, Amaral *et al.* [60] used predators reared in non-acidified water, whereas both predators and prey in the present experiment were reared under the same suite of

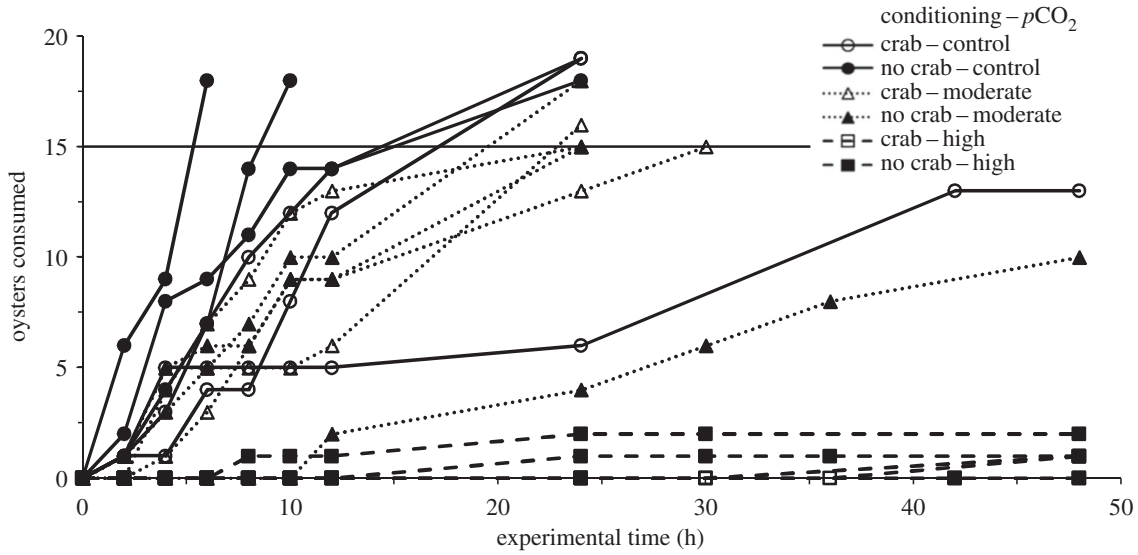

Figure 3. Oysters consumed versus time for each replicate predation trial. Reference line at 15 oysters consumed marks 75% mortality and trial termination.

control and acidified treatments. These unexpected findings highlight the importance of assessing the impact of acidification on predator behaviour, in addition to its impact on calcification rate and shell/skeletal properties.

To further explore why acidification reduced crab foraging rates, the impacts of acidification on each of the different crab behaviour and oyster consumption metrics were evaluated. Behaviours that remain consistent even in the high pCO_2 treatment can be considered resilient to changes in seawater pH. Crab general activity, agonistic behaviour, and number of predation attempts did not differ as a function of crab presence or acidification. Previous studies on the effects of acidification on the levels of general activity in crustaceans are mixed, with conflicting results concerning the mechanism driving the behavioural change. For example, acidification had no effect on general activity levels of two species of crayfish [61]. However, acidification reduced the time that a hermit crab (*Pagurus bernhardus*) spent in motion when either presented with an improved shell choice (approx. 25%; [48]) or exposed to prey cues (approx. 40%; [49]), and reduced the swimming ability of a penaeid shrimp (approx. 30%; [62]). Similar variation in activity response to pCO_2 is present in fishes, both across species and within species across temperatures [63–65]. The impact of pCO_2 on activity appears to be highly variable, but when changes in activity are induced by acidification, they are likely to have significant effects on predator–prey dynamics and community structure.

Agonistic behaviour was generally low across all treatments (approx. 1% of time observed) except in the two control and moderate pCO_2 replicate tanks with low rates of crab consumption of oysters, where agonistic interactions accounted for 8–13% of the time observed (figure 3). Acidification has been shown to invert the aggression and competitive dominance relationships between two species of damselfish competing for space on a coral reef [51] and conspecific agonistic interactions may be similarly susceptible to the influence of acidification. However, no such effect was observed in *P. herbstii*. Agonistic interactions observed in our study were mostly displays of dominance and very brief physical confrontations in which a single crab was consistently dominant. The experiment was explicitly designed to avoid resource depletion, and consequently may have dampened agonistic interactions among crabs, thereby making it challenging to identify differences in aggression across treatments. Additional experiments are needed to better understand the effect of acidification on *P. herbstii* aggression.

Although there was no effect of acidification on prey encounter rates, encounter rates in the high pCO_2 treatment were approximately one-third those in the other two treatments. De la Haye *et al.* [49] found that hermit crabs in acidified conditions were less successful at locating prey scent than those in the control treatment [49]. In their study, a non-food object was soaked in prey cue and then presented to the crab, thereby isolating scent as the only cue available to identify the food source. Multiple sensory cues have been shown to compensate for loss of olfaction in damselfishes [66], and this study maintained visual and tactile cues in addition to scent cues. Habitat choices made by settling damselfishes were significantly altered by acidification when only scent cues were presented, but those differences disappeared when a broader suite of sensory cues were provided [66]. Multiple senses appear to be capable of compensating, to some degree, for potential reduction in chemosensory ability. This study suggests that *P. herbstii* encounter rates of *C. virginica* are largely resilient to near-term acidification but may be reduced under extreme conditions.

Although encounter rates did not differ among the acidification treatments, acidification reduced total predator handling of prey and decreased the average time that predators spent unsuccessfully attempting to consume prey. These findings suggest that although acidification has minimal effect on the ability of predators to locate prey, predators are less persistent when they attempt to consume prey under acidified conditions. This could be a response to increased metabolic requirements associated with acidification. Penaeid shrimp were unable to maintain swimming efforts under highly acidified conditions [62]. Thus, crabs in acidified water may lack the capacity for prolonged predation attempts. However, we found no effect of acidification on several other energetically expensive behaviours (e.g. locomotion and aggression). A potential alterative mechanism explaining reduced crab handling of oysters could stem from GABA receptor excitation in *P. herbstii*, which could be disrupting cost–benefit processes. Suboptimal resource utilization after encountering prey has been previously observed in a hermit crab species [48]. De la Haye *et al.* [48] found that a hermit crab reduced shell switching from

inferior to optimal shells in elevated pCO_2 and concluded that acidification disrupts resource assessment and decision-making processes [48]. The role of GABA disruption as a mediating factor remains unclear as haemolymph Cl^- increased in acidified waters, which is the inverse of what has been observed in fish ([42] and references therein) and molluscs ([67] and references therein). Regardless of the mechanism, our results suggest that acidification disrupts the ability of predators to consume prey, and could consequently reduce transfer of energy to higher trophic levels.

It is unclear to what extent *P. herbstii* and *C. virginica* will adapt to future ocean acidification, which could result in greater or reduced mud crab foraging success on oysters under acidified conditions. Over sufficiently long timescales and barring extinction of either species, both species may adapt to CO_2-induced ocean acidification. In the near term, however, our results suggest that harm caused by ocean acidification to oysters and the reefs that they form may be at least partially offset by behavioural impairment of their crustacean predators. Yet, these results also suggest that the crustacean predators that demonstrated resilience (relative to molluscs) in early ocean acidification studies, because they are more capable of calcifying under acidified conditions, may indeed be vulnerable to acidification in other ways.

Acidification has been found to strongly impact calcification rates of individual organisms [15–24]. Generalizing the impacts of ocean acidification at population, community and ecosystem levels will require incorporating how other processes, such as predator foraging behaviour and prey avoidance of predators [35,60], are impacted by ocean acidification. Our study explores some of these other key processes and demonstrates that acidification-induced impairment of *P. herbstii* foraging on *C. virginica* offsets any potential benefit to the crabs that results from preying upon more weakly calcified oysters under acidified conditions. These findings have important implications for the management of crustacean fisheries and oyster reefs, which provide valuable ecosystem services such as providing nursery ground for economically valuable fishery species, stabilizing shorelines and removing anthropogenic nitrogen from eutrophied estuaries [68–70].

Ethics. The study used the minimum number of animals necessary to ensure scientific robustness. Under the Animals (Scientific Procedures) Act 1986, work with these organisms is not a licensable activity.

Data accessibility. Data are available at: http://www.bco-dmo.org/project/2152.

Authors' contributions. L.D., J.G., J.R. and M.P. designed the experiments. L.D. preformed the experiment and analysed predation data. I.W. preformed water chemistry analyses and J.R. analysed water chemistry data. L.D. and J.G. wrote early drafts of the manuscript and all authors contributed substantially to revisions.

Competing interests. We declare we have no competing interests.

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