Effects of estuarine acidification on predator–prey interactions

Valter Amaral[1,2,*], Henrique N. Cabral[1], Melanie J. Bishop[2]

[1]Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[2]Department of Biological Sciences, Macquarie University, North Ryde, New South Wales 2109, Australia

ABSTRACT: Short-term experiments exposing calcifying organisms to acidification have revealed altered growth and strength of their exoskeletons. We tested the hypothesis that multi-generational exposure to sustained estuarine acidification from runoff from acid sulphate soils (ASS) would: (1) reduce the shell strength of sessile or relatively immobile wild benthic invertebrates and (2) as a consequence render these invertebrates that rely on armour for anti-predator defence more susceptible to generalist benthic predators. First, we compared the force required to break the exoskeletons of *Saccostrea glomerata*, *Bembicium auratum*, and *Heloecius cordiformis* between replicate south-east Australian mangrove forests close to (acidified) and away from (reference) major ASS outflow drains. Second, we assessed differences in the susceptibility of oysters from acidified and reference forests to predation by the generalist muricid gastropod *Morula marginalba*. Mollusc shells were significantly weaker at ASS-affected than at reference sites, but the strength of crab carapaces was not influenced by acidification. Oysters from acidified sites were consumed by *M. marginalba* at a faster rate than oysters from reference sites in choice and no-choice experiments because *M. marginalba* required less time to drill through weaker shells. Many other predators such as crabs are generalist feeders that consume prey at rates inversely proportional to their shell strength. Hence, in the absence of effects of acidification on the ability of these predators to consume prey, molluscs at acidified sites may also be more susceptible to other such predators. This study highlights how human stressors can rapidly alter predator–prey interactions that have evolved over many years.

KEY WORDS: Acidification · Anti-predator defence · Armouring · Crabs · Macroinvertebrates · Oysters · pH · Predation

INTRODUCTION

Predation plays a central role in determining the structure of populations, communities, and ecosystems (e.g. Murdoch & Oaten 1975, Seitz et al. 2001). At the population level, predation can influence the biology and ecology of prey species by selecting for traits that lower the success of the predator in encountering, detecting, identifying, attacking, capturing, and/or consuming that species (Vermeij 1982). Some predators may, in return, have the capacity to evolutionarily respond to changes in anti-predator defences (Vermeij 1994). Nevertheless, over thousands of years, selection generally favours prey with anti-predator responses that are well adapted to the predatory selection regime (Vermeij 1987).

Increasingly, the selection of particular morphological and behavioural traits that have emerged over millennia is rapidly being reversed by human activities that modify these at time scales as short as years or even days (Byers 2002). Where these traits are important in anti-predator defence or predator success, major changes in predator–prey interactions can result (Byers 2002). For example, the continual expo-

sure of mammals and birds to human stimuli in urban areas can reduce their anti-predator responses involving vigilance behaviour through a process of habituation (e.g. Metcalf et al. 2000, Magle et al. 2005). In marine sediments, the induction of hypoxia can enhance the predatory mortality of burrowing, infaunal species by causing them to spend longer at, or close to, the sediment surface where they are more vulnerable to visually-feeding predators (e.g. Taylor & Eggleston 2000, Byers et al. 2010).

Within coastal ecosystems, bivalves, gastropods, and crustaceans generally have anti-predator responses that can be categorized as: (1) morphological barriers that increase handling time ('armoring'; Vermeij 1987); and/or (2) density or habitat refuges that reduce a predator's encounter rate ('avoidance'; Sponaugle & Lawton 1990). Species at the armoured end of the spectrum are typically on full display to predators and gain 'coexistence refugia' (Menge & Lubchenco 1981) due to their difficulty in being handled. This is possible because many benthic predators such as crabs, naticid and muricid gastropods, and fishes are generalist feeders, consuming those prey items that yield within a certain time (e.g. Fairweather & Underwood 1983, Brown & Haight 1992, Hartman 2000, Bishop et al. 2008). Among armoured species, processes that weaken shell thickness or strength may confer greater vulnerability to predation (e.g. Seed & Hughes 1995, Bishop & Peterson 2006, Newell et al. 2007).

Ocean acidification is among the anthropogenically enhanced processes that may modify the efficacy of exoskeletons in protecting animals from predation. In laboratory and field experiments, a high proportion of calcifying marine organisms has been observed to exhibit change in net calcification following transplant to CO_2-enriched waters, sometimes of a positive and sometimes of a negative direction (e.g. Ries et al. 2009, Rodolfo-Metalpa et al. 2011). As CO_2 concentrations increase, impacts to net calcification become increasingly negative, because any enhancement of growth and calcification by CO_2 is outweighed by the high rates of shell dissolution that occur at low pH (Rodolfo-Metalpa et al. 2011). Under such conditions, animals may lose their ability to adequately protect themselves against predators by using morphological barriers (Bibby et al. 2007).

In addition to CO_2 acidification, many estuaries are presently experiencing acidification from acid sulphate soils (ASS). ASS occur on all continents of the world (Dent & Pons 1995) and can locally reduce the pH of adjacent estuarine waters to as low as 2 to 6 where networks of drainage channels and flood-gates concentrate runoff from the adjacent land (Sammut et al. 1996, NSW DPI 2006). In many cases, exposure of estuarine ecosystems to ASS runoff has spanned several decades, providing an ideal model system in which to assess multi-generational impacts of low pH on calcifying organisms and their predator–prey interactions. Studies with oysters never before exposed to ASS runoff have shown that short-term exposure to acidified waters both in the laboratory and in the field can cause significant shell dissolution and perforation of juvenile oysters (Dove & Sammut 2007a,b). It is unclear whether bivalves, gastropods, and crustaceans that have been exposed to ASS runoff over multiple generations display the same response.

We investigated the long-term effects of estuarine acidification on the structural defences of mangrove invertebrates in estuaries of New South Wales (NSW), Australia. First, we tested for differences in the strength of the exoskeleton of: (1) sessile and mobile molluscs and (2) crabs, between areas close to and away from major ASS outflow drains. Sessile molluscs, mobile molluscs, and crabs represent the full spectrum of the armour-avoidance prey-defence continuum. Sessile molluscs rely almost entirely on armouring as protection against predation; mobile gastropods utilise a combination of armour and avoidance; and crabs have only a weak exoskeleton, depending more on avoidance (burial) or escape responses. We predicted that across all taxa, exoskeletons would be weaker in areas closer to drains, and that exoskeleton weakening would be most pronounced among sessile oysters, followed by gastropods and crabs, such that impact is also correlated with dependence on armouring. Second, we assessed whether a reduction of the strength of oyster shells leads to enhanced predation by a carnivorous muricid, the mulberry whelk *Morula marginalba*. *M. marginalba* is a generalist predator on intertidal rocky shores that displays highly variable handling times among its prey species according to their mechanical, behavioural, and chemical defences (Fairweather & Underwood 1983). On rocky shores, dead oysters are frequently found with a drill hole, indicative of predation by *M. marginalba* (Moran et al. 1984) or the mussel drill *Bedeva hanleyi*, another muricid gastropod that is locally common (Bishop et al. 2010). Using prey choice and no-choice laboratory experiments, we tested the hypothesis that oysters from sites close to drains would be preyed on at a greater rate than oysters from reference sites due to the shorter handling time required to penetrate the former.

MATERIALS AND METHODS

Strength of mollusc shells and crab carapaces

To test the hypothesis that exposure to acidified waters would weaken the exoskeleton of calcifying invertebrates, we collected: (1) gold-mouthed top shells *Bembicium auratum* and semaphore crabs *Heloecius cordiformis* from acidified and reference sites within each of the Hunter and Port Stephens estuaries, NSW, Australia during the summer of 2010; and (2) Sydney rock oysters *Saccostrea glomerata* from each of these 2 estuaries and a third estuary, the Hastings, during fall 2009. Within each estuary, we collected invertebrates from 2 acidified and 2 reference sites situated in intertidal mangrove forests, dominated by the grey mangrove *Avicennia marina* (Table 1). We measured water pH, temperature, and salinity at each sampling site at 8 randomly selected sampling times between April 2009 and February 2010 using a multi-parameter, handheld, water quality meter (CyberScan PCD 650, Eutech Instruments). Acidified sites were within 900 m of major ASS outflow drains, at locations where we had recorded depressed pH, and there was a history of low pH (Table 1). Reference sites were situated 2400 m from drains, at locations classified as being of low ASS runoff risk (Naylor et al. 1998), and at which we had not observed pH to drop below 7.7 (Table 1). All sites were of similar water temperature (22 to 23°C), but sites adjacent to drains were of slightly lower (~1 PSU) salinity (Table 1).

At each sampling site, *Saccostrea glomerata* formed dense aggregations attached to pneumatophores. *S. glomerata* and *Bembicium auratum,* which was found within oyster clumps, were collected from at least 15 randomly selected pneumatophores per site, at a mid-tidal elevation (mean low water +0.5 to 0.7 m). *Heloecius cordiformis* were hand-collected from the same mid-shore elevation. Upon collection, the maximal shell height of oysters and gastropods, and the maximal carapace width of crabs were determined using Vernier callipers (0.01 mm precision). The right (upper) valves (the shell-half most exposed to predators) of oysters were then oven-dried at 65°C for 24 h in preparation for strength testing. Oyster shells were dried prior to testing to prevent variable water retention by their highly heterogeneous surfaces from confounding measurements. Previous studies have shown that drying mollusc shells below 90°C does not affect shell resistance to crushing (Currey 1979). Gastropods and crabs, by contrast, had more homogenous surfaces that could be effectively towel-dried. These were tested wet and whole, because attempts to separate their flesh from their shell for drying caused damage to their exoskeleton.

We determined the force (Newtons) required to fracture the shells of oysters and gastropods and the carapaces of crabs using the Instron Universal testing system. Force was applied to each specimen via a probe travelling at 1.7 mm s^{-1} until the specimen failed. We used a 2 mm diameter probe for mollusc shells and a 1 mm diameter probe for crabs. Oyster right valves and crab carapaces were orientated so

Table 1. Mean (±SE) environmental conditions (Temp = temperature; salinity, pH) at each of our study sites in the year prior to our study (n = 8 sampling times). Within the Hunter (H), Port Stephens (P), and Hastings (S) estuaries, acidified (A1, A2) and reference (R1, R2) sites were selected on the basis of these measurements, previously recorded pH minima (pH min) and acid sulphate risk maps that categorise areas according to the probability that they are impacted by this disturbance (ASS risk; Naylor et al. 1998)

Site	Location	Latitude South	Longitude East	Temp (ºC)	Salinity	pH	pH min	ASS risk
HA1	Fullerton Cove	32° 50′ 37″	151° 48′ 34″	22 (±2)	26 (±2)	6.94 (±0.09)	~4[a]	High
HA2	Tomago Wetland	32° 51′ 07″	151° 46′ 05″	22 (±1)	26 (±1)	6.98 (±0.12)	~4[a]	High
HR1	Southern Ash Island	32° 51′ 43″	151° 47′ 03″	22 (±1)	26 (±2)	7.92 (±0.05)	~7[a]	Low
HR2	Northern Ash Island	32° 51′ 24″	151° 47′ 12″	23 (±2)	27 (±1)	7.92 (±0.04)	~7[a]	Low
PA1	Fenninghams Creek (entry)	32° 44′ 42″	152° 03′ 18″	22 (±1)	30 (±3)	6.52 (±0.27)	<5[b,c]	High
PA2	Fenninghams Creek (middle)	32° 45′ 11″	152° 03′ 06″	22 (±1)	30 (±2)	6.60 (±0.23)	<5[b,c]	High
PR1	Stuart's Island	32° 44′ 39″	152° 01′ 45″	23 (±1)	32 (±2)	7.93 (±0.11)	~6.8[c]	Low
PR2	4 km north of Stuart's Island	32° 45′ 38″	151° 59′ 40″	22 (±2)	31 (±2)	7.88 (±0.08)	~6.8[c]	Low
SA1	Fernbank Creek	31° 24′ 28″	152° 31′ 25″	21 (±2)	28 (±3)	6.75 (±0.23)	<4[d]	High
SA2	Maria River	31° 23′ 41″	152° 50′ 56″	22 (±1)	28 (±2)	6.85 (±0.24)	~2[d]	High
SR1	Settlement Point	31° 24′ 13″	152° 53′ 45″	22 (±1)	29 (±3)	7.72 (±0.11)	~6.5[d]	Low
SR2	Limeburners Creek	31° 24′ 05″	152° 53′ 18″	21 (±2)	30 (±2)	7.82 (±0.10)	~6.5[d]	Low

[a]NSW DPI (2008); [b]NSW DPI (2006); [c]NSW DPI (2009); [d]Tulau (1999)

that their plane was perpendicular to the probe, which was in turn directed at their centre. For gastropods, the vertical probe applied force to the suture between 2 whorls halfway up the gastropod shell. The maximum load on each specimen immediately prior to failure was taken as a measure of strength and was correlated to the height of oyster and gastropod shells or the width of crab carapaces.

Within each estuary, we compared the relationship between the size and exoskeleton strength of each invertebrate species between acidified and reference sites, using linear regression. Replicate sites within estuaries were pooled in analyses because 2-tailed Student's *t*-tests (Zar 1984) revealed that the regression slopes between strength and size of exoskeletons did not differ between these (Table 2). The regression slopes were compared between acidified and reference treatments within each estuary using 2-tailed Student's *t*-tests. The null hypothesis was that the regression slopes would not significantly differ. Analyses were considered significant at $\alpha = 0.01$ to correct for the multiple comparisons.

Predation experiments

We conducted choice and no-choice predation experiments to test the hypothesis that exposure of *Saccostrea glomerata* to ASS-affected waters over many generations would increase their susceptibility to predation. Oysters, 25 to 30 mm in shell height

(~12 mo old) and sourced from the acidified and reference sites within the Port Stephens Estuary, were offered to individual *Morula marginalba* that were 20 to 22 mm in length and collected from Long Reef (33.738° S, 151.310° E) NSW, Australia, a coastal site that is not exposed to ASS runoff. We used 25 to 30 mm oysters in our experiments because this size class is: (1) numerically dominant at many of our study sites (Amaral et al. 2011) and (2) may be particularly susceptible to enhanced predatory mortality following shell-weakening because it is towards the upper size limit of *Saccostrea* spp. that *Morula* spp. can handle (Taylor 1990, Koh-Siang 1993). The size range of *M. marginalba* was selected based on the availability of animals at the time of our study, and on previous observations that this size range has greater impact on oyster populations than smaller size classes (Moran 1980). The method by which *M. marginalba* bores a hole in prey species using its radula and protease enzymes is, for a given predator size, independent of the predator's own shell strength. Hence, we assumed that the predator's response would be independent of any shell dissolution that it itself succumbs to at acidified sites.

The oysters used in the experiments were single individuals, separated from one another and scrubbed to remove fouling organisms that may confound experimental results. Individual oysters, not clumps, were used so as to allow us to evaluate selection of single prey items, holding density constant through replacement. Because muricid gastropods prey by

Table 2. Results of 2-tailed Student's *t*-tests comparing the slopes of linear regressions describing the relationship between strength and invertebrate size (oysters, gastropods: shell height; crabs: carapace width) within acidified (A1, A2) and reference (R1, R2) sites of the Hunter River (H), Port Stephens (P) and Hastings (S) estuaries. Within each estuary, sites of a particular treatment (i.e. acidified or reference) were initially compared (Differences between sites). Where no differences were found, data were pooled and differences between acidified (A) and reference (R) treatments were tested at the estuary level (Differences between treatments — pooled data). Terms significant at $\alpha = 0.01$ are shown in **bold**. All data were analysed untransformed

Sites	Oysters			Gastropods			Crabs		
	t	df	p	*t*	df	p	*t*	df	p
Differences between sites									
HA1 vs. HA2	0.05	219	>0.959	1.65	267	>0.100	0.22	167	>0.822
HR1 vs. HR2	0.31	556	>0.753	0.93	616	>0.351	0.2	212	>0.844
PA1 vs. PA2	0.24	500	>0.807	0.32	220	>0.750	0.36	128	>0.717
PR1 vs. PR2	0.01	336	>0.989	0.09	504	>0.924	0.58	153	>0.562
SA1 vs. SA2	0.18	336	>0.859		Not sampled			Not sampled	
SR1 vs. SR2	0.1	311	>0.919		Not sampled			Not sampled	
Differences between treatments — pooled data									
HA vs. HR	3.39	559	**<0.001**	2.89	807	**<0.004**	0.09	383	>0.930
PA vs. PR	2.79	896	**<0.006**	4.03	728	>0.027	0.21	285	>0.836
SA vs. SR	3.26	815	**<0.002**		Not sampled			Not sampled	

drilling through the right valve of bivalves, rates of predation should not have been unduly influenced by our use of single rather than clumped oysters. Prey and predators were held separately in 40 l aquaria prior to experimentation (~1 wk). Both holding tanks and the smaller 10 l aquaria used for experimental trials contained filtered (<750 µm) seawater maintained at 22 ± 1°C using electric heaters and a salinity of 32. Small rocks were introduced to aquaria to provide substrate for animals. White fluorescent tubes (~80 lux) provided a photoperiod of 14 h light:10 h dark, approximating natural conditions in NSW at the time of the experiment. Experimental tanks were only partially filled with water to allow *Morula marginalba* to utilise both emersed and submerged habitat. To prevent *M. marginalba* from escaping, we applied a 2 cm wide petroleum jelly band to the wall of mesocosms, 5 cm above the water surface.

Prior to the start of each experiment, animals were fed in excess every other day — oysters with a commercial shellfish diet of 4 marine microalgae (Shellfish Diet 1800®, Reed Mariculture), and gastropods with 14 to 17 mm *Saccostrea glomerata* obtained from Port Stephens. Just before each feeding, the water within each aquarium was changed. We continued to feed oysters throughout the experiment, but the feeding of whelks was discontinued 48 h before the commencement of a trial, at which time whelks were placed in 10 l experimental mesocosms (one whelk per tank) for acclimation prior to the addition of prey items. Starving whelks immediately prior to each trial standardized hunger levels.

First, to test whether *Saccostrea glomerata* from ASS-exposed sites are more susceptible to predation by *Morula marginalba* than *S. glomerata* from reference sites, we conducted choice experiments in which we simultaneously offered equal numbers (3) of oysters from acidified (right valve marked with red nail polish) and reference sites to a single *M. marginalba* (n = 18 replicate trials). Pilot studies indicated that marking oysters with nail polish did not influence predator selection of prey. To maintain independence of replicates, animals and substrata were used only once. Oysters were checked for predation every 12 h, for a period of 10 d. At each observation time, dead (entire, without drill hole) or consumed (with drill hole) oysters were removed from aquaria and replaced with a prey item of similar type. We chose to maintain a constant density of prey items because *M. marginalba* is known to exhibit density-dependent rates of predation on at least some of its prey items (Moran

1985). Where *M. marginalba* were found sitting on an oyster, the oyster and whelk were not disturbed. We tallied the total number of oysters of each type that were consumed over the 10 d period, the duration of which was chosen based on rates of prey consumption by *M. marginalba* observed on rocky intertidal shores (Moran 1985). A 1-tailed paired *t*-test (n = 18 trials) evaluated the hypothesis that more oysters from acidified than reference sites would be consumed.

Second, to assess whether in choice experiments any differences in rates of predation on ASS-exposed and reference oysters were due to active prey selection by *Morula marginalba*, or simply due to a reduced handling time for drilling through the weakened shells of acidified oysters, we also conducted no-choice experiments (n = 18 replicate trials of each type). In these, we offered 6 oysters either from acidified sites or from reference sites to a single *M. marginalba*. Experimental conditions were otherwise similar to those in choice experiments. Every 12 h we checked oyster numbers and replaced any dead or consumed individuals. We terminated the trials after 10 d. Under the null hypothesis of no preference for one source of oyster over the other, the expected numbers of acidified or reference oysters consumed by *M. marginalba* in choice experiments were:

$$E_A = N_T[M_A / (M_A + M_R)]$$

$$E_R = N_T[M_R / (M_A + M_R)]$$

where M_A and M_R are, respectively, the numbers of acidified and reference oysters consumed when each is presented on its own (in equal numbers) and N_T is the total number of oysters consumed when offered together in choice experiments. We estimated M_A and M_R by summing the number of each species consumed in monospecific trials across independent replicates. We used χ^2 goodness-of-fit tests to compare the expected numbers of oysters consumed, E_A and E_R, with the numbers of oysters consumed when presented together (N_A, N_R for oysters from acidified and reference sites, respectively; $N_A + N_R = N_T$). Because these χ^2 tests are subject to excessive Type I error (see Liszka & Underwood 1990), only nonsignificant results could be interpreted.

Among no-choice trials, differences in the number of oysters consumed and the average time spent by *Morula marginalba* consuming them were compared between acidified and reference oysters using 2-sample, 1-tailed *t*-tests.

RESULTS

Strength of mollusc shells and crab carapaces

Within estuaries, regression slopes between animal size and exoskeleton strength did not differ between replicate sites for any of the 3 species considered (Table 2). Consequently, sites could be pooled prior to comparison of treatments (i.e. acidified vs. reference). Analysis of pooled data revealed that within each estuary, the shells of oysters and gastropods were weaker at acidified than reference sites (Figs. 1 & 2), although this general trend was not statistically
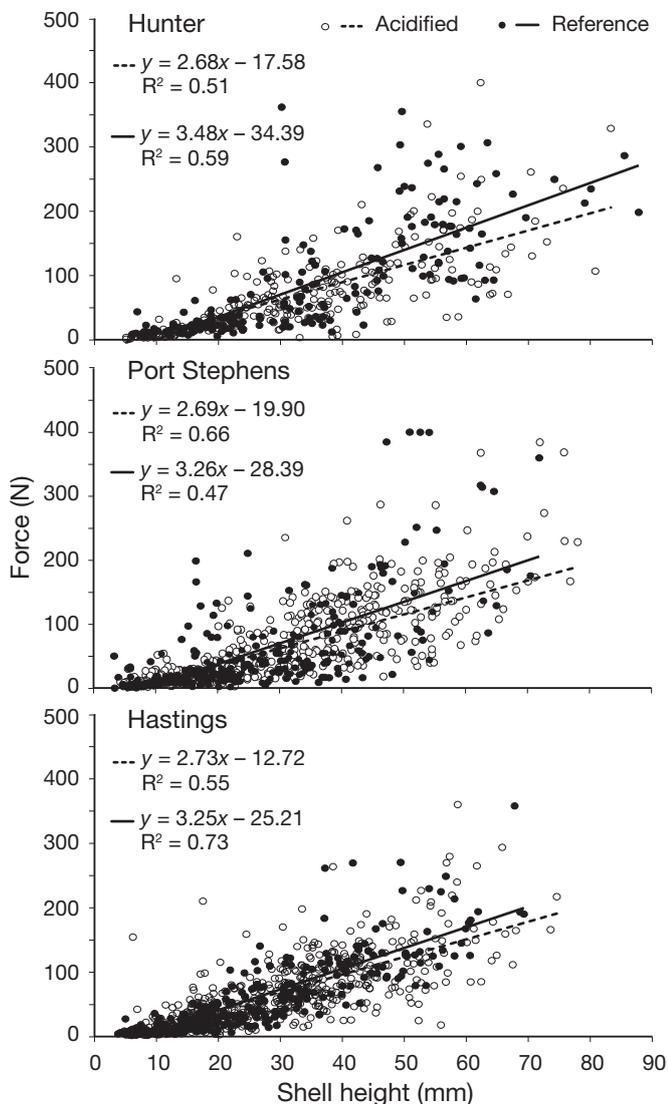
Fig. 1. Force (in Newtons) required to break the shells of *Saccostrea glomerata*. Within estuaries, data were pooled across replicate sampling sites within a treatment (acidified or reference) because regression slopes did not significantly differ
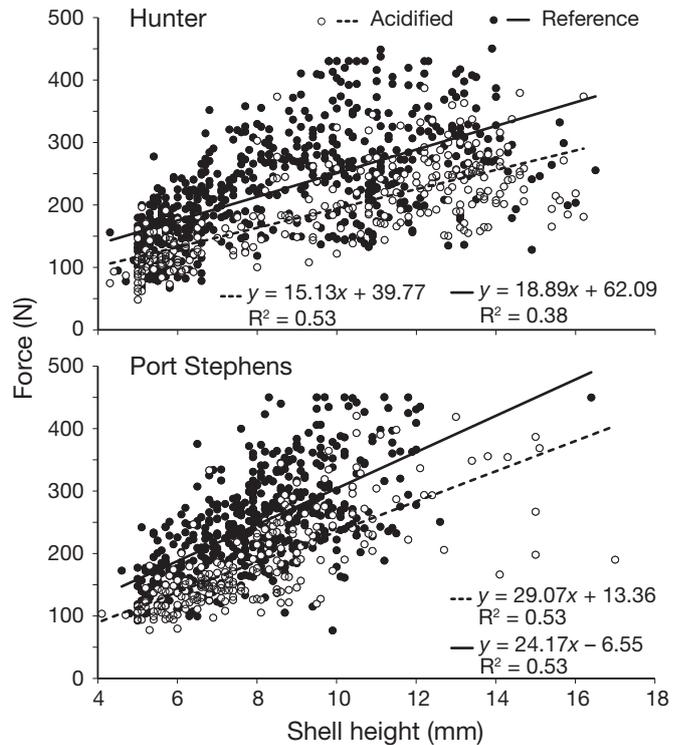
Fig. 2. Force (in Newtons) required to break the shells of *Bembicium auratum*. Within estuaries, data were pooled across replicate sampling sites within a treatment (acidified or reference) because regression slopes did not significantly differ

significant for gastropods within Port Stephens (Table 2, Fig. 2). By contrast, the strength of crab carapaces did not differ between acidified and reference sites in either of the estuaries (Fig. 3, Table 2). For both oysters and gastropods, the difference in shell strength between acidified and reference sites increased with animal size (Figs. 1 & 2).

Effect of oyster exposure to ASS runoff on susceptibility to predation by a gastropod

On average, across all trials, a single *Morula marginalba* completely consumed 2.5 ± 0.1 (mean ± SE) oysters within the 10 d of the experiment. In 9 of the 18 choice trials, *M. marginalba* consumed more *Saccostrea glomerata* from acidified than reference sites. In 8 of the 18 trials, *M. marginalba* consumed equal numbers of oysters from different sources, and in only 1 trial were more oysters from the reference site consumed. Consequently, across all 18 choice trials, *M. marginalba* consumed significantly more oysters from acidified (1.7 ± 0.2 ind. trial^{-1}) than reference (1.0 ± 0.1 ind. trial^{-1}) sites (1-tailed paired *t*-test: $t = 2.92$, df = 17, $p < 0.005$; Fig. 4), overall by 67 %.
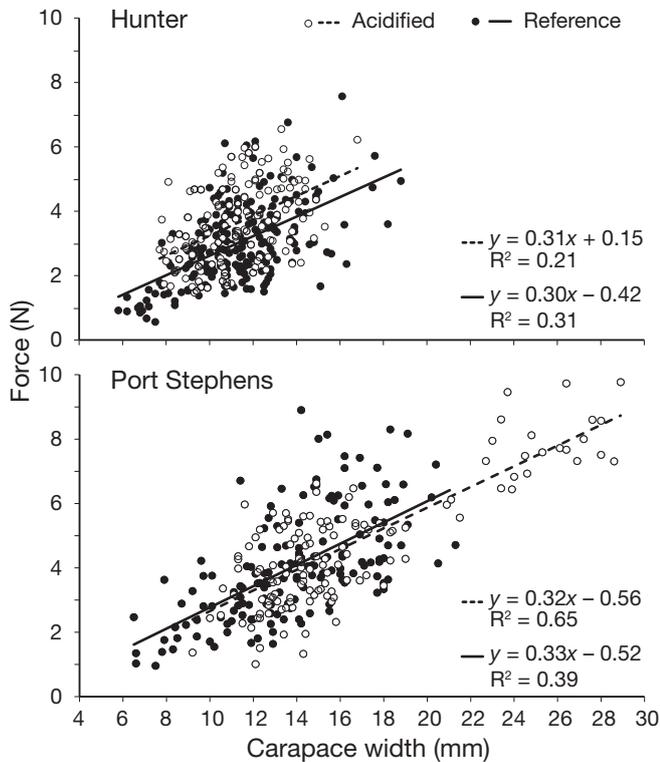
Fig. 3. Force (in Newtons) required to break the carapaces of *Heloecius cordiformis*. Within estuaries, data were pooled across replicate sampling sites within a treatment (acidified or reference) because regression slopes did not significantly differ
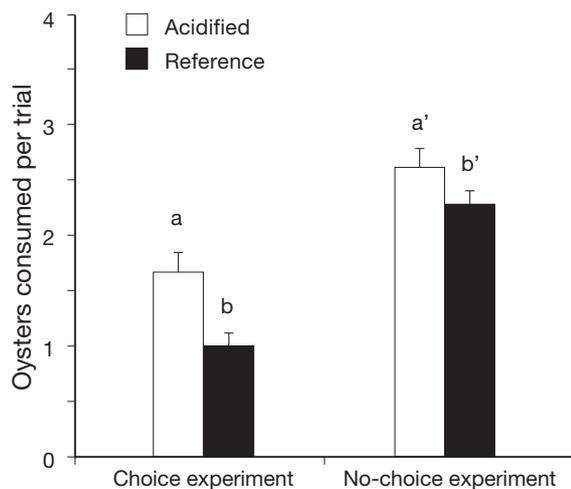

Fig. 4. Mean (+SE) number of *Saccostrea glomerata* consumed by *Morula marginalba* during 10 d trials in which predators were offered equal numbers of prey from acidified and reference sites (choice experiment) or were offered only a single prey type (no-choice experiment). Oysters were replaced whenever consumed or dead to maintain a constant density of 6 oysters per replicate. Bars that do not share letters are significantly different at p < 0.05. n = 18

Among the 18 choice trials, *M. marginalba* attacked an acidified oyster first in half the trials (9). Despite the identical probability of a whelk attacking an acidified or reference oyster first, in 15 of the 18 trials the second oyster consumed was from an acidified site. Of the 12 whelks that consumed a reference oyster as either their first or second prey item, only 5 went on to consume additional (3 to 4 total) prey. By contrast, 5 of the 6 whelks that consumed acidified oysters as both their first and second prey items went on to consume additional oysters. No whelk was observed to consume 2 reference oysters in a row, whereas whelks consumed 2 acidified oysters in a row in half (9) of the trials.

Among the no-choice trials, *Morula marginalba* also consumed more oysters sourced from acidified (2.6 ± 0.2 ind. trial^{-1}) than reference (2.3 ± 0.1 ind. trial^{-1}) sites (1-tailed 2-sample *t*-test: $t = 1.69$; df = 34, p = 0.05; Fig. 4). Consequently, we found no significant difference in the proportionate contribution of acidified and reference oysters to the total consumed between choice and no-choice trials ($\chi^2 = 1.6$, df = 1, p = 0.21). On average, in no-choice experiments, the time taken for *M. marginalba* to handle one oyster was ~20% shorter for oysters from acidified (36 ± 2 h) than reference (45 ± 2 h) sites (1-tailed 2-sample *t*-test: $t = -3.38$; df = 34, p = 0.001).

DISCUSSION

Previously, shell dissolution has been observed among transplanted *Saccostrea glomerata*, after only 6 h exposure to ASS-affected waters in the laboratory (Dove & Sammut 2007a), and after 40 d exposure in the field (Dove & Sammut 2007b). Similar shell dissolution of gastropod shells has also been noted in acidified estuaries (Marshall et al. 2008). Based on these results, we hypothesized that multi-generational exposure of wild mangrove benthic invertebrates to estuarine acidification would reduce the strength of their exoskeleton. We also hypothesised that effects would be greater among species that are sessile or relatively sedentary than among those that are highly mobile and, among sessile invertebrates almost entirely dependent on armouring as an anti-predator defence, as this would increase susceptibility to predation. As predicted, within each of the estuaries sampled, the forces required to break mollusc shells were lower for molluscs from acidified than reference sites throughout the whole range of molluscs' sizes. However, we found no evidence that estuarine acidification reduces the strength of crab carapaces.

Among sessile oysters, a reduction in shell strength resulted in a greater rate of predatory mortality.

Interestingly, the reduction in the shell strength of animals from acidified sites was of lower magnitude for *Saccostrea glomerata* than for *Bembicium auratum*. This is contrary to our predictions that the shells of sessile organism would be more impacted than those of animals with an ability to move out of ASS-affected waters. Whereas oysters remain attached to hard substrate following settlement, gastropods of several species, including *B. auratum*, can undergo up-shore migration during high tides to avoid predators, moving down the shore only when the tide retreats (e.g. Fairweather 1988, Reid 1988). Instead the shells of *S. glomerata* may be more resistant to dissolution by acidic ASS runoff than the shells of *B. auratum*. Whereas the shells of adult oysters are predominantly comprised of calcite (Stenzel 1964), the shell of *B. auratum* is of the more soluble aragonite (Taylor & Reid 1990). Additionally, *S. glomerata*, which must rely almost entirely on shell thickening as anti-predator defence, might allocate more energy and resources to maintenance of the shell thickness under acidified conditions. Both oysters and gastropods are able to increase shell production in response to environmental pressure, such as predation, pollution, and mechanical stress (Currey & Hughes 1982, Bibby et al. 2007, Newell et al. 2007). Mobile *B. auratum*, which combine armouring with avoidance strategies of anti-predator defence might not need to up-regulate shell-building to the same degree.

By contrast to the molluscs, mangrove crabs did not display weakened exoskeletons at the acidified sites. This may be due to a thick epicuticle, absent in molluscs, that isolates crustaceans from the exterior environment and allows them to regulate their internal pH and carbonate system in response to changes in water acidification (Cameron 1985, Ries et al. 2009). In a previous study, crabs were able to increase calcification rates while molluscs exhibited shell dissolution in response to CO_2-induced acidification (Ries et al. 2009). Additionally, mangrove crabs may also be better able to withstand estuarine acidification as a consequence of their behaviour. For example, burrowing crabs can remain inside plugged burrows during the rising tide and thus avoid direct exposure to strongly ASS-acidified waters, while others have dispersion abilities that allow them to simply remain up on the shore above the water line (e.g. Eshky et al. 1995, Skov & Hartnoll 2001). Previously, we found no difference in the abundances or size-structures of crabs between acidified and reference sites (Amaral et al. 2011) and Russell & Helmke (2002) reported that acidic waters from ASS

runoff did not negatively affect the survival of mud crabs *Scylla serrata* in the wild.

Overall, the impact of estuarine acidification on the shell strength of molluscs was smaller than predicted from previous short-term experiments exposing organisms to runoff from ASS soils (Dove & Sammut 2007a,b). In the laboratory, *Saccostrea glomerata* suffered shell degradation, soft tissue lesions and reduced filtration rates following just 6 h of exposure to water of pH 5.1 (Dove & Sammut 2007a). In the field, up to 85% of small oysters were observed to have shell lesions following 2 mo of exposure to waters of average pH 5.47 (Dove & Sammut 2007b). The weaker effects observed in the present study may be because it was conducted in a relatively dry period, during which the acidified sites had a pH of 6.52 to 6.98. During rainfall periods, when large amounts of runoff from ASS enter estuaries, pH can drop to 2 to 5 (Sammut et al. 1996, Russell & Helmke 2002, Dove & Sammut 2007b). Organisms may exhibit some capacity to recover in dry periods following major acidification events. Alternatively, organisms exposed to ASS-acidification over many generations may be able to adapt behaviourally and/or physiologically to the stressor. Mount et al. (1990) also reported that fish exposed for multiple generations to ASS runoff developed long-term physiological resistance to acidification.

Despite the relatively weak effect of acidification on the shell strength of oysters, we found that this translated into enhanced susceptibility to predatory mortality by a generalist benthic predator. Consistent with previous observations that even small changes in the structural defences of invertebrates can lead to substantially increased susceptibility to predation (e.g. Seed & Hughes 1995, Cotton et al. 2004), weaker-shelled oysters from acidified sites were 67% more susceptible to predation by the muricid gastropod *Morula marginalba* than stronger-shelled oysters from reference sites. Three main lines of evidence suggest that the mechanism by which *M. marginalba* consumed more oysters from acidified than reference sites was primarily based on the biomechanics of shell strength. First, in no-choice experiments, we observed the handling time of an acidified oyster was on average 20% less than for a reference oyster. Second, even among no-choice trials, *M. marginalba* consumed more acidified than reference oysters, and in a similar proportion to that in choice trials. Third, in choice experiments *M. marginalba* attacked oysters in random order, with acidified and reference oysters equally as likely to be consumed first. Therefore, it is likely that the weakening

of the shells of *Bembicium auratum* at the acidified sites would also lead to greater rates of predatory mortality amongst their populations, although predator avoidance behaviour in marine gastropods might compensate for a weakened shell (Cotton et al. 2004).

In addition to muricid gastropods, many species of crab consume prey items in inverse proportion to their shell strength (e.g. Hughes & Elner 1979, Bishop & Peterson 2006). Crabs are among the most common predators of molluscs (e.g. Juanes 1992, Seed & Hughes 1995, Mascaro & Seed 2001), and several large species, including *Scylla serrata*, are common within the estuaries sampled in the present study. These species may also gain advantage over oysters and gastropods with weakened shells by exposure to estuarine acidification. Such increased susceptibility of weakened oysters and gastropods to predation by a multitude of generalist predators may account for the reduced abundance and skewed size-frequency distributions we have found for these species at acidified sites (Amaral et al. 2011).

In our predation experiments, we used predatory gastropods sourced from areas unaffected by ASS runoff. Nevertheless, given our demonstration of weakened shells among *Bembicium auratum* at acidified sites, *Morula marginalba* shells would likely be weakened, too. We expect that had we used *M. marginalba* from acidified sites, the outcome of experiments would not have been altered, as the gastropod's capacity to consume prey items is driven by its size but not its own shell strength. Crabs, by contrast, risk mechanical damage in opening large prey, and take this into account when choosing prey items (Juanes 1992, Seed & Hughes 1995). Hence crabs that have chelae that are weakened by acidification might not experience the same benefit of weakened prey items as muricid gastropods. In the present study, we found that the carapace of *Heloecius cordiformis* was not weakened by acidification. Further studies would be required to ascertain whether predatory crabs, such as the portunids and *Scylla serrata*, similarly do not suffer the same reduction in exoskeleton strength as their molluscan prey items.

In summary, our investigations clearly demonstrate the potential for a human-mediated stressor, estuarine acidification, to alter predator−prey interactions that have evolved over thousands of years. Sustained exposure to acidic runoff from ASS reduced the exoskeleton strength of oysters and gastropods, rendering them more susceptible to generalist predators that consume prey in proportion to their shell strength. More mobile crabs, by contrast, did not suffer any reduction in exoskeleton strength, and if this pattern extends to predatory crabs, they may benefit from prey with weakened shells in predatory interactions. The present study highlights the importance of not just examining ecological impacts of stressors to individual species, but also to tropho-dynamics, as impacts can clearly propagate through ecological interactions.

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