

Multiple stressors associated with acid sulfate soil effluent influence mud crab *Scylla serrata* predation on Sydney rock oysters *Saccostrea glomerata*

Cassandra N. Glaspie^{A,B,C} and Rochelle D. Seitz^A

^AVirginia Institute of Marine Science, College of William & Mary, Department of Biological Sciences, PO Box 1346, Gloucester Point, VA 23062, USA.

^BOregon State University, Department of Fisheries and Wildlife, 104 Nash Hall, Corvallis, OR 97731, USA.

^CCorresponding author. Email: glaspiec@oregonstate.edu

Abstract. Studies of long-term exposure to multiple stressors on predator–prey interactions are necessary to determine the effect of coastal degradation on organisms that have had generations to adapt and acclimate to change. In New South Wales, Australia, a natural gradient of multiple stressors produced by acid sulfate soil effluent was used to determine the impact of exposure to multiple stressors on predator–prey dynamics between mud crabs *Scylla serrata* and Sydney rock oysters *Saccostrea glomerata*. Wild oysters were collected from two polluted and two reference sites that varied in their distance away from a flood gate that acted as a point source of water with low salinity, low pH and low alkalinity. Oysters from sites affected by multiple stressors and those from reference sites were offered to mud crabs in 48-h laboratory no-choice feeding trials. Oysters from affected sites had lower mortality than those from a reference site that was farthest from the source of polluted water. Linear models containing distance from flood gate best explained oyster mortality. Differences in rates of mortality were due to the decreased time crabs spent foraging on affected oysters. Long-term exposure to acid sulfate soil effluent alters trophic dynamics between predators and prey, which may have consequences for coastal food webs.

Additional keywords: Australia, bivalve, pollution, predator–prey.

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Introduction

Humans depend on the coast for protection, fisheries, biogeochemical cycling, tourism, recreation and many other ecosystem services (Barbier *et al.* 2011). As a result of the close tie between humanity and the coast, over 41% of the human population lives within 100 km of the coastline (Martínez *et al.* 2007). This means that coastal systems are exposed to a wide variety of stressors from the land and from the sea, in particular pollutants and stressors related to coastal development (Lotze *et al.* 2006; Halpern *et al.* 2007; Dachs and Méjanelle 2010). Globally, 28% of the coastline has been converted to agriculture or urban landscape (Martínez *et al.* 2007). However, wetlands are disappearing slightly faster, with 35% of mangroves and over 50% of marshes lost globally due to human activities (Valiela *et al.* 2001; Millennium Ecosystem Assessment 2005).

Severely degraded environments such as wetlands rarely experience only a single stressor; it is more likely that they will experience multiple stressors at the same time, which can interact to produce unpredictable and sometimes drastic consequences for biota. Very few stressors exhibit additive effects on populations or communities, whereas antagonistic or synergistic effects of multiple stressors are both fairly common (Darling and Côté 2008), and synergistic effects of multiple

stressors tend to dominate in the marine realm (Burkepile and Hay 2006; Crain *et al.* 2008). Synergisms between stressors are particularly devastating because they have unpredictable consequences for ecosystem stability, and may even lead to regime shifts (Paine *et al.* 1998; Sala *et al.* 2000; Davis *et al.* 2010).

A thorough assessment of the impact of anthropogenic stressors on ecosystem stability will require close examination of the effect of multiple stressors on predator–prey interactions. The majority of multiple stressors studies are done in the laboratory and focus on individual species, rather than interactions between trophic levels (Walther *et al.* 2002; Crain *et al.* 2008). When predators and prey are exposed to multiple stressors, morphological and behavioural responses of both species combine additively, synergistically or antagonistically, further complicating ecosystem-scale responses to human disturbance. For example, acidification and high temperatures weakened shells in *Littorina littorea* and claw strength in *Carcinus maenas*, resulting in no net effect on predator–prey interactions between crabs and snails (Landes and Zimmer 2012). However, temperature and CO₂ acted synergistically to increase predation rates and decrease predator selectivity in reef fish (Ferrari *et al.* 2011). Lastly, larval topmelt anti-predator aggregative behaviour decreased additively with habitat

degradation and pesticide exposure (Renick *et al.* 2015). As predators can have a disproportionately large effect on ecosystem functioning (Jackson *et al.* 2001), it is important to consider the effect of multiple stressors on predator–prey interactions.

Time scale is also a major component in ecosystem response to abiotic drivers. Studies regarding the effects of stressors generally involve short-term exposure in the laboratory (Bibby *et al.* 2007; Landes and Zimmer 2012). However, recent literature suggests that these studies often do not agree with more realistic long-term and field-based studies (Green *et al.* 2004; Amaral *et al.* 2012a). This is likely because organisms exhibit the capacity to adapt or acclimate to stress (Form and Riebesell 2012; Benner *et al.* 2013; Calosi *et al.* 2013a). Studies that focus on natural gradients in multiple stressors, such as those at CO₂ seeps (Calosi *et al.* 2013b; Kroeker *et al.* 2013), marine volcanoes (Fabricius *et al.* 2011) and areas of upwelling (Wootton *et al.* 2008), avoid some of the problems associated with short-term studies that involve manipulation of stressors in the laboratory, and the results of such studies are likely more applicable to long-term change in natural systems.

Estuaries with acid sulfate soils exhibit persistent and severe gradients in multiple stressors that can be used to examine anthropogenic impacts on coastal marine communities. Acid sulfate soils are formed by previously deposited marine sediments containing sulfate-reducing bacteria and iron pyrite, which when oxygenated form sulfuric acid (Cook *et al.* 2000; Mosley *et al.* 2014). Development and agricultural activity increase the production of sulfuric acid and its accumulation in drainage ditches (Dent 1986; Cook *et al.* 2000). Acidic water is then distributed to surrounding water bodies via outflow drains, exposing aquatic organisms to multiple stressors including low salinity, acidification and toxic metals (Wilson and Hyne 1997; Mosley *et al.* 2014).

This study focuses on a natural gradient of multiple stressors produced by acid sulfate soil runoff in the Hastings Estuary, an intertidal mangrove forest in New South Wales (NSW), Australia. Acidification in the Hastings Estuary is exacerbated by drainage and development projects from the 1960s and 1970s, which led to accumulation of acidified water in drainage ditches and caused acute and chronic drops in pH, especially near flood gates at the confluence of these drainage ditches and the estuary proper. Acid sulfate soils in the area lower the pH of estuarine waters near flood gates by 3 units or more after heavy rain, when acidic water is flushed into the estuary. Areas polluted by acid sulfate soils also exhibit lower and more variable salinity (Amaral *et al.* 2011), increased concentration of toxic metals (Nath *et al.* 2013) and lower alkalinity.

Despite exposure to polluted water for several decades, the Hastings Estuary supports populations of the Sydney rock oyster *Saccostrea glomerata* (Amaral *et al.* 2011). *S. glomerata* is a bivalve species endemic to Australia and New Zealand that supports a valuable commercial fishery and aquaculture industry (Neill 1993; Amaral *et al.* 2011; Parker *et al.* 2011). Acid sulfate soil runoff may influence predator–prey dynamics of *S. glomerata* in estuaries like the Hastings. Acidification compromises shell integrity and could therefore affect survival rates; thus, bivalve molluscs are expected to be some of the most sensitive organisms to changes in pH (Beniash *et al.* 2010; Kroeker *et al.* 2014). *S. glomerata* near areas of acid sulfate soil drainage are less

abundant (Amaral *et al.* 2011) and grow at approximately half the rate of oysters at reference sites (Amaral *et al.* 2012b). Oysters from sites at the greatest risk of acid sulfate soils have weaker shells than those from reference sites, which may increase mortality due to predation (Amaral *et al.* 2012a). Amaral *et al.* (2012a) observed interactions between juvenile *S. glomerata* and a drilling gastropod, the mulberry whelk *Morula marginalba*, and concluded that oysters from polluted sites are consumed by gastropods at a faster rate than those from reference sites because *M. marginalba* are more efficient at drilling through oysters' weakened shells (Amaral *et al.* 2012a).

Oyster shell thinning may also benefit decapod crustaceans such as the mud crab *Scylla serrata*. The interaction between oysters and large predatory crabs is of particular interest because, unlike drilling gastropods, *S. serrata* is capable of consuming all sizes of *Saccostrea glomerata*, including reproductive adults (Underwood and Barrett 1990; Anderson and Connell 1999). *Scylla serrata* is found in mangrove estuaries in the Indo-West-Pacific region, where it supports a profitable fishery. *S. serrata* is tolerant of variable water quality, including events of increased acid sulfate soil runoff (Russell and Helmke 2002), and preys mainly upon molluscs (Hill 1976); thus, it is likely to prey upon oysters from polluted sites and is an ideal predator for the current study.

This study examined the effect of multiple stressors produced by acid sulfate soil runoff on the interaction between Sydney rock oysters *Saccostrea glomerata* and mud crabs *Scylla serrata*. We used controlled laboratory experiments to compare crab foraging behaviour on populations of oysters collected from affected and reference sites in the Hastings Estuary in NSW, Australia. We hypothesised that crabs preying on oysters from affected sites would have shorter handling times than those preying on oysters from reference sites. Consequently, we expected a greater rate of consumption of oysters from affected v. reference sites.

Materials and methods

Oysters and crabs

Sydney rock oysters (*Saccostrea glomerata*) for use in predation experiments were collected from four sites within the Hastings Estuary, NSW, Australia. Two of the collection sites (hereafter, affected) were within 2.5 km of a flood gate in Fernbank Creek where waters affected by acid sulfate soils are channelled into the estuary through the mouth of the creek (Fig. 1). These sites have been previously identified as sites of chronic acidification (Tulau 1999; Amaral *et al.* 2011; Amaral *et al.* 2012a; Dove and Sammut 2013) and during our study had one or more pH readings below 5.5 and orange-red sediment in the intertidal zone, an indication of pollution by acid sulfate soil runoff (Amaral *et al.* 2012b). Previous studies have reported pH as low as 4 near the first of our affected sites, whereas near the second, pH values as low as 2 have been recorded following rainfall events (Tulau 1999). Two of the collection sites (hereafter, reference) were at least 3 km from flood gates and situated in stretches of the estuary identified as being of low risk of acid sulfate soil (Tulau 1999). During our study, reference sites had pH values that exceeded 6.3 and were free of orange-red discolouration. The pH, temperature and salinity of study sites were measured once at high tide, once at low tide and three times at the time of oyster

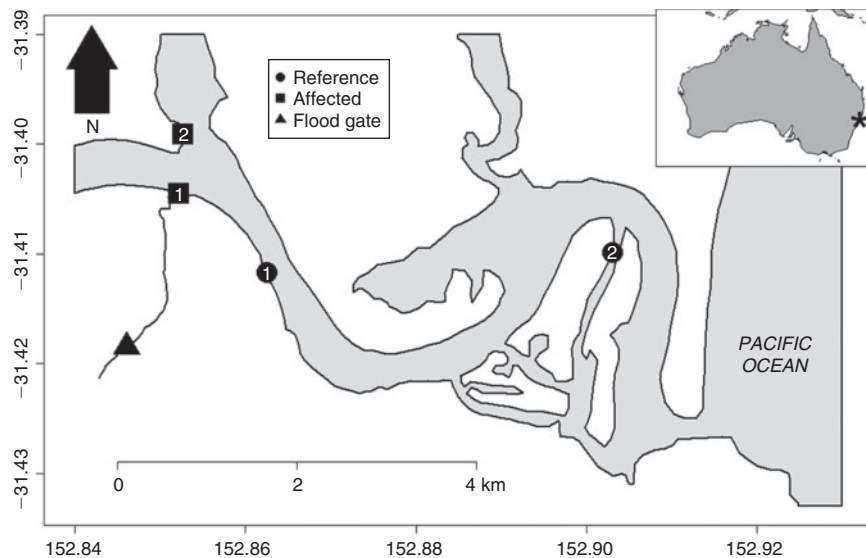


Fig. 1. Map of study sites in the Hastings Estuary, New South Wales, Australia. The black triangle denotes the location of the flood gate in Fernbank Creek. Affected sites (squares) were located close to the flood gate and directly in the path of the effluent, which enters the estuary from the mouth of Fernbank Creek. Reference sites (circles) were farther downriver of sites exposed to acid sulfate soil runoff. Inset: map of Australia with asterisk indicating the location of Hastings Estuary.

collection using a Horiba U-50 series multi-parameter water quality meter (Horiba Instruments, Irvine, CA, USA). Dissolved oxygen was measured once at low tide. Four water samples from each site were brought back to the laboratory and alkalinity was measured using an Aquarium Pharmaceuticals carbonate hardness test kit (Aquarium Pharmaceuticals, Chalfon, PA, USA).

Clumps of 14–25 oysters of shell height 18.7–68.6 mm were collected from each study site in July 2014 (during the Austral winter), transported back to Macquarie University in Sydney, NSW, and allowed to acclimate to laboratory conditions for 2 weeks before the start of the experiment. Conditions in the laboratory were as follows: water temperature 20°C, salinity 35 and lighting regime of 12 h of light and 12 h of dark. During this acclimation time, the water temperature was raised to 26°C, which was the temperature at which crabs would feed. Oysters were fed algal paste throughout the experiment, and water was changed twice per week.

Adult male mud crabs (*Scylla serrata*) for use in experiments were obtained commercially from Northern Queensland and ranged in size from 74.5- to 78.0-mm carapace width. Crabs were acclimated to the laboratory over a period of 2 weeks in communal tanks (dimensions: length, 760 cm; width, 250 cm; height, 20 cm). Water temperature was slowly raised until crabs were feeding, which occurred at 26°C. Crabs were fed fish, mussels or oysters in excess, and water was changed every other day.

None of the crabs used in experiments had been previously exposed to waters polluted by acid sulfate soil, nor were they exposed to polluted water throughout the course of the experiment. Whereas crustaceans may experience physiological, morphological and behavioural consequences of acidification (Small *et al.* 2010; Donohue *et al.* 2012; Small *et al.* 2015), *Saccostrea serrata* is tolerant of acidification events and is commonly found in estuaries polluted by acid sulfate soil effluent (Russell and Helmke 2002); thus, it was considered

an ideal predator for the current study, despite lack of exposure to the same stressors as its experimental prey.

Predation experiments

No-choice feeding trials, in which each crab was offered oysters from a single source site (which in turn had varying exposure to multiple stressors), assessed how consumption rates varied according to oyster source conditions. Oysters were exposed to mud crab predation in 60-L mesocosm tanks (dimensions: length, 60 cm; width, 36 cm; height, 28 cm) half filled with filtered seawater and maintained at the temperature, salinity and lighting regime described above. Tanks were randomly assigned oysters from either affected or reference sites (two oyster source sites nested within each, $n = 6$ tanks per site); however, all mesocosms were conducted in the same unpolluted, filtered seawater. Each tank received 1–2 oyster clumps to give a similar number of oysters among tanks (mean 18.7 ± 4.3 s.d.). Oysters were counted before addition to tanks. A single crab was added to each tank and the number of live oysters (i.e. intact, uncrushed oysters that were not exhibiting gaping behaviour) remaining at the end of 48 h was counted. Trials were run in blocks of four tanks because of space limitations; however, all trials were run within 2 weeks and an ANCOVA including source site conditions (affected *v.* reference) and nested sites as main effects and day as a covariate indicated that there was no effect of time ($F_{1,19} = 2.005$, $P = 0.17$), so each trial was treated as an independent replicate.

For half of the trials ($n = 3$) predator behaviour was recorded using an infrared-sensitive camera system. A red spotlight was used to improve night-time video quality without disrupting crab behaviour (Cronin and Forward 1988). Raw footage videos of predator behaviour were analysed. The total amount of time a crab spent foraging (manipulating oyster clumps) and feeding

(engaging an oyster with mouth parts) was recorded, as well as the number of encounters with oysters (instances where the crab picked up an oyster with its claws). The average handling time for each mesocosm trial was calculated by dividing the total time a crab spent manipulating or eating an oyster (s) by the number of encounters.

It was not possible to use a different crab for each trial because of space requirements, nor was it possible to use each crab the same number of times because of losses throughout the experiment. Crabs were used between one and three times, and crabs were randomly assigned to trials so there was no bias inherent in the re-use of crabs. To track individual behaviour over time and account for learning or individual differences, crabs were assigned numbers and crab identity was recorded for each experiment. An ANCOVA including source site conditions (affected *v.* reference) and nested sites as main effects and individual crab identity as a covariate indicated that there was no difference in behaviour of individual crabs ($F_{8,10} = 2.02$, $P = 0.15$). Similarly, an ANCOVA including the number of times a crab was used as a covariate indicated that there was no trend of increased predation on subsequent use ($F_{1,19} = 1.871$, $P = 0.19$) indicating crabs did not exhibit learning behaviour; thus, each trial was treated as an independent replicate.

Statistical analysis

The degree to which mortality due to predation ($n = 6$), pH ($n = 5$), alkalinity ($n = 4$), salinity ($n = 5$), handling time ($n = 3$), foraging time ($n = 3$) and shell height ($n = 24$ clumps) differed between sites was assessed using nested ANOVAs with two factors: oyster source environmental condition (affected *v.* reference) and site (two levels, nested within each). The assumptions of parametric tests were assessed visually using residual plots and quantile-quantile plots. Oyster mortality data were logit-transformed, handling time data were quartic-root transformed, and foraging time data were square-root transformed to meet assumptions. For all two-group comparisons, Cohen's d is reported as a measure of effect size. Tukey's Honest Significant Difference (HSD) tests were performed for *post-hoc* comparisons. Trends between oyster mortality due to predation and handling time or foraging time were examined using Pearson's product-moment correlation.

Prior to experimentation, a set of candidate linear regression models were developed to represent several hypotheses regarding the effects of environmental factors on oyster mortality. Of these models, only those that did not contain collinear variables were included in the final analysis. Akaike's Information Criterion (AIC), which allows for the comparison of multiple models (Burnham and Anderson 2002), was used to determine the candidate model that best balances parsimony and goodness-of-fit. A correction (AICc) was used for low sample size (Burnham and Anderson 2002). All analyses were conducted in R statistical software (*ver.* 3.0.2, R Foundation for Statistical Computing: Vienna, Austria).

Results

Site characterisation

During the study, reference sites had significantly higher pH ($F_{1,2} = 18.38$, $P = 0.050$, $d = 0.82$) than affected sites (Fig. 2).

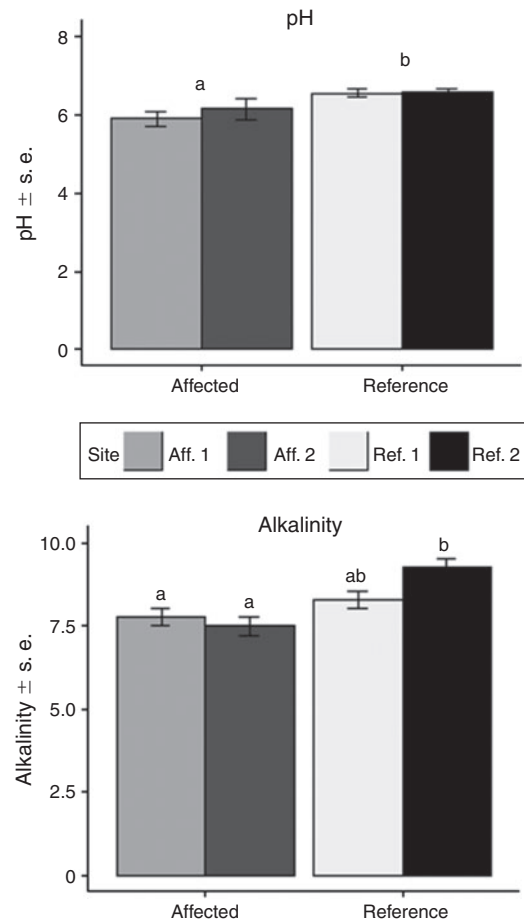


Fig. 2. Mean (± 1 s. e.) oyster source site pH ($n = 5$) and alkalinity ($n = 4$) in Hastings Estuary, New South Wales, Australia. Sites were considered Affected (Aff.) or Reference (Ref.). Letters above bars denote significant difference at $\alpha = 0.05$.

Over the 3-week period of monitoring, affected sites also had more variable pH values (range 5.00–6.63) than reference sites (range 6.36–6.80). Alkalinity differed significantly between nested sites ($F_{2,12} = 3.9$, $P = 0.049$); one reference site (Reference 2) had higher alkalinity than both affected 1 ($P = 0.008$) and 2 ($P = 0.024$) (Fig. 2). The alkalinity of sites was positively correlated with distance from the flood gate (Table 1; $r = 0.81$, $n = 16$, $P = 0.0001$). There was no difference in salinity between affected and reference sites ($F_{1,2} = 3.41$, $P = 0.21$) or between sites nested within these ($F_{2,12} = 2.716$, $P = 0.11$).

Predation experiments

At the end of the experiment, all dead oysters displayed signs of shell damage (e.g. cracks, chips) so changes in live oyster numbers from the start to the end of the experiment were considered to be caused by predation. Of the 24 trials, 12 had no mortality of oysters, with eight of these from affected sites. In one trial, in which crabs were offered oysters from a reference site, 100% of the oysters were eaten.

Overall, across all trials, there were differences in oyster mortality between the individual nested sites ($F_{2,20} = 7.99$,

Table 1. Environmental data for the four oyster source sites in Hastings Estuary, New South Wales, Australia, during July 2014
Affected sites and Reference site values are means (± 1 s. e.) of pH ($n = 5$), temperature (Temp, $n = 5$), salinity ($n = 5$) and dissolved oxygen (DO, $n = 1$). Distance refers to the distance between the site and the flood gate

Site	Latitude	Longitude	pH	Temp (°C)	Salinity	DO (mg L ⁻¹)	Distance (km)
Affected-1	-31°24'16"	152°51'08"	6.14 \pm 0.29	15.01 \pm 1.06	32.1 \pm 0.72	6.56	2.01
Affected-2	-31°23'57"	152°51'10"	5.89 \pm 0.20	16.24 \pm 0.04	31.8 \pm 0.58	8.45	2.76
Reference-1	-31°24'42"	31°24'42"	6.55 \pm 0.09	17.21 \pm 0.04	33.9 \pm 0.09	4.96	3.54
Reference-2	-31°24'36"	152°54'11"	6.57 \pm 0.07	18.18 \pm 0.02	35.4 \pm 0.18	8.63	8.24

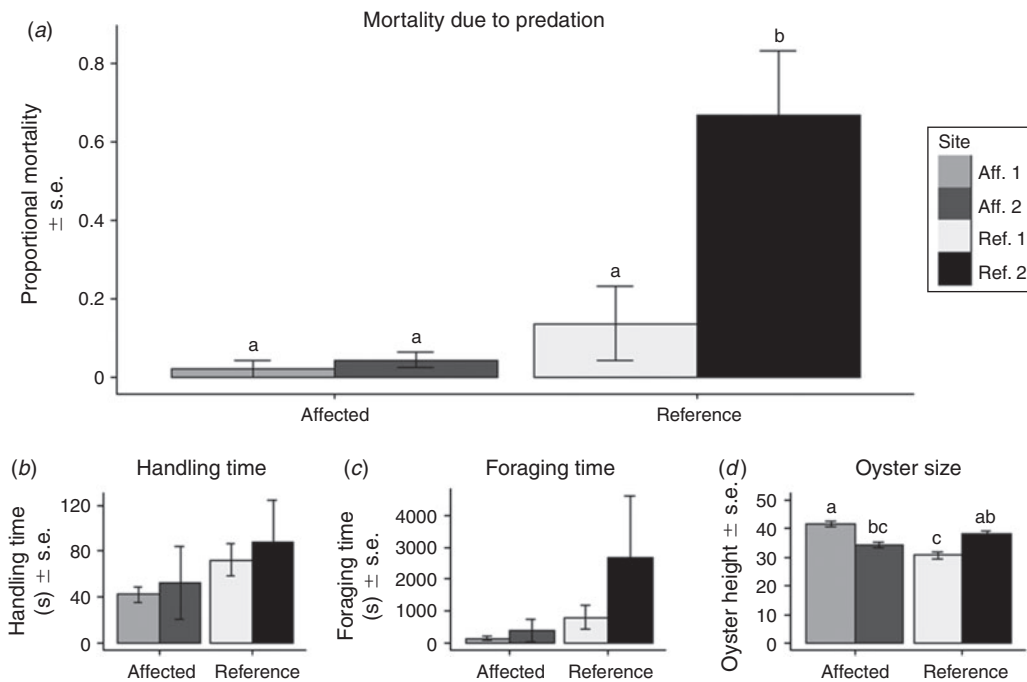


Fig. 3. Mean (± 1 s. e.) (a) oyster mortality ($n = 6$), (b) crab handling time ($n = 3$), (c) crab foraging time ($n = 3$) and (d) oyster size ($n = 24$) for oysters from each of the four source sites. Sites were considered Affected (Aff.) or Reference (Ref.). Letters above bars denote significant differences at $\alpha = 0.05$.

$P = 0.003$), though there were no observed differences between sites classified as affected and those classified as reference ($F_{1,2} = 1.36$, $P = 0.36$). Mortality was greater at one of the reference sites, Reference 2, than at any of the other sites (v. Affected 1, $P = 0.003$, $d = 0.71$; v. Affected 2, $P = 0.018$, $d = 0.69$; v. Reference 1, $P = 0.003$, $d = 0.58$) (Fig. 3a).

Video analysis revealed that all but one crab spent time feeding or foraging. There was no difference in handling time between affected and reference sites ($F_{1,16} = 0.76$, $P = 0.40$) or between nested sites ($F_{2,16} = 2.386$, $P = 0.12$) (Fig. 3b). There was some evidence that crabs spent more time foraging on oysters from reference sites than those from affected sites ($F_{1,2} = 14.10$, $P = 0.064$, $d = 0.28$), with no between-site differences in foraging time ($F_{2,8} = 0.39$, $P = 0.69$) (Fig. 3c). Oyster mortality and foraging time were positively correlated ($r = 0.65$, $n = 12$, $P = 0.023$), and mortality and handling time displayed a weak, though non-significant, positive relationship ($r = 0.56$, $n = 12$, $P = 0.058$). Foraging time was positively correlated with

distance from flood gate ($r = 0.58$, $n = 12$, $P = 0.048$), but there was no evidence for a correlation between handling time and distance from flood gate ($r = 0.34$, $n = 12$, $P = 0.27$).

Contribution of environmental variables

Oysters from sites classified as affected were no different in size than those from sites classified as reference ($F_{1,2} = 0.49$, $P = 0.44$), though there were differences between individual nested sites ($F_{2,92} = 19.17$, $P < 0.0001$) (Fig. 3d).

Many environmental variables were correlated, including pH, alkalinity, salinity and distance from flood gate. Linear models containing collinear variables were excluded from analysis. Remaining models included the intercept-only model (H_{Int}); models of oyster mortality containing the single predictor variables pH (H_{pH}), alkalinity (H_{Alk}), distance from flood gate (H_{Dist}) or shell height (H_{Hght}); and a model expressing oyster mortality as a function of temperature, salinity and dissolved oxygen (H_{Env}) (Table 2).

Table 2. Results of corrected Akaike's Information Criterion (AICc) analysis on models of oyster mortality

Models are listed in order of support, from most likely to be the best of the candidate set to least likely. Weights correspond to the probability of the model being the best of the candidate set. Model estimates represent modelled increases in mortality due to predation (expressed as proportional mortality) as a function of the combinations of predictor variables, and have been back-transformed (models were fit using a logit-transformation)

Model	Explanatory variable	AICc	Delta	Weight	Model estimate	P	R ²
H _{Dist}	Distance	121.69	0.00	0.73	0.77	<0.001	0.56
H _{Alk}	Alkalinity	123.97	2.29	0.23	0.99	<0.001	0.52
H _{Env1}	Salinity	127.33	5.64	0.04	0.85	0.08	0.57
	Temperature				0.49	0.97	
	Dissolved oxygen				0.74	0.02	
H _{pH}	pH	137.31	15.63	<0.01	1.00	0.05	0.16
H _{Int}	Intercept	138.98	17.30	<0.01			
H _{Hght}	Shell height	141.48	19.79	<0.01	0.52	0.73	0.01

The model containing distance from flood gate was selected using AICc as the best of the candidate set of models for oyster mortality (Table 2). This model had a 73% probability of being the best of the candidate set. The model containing alkalinity also had support, with a 23% probability of being the best model of the set, whereas the model containing pH had little support.

Discussion

Contrary to our hypothesis, mud crab predation was greater on oysters from reference than affected sites. We had expected that, as in previous studies, oysters that had been exposed to multiple stressors including low pH, low salinity and low alkalinity would be thinner shelled (Amaral *et al.* 2012a) and smaller (Sanford *et al.* 2014) than oysters exposed to less stressful conditions, and that this would render them more susceptible to predation by crabs, as has been shown previously for gastropod oyster drills (Amaral *et al.* 2012a; Sanford *et al.* 2014). Instead, in the present study, oysters displayed site-specific differences in shell size that were unrelated to the stressors and which did not follow patterns of predation.

Mortality rates are expected to be negatively correlated with handling time of prey, as spending more time manipulating and consuming prey should slow down the predator and decrease mortality due to predation (Amaral *et al.* 2012a). This feature of predator-prey interactions was not observed in the current study, where there was weak evidence for a positive correlation between oyster mortality and handling time. This may be a function of predator preferences, as handling time may not only depend on the shell strength, but also on the amount of time a predator is willing to spend on a prey resource. Predator foraging time was positively correlated with oyster mortality and the distance of the oyster collection site from a flood gate, providing a potential mechanism for observed trends in oyster mortality. The closer the collection site of oysters was to the flood gate, the less time a predator spent investigating prey resources, leading to lower mortality rates.

One possible reason that crabs foraged less on oysters from sites close to than away from the flood gate is a lower nutritional value of affected oysters. Optimal foraging theory is based on the premise that predators make choices to maximise energy

intake, and thus increase fitness (Pyke 1984). Some invertebrates select food resources based on specific nutritional needs (Mayntz *et al.* 2005). If a prey item is in poor condition, it follows that a predator may decide not to exert the energy it would take to consume it. Extreme stress, such as that experienced by these oysters in an estuary polluted by acid sulfate soil runoff, may result in bivalves allocating extra resources to shell growth, resulting in fewer resources available for tissue growth or maintenance (Lannig *et al.* 2010; Hiebenthal *et al.* 2012; Hiebenthal *et al.* 2013). Even small changes in prey condition can lead to unexpectedly drastic changes to prey value, such that prey may even exhibit toxic qualities (Mitra and Flynn 2005). In addition, areas with acid sulfate soil runoff have higher concentrations of metals that may be bioavailable and contaminate oyster tissue (Nath *et al.* 2013). The stress that *Saccostrea glomerata* experience in polluted estuaries, along with toxicity due to accumulated metals, may be enough to reduce their condition to the point that they are no longer a preferred prey resource for large predatory crabs such as *Scylla serrata*.

Alternatively, the reduced predation rates of crabs on oysters from water polluted by acid sulfate soil may be caused by changes in oyster behaviour. Among bivalves, a common short-term response to stress is to close valves and cease pumping (Ortmann and Grieshaber 2003; Anestis *et al.* 2007). Valve closure and cessation of pumping activity reduces detection by predators, likely because chemical cues of the bivalve's presence are no longer being leaked into the environment (Weissburg and Zimmer-Faust 1993; Nakaoka 2000; Smeed and Weissburg 2006; Hay 2009). Many marine species use chemical cues to detect prey, from urchins to sea birds (Hay 2009). Crabs rely heavily on chemical cues from their environments, including for foraging (Weissburg and Zimmer-Faust 1993; Hay 2009). If the oysters from polluted portions of the estuary were exhibiting prolonged periods of valve closure, the crabs may not have been able to detect the presence of live prey. This may have decreased the amount of time a crab was willing to spend foraging among oysters, and this may have been another factor driving the reduced mortality in oysters from sites that were heavily polluted by acid sulfate soil runoff.

Although this study provides information on relative rates of predation for crabs foraging on either oysters that were exposed to acid sulfate soil runoff or those that were not, crabs in natural

environments would likely have a choice of prey items. This study does not address whether crabs would consume affected or reference oysters preferentially, if given the choice between the two. A multiple-choice feeding experiment would address changes in predator preference and helps determine if predators avoid feeding when a portion of the prey are affected by multiple stressors, or if they are able to choose which prey items are less affected by stress. This information will elucidate the scale at which shifts in predator behaviour may operate. For example, if mud crabs avoid feeding when stressed prey are nearby, these predators may become functionally extinct from large portions of estuaries characterised by acid sulfate soil runoff.

Many predators exhibit physical and behavioural responses to stressors, especially low pH (Kroon 2005; Cripps *et al.* 2011; Ferrari *et al.* 2011; Dixon *et al.* 2015). Although the effect of exposure to acid sulfate soil runoff on crabs was not a focus of this study, the results remain applicable to crab-oyster interactions in areas that experience episodic intrusion of acid sulfate soil runoff, because *S. serrata* are tolerant of such events. Although *S. serrata* tend to be less active during extremely low pH, there is no evidence for migration out of affected areas or a cessation of feeding activity during acid sulfate soil leaching events (Russell and Helmke 2002). Productive fisheries for *S. serrata* exist in areas that experience daily drops in pH to 4 or less (Russell and Helmke 2002). Crabs used in this study may or may not have lived in affected estuaries, but given the resilience of this species in regions experiencing acid sulfate soil runoff, we would not expect exposure to the salinity, pH or alkalinity levels observed in this study to make a noticeable difference in the behaviour of *S. serrata*.

However, very little is known about the effect of low pH on predator-prey interactions involving crustaceans. Similar to the results of the current study, there were negative effects of CO₂ acidification on Atlantic mud crab *Panopeus herbstii* handling time and consumption of Eastern oysters *Crassostrea virginica* that outweighed the greater susceptibility of thinner shelled bivalves to predation (Dodd *et al.* 2015). This decrease in predation was attributed to negative effects of acidification on mud crab detection of prey. However, it is also possible that mud crabs failed to forage because of a perceived decrease in prey nutritional value. Examining interactions between *Scylla serrata* and *Saccostrea glomerata* in acidified water will elucidate the mechanism behind the observed decreases in predator foraging activity in affected crab-oyster systems.

Conclusions

A natural gradient in pH, salinity and alkalinity produced by acid sulfate soil runoff was used to examine the effects of long-term exposure to multiple stressors (including low salinity, pH and alkalinity) on the suitability of oysters as a prey resource for large predatory crabs. Encounters between mud crabs *Scylla serrata* and affected Sydney rock oysters *Saccostrea glomerata* resulted in lower rates of mortality as compared to oysters from reference sites farther from a point source of water polluted by acid sulfate soil runoff. Differences in rates of mortality were largely due to the decreased time crabs spent foraging on affected oysters. Crab disinterest in prey exposed to multiple

stressors could be due to low nutritional value or valve closure, which limits environmental cues of prey presence.

A decrease in predator foraging on oysters experiencing episodic inundation by acid sulfate soil runoff may result in unpredictable changes in coastal marine food webs. Stressor-induced changes in food web structure, driven by altered prey quality or predator behaviour, may sever the connection between the benthos and upper trophic levels, which in turn may have drastic consequences for ecosystem function and commercial fisheries in coastal regions. Elucidating the mechanisms behind changes in trophic interactions due to multiple stressors such as acidification and salinity is the first step towards making viable predictions and conservation actions that may preserve these ocean resources for future generations.

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