CAN OYSTERS PROVIDE A REFUGE TO COASTAL BIODIVERSITY IN A CHANGING WORLD?

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GENERAL SUMMARY

Climate change is forcing species to adapt to the rapidly changing environment, migrate, or face extinction. Ecosystem engineers can ameliorate environmental stress experienced by associated organisms, and may provide climate refugia for biodiversity. However, for their conservation to be an effective strategy for climate change adaptation, we need to know where, when and how ecosystem engineers have the greatest influence on biodiversity. This thesis focuses on ecosystem engineering by intertidal *Saccostrea* oysters, examining (1) how the mechanisms of facilitation by oysters change across environmental gradients, (2) whether specific *Saccostrea* populations are more resilient to temperature extremes, (3) whether greater resilience is driven by sub-cellular stress responses to high temperatures, and (4) how intraspecific variation in key structural traits of oysters influence their capacity to ameliorate temperature extremes. Manipulative experiments replicated across 900km of coastline revealed that although provision of structure by oysters was a key mechanism by which they facilitated biodiversity, at warmer sites amelioration of heat and desiccation stress was an increasingly important mechanism of facilitation, whereas in cooler climates, amelioration of predation was more important. Oysters selectively bred for fast growth and disease resistance were more susceptible to rising temperatures than unselected oysters, and consequently, were less effective microhabitat refugia to invertebrates under scenarios of warming. However, selectively bred oysters demonstrated greater upregulation of genes involved in maintaining cellular homeostasis under warmer climate scenarios, suggesting that breeding programs targeting climate resilience may successfully increase the resilience of *Saccostrea*. The structural traits of oyster habitat influenced their capacity to ameliorate climate, with vertically but not horizontally orientated oysters alleviating physical stress experienced by associated species. Overall, *Saccostrea* appear to have the capacity to endure the predicted temperature

increases for the coming decades, and where they form dense, vertically orientated habitat, their conservation can provide a climate-adaption strategy for coastal biodiversity.

STATEMENT OF CANDIDATE

I declare that the work in this thesis, titled "Can oysters provide a refuge to biodiversity in a changing world", has not been previously submitted for, or does not comprise a component of, any other degree at Macquarie University or any other university or institution. The research in this thesis is original material and has been written in my own words.

Any assistance I received with my research or the preparations of chapters contained within this thesis have been acknowledged in the *Statement of contributions to chapters* section.

Dominic McAfee

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STATEMENT OF CONTRIBUTIONS TO CHAPTERS

CHAPTER ONE – GENERAL INTRODUCTION

I completed the literature review and writing of this chapter with constructive feedback from my supervisor, Melanie Bishop.

CHAPTER TWO – THE MECHANISMS BY WHICH OYSTERS FACILITATE INVERTEBRATES VARY ACROSS ENVIRONMENTAL GRADIENTS

Dominic McAfee, Melanie J. Bishop

My contribution: concept and design – 60%; data collection – 100%; analysis and interpretation – 80%; manuscript drafting – 100%; critical revision – 60%

Melanie Bishop assisted with the conceptual design, data interpretation, and critical revision of this chapter.

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CHAPTER THREE – FAST GROWING OYSTERS SHOW REDUCED CAPACITY TO PROVIDE A THERMAL REFUGE TO INTERTIDAL BIODIVERSITY AT HIGH TEMPERATURES

Dominic McAfee, Wayne A. O'Connor, Melanie J. Bishop

My contribution: concept and design – 60%; data collection – 100%; analysis and interpretation – 80%; manuscript drafting – 100%; critical revision – 60%

Melanie Bishop assisted with the conceptual design, data interpretation, and critical revision of this chapter, and Wayne O'Connor contributed to the data interpretation and critical revision.

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CHAPTER FOUR – INTRASPECIFIC DIFFERENCES IN THE TRANSCRIPTIONAL STRESS RESPONSE OF TWO POPULATIONS OF SYDNEY ROCK OYSTER INCREASE WITH RISING TEMPERATURES

Dominic McAfee, Vivian Cumbo, Melanie J. Bishop, David A. Raftos

My contribution: concept and design – 40%; data collection – 100%; analysis and interpretation – 70%; manuscript drafting – 100%; critical revision – 60%

All authors contributed to the concept, design, and critical revision of this chapter. Vivian Cumbo and David Raftos contributed to the data interpretation.

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CHAPTER FIVE – ARE ALL OYSTER BEDS CREATED EQUAL? TRAIT-MEDIATED CLIMATE AMELIORATION BY OYSTERS

Dominic McAfee, Melanie J. Bishop, Gray A. Williams

My contribution: concept and design – 60%; data collection – 100%; analysis and interpretation – 80%; manuscript drafting – 100%; critical revision – 40%

Gray Williams and Melanie Bishop contributed to the experimental design, data analysis and interpretation and critical revision of this chapter.

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CHAPTER SIX – DISCUSSION

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 CLIMATE REFUGIA

Globally, anthropogenic climate change is increasing the severity and frequency of extreme weather events (Pachauri et al. 2014). The warming climate is having a profound influence on natural communities, with resultant shifts in biogeography and phenology recorded for virtually all major taxa, in all environments (Parmesan and Yohe 2003, Parmesan 2006). While the impacts of climate change are multifarious and synergistic, rising temperature is perhaps the greater challenge to biodiversity as it underpins biological function (Pörtner and Farrell 2008, Schulte 2015) and determines the environmental context in which species interactions take place (Bertness and Callaway 1994). With mean global surface temperatures predicted to increase by as much as 3.7ºC this century (scenario PCP8.5: Pachauri et al. 2014), avoiding mass species extinctions may depend on management strategies that assist the adaptation of species to rising temperatures.

For species to persist within their optimal thermal range they must either shift their distribution (Parmesan & Yohe 2003), adapt their physiology or phenology (Hughes 2000), or adopt thermoregulatory behaviour (Marshall et al. 2010). Human encroachment and habitat fragmentation severely limit the natural capacity of many species to migrate with the changing climate (Travis 2003), while the predicted rate of warming is likely to far outpace the evolutionary response of most species (Parmesan 2006). In physically harsh environments persistence of biodiversity may rely on access to habitats that provide a refuge from the extremities of climate (Crain and Bertness 2006). Behavioural selection of habitat that ameliorates adverse conditions may provide the most feasible mechanism for species to adapt with the changing climate (Marshall et al. 2010), and, therefore, management of such refugia can provide a climate-adaptation strategy for biodiversity (Morelli et al. 2016).

Climate change refugia are physical features of the environment that ameliorate climatic extremes, thereby providing some climatic stability through time (Morelli et al. 2016). Abiotic structures, such as complex topography that provides a diversity of microclimates (i.e mountains, valleys, substrate crevices, rock pools), can provide climate refugia (Dobrowski 2011, Morelli et al 2016). Similarly, biological structures, such as the physical habitat provided by ecosystem engineers (i.e. trees, coral reefs: Jones et al. 1994, 1997), can provide refugia, and unlike abiotic features they can grow and respond to the changing environment (Ridge et al. 2015).

1.2 ECOSYSTEM ENGINEERING

Traditionally, ecologists had focused on the role of negative species interactions, such as competition and predation, in structuring ecological communities, with positive interactions largely ignored (Bruno et al. 2003). However, over the past two decades much research has recognised the important role facilitation plays in structuring communities, by expanding the realised niche of species (Bertness and Callaway 1994, Stachowicz 2001). Positive species interactions occur when an organism modifies the environment making conditions more favourable for the persistence of other organisms, with resultant increases in the fitness of individuals and distribution of species (see Stachowicz 2001, Bruno et al. 2003).

Autogenic ecosystem engineers are organisms who grow physical structures that modify the availability of resources to other organisms (Jones et al. 1994). The structures formed by autogenic ecosystem engineers create or modify habitat, producing a new structural state relative to the unmodified surrounds, and changing the landscape in which species interactions take place (Jones et al. 1994, 1997). Though the scale of an ecosystem engineer's impact on local biodiversity is both a property of their population density and the temporal and spatial extent of their population (Jones et al. 1997), where their physical structures increase

habitat complexity they typically positively influence biodiversity (Wright and Jones 2004). Conversely, where ecosystem engineers are removed there may be large negative cascading effects (Coleman and Williams 2002).

Ecosystem engineered habitat may influence biota directly, through the provision of physical living space, or indirectly via amelioration of environmental stress (Jones et al 1994, 1997). Engineered structures interact with the abiotic environment by physically disrupting the flow of energy, potentially dissipating the severity of an environmental stress (i.e. solar radiation, wind speed, wave energy) and, therefore, maintaining more abiotically stable conditions (Callaway and Walker 1997, Bertness and Leonard 1997, Silliman et al. 2011). Furthermore, these physical structures can disrupt predator-prey interactions, where the physical characteristics of the engineered habitat provide predator-free space for prey (Bertness and Callaway 1994). However, the nature by which an ecosystem engineer interacts with associated species will ultimately be governed by the background abiotic and biotic environmental context (Bertness and Callaway 1994). Therefore, the successful use of ecosystem engineers as a conservation strategy to enhance the resilience of biodiversity to climate change will depend on knowledge of the environmental conditions under which positive species interactions are greatest (Wright and Jones 2006).

Ecological theory predicts that species interactions will switch from negative, competitive interactions in environments with relatively benign stress, to positive interactions as abiotic stress or consumer pressure increases (Bertness and Callaway 1994). In physically extreme environments, ecosystem engineers are predicted to facilitate biodiversity by ameliorating abiotic stressors such as extreme temperature (Arroyo et al. 2003, McAfee et al. 2016), desiccation stress (Gomez-Aparicio et al. 2004, Cavieres et al. 2006, Silliman et al. 2011) and physical disturbance (Bruno and Kennedy 2000). As physical stress decreases and consumer pressure becomes the predominant stressor, the primary mechanism of facilitation is

predicted to change to associational defences through provision of predation refugia and by moderating interference competition (Bertness and Callaway 1994, Grabowski and Powers 2004). In reducing the environmental stress that limits species distributions, ecosystem engineers can expand the realised niche of species outside their fundamental niche (Bulleri et al. 2016), and may be the sole determinant of biodiversity in extremely stressful environments (Silliman et al. 2011, McAfee et al. 2016). As climate related stressors intensify, the strength of positive interactions is predicted to increase, hence ecosystem engineers may be even more critical to sustaining biodiversity in the future, affording associated organisms more time to adapt with the changing environment (Crain and Bertness 2006).

Ecosystem engineers exhibit considerable inter- and intra-specific variation in their expression of traits, and where an engineer's traits do not sufficiently disrupt the limiting environmental stress, the mere presence of engineered habitat may not necessitate facilitation (Irving and Bertness 2009, Harley and O'Riley 2011, He et al. 2012). The capacity of an ecosystem engineer to facilitate biodiversity will differ with variation in the key structural traits responsible for habitat amelioration, with positive species interactions generally increasing with the density (van Hulzen et al. 2007, Bishop et al. 2012), height (Bell & Westoby 1986, Irving and Bertness 2009), and architectural complexity (Drezner 2006) of the ecosystem engineer. In extremely harsh environmental conditions trait-dependent thresholds for facilitation may occur. For example, Bruno and Kennedy (2000) observed that facilitation of plant species by intertidal *Spartina* cordgrass was patch-size dependent, with sufficient substrate stabilisation for seedling establishment only occurring in high flow environments when *Spartina* patches were above a critical size. Furthermore, the direct structural, or indirect abiotic or biotic change ecosystem engineers provide, may feedback to the engineer itself and potentially determine whether their own population will expand or decline (Jones et al. 2010). Identifying which structural traits produce positive feedbacks for the engineering species will

assist restoration projects to achieve self-perpetuating engineered habitat, subsequently increasing the sustainability of the project by reducing the need for direct human intervention (Byers et al. 2006, Jones et al. 2010).

Similar to their structural traits, ecosystem engineers can display considerable variation in their environmental tolerance (Parker et al. 2012, He et al. 2012). Among geographically separated populations, intraspecific variability in temperature tolerance can be high (Fangue et al. 2006, Sorte et al 2011), potentially due to genetic or phenotypic variation in their cellular function (Schulte 2015). Such inherent variation within species to environmental stress suggests that selective breeding programs could be used to select for greater resilience to climate stressors in populations of ecologically important species (van Oppen et al 2015). Though selective breeding for increased production and environmental resilience is common for commercial species, applying such management practices for achieving ecological goals has seldom been discussed (but see Jones and Monaco 2009, van Oppen et al. 2015). Selectively breeding ecosystem engineers for enhanced environmental resilience may be particularly important for aiding the restoration of highly degraded environments where evolutionary adaptions do not meet contemporary selection pressures (Jones and Monaco 2009), and where the rate of environmental change is likely to far outpace the evolutionary response of foundation species (van Oppen et al. 2015). However, the unforeseen evolutionary trade-offs that result from selecting for specific trait enhancement may ultimately lead to populations that are maladapted for survival in the wild (Stearns 1989, van Oppen et al. 2015). For example, selecting traits to enhance the environmental resilience of a species can trade-off against their reproductive potential (Ghalambor et al. 2004), or against different life stages, such that selection may confer an advance for juveniles at the expense of the adult's performance (Thompson et al. 2015). Therefore, the physiological consequences of selection should be investigated at both the sub-cellular and ecological level, to determine how this may

influence organism- to ecosystem-level functioning. Importantly, where restoration projects target climate amelioration, managers will need to prioritise ecosystem engineer populations with the greatest resilience and adaptive capacity to climate change (Keppel and Wardell-Johnson 2012).

Given their capacity to facilitate biodiversity through modification of the abiotic environment, restoration of ecosystem engineers may provide a mechanism for transitioning degraded ecosystems from an abiotically limited state to a desired biological state (Byers et al. 2006). While engineered habitat is predicted to be increasingly important as temperatures rise (Crain and Bertness 2006), the long term success of projects targeting climate-adaptation may depend on knowledge of the structural traits important for climate amelioration, and which populations of engineers display the greatest environmental resilience (Keppel and Wardell-Johnson 2012). Particularly where engineering feedbacks are achieved, the restoration of ecosystem engineers may provide the most cost-effective and sustainable means of providing a climate adaptation strategy (Byers et al. 2006, Jones et al. 2010). However, few studies have directly investigated how intraspecific variation influences an engineer's capacity to provide climate refugia. And furthermore, detailed information on the number and types of resources an ecosystem engineer modifies, and how this capacity varies spatially, is seldom available (Jones et al. 1997). Such knowledge will increase our predictive understanding of when and where ecosystem engineers will provide the greatest benefit to biodiversity, and could therefore be considered as a conservation target.

1.3 LIVING TOUGH IN THE INTERTIDAL

Exposed to both terrestrial and marine conditions, organisms living in the intertidal must endure extreme physical and biological stress that can fluctuate sharply with the rise and fall of the tide (see Thompson et al. 2002, Helmuth et al 2006). At low tide, intertidal organisms

may be exposed to extreme temperature, desiccation stress, and terrestrial predators (i.e. birds, mammals). Whereas at high tide, intertidal organisms are exposed to aquatic predators such as fish and crabs, and physical disturbance by wave action (Paine 1974, Paine and Levin 1981). Consequently, the upper distribution of species is typically limited by their physiological tolerance to temperature and desiccation stress (Stillman and Somero 2000), while biological interactions such as predation and competition for space often determine lower distributional limits (Connell 1972, Paine 1974, Underwood 1981). Furthermore, rising sea-levels and the increased frequency of storm events have not only intensified physical disturbance from wave action, but have resulted in increasing anthropogenic armouring of coastlines with vertical seawalls (Doody 2004). Trapped between the rising sea and human encroachment, this "coastal squeeze" is reducing the availability of habitat in the intertidal, and with it the potential for species to naturally respond to climate change.

As a consequence of their exposure to extreme diurnal fluctuations in climate over short spatial and temporal scales, many intertidal organisms already live close to their physiological threshold with limited capacity to respond to further temperature increase (Stillman 2003, Somero 2010). On rocky shores, when low tides coincide with extreme midday temperature events, heat-related mass mortality of entire intertidal communities can occur (Tsuchiya 1983, see Helmuth et al 2002). Local heat stress is determined by the complex interplay between aerial exposure, air and sea-temperatures as well as topographic features that determine local microclimate (Helmuth et al. 2002, 2006). Therefore, predicting which communities are most vulnerable to climate change can be difficult. Nevertheless, intertidal habitats are predicted to be among the most heavily impacted by climate change, with mass mortality events predicted to increase as atmospheric temperatures rise (Thompson et al. 2002, Hobday et al. 2006).

For intertidal ectotherms, whose body temperatures typically conform to substrate temperatures, surviving extreme heat events may depend on their ability to exploit thermal

refuges (Marshall et al. 2013, Ng et al. 2016). Shading provided by convoluted rock surfaces, crevices, and rock pools can provide important thermal refugia that can substantial lower body temperatures relative to those in unshaded habitats (e.g. by 11^{o}C: Marshall et al. 2013). Similarly, the three-dimensional structure ecosystem engineers introduce into intertidal habitats not only increases the surface area available for attachment and foraging, but can also dampen the extremities of heat and desiccation stress experienced by associated organisms (Bertness et al. 1999). Unlike topographic features, however, ecosystem engineers can rapidly transform relatively featureless habitat, and their distribution can shift with the rising sea (Ridge et al. 2015).

Positive species interactions in intertidal systems have been associated with a diversity of habitat forming organisms such as macroalgae (Bertness et al. 1999, Leonard 2000), marsh grasses (see Bertness and Leonard 1997, Bruno and Kennedy 2000), mangroves (Kathiresan and Bingham 2001), barnacles (Kawai and Tokeshi 2004, Cartwright and Williams 2012), and bivalves (Seed 1996, Cole 2010, McAfee et al. 2016). During aerial exposure, epibenthic ecosystem engineers can provide shading that reduces temperature variability, deflect desiccating winds, and block harmful ultraviolet radiation (Bertness and Leonard 1997, Thompson et al. 2002, Silliman et al. 2011). At high tide the complex interstitial spaces within engineered habitat can alleviate predation pressure for inhabiting prey, increase the foraging efficiency of intermediate predators by reducing interference competition, and provide important nursery habitat for juvenile fish (Kathiresan and Bingham 2001, Grabowski 2004, Grabowski and Powers 2004). The strength of positive interactions caused by abiotic amelioration in the intertidal typically increase with height on the shore (Bertness 1989) and with decreasing latitude (Leonard 2000). Similarly, facilitation by associational defence is predicted to be greatest where physiological conditions are relatively benign and consumer pressure is the dominant stress (Bertness and Callaway 1994).

Predicting where climate refugia will be most important for conserving intertidal biodiversity is complicated by the fact that heat stress in the intertidal does not follow a linear relationship with latitude, but instead, is characterised by a complex spatio-temporal mosaic of thermal hot spots (Helmuth et al. 2002). Furthermore, an ecosystem engineer's capacity to provide a refuge from stress will depend on whether its structural traits sufficiently ameliorate the limiting abiotic and/or biotic stress, and whether its population density is sufficient to ensure its own persistence (Bertness and Leonard 1997, Jones et al. 2010). These spatial, temporal and structural considerations all influence species interactions, complicating predictions of where ecosystem engineers will provide the greatest benefit to biodiversity. Experiments that test how the mechanisms of ecosystem engineering vary across environmental gradients, and how different associated taxa interact with engineered habitat, will aid management strategies that target the amelioration of specific environmental stressors, or aid to conserve the most vulnerable communities.

1.4 HABITAT FORMING BIVALVES

Habitat forming bivalves (i.e. oysters and mussels) are important ecosystem engineers in the intertidal where they encrust rocky shorelines, or can provide some of the only hard structure in soft-sediment environments (Stephens and Bertness 1991, Seed 1996, Beck et al. 2011). Where oysters and mussels aggregate, their hard shells form a complex habitat matrix that greatly increases the surface area available for epibiont attachment and benthic grazing, and provides interstices between shells in which infauna can seek refuge from predators and physical disturbance (see Gutierrez et al. 2003). The convoluted, three-dimensional structure of bivalve habitat can ameliorate the extremities of heat and desiccation stress by providing shading and trapping water during low tide aerial exposure (Stephens and Bertness 1991, Silliman et al. 2011, McAfee et al. 2016). Large bivalve aggregations can attenuate wave

energy, which helps to stabilise neighbouring shoreline and benthic habitats, and provide sheltered marine conditions for juvenile fish (see Grabowski et al. 2012). Following death, the structural legacy of bivalve shells continue to facilitate biodiversity, and the structural change following valve disarticulation can provide novel habitat relative to live bivalves that may further increase facilitation (Hastings et al. 2007, Summerhayes et al. 2009a, Tomatsuri & Kon 2017). Furthermore, the precipitation of calcium carbonate following shell dissolution can locally regenerate alkalinity, potentially increasing bivalve recruitment (Green et al. 2009) and buffering corrosive conditions for settled spat (Waldbusser et al. 2013). Where live bivalves aggregate, their filter feeding activity can improve water quality by removing seston from the water column, cycle nutrients to the benthos, and modify patterns of larval recruitment (Underwood and Fairweather 1989, Newell 2004). Where present, bivalve habitat generally increases local biodiversity by several orders of magnitude relative to bare, neighbouring habitat, and can be the sole determinant of biodiversity in stressful habitats (Seed 1996, Silliman et al 2011, McAfee et al. 2016, Bateman & Bishop 2017). Therefore, devastating impacts on biodiversity and water quality can be expected where bivalves are completely removed from the environment (Coleman and Williams 2002).

Oysters have long been recognised for their economic value and are intensively farmed for aquaculture across the globe. The ecological benefits of oyster habitat have, however, been largely overlooked due to "collective amnesia" as to the ecological role these habitats historically played, prior to over-exploitation during the industrial revolution (Beck et al. 2011, Alleway and Connell 2015). Oyster reef habitat was once ubiquitous on temperate and subtropical shorelines around the world, forming substantial calcareous reefs that likely performed a similar functional role to that of extant coral reefs (Beck et al. 2011). These reefs were rapidly razed from the marine landscape during the industrial revolution as a result of over-extraction for food and lime, and rapid catchment land-use change that degraded the

quality of coastal waters (Ogburn et al. 2007, Beck et al. 2011). Today, it is estimated that 85% of global oyster habitat has been completely lost or is functionally extinct, with over 99% of historic reefs lost in Australia (Beck et al. 2011).

In Australia, the relatively recent recognition of the ecological significance of this lost habitat has seen a recent surge of interest in restoring shellfish reefs for their ecosystem services (Gillies et al. 2015). Restoring oyster reefs to recover ecosystem services has long been appreciated in North America (Peterson et al. 2003, Luckenbach et al. 2005, Grabowski and Peterson 2007), and the case for restoring their ecological services has been bolstered by economic valuations of restored oyster reef of USD$5500 - $99,000 per hectare/yr (Grabowski et al. 2012) - a value that far exceeds the worth of the fishery alone. This ecosystem-based approach to conserving marine biodiversity potentially provides one of the most sustainable, cost-effective management strategies for assisting the adaption of marine ecosystems with climate change (Byers et al. 2006). A financial return on the investment of restoring oyster habitat has been estimated at just five years due to improvements to wild fish stocks alone (Creighton et al. 2015), and where oyster populations achieve self-perpetuating densities there will be minimal ongoing investment or direct human intervention required (Byers et al. 2006). Nevertheless, there is little margin for error for restoration projects as funding is inevitably limited and failed restoration efforts will likely deter future investment. Therefore, experiments are needed that advance ecosystem engineering theory so that coastal managers can reliably predict where conservation and/or restoration of habitat forming bivalves will have the highest ecological value (Crain and Bertness 2006). Specifically, experiments are needed that investigate (1) how the mechanisms of ecosystem engineering by oysters vary across environmental gradients, (2) the key functional traits of oysters that maintain positive species interactions during stressful events, and (3) if there are specific populations of oysters that are more resilient to environmental stress and should therefore be considered for restoration efforts.

1.5 SYDNEY ROCK OYSTERS

The Sydney rock oyster, *Saccostrea glomerata* (Gould 1850), is a predominantly intertidal oyster broadly distributed along the east Australian coastline (approximately 25º17'S to 38º09'S) where it is abundant on sheltered rocky shorelines and attached to pneumatophores in mangrove forests (Summerhayes et al. 2009b, Bishop et al. 2010, McAfee et al. 2016). At mid-intertidal elevations, *S. glomerata* commonly forms complex, three-dimensional habitat that supports biodiverse invertebrate communities (Summerhayes 2009a, Wilkie et al. 2012), and aids the persistence of many thermally-sensitive species on rocky shorelines with extreme temperature stress (McAfee et al. 2016). During low-tide aerial exposure, species retreating to *S. glomerata* habitat typically experience more humid and thermally stable microclimates than adjacent oyster-free habitat, with interactions between *S. glomerata* and associated invertebrates sufficient to interrupt biogeographic patterns in invertebrate assemblages seen in bare habitat (McAfee et al. 2016). In modifying the abiotic environment and dampening the climatic extremes experienced by inhabiting species, *S. glomerata* habitat may serve as a climate refugia to associated biodiversity, affording inhabitants more time to adapt with the changing conditions (Byers et al. 2006, Keppel and Wardell-Johnston 2012).

As well as their ecological significance, *S. glomerata* is a commercially important aquaculture species that is farmed on intertidal racks in the sheltered bays of New South Wales (NSW), Australia (O'Connor et al. 2008). Oyster aquaculture is NSW's most valuable fishery, however *S. glomerata* production has continued to decline from peak harvests in the late 1970s due to the catastrophic impact of QX disease (caused by the protistan parasite *Marteilia sydneyi*), which results in 97% mortality of infected stock, and the resultant rise of Pacific oyster (*Crassostrea gigas*) aquaculture (Nell et al. 2000, O'Connor et al. 2008). To improve commercial production of *S. glomerata* a selective breeding program was established by NSW

Fisheries in 1990, mass selecting *S. glomerata* for fast growth and QX disease resistance (Nell et al. 2000, O'Connor and Dove 2009). The program successfully developed QX disease resistance and increased the growth rate of selected oysters by 36% relative to unselected controls in the absence of the disease, with the difference increasing when QX disease is present (NSW DPI 2014). Over seven generations, this selection also appears to have inadvertently conferred an increased resilience to ocean acidification, with selected *S. glomerata* larvae displaying greater survivorship, developmental rates, and shell production in comparison to unselected, wild-type larvae (Parker et al. 2011, 2012). Furthermore, an analysis of the sub-cellular response of these same populations to elevated CO_2 detected a greater intracellular stress response to acute acidification stress, and greater trans-generational acclimation potential in the selected *S. glomerata* relative to unselected controls (Goncalves et al. 2016).

Though *S. glomerata* populations remain common on the rocky intertidal shorelines of Australia's sheltered east coast estuaries (McAfee et al. 2016), just two hundred years ago *S. glomerata* formed large intertidal and subtidal reefs in most of the estuaries of New South Wales and Southern Queensland (Gillies et al. 2018). Following European settlement of Australia, oyster reefs were intensively harvested for food and their shells were burnt to manufacture lime (Ogburn et al. 2007). Within a hundred years of colonisation, virtually all of Australia's east coast temperate estuaries had experienced at least some oyster harvesting (Kirby 2004). Today, only six extant *S. glomerata* reef systems remain from at least sixty known historically harvested locations, with an estimated 92% of *S. glomerata* reefs lost (Gillies et al. 2018). Including reefs formed by Mud Oysters (*Ostrea angasi*), Australian oyster reefs are currently estimated at less than 1% of their historic abundance, and are considered ecologically functionally extinct (Ogburn et al. 2007, Beck et al. 2011). Given the historic extent of this habitat, its important ecosystem functions, and the favourable growing conditions highlighted by the persistence of an aquaculture industry, the restoration potential for *S.*

glomerata is enormous (Gillies et al. 2015). The increased performance of selectively bred *S. glomerata* larvae under scenarios of ocean acidification suggests that selection for fast growth and disease resistance has enhanced their inducible cellular stress response, potentially conferring greater environmental resilience to other environmental stressors (Parker et al. 2011, 2012, Goncalves et al. 2016). *S. glomerata* populations with enhanced environmental resilience could potentially benefit conservation and/or restoration efforts. However, much is unknown of the other potential trade-offs from selective breeding, with the redistribution of energy to enhance selected traits potentially undermining the performance of other important metabolic activities or life-stages (van Oppen et al. 2015). For example, a recent proteomic comparison of these same populations suggested that the increased resilience of selected *S. glomerata* juveniles may have come at a cost to their performance as adults, with increased cellular dysfunction relative to unselected adults (Thompson et al. 2015). Furthermore, these studies have all been laboratory based, and as such little is known about how these selected oysters will perform in the wild, and how resilient they will be to rising atmospheric temperatures.

1.6 RESEARCH AIMS

Given the rate of environmental change is likely to outpace the adaptive response of many intertidal species, conservation strategies that assist the climate adaption of ecological communities will be critical to minimising biodiversity loss. My thesis investigates the potential for the conservation and restoration of Sydney rock oysters to provide climate refugia to coastal biodiversity on Australia's eastern seaboard. This research expands on the study by McAfee et al. (2016) that found spatial variation in the positive interactions between *S. glomerata* and intertidal invertebrates generally increased with climatic stress. The four data chapters comprising this thesis go further by addressing key knowledge gaps of how the mechanisms of ecosystem engineering by oysters change with environmental context, how the

habitat structure of oysters interacts with associated invertebrates and influences survivorship of the oysters themselves, and how *S. glomerata* is likely to perform in a warmer world.

The mechanisms by which oysters facilitate biodiversity may vary from direct habitat provision, amelioration of abiotic stress, provision of predator-free space, or a combination of these mechanisms. However, the nature of species interactions will ultimately be determined by the background environmental context (Bertness and Callaway 1994). In Chapter Two I present a manipulative field experiment distributed over 900km of coastline that investigates how the mechanisms of invertebrate facilitation by *S. glomerata* change with environmental context. I use caging and shading structures that mimic the environmental amelioration provided by *S. glomerata* habitat to partition the mechanisms by which oysters facilitate invertebrate taxa. The outcomes of this study will help identify the environmental conditions under which oysters will have the greatest influence on the richness and abundance of invertebrate taxa, and where the amelioration of abiotic stress, alleviation of biotic pressure, or direct habitat provision is the driving mechanism of invertebrate facilitation.

Identifying specific populations of ecosystem engineering species that are more resilient to environmental stress may enhance conservation efforts targeting climate adaptation (Keppel and Wardell-Johnson 2012). However, intraspecific variation in the environmental resilience and habitat production of ecosystem engineers has seldom been investigated. Chapters Three and Four expand on the existing literature that compares the environmental resilience of *S. glomerata* populations that have either been selectively bred for fast growth and disease resistance, or have not been under selection (i.e. Parker et al. 2011, 2012, Thompson et al. 2015, Goncalves et al. 2016), by exposing these two *S. glomerata* "breeding-lines" to increasing temperature stress in the wild for the first time. Chapter Three describes two manipulative field experiments that expose these oyster breeding-lines to an artificial temperature gradient in the wild to compare their capacity to persist and form habitat under

warmer temperatures. In Chapter Four, an identical experimental design is used to investigate differences in the sub-cellular stress response of each *S. glomerata* breeding-line to the temperature gradient, with qPCR used to analyse the transcriptional profiles of each breeding-line. Combined, this genes-to-ecosystem approach will improve our understanding of how sub-cellular processes can influence ecological outcomes, and whether selective breeding for market production has produced oysters that may also benefit restoration projects.

Intraspecific variation in the structural traits of ecosystem engineers can be considerable, and as such the magnitude of stress amelioration by engineered habitat should vary according to variation in their key structural traits. Chapter Five describes a rocky shore mesocosm experiment that investigated how the capacity of oysters to ameliorate maximum temperatures and hence, environmental stress experienced by associated species, varies with the structural configuration of oyster habitat. By identifying which structural traits of oyster habitat need to be restored to ensure temperature amelioration, this research will assist projects aimed at providing climate refugia to associated biodiversity.

In my final chapter (Six) I synthesise the outcomes of my four data chapters. I discuss the future of species interactions between oysters and associated communities in a warmer world, and more generally between engineering species and their ecological communities, and emphasise how coastal managers may apply these outcomes to enhance the probability of successful restoration projects.

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CHAPTER TWO

THE MECHANISMS BY WHICH OYSTERS FACILITATE INVERTEBRATES VARY ACROSS ENVIRONMENTAL GRADIENTS

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2.1 Abstract

The effective use of ecosystem engineers to conserve biodiversity requires an understanding of the types of resources an engineer modifies, and how these modifications vary with biotic and abiotic context. In the intertidal, oysters engineer ecological communities by reducing temperature and desiccation stress, enhancing the availability of hard substrate for attachment, and by ameliorating biological interactions such as competition and predation. Using a field experiment manipulating shading, predator access and availability of shell substrate at four sites distributed over 900km of east Australian coastline, we investigated how the relative importance of these mechanisms of facilitation vary spatially. At all sites, and irrespective of environmental conditions, the provision of hard substrate by oysters enhanced the abundance and richness of invertebrates, in particular epibionts (barnacles and oyster spat) and grazing gastropods. Mobile arthropods utilised the habitat provided by disarticulated dead oysters more than live oyster habitat, whereas the abundance of polychaetes and bivalves were much greater in live oysters, suggesting the oyster filter-feeding activity is important for these groups. As maximum temperatures increased, shading by oysters had an increasingly large effect on biodiversity, whereas in cooler estuaries, the provision of a predation refuge by oysters played a more important role. Such knowledge of how ecosystem engineering effects vary across environmental gradients can help inform management strategies targeting ecosystem resilience via the amelioration of specific environmental stressors, or conservation of specific community assemblages.

2.2 Introduction

Ecosystem engineers - organisms that create, modify, maintain or destroy habitats - are increasingly the targets of ecosystem-based management (Byers et al. 2006, Crain and Bertness 2006). At landscape scales incorporating engineered and non-engineered space, ecosystem engineers tend to enhance biodiversity by increasing habitat heterogeneity and niche space (Jones et al. 1994, 1997). However, at smaller scales, ecosystem engineers may have positive, negative or neutral effects on the abundance of individual species, and hence overall species abundance and richness (Jones et al. 1994, 1997). Whether impacts of ecosystem engineers are positive or negative may be determined by their density, the longevity of the habitat modifications they cause, the number and types of resource flows that they modify, as well as the species assemblage that depends on these flows (Jones et al. 1997, 2010). Understanding how each of these factors affects ecosystem engineering is critical for the effective use of ecosystem engineers in conservation and management (Crain and Bertness 2006, Hastings et al. 2007).

At small scales, positive effects of ecosystem engineers on biodiversity may arise where they enhance the availability of habitat or provide refugia from physical stressors, such as temperature extremes and desiccation, or biotic stressors, such as competition and predation (Jones et al. 1994, 1997). In reducing the impact of stressors, ecosystem engineers can expand the realised niche of associated species into areas where conditions would otherwise result in their extirpation (Bulleri et al. 2016). Consequently, the positive effects of ecosystem engineers on associated biota often increase with physical stress and/or biotic pressure (Bertness and Callaway 1994, Gomez-Aparicio et al. 2004, He et al. 2013). Studies addressing the so-called stress gradient hypothesis (Bertness and Callaway 1994) typically focus on how the overall interaction strength of ecosystem engineering varies across environmental stress gradients (e.g. Silliman et al. 2010, McAfee et al. 2016). Such studies

rarely consider the specific mechanisms by which ecosystem engineers alter resources. Understanding causes of spatial variation in the number and types of resources that are directly or indirectly controlled by ecosystem engineers, the ways these resources are controlled, and the number of other organisms that depend on these resources, is also essential to understanding ecosystem engineer impact (Jones et al. 1997). Multi-factor experiments that manipulate the environment in the absence of ecosystem engineers to mimic the multiple possible pathways of effects could be useful in this respect (Jones et al. 1997), but are seldom replicated across environmental stress gradients (reviewed by Jones et al. 2010).

Habitat-forming bivalves, such as oysters and mussels, are important marine ecosystem engineers that support dense and diverse fish and invertebrate communities (Grabowski et al. 2012, Bateman and Bishop 2017). Habitat-forming bivalves facilitate ecological communities via several mechanisms: their complex three-dimensional structures provide surface area for epibiont attachment and grazing (Minchinton and Ross 1999, Summerhayes et al. 2009), and interstices which shelter fish and invertebrates from predators and, in the intertidal, physical stressors, such as temperature and desiccation stress (Grabowski 2004, Silliman et al. 2010, McAfee et al. 2017). Additionally, as filter feeders, habitat-forming bivalves can influence community structure by modifying water quality, driving benthic-pelagic coupling, and/or ingesting or otherwise influencing settlement patterns of larvae (Nelson et al. 2004, Newell 2004, Wilkie et al. 2013). Whereas mechanisms of facilitation that are based on filter feeding are specific to live oysters, those that are based on structural features may persist long after death (Summerhayes et al. 2009, Ridge et al. 2015). The relative importance of these various mechanisms in facilitating associated species is likely to vary among functional groups according to their resource requirements and susceptibility to stressors (see Maestre et al. 2009). For example, epifauna may respond more strongly to the structural features of oysters,

whereas infauna, many of which feed on detritus, may respond more strongly to their biodeposition (McAfee et al. 2016).

Although the important role of oysters in facilitating biodiversity has long been recognised (e.g. Wells 1961), studies using appropriate manipulative experiments to partition facilitation into outcomes of abiotic and biotic stress amelioration are notably lacking. Most studies have focused on only one or the other of these mechanisms, and within a small geographic range (e.g. Grabowski 2004, Summerhayes et al. 2009, Padilla 2010). Abiotic stressors, such as high temperatures and desiccation, and biotic stressors, such as predation and competition, may be expected to vary spatially (Freestone and Osman 2011, Lavender et al. 2017), with the magnitude of biotic interactions becoming increasingly important as the magnitude of abiotic effects decreases (Mittelbach et al. 2007). We expect that the mechanisms by which oysters sustain associate invertebrates will vary spatially according to the magnitude of biotic and abiotic stressors, and vary among taxa according to the identity of stressors to which they are most susceptible. Specifically, we predict that although oysters will play an important role in providing substrate for invertebrates, and in particular sessile and grazing species, at all sites, their role in ameliorating heat stress will increase with increasing ambient temperature, while their role in ameliorating predation pressure will decrease and that the magnitude of these effects will vary among functional groups.

Here, we assess how the mechanisms by which oysters maintain associated invertebrate communities vary spatially with abiotic and biotic stressors, and vary among functional groups of organisms, that differ in their resource requirements. We manipulate oyster presence, shading, and predator access at four sites, spanning 900km of coastline to test the predictions that; (H_1) at all sites, and in all shading and caging treatments, oysters will enhance the richness and abundance of invertebrates as compared to bare habitat; (H_2) the physical structure of oyster habitat will enhance the abundance of epifauna, irrespective of whether oysters are live

or dead, but live oysters with biodepositional capacity will be required to facilitate infaunal organisms; (H_3) the effect of shading and of caging on invertebrate communities will be greater in plots without than with oysters, consistent with a role for oysters in ameliorating temperature extremes and predation; (H_4) in the absence of oysters, the effect of the shading treatment will increase and of the caging treatment will decrease as the maximum temperature of sites increases; and (H_5) in the presence of oysters, relationships between the maximum temperature of sites, and the effect of the shading and caging treatments will be weakened due to the role of oysters in ameliorating temperature and predation pressure. Additionally, we explore how the different taxonomic groups of invertebrates separately respond to abiotic and biotic stress. We interpret stronger effects of shading on invertebrate communities in the absence than the presence of oysters as evidence that facilitation of invertebrates is at least partially driven by amelioration of temperature and/or desiccation stress. Similarly, we interpret stronger effects of caging in the absence than the presence of oysters as evidence that facilitation is at least partially driven by protection of invertebrates from predators. Alternatively, if effects of oysters are primarily due to enhancement of substrate for attachment, we expect that shading and caging would have the same effect on habitat plots with bare, dead or live oysters, and the live or dead oysters would support more invertebrates of more taxa, than bare habitat.

2.3 Materials and methods

Field sites

In September 2014 (Austral spring), we established a three-factor fully orthogonal experiment at each of four field sites. The four sites spanned ~6° of latitude and 900km of the east Australian coast (Fig. 1), with two sites, the Bellinger River (30.5^{o}S, 153.02^{o}E) and the Hastings River (31.43^{o}S, 152.9^{o}E) on the mid-north coast of New South Wales, and the other two, the Clyde River (35.71^{o}S, 150.18^{o}E) and the Bermagui River (36.44^{o}S, 150.06^{o}E), on

the south coast (Fig. 1). We have previously demonstrated spatial variation in oyster facilitation of invertebrates across this stretch of coastline, with effect sizes generally increasing with decreasing latitude (McAfee et al. 2016). Sites were established at a mid-intertidal height (0.6–0.9m above Indian Spring Low Water) of *Avicennia marina* mangrove forests, where clumps of the native Sydney rock oyster, *Saccostrea glomerata*, naturally attach to unshaded pneumatophores (mangrove peg roots, that are up to ~ 20 cm in height) extending seaward of the mangrove canopy. All sites were within 2km of the estuary mouth, with ~1.5 m semi-diurnal tidal ranges and salinities that remained close to 35 ppt.

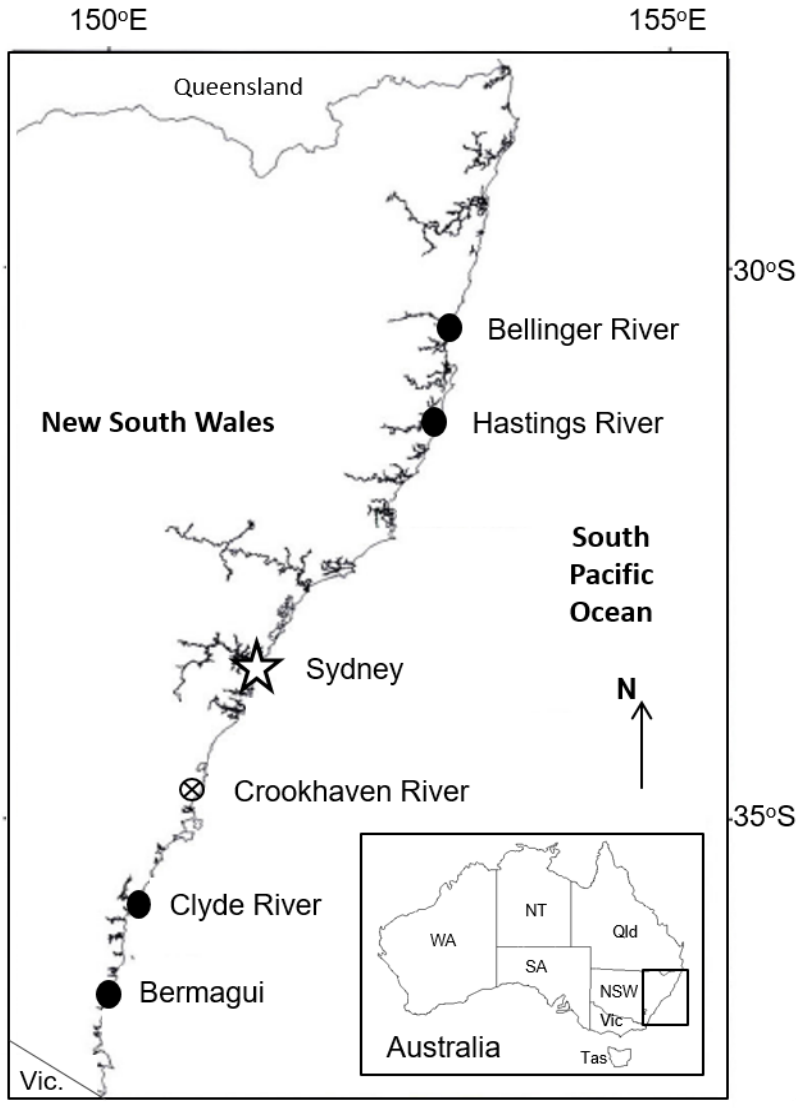

Fig. 2. Location of the four estuaries (black circles) where experimental structures were deployed along the coastline of New South Wales, Australia. Oyster habitat was collected from Crookshaven River (crossed circle).

Experimental design

At each site, sixty habitat patches that were 30 x 30 cm in area, and contained at least 20 pneumatophores were established at least 50 cm apart. These were cleared of all epifauna and naturally occurring oysters and randomly assigned to one of 12 treatments, to give $n = 5$ replicate plots per treatment. On the same day as clearing, plots were either shaded or unshaded, open or closed to predators, and received either live, dead or no oysters in a fully orthogonal design.

Plots assigned to the shading treatment received a 30 x 30 cm square of shade cloth (Coolaroo 70% UV block) attached at a 30 x 30 cm square of 2.5 x 2.5 cm square wire mesh to maintain rigidity. The shade cloth was suspended 25 cm above the substrate surface by attachment to four 60 cm-long and 3 cm-diameter PVC posts that were sunk ~35 cm into the sediment. Pilot studies, comparing invertebrate colonization over 6 weeks between plots receiving the square mesh, which provided the structure of the shading treatment without the shading, and undisturbed control plots indicated that there were no experimental artefacts associated with the method of shading that needed to be controlled for (ANOVA: $F_{1,12} = 2.11$, $P = 0.16$). Consequently, unshaded plots were marked only with the four PVC posts.

Large vertebrate (e.g. birds, fish) and invertebrate (e.g. crabs) predators were excluded from plots using 30 x 30 x 30 cm cages constructed of 2.5 x 2.5 cm square wire mesh. This mesh size allowed migration of most mobile species of benthic macroinvertebrates found at our sites, but in doing so, also allowed passage of some smaller predators, such as flatworms and muricid and naticid gastropods. The cages did not have mesh on their bottoms, and instead, the sides of the cages were sunk ~5 cm into the sediment such that they excluded all but the

deepest burrowing infaunal predators and stood 25 cm above the substrate. Cages were secured to the four PVC posts marking each plot. In pilot studies, we assessed caging artefacts on invertebrate colonization with partially open cages (large sections of mesh were removed), which mimicked the caging structure but allowed predator (i.e. fish) access. There was no difference in invertebrate communities between plots receiving partial cages and no structure (i.e. undisturbed; $F_{1,12} = 1.14$, $P = 0.35$). Consequently, the plots open to predators were free of mesh.

The dead and live oyster habitat used in experiments was sourced from a common site, Crookhaven River, Greenwell Point, NSW (34.9^{o}S, 150.73^{o}E; Fig. 1), from which translocations are permitted due to the absence of QX oyster disease (NSW DPI, pers. comm.). Live clumps of oysters were collected from amongst pneumatophores three days prior to deployment at the four field sites. To produce dead oyster habitat that did not differ in structure from live oysters, we also collected some live oyster clumps six weeks prior to experimental deployment, that were left to dry and die through continuous air exposure. We removed dead oyster meat where this could be achieved without altering the oyster clump structure. Any remaining meat was removed by terrestrial invertebrates during the 6 week period of air exposure. Prior to deployment, live and dead oyster habitat was defaunated by hand removing visible invertebrates and immersing clumps in freshwater for 24 hours to kill inaccessible infauna. As oysters are able to keep their valves closed for several weeks to avoid unfavourable environmental conditions (La Peyre et al. 2009), this method did not compromise the survivorship of live oysters. All oyster clumps were of similar weight (340 ± 6 g; mean ± SE) and size (9 – 13 cm height; 10 – 15 cm width) and were within the size range of naturally occurring oyster clumps at each site. For dead oyster habitat, we standardised weight and size prior to death to ensure shell volume was similar to live oyster habitat. Each clump was attached to the end of a 20.5 cm wooden chopstick using non-toxic two-part

marine epoxy (Vivacity Engineering Pty. Ltd.). The chopsticks were designed to mimic the pneumatophores to which wild oysters naturally attach (Bishop et al. 2012) and were depressed ~15 cm into the sediment so that the base of the oyster clump rested on the sediment surface level with oyster clumps occurring naturally outside the plots. Plots assigned to the live or dead oyster treatment received a single clump of oysters centrally positioned within the plot, within the range (6-18 clumps/m^2; pers. observ.) found at each site. Plots without oysters received a single chopstick.

In each of the four treatments without oysters, a single iButton data-logger (DS1921G Thermochron iButton) was deployed in three randomly selected plots to record the effects of shading, caging, and their interaction on temperature. iButtons were not deployed in oyster habitat because: (1) we had an insufficient number of loggers to sample both habitats; (2) previous sampling at 32 sites spanning the NSW coastline documented temperature differences between habitat with and without oysters (McAfee et al. 2016); and (3) our focus here was on documenting environmental differences among sites that may influence the role of oysters and in demonstrating the efficacy of the shading treatment in reducing temperature in the absence of oysters. iButtons were programmed to record temperatures at 20 minute intervals (accuracy: 0.5°C) and waterproofed using Plastidip rubber coating (Performix Brand: McAfee et al. 2016). iButtons were secured to the end of 20.5 cm chopsticks to represent invertebrates positioned on pneumatophores. Chopsticks were sunk ~10 cm into the sediment until the iButton was positioned at a similar height to the neighbouring pneumatophores. At the experiment's conclusion maximum temperature was determined for each iButton.

In January 2015, four months after the establishment of experimental interventions, the colonization of macroinvertebrates (> 500 µm diameter) to experimental plots was assessed using destructive sampling. Within each plot, all pneumatophores and oysters were carefully

collected, so as not to dislodge their associated invertebrate communities, and these and any epifaunal invertebrates on the sediment surface within the plot were bagged together for transport back to the laboratory for further analysis. Live pneumatophores were cut at the substrate surface. Crabs on the sediment surface were identified and enumerated *in situ* due to their high mobility which hampered destructive sampling. Invertebrates on sediment, pneumatophores and oysters were pooled in analyses as many species move between microhabitats (Bishop et al. 2009, Hughes et al. 2014). Two shaded plots and two caged plots had damaged structures and were excluded from analysis.

In the laboratory, the contents of each bag was washed over a 500 μm sieve, with mobile invertebrates separated from oysters and pneumatophores and preserved in 70 % ethanol until time permitted identification and enumeration. Sessile invertebrates, such as barnacles, that could not easily be removed from oysters or pneumatophores without damage were counted on the structures themselves with aid of a magnifying glass. Fauna were identified to mixed taxonomic level (polychaetes to family; crustaceans to genus; molluscs to species) and enumerated by taxon under a dissecting microscope.

Data analysis

First, to assess spatial variation in the temperature of the four sites, and the extent to which shading or caging ameliorates this, we ran a three-way ANOVA with the factors shading (2 levels, fixed: shaded, unshaded), caging (2 levels, fixed: caged, uncaged), and site (4 levels, random) on the maximum temperatures recorded by iButtons. Second, to assess spatial variation in the mechanisms of facilitation of invertebrates, ANOVAs tested for effects of oyster habitat (3 levels, fixed: absent, dead, live), shading, predator exclusion (i.e. caging), and site on the total abundance and taxon richness of all invertebrates (i.e. sessile and mobile combined), as well as on the abundance of key functional groups (sessile organisms [barnacles and oyster spat], gastropods, mobile arthropods, and infaunal bivalves [excluding

oyster spat], and polychaetes). Data were log transformed prior to analyses to ensure that they satisfied assumptions of normality and homogeneity of variance (confirmed using Levene's test). Sources of significant effects of oyster habitat, site and their interaction with other factors were identified using post-hoc Tukey's tests.

Third, to test the hypotheses (H_4) that in the absence of oysters, effects of shading would increase and effects of predation would decrease with temperature, and (H_5) in the presence of live or dead oysters these relationships would weaken, linear regressions were run between maximum temperatures and log-response ratios between shaded and unshaded, or caged and uncaged treatments. Ratios were calculated using the mean abundance or mean richness of the five replicates of each treatment at each site, and were regressed against the mean maximum temperature recorded from the corresponding bare treatment. Two-tailed t-tests compared the slope of the regression lines between each habitat. Linear regressions were similarly run between the log response ratios calculated using the abundances of the key functional groups and maximum temperatures.

All analyses were conducted in SPSS 24.

2.4 Results

Invertebrate colonisation

Across the four study sites, 79 invertebrate taxa were observed of which 34 were found in bare habitat, 71 in live oyster habitat and 61 in dead oyster habitat (Online Resource 1: Table 1). The most abundant taxa were the barnacles (*Austrominius covertus* and *Chthamalus antennatus*; 41% and 8% of total abundance respectively), the gastropods *Bembicium auratum* (12%) and *Patellioda mimula* (5%), the collembolid *Anurida maritime* (9%),

Saccostrea glomerata oyster spat (6%), Phoxocephalidae amphipods (5%) and Spionidae polychaetes (3%). Of the 52,850 invertebrates that colonised the experimental plots, 14% were found in bare habitat, and 42% in live and 44% in dead oyster habitat.

Effects of habitat

Of the four factors considered – habitat, caging, shading and site – habitat explained the greatest proportion of variation in the abundance and taxon richness of invertebrates among plots (Online Resource 1: Table 2). Consistent with our hypothesis (H_1) that oysters would enhance the richness and abundance of invertebrates, at each of the four sites, and irrespective of caging or shading treatment, live and dead oyster habitat supported more abundant and taxon rich invertebrate communities than bare habitat (post-hoc tests, sig. Hab x Si, Hab x Ca, Hab x Sh interactions, Online Resource 1: Table 2, Fig. 1-2). At each site, and within each of the shading and caging treatments, there was no significant difference in the total abundance or taxon richness of invertebrates between live and dead oyster habitats (post-hoc tests, sig. Hab x Si, Hab x Ca, Hab x Sh interactions, Online Resource 1: Table 2, Fig. 1, 2).

With the exception of sessile invertebrates, each of the functional groups was significantly more abundant in live or dead oysters than in bare habitat at each site, and in each caging and shading treatment (post hoc tests, sig. Hab x Si, Hab x Ca, Hab x Sh interactions, Online Resource 1: Table 3). At one site (Clyde), the abundance of sessile invertebrates (dominated by barnacles, that settled on pneumatophores in both bare and oyster treatments, as well as on live and dead oyster shells) did not differ between bare and oyster habitats (post-hoc test, sig. Hab x Si interaction, Online Resource 1: Table 3). As hypothesised (H_2), differences in invertebrate abundances between live and dead oyster treatments varied among functional groups. The epifaunal groups, sessile invertebrates and gastropods, were similarly abundant

in live and dead oyster habitat, while mobile arthropods were more abundant in dead than live oysters (post-hoc tests, sig. Hab x Si, Hab x Ca, Hab x Sh interactions, Online Resource 1: Table 3). By contrast, live oysters, supported more of the predominantly infaunal groups, polychaetes and bivalves, than dead oysters within at least some sites and treatments (post hoc-tests, sig. Hab x Si, Hab x Ca, Hab x Sh interactions; Online Resource 1: Table 3).

Effects of shading

Maximum temperatures in unshaded bare plots differed between each of the sites, by as much as 6.7 ^{o}C (post-hoc test, sig. Sh x Si, Online Resource 2: Table 1; Fig. 2). At each of the sites except Bermagui (the coolest site), shading reduced maximum temperature, reducing among-site variability in this variable (post-hoc test, sig. Shading x Site, Online Resource 2: Table 1; Fig. 2).

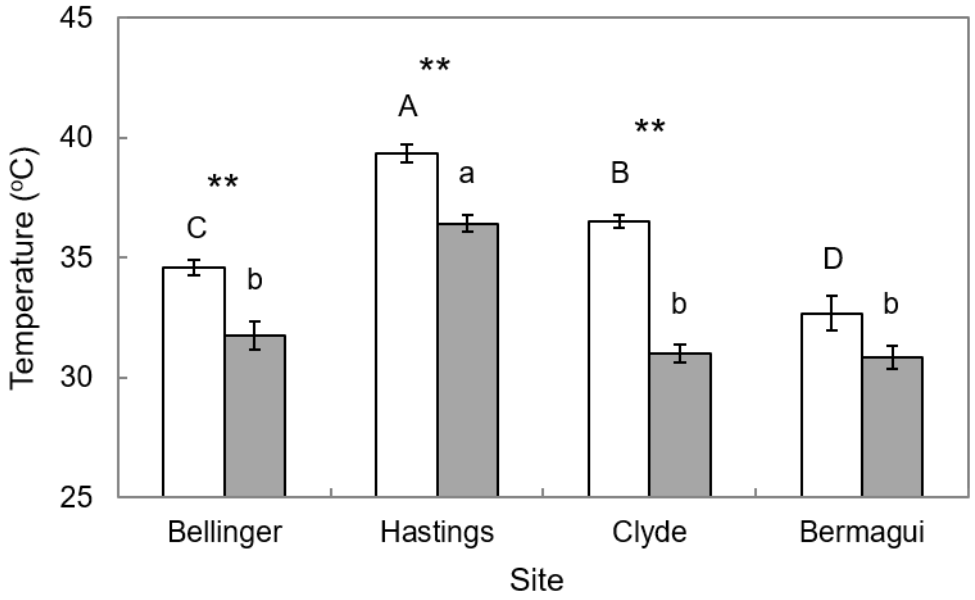

Fig. 2. Mean (±SE) maximum temperature recorded at four sites of New South Wales Australia, in unshaded (white bars) and shaded (grey bars) plots without oysters. Sites are arranged from left to right according to increasing latitude. Significant differences ($\alpha = 0.05$) between shading treatments at each site are marked with **, and among sites are marked with letters within unshaded (upper case) and shaded (lower case) treatments. n = 3 per treatment.

Consistent with the hypothesis (H_3) that effects of shading would be greater in bare than live or dead oyster habitat, we found that in bare habitat, shading significantly increased (as compared to otherwise similar unshaded plots) the total taxon richness of invertebrates and the abundance of gastropods and polychaetes, but in live or dead oyster habitats, shading had no or negative effects on these variables (post-hoc tests, sig. Hab x Sh or Hab x Sh x Ca interactions, Online Resource 1: Table 2,3). In bare habitats, shading increased taxon richness by 38% in uncaged plots, though in caged plots, effects of shading were not significant (post-hoc test, sig. Hab x Sh x Ca interaction, Online Resource 1: Table 2). Whereas shading had no effect on species richness or polychaete abundance in live or dead oyster habitats, shading had negative effects on gastropod abundance in plots with live or dead oysters (post-hoc tests, sig. Hab x Sh interaction, Online Resource 1: Table 3). Effects of shading on total abundance did not vary as a function of habitat or of caging, but instead varied among sites (sig. Sh x Si interaction, Online Resource 1: Table 2). At Hastings, the hottest site, a significantly greater total abundance was found in shaded than unshaded treatments, with no difference detected at any other site (post-hoc tests, sig. Sh x Si interaction).

Linear regressions supported our hypotheses (H_4) that in bare habitats positive effects of shading would increase with temperature, but (H_5) in habitats with live or dead oysters, the relationship between the effects of shading and temperature would be weaker. In bare habitats, as temperatures increased so did the effect of shading structures, with the log-response of total invertebrate abundance and taxon richness in shaded as opposed to unshaded treatments increasing significantly with maximum temperatures (Online Resource 2, Table 2; Fig. 3). In live oyster habitat, neither log-response ratios between shaded and unshaded treatments for total abundance nor richness displayed a relationship with temperature (Online Resource 2, Table 2; Fig. 3), with the relationship for taxon richness significantly differing between the live oyster and bare habitats (Online Resource 2, Table 3).

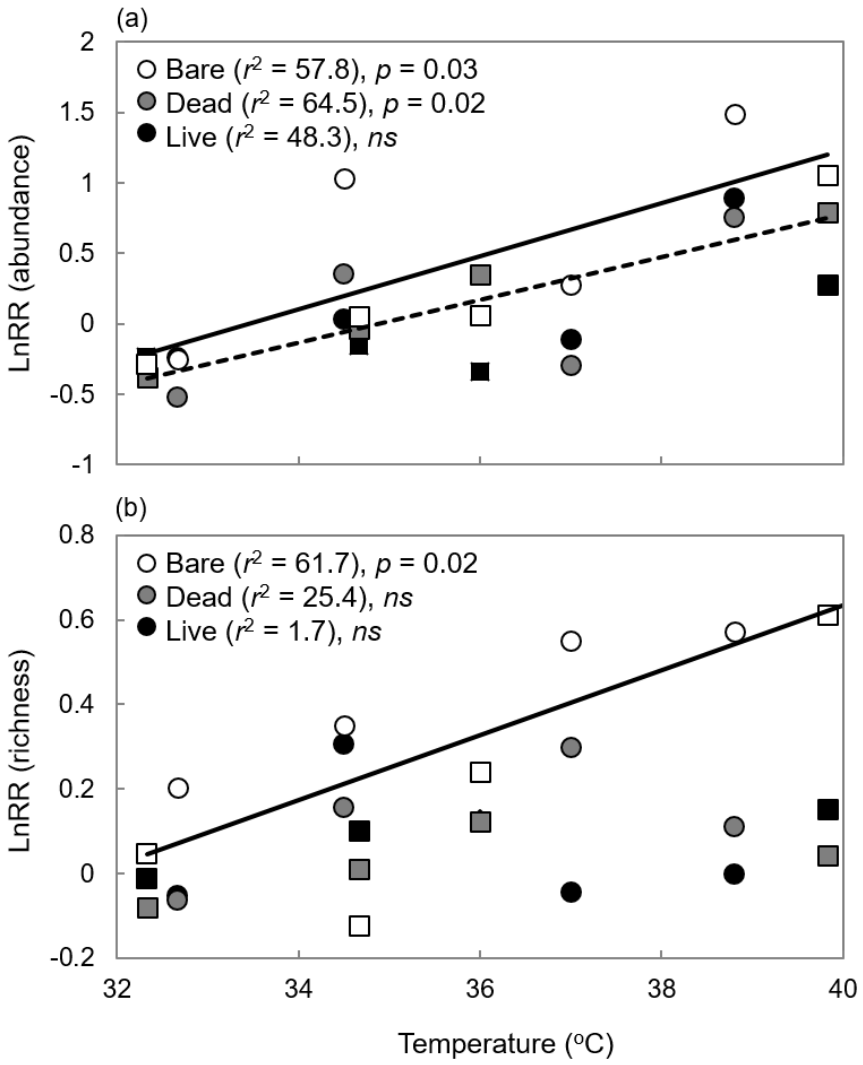

Fig. 3. Relationship between mean maximum temperature and mean log response ratio between shaded and unshaded treatments for (a) invertebrate abundance and (b) taxon richness, in bare (white symbols; solid trendline), dead oyster (grey symbols; dashed trendline) and live oyster (black symbols) habitat that either received predator excluding cages (squares) or was exposed to predators (circles). Mean log-response ratios were calculated from n = 5 replicate habitats per site. The r^2 and significance of relationships are described at the top of each panel with non-significant relationships denoted (*ns*). Only significant relationships have trendlines displayed.

However, in dead oyster habitat the relationship for total abundance was significant and did not differ from the relationship found in bare habitat (Online Resource 2, Table 2-3), although the relationship was non-significant for richness (Fig. 3). At functional group level, a positive relationship between temperature and the log-response ratio of abundance in

shaded as opposed to unshaded bare plots was found for sessile organisms and gastropods, but no relationship was found for mobile arthropods, bivalves or polychaetes (Online Resource 2, Table 4, Fig. 5). In live and dead oyster habitat, consistent with the role of oysters ameliorating maximum temperatures (H_5), we detected no relationship between temperature and the effect of shading structures for any of the functional groups (Online Resource 2, Table 4).

Effects of caging

As with shading, and consistent with our hypotheses (H_3), caging had a greater effect on invertebrate colonisation in bare than live or dead oyster habitats. Caging increased total invertebrate abundance in bare habitats by 48% relative to uncaged plots (post-hoc test, sig. Hab x Ca interaction, Online Resource 1: Table 2), and significantly increased taxon richness in unshaded bare habitats by 43%, although there was no difference between caged and uncaged bare plots that were shaded (post-hoc tests, sig. Hab x Sh x Ca interaction; Online Resource 1: Table 2). By contrast, there was no effect of caging in habitat with live or dead oysters. Similarly, caging had positive effects on the functional group abundances for sessile invertebrates, mobile arthropods and gastropods in bare habitats, with no influence detected in live or dead oyster habitat (post-hoc tests, sig. Hab x Ca interaction, Online Resource 1: Table 3). Caging did not influence polychaete or bivalve abundance (post-hoc tests, sig. Hab x Ca interaction, Online Resource 1: Table 3).

Consistent with the hypothesis that in bare habitat, effects of caging would decrease as temperature stress increased (H_4), the log-response of total abundance in caged vs uncaged bare habitat decreased significantly with increasing temperature (Online Resource 2, Table 2; Fig. 4). However, as expected (H_5) there was no relationship between these variables in dead or live oyster habitats (Online Resource 2, Table 2; Fig. 4a), resulting in a significant

difference in relationship between live or dead oyster and bare habitats (Online Resource 2, Table 3). By contrast, the influence of caging on taxon richness bore no relationship with maximum temperature in any of the three habitats (Online Resource 2; Table 2-3; Fig. 4b).

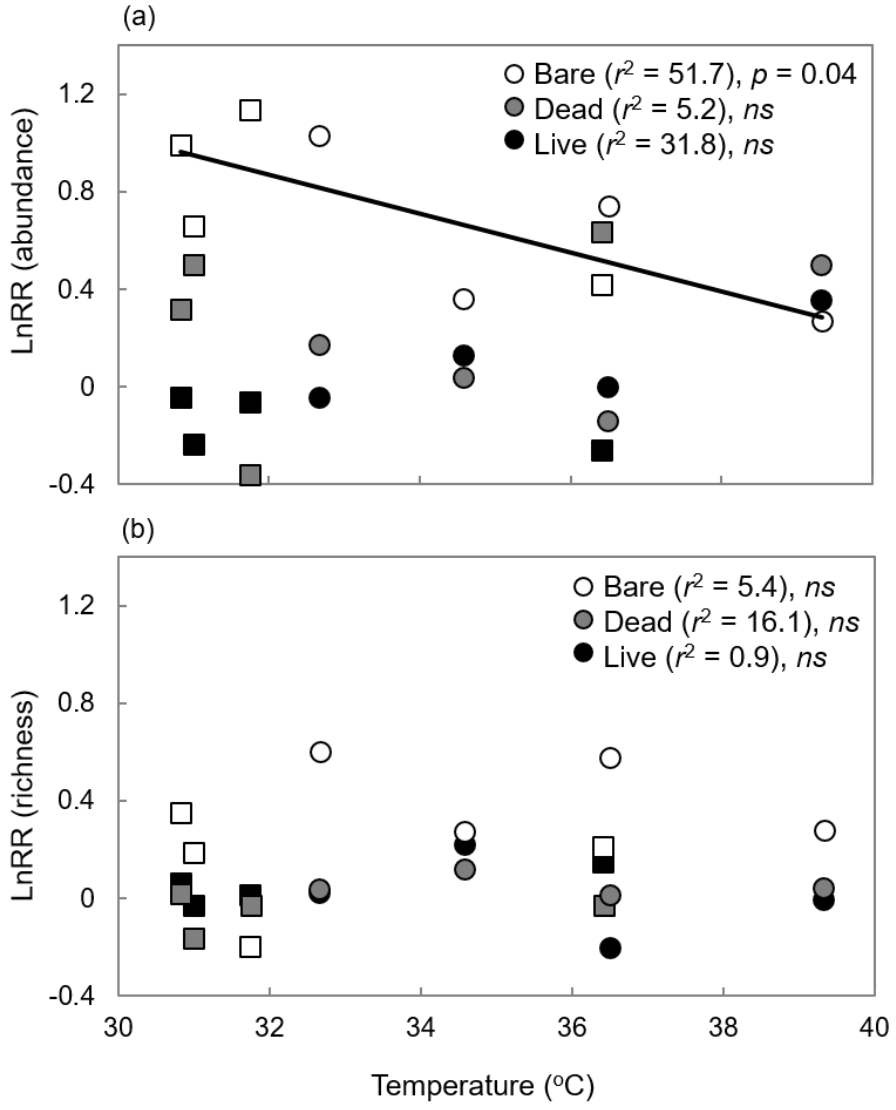

Fig. 4. Relationships between mean maximum temperature and the mean log-response ratio of (a) invertebrate abundance and (b) taxon richness between caged and uncaged habitats, recorded from bare (white symbols), dead oyster (grey symbols) and live oyster (black symbols) habitat that either received shading structures (squares) or no shading (circles). Mean log-response ratios were calculated from n = 5 replicate habitats per site. The r^2 and significance of relationships are described at the top of each panel with non-significant relationships denoted (*ns*). Only significant relationships have trendlines displayed.

The log-response ratio for sessile organisms in caged versus uncaged plots of bare habitat had a significant negative relationship with maximum temperature (Online Resource 2, Table 4; Fig. 5). No relationship between the effect of caging and maximum temperature was, however, found in live or dead oyster habitat (Online Resource 2, Table 4; Fig. 5). By contrast, the log response ratio for gastropod abundance in live habitat was shown to have a significant positive relationship with increasing temperature (Online Resource 2, Table 4; Fig. 5), but no relationship was found in dead oyster or bare habitats. No other organismal group showed a significant relationship between caging log-response ratios and temperature (Online Resource 2, Table 4; Fig. 5).

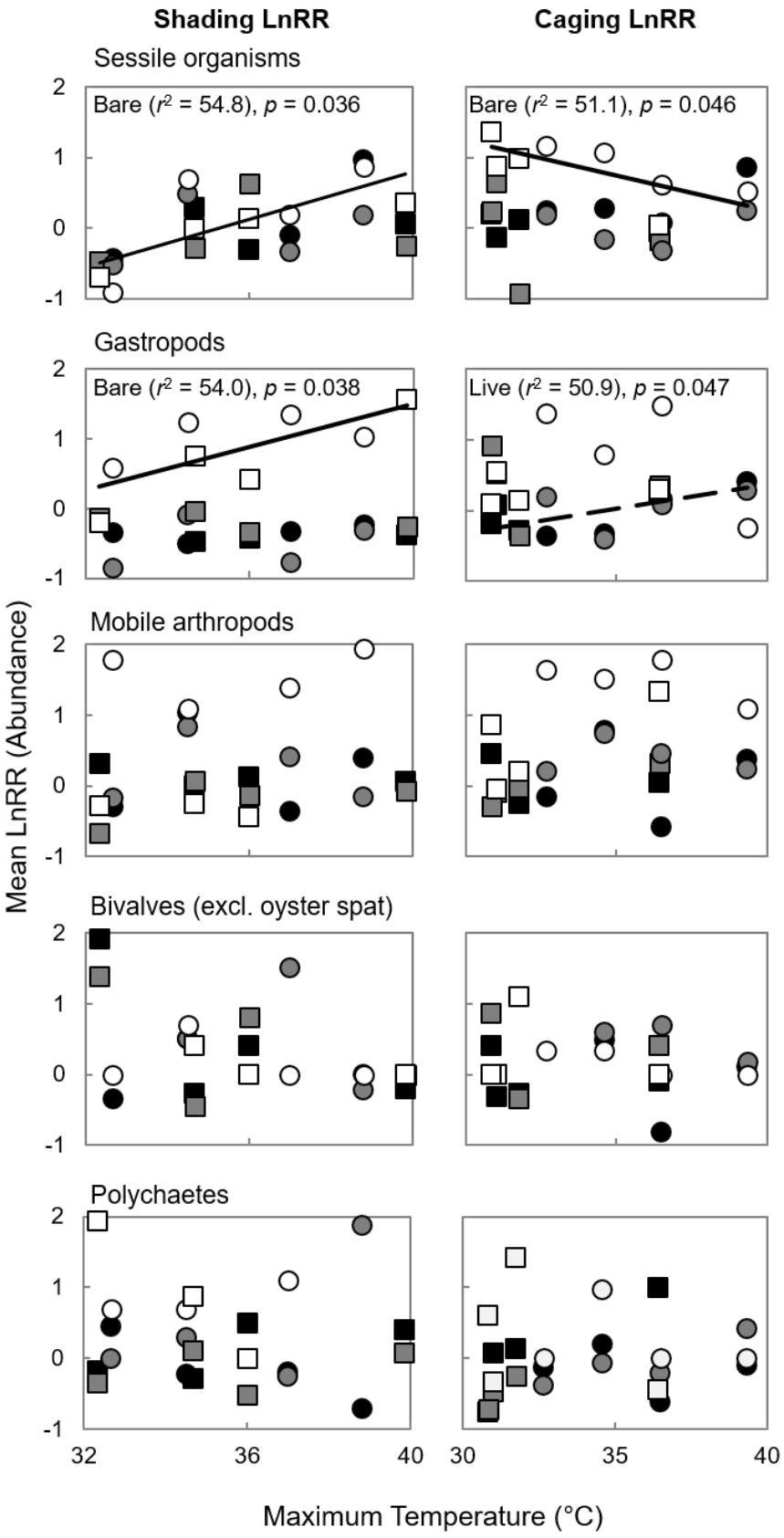

Fig. 5. Relationships between maximum temperatures and the mean log-response ratio of invertebrate abundance between shaded and unshaded habitats, and between caged and uncaged habitats, within bare (white symbols;

solid trendline), dead oyster (grey symbols), and live oyster (black symbols; dashed trendline) habitat that either received shading structures (squares) or no shading (circles). Mean log-response ratios were calculated from n = 5 replicate habitats per site. The r^2 and trendlines of significant relationships are displayed.

2.5 Discussion

Previous studies have tested predictions of the Bertness and Callaway (1994) stress gradient hypothesis by comparing the overall interaction strength of ecosystem engineering across environmental stress gradients (e.g. Silliman et al. 2010, McAfee et al. 2016). Although the majority of these studies have supported the hypothesis (reviewed by Michalet et al. 2006, Callaway 2007), a number of exceptions have been found (Callaway 2007), suggesting that some refinement of the model is needed (Maestre et al. 2009). It has been proposed that consideration of the traits of the species involved and characteristics of the stress factor may assist in refining predictions of species interactions (Maestre et al. 2009). Our study partitioned the mechanisms by which oysters facilitate invertebrate abundance and taxon richness into provision of substrate, shading and amelioration of predation, and assessed spatial variation in the importance of these mechanisms in facilitating individual functional groups as well as community metrics across an environmental gradient. We demonstrate that the mechanisms by which oysters facilitate invertebrate abundance and species richness vary spatially with variation in the key stressors to which invertebrates are exposed. Although oysters enhanced invertebrate abundance and taxon richness at all sites, the contribution of shading by oysters to the enhancement of biodiversity was limited to sites attaining highest maximum temperatures, and the role of oysters in mitigating predation pressure increased with decreasing abiotic stress. These results highlight the importance of matching mechanisms of facilitation to gradients in the specific environmental stressors of organisms.

Molluscs may enhance biodiversity both directly, via substrate provision, and indirectly via stressor amelioration, or via their grazing and biodeposition (Gutiérrez et al. 2003). This study did not directly measure temperature stress to invertebrates or rates of their predatory mortality in the presence versus the absence of oysters. Instead, the stronger effect of shading on invertebrate abundance and richness in the absence than the presence of oysters was taken as evidence that temperature amelioration was among the mechanisms by which oysters facilitate invertebrates. Similarly, the stronger effect of caging on invertebrate abundance in the absence than the presence of oysters was considered evidence that provision of predator refuge is among the mechanisms of facilitation in our study system. Positive effects of oyster additions on invertebrate abundance and, in some instances, richness, irrespective of caging or shading treatments, indicated that amelioration of temperature and desiccation stress alone could not explain the high abundances of invertebrates associated with oysters, and provision of substrate and/or functions such as filtration and biodeposition also contributed. Coupling the manipulations of stressors performed in the present study with direct measurements of mortality and sublethal stress to associate organisms would provide a more comprehensive assessment of stressor amelioration. Overall, consistent with the well documented role of habitat-forming bivalves in facilitating invertebrate communities (Seed 1996, McAfee et al. 2016, Bateman and Bishop 2017), we found oysters supported up to twenty times the abundance and three times the species richness of invertebrates than in otherwise similar bare habitat.

Effects of oyster substrate addition were common to all sites, and instead varied with organismal functional group. Consistent with the primary use of oysters by epifauna as a substrate for attachment and grazing, in most instances sessile organisms, dominated by barnacle and oyster spat, and gastropods, dominated by the grazer (Reid 1988) *Bembicium auratum*, did not display any difference in abundance between live and dead oyster

treatments. Where differences did occur between live and dead oyster treatments, these groups were generally more abundant on dead oyster shell, consistent with a direct response to the increased surface area for attachment provided by shell disarticulation (Gutiérrez et al. 2003, Summerhayes et al. 2009). Infaunal polychaetes and bivalves were, on the other hand, more abundant on live than dead oyster habitat, although even dead oysters increased the abundance of polychaetes 13-fold above bare habitat. Although mangrove roots and trunks provide surface area for attachment and grazing at our study sites, competition for this resource can be intense (Branch and Branch 1980), such that oysters enhance the availability of limited resource. Live oysters may support greater abundances of infauna that dead oysters as a result of their biodeposition of nutrient rich faeces and pseudofaeces (Newell 2004).

In contrast to the spatially consistent effect of substrate addition on invertebrates, effects arising from amelioration of abiotic and biotic stressors displayed marked spatial variation. As temperatures increased, the magnitude of the difference in invertebrate communities between shaded and unshaded plots increased significantly when oysters were absent, but did not differ between shading treatments when oysters were present, indicating an increasing role for oyster shading in invertebrate facilitation as temperature increased. Temperature exerts a strong structuring influence on intertidal communities, with many species' upper intertidal distribution limited by temperature and desiccation stress (Helmuth et al. 2006). Provision of cool and moist microhabitats can enable organisms to persist in environments in which they would otherwise not persist (Keppel et al. 2012). Although the amelioration of temperature by oysters was not directly measured in this study, our previous research along this coastline found that during midday low tides shading by oysters can reduce maximum temperatures by over 4.5 ^{o}C below that of adjacent bare habitat (McAfee et al. 2016). Despite the hard exterior of gastropods and sessile invertebrates, such as barnacles, which protects them against desiccation stress (McMahan 1990), the abundance of these groups displayed a

particularly strong negative relationship with temperature in bare habitats that was disrupted by the presence of oysters.

At the coolest site, shading did not influence invertebrate communities, and amelioration of predation appeared the more important mechanism by which oysters facilitated invertebrates. Differences in invertebrate abundances between caged and uncaged plots without oysters decreased as temperature increased, but there was no relationship between temperature and the magnitude of the difference in plots with oysters, suggestive of an increasing influence on predator amelioration as temperature decreased. While we did not explicitly quantify biotic stress in this experiment by documenting predator abundances or rates of predatory mortality, biological interactions are expected to become the dominant community structuring influence as abiotic stress diminishes (Bertness and Callaway 1994). The complex, three-dimensional structure of oysters provides inhabitants with protection from larger predators (i.e. fish, birds), although activity of mesopredators may be increased due to reductions in interference competition (Grabowski and Powers 2004), or protection from higher order predators (Grabowski 2004).

Understanding how ecosystem engineers modify abiotic factors and biotic processes is required for predicting their influence on species distributions (Wright and Jones 2006). We have shown that facilitation of invertebrates by oysters results from a combination of habitat provision and stressor amelioration, and that the relative importance of these mechanisms varies spatially according to the magnitude of environmental stressors. In demonstrating how the mechanisms of facilitation vary spatially across environmental stress gradients, according to variation in the identity of key environmental stressors, our study provides a rare example of the processes that underlie the stress gradient hypothesis (Bertness and Callaway 1994). Further, in demonstrating how organismal groups respond differently to these individual stressors, our results suggest that tests of the stress gradient hypothesis may be strengthened

through consideration of how the traits of organisms match to primary stressors (see also Maestre et al. 2009).

Knowledge of how mechanisms of ecosystem engineering vary across environmental gradients could be helpful in designing restoration projects targeting amelioration of specific stressors or conservation of particularly vulnerable taxa. For example, with knowledge that an ecosystem engineer has a particularly important role in ameliorating predation pressure in abiotically benign environments, restoration projects at such sites could focus on creating those configurations and morphologies that are particularly effective at disrupting top-down processes (see Grabowski 2004, Grabowski et al. 2008). Conversely, in environments where ecosystem engineers are more important in mitigating temperature stress, different morphologies may be most effective. Additionally, by considering how the traits of individual species may influence their susceptibility to stressors, conservation efforts may be targeted at specific ecosystem engineers that ameliorate those specific stressors to taxa of interest. By understanding how ecosystem engineering activities vary with environmental context, we can predict where conservation and/or restoration efforts can provide the greatest benefits to biodiversity and ecosystem resilience (Wright and Jones et al. 2006).

2.6 Acknowledgements

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Supplemental material: The mechanisms by which oysters facilitate invertebrates vary across environmental gradients

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Online Resource 1. Sources of spatial variation in invertebrate abundance and taxon richness, and the abundance of major functional groups.

Table 1. Summary of the invertebrates identified at each of the four sites and the habitats in which they were recorded.

Taxon	Bellinger	Hastings	Clyde	Bermagui
ANNELIDA				
CLASS POLYCHAETA				
Capitellidae	-	Live	-	-
Nephtyidae	-	-	-	Live, Dead

Nereidae	All	Live, Dead	Live, Dead	All
Phyllodocidae	-	Live, Dead	Live, Dead	Live, Dead
Sabellidae	Live, Dead	Live, Dead	Live, Dead	All
Serpulidae	All	All	-	Live
Spionidae	All	Live, Dead	Live, Dead	All
Syllidae	All	Live, Dead	Live, Dead	Live, Dead
Terebellidae	Live, Dead	Live, Dead	Live	Live, Dead
ARTHROPODA				
CLASS ARACHNIDA				
Halacaridae	Dead	Live, Dead	Live, Dead	Live, Dead
CLASS COLLEMBOLA	All	Live, Dead	Live, Dead	Live, Dead
CLASS MALOCOSTRACA				
Heloeciidae				
Heloecius cordiformis	-	Bare	Live, Bare	Dead, Bare
Hymenosomatidae				
Halicarcinus ovatus	-	-	Live, Dead	-
Paguridae				
Pagurus sinuatus	Live, Dead	-	Live, Dead	Live, Bare
Pilumnidae				
Pilumnus sp.	Live, Dead	Dead	-	Live, Dead
Sesarmidae				
Parasesarma erythodactyla	All	Live, Dead	All	All
Varunidae				
Helograpsus haswellianus	Live, Dead	Dead	Live, Dead	Live, Dead
Paragrapsus laevis	Live, Dead	Live, Dead	All	All
Order Amphipoda				
Corophiidae	Live, Dead	Live, Dead	Live, Dead	Live, Dead
Oedicerotidae	Dead	-	Live	Live
Phoxocephalidae	All	Live, Dead	All	All
Order Isopoda	All	All	All	All
Suborder Dendrobranchiata	-	-	Live, Dead	Dead
CLASS MAXILLOPODA				
Austrobalinidae				
Austrminius sp.	All	All	All	All
Chthamalidae				
Chthamalus antennatus	-	-	Live, Dead	Dead
CNIDARIA				
Order Actiniaria	Live	-	-	-
MOLLUSCA				
CLASS POLYPLACOPHORA				
Chitonidae	Live	Live	-	-
CLASS GASTROPODA				
Assimineidae				
Cryptassiminea buccinoides	Live, Dead	Live, Dead	-	-
Batillaridae				
Batillaria australis	Live	-	All	All
Pyrazus ebeninus	Bare	-	All	All
Ellobiidae				
Cassidula sp.	Dead	-	-	-
Ophicardelus ornatus	-	-	Bare	Bare

Iravadiidae				
Nozeba topaziaca	-	-	-	Live
Littorinidae				
Bembicium auratum	All	All	All	All
Littoraria luteola	All	Live	Dead, Bare	
Lottidae				
Patelloida mimula	All	Live, Dead	Live, Dead	All
Nassariidae				
Nassarius jonasii	-	-	-	Bare
Tritia burchardi	-	-	Live, Dead	Dead
Naticidae				
Conuber sordidium	All	Live, Dead	All	Dead
Neritidae				
Nerita atramentosa	Live	-	-	Dead
Siphonariidae				
Siphonaria denticulata	-	Live	-	Dead
Tornidae				
Pseudoliotia micans	Live, Dead	Live, Dead	All	Live, Dead
Trochidae				
Austrocochlea porcata	All	Dead	Live, Dead	All
CLASS BIVALVIA				
Glauconomidae				
Glauconome radiata	-	-	Live	-
Hiatellidae				
Hiatella arctica	-	-	-	Live
Lasaeidae				
Lasea australis	Live, Dead	Live, Dead	Live, Dead	All
Laternulidae				
Laternula sp.	All	Live, Dead	Live, Dead	Live
Mytilidae				
Mytilus galloprovincialis	-	Live	-	Live
Trichomya hirsuta	Live	Live, Dead	Live, Dead	Live, Dead
Xenostrobus securis	Live, Dead	Dead	All	Live, Dead

Table 2. Four-way univariate ANOVAs examining sources of spatial variation in the total abundance and taxon richness of invertebrates among habitats (Hab: live oyster, dead oyster, oyster-free bare), shading (Sh: shaded, unshaded) and caging (Ca: caged, uncaged) treatments, at each of four sites (Si). Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold. n = 5.

		Abundance			Richness		
Source	**df**	**MS**	***F***	**P**	**MS**	***F***	**P**
Hab	2	17.14	306.6	**0.001**	9.05	796.0	**0.001**
Sh	1	0.35	6.2	**0.014**	0.22	19.7	**0.001**
Ca	1	0.89	15.9	**0.001**	0.14	12.0	**0.001**
Si	3	6.39	114.4	**0.001**	0.26	22.9	**0.001**
Hab x Sh	2	0.14	2.4	0.089	0.06	5.4	**0.005**
Hab x Ca	2	0.42	7.6	**0.001**	0.09	7.9	**0.001**
Hab x Si	6	0.88	15.8	**0.001**	0.07	6.7	**0.001**
Sh x Ca	1	0.07	1.3	0.260	0.04	3.6	0.061
Sh x Si	3	0.67	12.0	**0.001**	0.04	3.2	**0.024**
Ca x Si	3	0.03	0.6	0.646	0.01	0.8	0.507
Hab x Sh x Ca	2	0.06	1.1	0.340	0.03	2.3	0.051
Hab x Sh x Si	6	0.02	0.4	0.903	0.01	1.3	0.244
Hab x Ca x Si	6	0.07	1.2	0.307	0.20	1.4	0.228
Sh x Ca x Si	3	0.05	0.9	0.438	0.01	1.1	0.336
Hab x Sh x Ca x Si	6	0.03	0.5	0.835	0.01	0.5	0.799
Res	191	0.06			0.01		

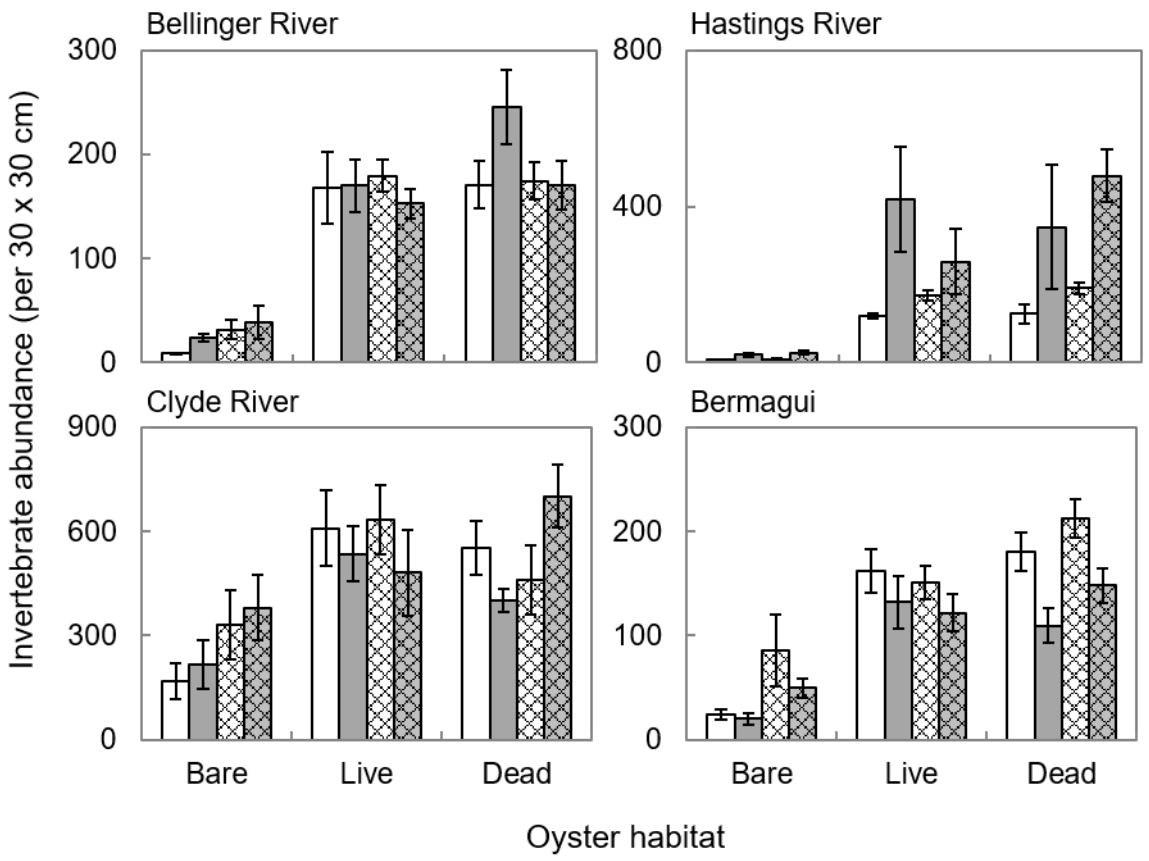

Fig. 3. Mean (±SE) total invertebrate abundance in experimental plots at each of four sites that received one of three habitat treatments (Bare, Live, Dead), that were shaded (grey bars) or unshaded (white bars), and open (no patterning) or closed to (crossed patterning) predators,. n = 5.

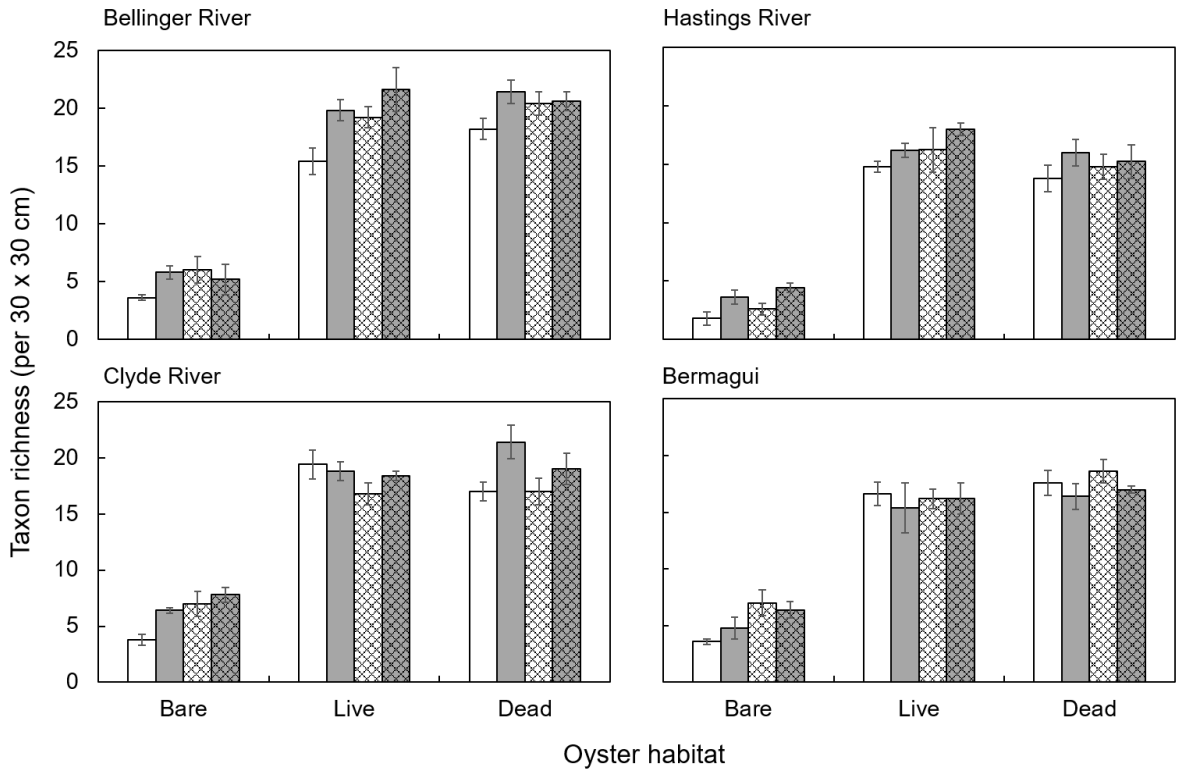

Fig. 2. Mean (±SE) taxon richness of invertebrates in experimental plots that received one of three habitat treatments (Bare, Live, Dead), that were shaded (grey bars) or unshaded (white bars), and open (no patterning) or closed to (crossed patterning) predators, at each of the four sites. n = 5.

Table 3. Four-way univariate ANOVAs examining sources of spatial variation in the major functional groups of invertebrates among habitats (Hab: live oyster, dead oyster, oyster-free bare), and shading (Sh: shaded, unshaded) and caging (Ca: caged, uncaged) treatments, at each of four sites (Si). Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold. n = 5.

		Sessile			Gastropods			Arthropods			Bivalves			Polychaetes		
Source	**df**	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**
Hab	2	7.35	69.32	**0.001**	21.48	329.73	**0.001**	30.77	288.56	**0.001**	5.17	105.77	**0.001**	29.04	361.68	**0.001**
Sh	1	0.01	0.08	0.777	<0.01	0.01	0.916	1.86	17.48	**0.001**	0.07	1.47	0.227	0.31	3.92	**0.049**
Ca	1	0.91	8.60	0.004	0.44	6.81	**0.010**	1.43	13.42	**0.001**	0.01	0.06	0.811	0.01	0.13	0.720
Si	3	21.33	201.2	**0.001**	0.50	7.68	**0.001**	1.32	12.45	**0.001**	0.69	14.30	**0.001**	1.97	24.66	**0.001**
Hab x Sh	2	0.03	0.26	0.769	1.75	26.87	**0.001**	0.01	0.12	0.886	0.02	0.43	0.653	0.21	2.65	**0.051**
Hab x Ca	2	0.44	4.18	**0.017**	0.21	3.28	**0.040**	0.25	2.31	**0.044**	0.04	0.83	0.440	0.26	3.24	**0.041**
Hab x Si	6	0.28	2.64	**0.017**	0.38	5.82	**0.001**	0.67	6.29	**0.001**	0.25	5.12	**0.001**	0.31	3.94	**0.001**
Sh x Ca	1	0.07	0.69	0.407	0.01	0.12	0.732	0.45	4.26	**0.040**	0.02	0.37	0.541	0.02	0.22	0.639
Sh x Si	3	0l43	4.10	**0.008**	0.08	1.24	0.298	0.85	7.97	**0.001**	0.05	1.05	0.371	0.09	1.16	0.325
Ca x Si	3	0.12	1.12	0.341	0.13	2.09	0.103	0.12	1.09	0.354	0.04	0.86	0.462	0.10	1.25	0.293
Hab x Sh x Ca	2	0.02	0.24	0.784	0.17	2.56	0.080	0.03	0.27	0.765	0.02	0.33	0.718	0.14	1.82	0.166
Hab x Sh x Si	6	0.05	0.45	0.843	0.06	0.99	0.430	0.09	0.81	0.566	0.06	1.29	0.265	0.09	1.09	0.368
Hab x Ca x Si	6	0.10	0.98	0.437	0.09	1.42	0.208	0.19	1.79	0.104	0.02	0.51	0.803	0.08	1.00	0.426
Sh x Ca x Si	3	0.17	1.64	0.181	0.01	0.14	0.935	0.18	1.71	0.166	0.27	5.52	**0.001**	0.01	0.08	0.969
Hab x Sh x Ca x Si	6	0.06	0.58	0.744	0.05	0.86	0.529	0.19	1.75	0.111	0.10	2.11	0.054	0.13	1.60	0.148
Res	192	0.11			0.06			0.11			0.05			0.08		

Online Resource 2. Analyses of spatial variation in the maximum temperature of bare habitat, and of the relationships between temperature and the log-response ratios for the effects of shading or caging on invertebrates.

Table 1. Three-way univariate ANOVA examining sources of spatial variation in the maximum temperatures recorded from bare (oyster-free) habitat that received shading (Sh: shaded, unshaded) and caging (Ca: caged, uncaged) treatments at each of four sites (Si). Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold. n = 3.

Source	**df**	**MS**	***F***	**P**
Sh	1	0.940	93.3	**0.001**
Ca	1	0.005	0.5	0.491
Si	3	0.595	59.1	**0.001**
Sh x Ca	1	0.004	0.3	0.557
Sh x Si	3	0.055	5.4	**0.004**
Ca x Si	3	0.008	0.8	0.493
Sh x Ca x Si	3	0.006	0.6	0.616
Res	32	0.010		

Table 2. Linear regressions between maximum temperatures and the log-response ratios between shaded and unshaded (SHADING), or caged and uncaged (CAGING) treatments, for the total abundance and taxon richness of invertebrates in each habitat type. Significant results (at $\alpha = 0.05$) are highlighted in bold.

		Abundance			Taxon richness		
Source	**df**	**r^2**	**P**	**Equation**	**r^2**	**P**	**Equation**
SHADING							
Bare	6	57.8	**0.029**	y = 0.19x - 6.301	61.7	**0.021**	y = 0.08x - 2.440
Live	6	48.3	0.056	y = -3.68x + 0.100	1.7	**0.755**	y = -0.14x + 0.010
Dead	6	64.5	**0.016**	y = -5.27x + 0.151	25.4	0.203	y = -0.75x + 0.021
CAGING							
Bare	6	51.7	**0.044**	y = 3.06x + -0.188	5.4	0.579	y = -0.36x + 0.019
Live	6	31.8	0.145	y = -1.25x + 0.036	0.9	0.826	y = 0.16x – 0.004
Dead	6	5.2	0.586	y = -0.67x + 0.026	16.1	0.324	y = -0.36x + 0.001

Table 3. t-tests assessing significant differences between habitats in the slope of the linear regressions between maximum temperatures and log-response ratios between shaded and unshaded (SHADING), or caged and uncaged (CAGING) treatments, for the total abundance and taxon richness of invertebrates. Significant results (at $\alpha = 0.05$) are highlighted in bold.

	Abundance			Richness		
Source	**df**	***t***	**P**	**df**	***t***	**P**
SHADING						
Live vs Dead	12	-0.754	0.465	12	-0.424	0.499
Bare vs Live	12	-1.079	0.303	12	-2.297	**0.040**
Bare vs Dead	12	-0.422	0.650	12	-1.819	0.179
CAGING						
Live vs Dead	12	-0.424	0.679	12	-0.745	0.470
Bare vs Live	12	3.169	**0.008**	12	-0.249	0.542
Bare vs Dead	12	2.515	**0.027**	12	-0.627	0.807

Table 4. Linear regressions between maximum temperatures and the log-response ratios between shaded and unshaded (shading), or caged and uncaged (caging) treatments, for the abundance of each of the functional groups of invertebrates. Significant results (at $\alpha = 0.05$) are highlighted in bold.

		Shading			Caging		
Source	**df**	**r^2**	**P**	**Equation**	**r^2**	**P**	**Equation**
Sessile organisms							
Bare	6	54.8	**0.036**	y = -5.93x + 0.168	51.1	**0.046**	y = 4.16x + -0.097
Live	6	28.8	0.170	y = -3.43x + 0.098	33.9	0.130	y = -1.74x + 0.057
Dead	6	6.7	0.537	y = -1.57x + 0.042	0.7	0.847	y = 0.40x + -0.013
Gastropods							
Bare	6	54.0	**0.038**	y = -4.74x + 0.156	0.7	0.849	y = 1.12x + -0.016
Live	6	0.3	0.902	y = -0.28x + -0.002	50.9	**0.047**	y = -2.36x + 0.068
Dead	6	0.1	0.967	y = -0.42x + 0.002	3.6	0.652	y = 1.12x + -0.027
Mobile arthropods							
Bare	6	31.1	0.151	y = -7.38x + 0.234	30.9	0.153	y = -3.07x + 0.121
Live	6	0.2	0.910	y = -0.88x + 0.001	0.4	0.879	y = -0.23x + 0.009
Dead	6	2.9	0.686	y = -0.98x + 0.028	38.5	0.101	y = -2.01x + 0.067
Bivalves							
Bare	6	6.3	0.549	y = -1.70x + 0.047	12.7	0.387	y = 1.74x + -0.044
Live	6	22.1	0.239	y = -2.45x + 0.084	1.2	0.795	y = -1.26x + 0.028
Dead	6	0.1	0.946	y = -0.08x + 0.010	5.8	0.567	y = -1.76x + 0.055
Polychaetes							
Bare	6	19.4	0.275	y = -3.69x + 0.138	11.9	0.401	y = 2.81x + -0.074
Live	6	1.0	0.814	y = -3.11x + 0.005	4.67	0.670	y = -1.32x + 0.038
Dead	6	19.8	0.269	y = -4.20x + 0.122	9.91	0.448	y = -2.20x + 0.053

CHAPTER THREE

FAST GROWING OYSTERS SHOW REDUCED CAPACITY TO PROVIDE A THERMAL REFUGE TO INTERTIDAL BIODIVERSITY AT HIGH TEMPERATURES

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Fast-growing oysters show reduced capacity to provide a thermal refuge to intertidal biodiversity at high temperatures

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Abstract

1. Ecosystem engineers that modify the thermal environment experienced by associated organisms might assist in the climate change adaptation of species. This depends on the ability of ecosystem engineers to persist and continue to ameliorate thermal stress under changing climatic conditions—traits that may display significant intraspecific variation.
2. In the physically stressful intertidal, the complex three-dimensional structure of oysters provides shading and traps moisture during aerial exposure at low tide. We assessed variation in the capacity of a faster- and slower-growing population of the Sydney Rock Oyster, *Saccostrea glomerata*, to persist, form three-dimensional structure and provide a cool microhabitat to invertebrates under warmer conditions.
3. The two populations of oysters were exposed to a temperature gradient in the field by attaching them to passively warmed white, grey and black stone pavers and their growth, survivorship and colonisation by invertebrates was monitored over a 12-month period.
4. Oysters displayed a trade-off between fast growth and thermal tolerance. The growth advantage of the fast-growing population diminished with increasing substrate temperature, and at higher temperatures, the faster-growing oysters suffered greater mortality, formed less habitat, and were consequently less effective at ameliorating low-tide air temperature extremes than slower-growing oysters. The greater survivorship of slower-growing oysters, in turn, produced a cooler microclimate which fed back to further bolster oyster survivorship. Invertebrate recruitment increased with habitat cover and was greater among the slower than the faster-growing population.
5. Our results show that the capacity of ecosystem engineers to serve as microhabitat refugia to associated organisms in a warming climate displays marked intraspecific variation. Our study also adds to growing evidence that fast growth may come at the expense of thermal tolerance.

KEYWORDS
climate change adaptation, density dependence, ecosystem engineer, facilitation, refugia, stressor, temperature, trade-off

1 | INTRODUCTION

Anthropogenic climate change is warming the planet and increasing the frequency and severity of extreme heat events (Kharin, Zwiers, Zhang, & Hegerl, 2007). Organisms already living at temperatures close to their thermal maxima are most vulnerable to warming, particularly those possessing limited ability to physiologically adapt to or migrate with the changing climate (Somero, 2010). For these organisms, microhabitats that are cooler than the surrounding environment may serve as critical refugia, enabling them to persist under otherwise unfavourable conditions and affording them more time to adapt (Scheffers, Edwards, Diesmos, Williams, & Evans, 2014).

Abiotic features of the environment, such as topography, and biotic components, such as ecosystem engineers, can produce microhabitat refugia for associated species (Morelli et al., 2016). Physical ecosystem engineers build structures that modify the availability of resources to, and the environmental conditions experienced by, associated organisms (Jones, Lawton, & Shachak, 1994). Consequently, they may facilitate ecological communities in stressful environments in which they would not otherwise persist (He & Bertness, 2014; McAfee, Cole, & Bishop, 2016). In order to serve as refugia from a warming climate, ecosystem engineers must persist and continue to ameliorate temperature to an extent that is tolerable by associated organisms.

Ecosystem engineers may, conceivably, display considerable intraspecific variation in their ability to persist and ameliorate temperature stress under warmer conditions. Within species, individuals can vary in their thermal tolerance (Sorte, Jones, & Miller, 2011), according to phenotypic and genetic variation in mitochondrial function (Schulte, 2015). Further, the capacity of ecosystem engineers to ameliorate environmental stressors can vary according to intraspecific differences in their morphological traits (e.g. Harley & O'Riley, 2011; Irving & Bertness, 2009). Body size is a potential predictor of inter-individual variation in capacity for thermal adaptation, as animal body size generally decreases with latitude (Bergmann 1847, revisited by Blackburn, Gaston, & Loder, 1999). Whereas at low temperatures, the fast growth of ecosystem engineers may be beneficial for their formation of complex structures that ameliorate environmental stressors, at high temperatures, increased rates of mortality may offset this effect of growth on habitat formation. Studies directly assessing intraspecific variation in the capacity of ecosystem engineers to act as thermal refugia are needed.

In intertidal habitats, where mid-day low tides expose inhabitants to extreme heat and desiccation stress (Helmuth, Mieszkowska, Moore, & Hawkins, 2006) and many organisms already live at or close to their upper thermal limit (Somero, 2010), the persistence of microhabitat refuges may be particularly critical in facilitating climate change adaptation of ecological communities. Oysters and mussels have complex shell structures that provide shading and trap moisture at low tide (Gutiérrez, Jones, Strayer, & Iribarne, 2003; McAfee et al., 2016; Silliman et al., 2011). Among the invertebrates that live in the interstices between their shells (Cole, 2010; Summerhayes, Bishop, Leigh, & Kelaher, 2009), many are unable to persist on intertidal shores in their absence (Silliman, Bertness, Altieri, Griffin, Bazterrica, Hidalgo, and Reyna, 2011). On Australia's east coast, the Sydney rock oyster, *Saccostrea glomerata* (Gould 1850), can reduce local air temperatures on rocky shores by over 4.5°C (McAfee, Cole, and Bishop, 2016).

Here, we compare the capacity of two populations of *S. glomerata* —a fast-growing and a slow-growing—to persist, grow and provide microhabitat refugia for intertidal invertebrates in a warming climate. We use passively warmed settlement panels (see Kordas, Dudgeon, Storey, & Harley, 2015; Lathlean & Minchinton, 2012) to manipulate temperature in the field, as the warming of bare rock and resultant evaporative water loss during low-tide solar irradiation is among the major sources of stress to organisms on the mid-intertidal rocky shore (Lathlean, 2014). We hypothesise that due to a trade-off between growth and thermal tolerance, as temperature increases, the faster-growing population will display decreased survival as compared with the slower-growing population, and lose its growth advantage, resulting in reduced habitat provision. We hypothesise that as habitat provision and oyster density decrease, so too will oyster amelioration of extreme air temperatures, resulting in fewer individuals, of fewer invertebrate species living among the faster- than the slower-growing oysters.

2 | MATERIALS AND METHODS

2.1 | Study system

Experiments were conducted at three sites within Port Stephens estuary, New South Wales, Australia (32.708°S, 152.19°E): Cromarty Bay (hereafter Cromarty), North Arm Cove (NAC) and Tilligerry Creek (Tilligerry). Port Stephens is located in the middle of the distribution of *S. glomerata*, which extends from subtropical Queensland to the temperate waters of Victoria (Nell, 2001). Study sites had semi-diurnal tides of *c.* 1.5 m range, mean salinities greater than 29.7 (Wolf & Collins, 1979), and were free of QX oyster disease (NSW DPI, 2014). The mean daily maximum air temperature in January 2014, the hottest month of our study, was 26.8°C, and the mean daily minimum air temperature for July 2014, the coldest month of our study, was 10.1°C (Bureau of Meteorology, 2016). Water temperatures ranged from a mean 19.1°C in August to 24.3°C in February (World Sea Temperatures, 2016).

Our experiments utilised two different breeding lines of oysters produced by the *S. glomerata* aquaculture industry, and sourced from nearby oyster leases in Port Stephens. The faster-growing population were the B2 breeding-line (hereafter, "fast-growing"), mass-selected for fast growth and QX disease resistance, over five generations (NSW DPI, 2014). The slower-growing population (hereafter, "slow-growing") were hatchery spawned oysters that had not been subjected to this same selective pressure. The two populations were genetically distinct, with the selectively bred population, interestingly, of higher genetic diversity (Thompson, Stow, & Raftos, 2017).

2.2 | Oyster growth, survivorship, and habitat formation across a thermal gradient

To examine the effect of substrate temperature on the survivorship and growth of oyster populations, and their facilitation of invertebrates, an artificial temperature gradient was created using painted white, grey and black stone pavers (300 × 300 × 17 mm). Following 30 min of exposure to 27°C sun, the average temperature of white pavers was 26°C, of grey pavers was 30°C and of black pavers was 39°C (infrared camera, Testo i80). All paver colours produced temperatures within the natural range that *S. glomerata* experiences on rocky intertidal shores during summer low tides (McAfee, Cole, and Bishop, 2016), with the white pavers of similar temperature to a rocky shore adjacent to our study site. Across its distribution, the rock type on which *S. glomerata* can be found ranges from pale sandstone to dark basaltic rock (D. McAfee pers. obs.). Each paver received two coats of low-sheen paint (Dulux Weathershield), and three coats of clear non-toxic pond sealer (Crommelin Waterproofing) to ensure a homogenous surface chemistry.

At each site, 18 pavers of each colour were deployed, giving a total of 54 pavers per site. Six pavers per colour were randomly selected to receive either slow-growing or fast-growing oysters, while the other six remained bare, serving as controls. Each paver assigned to an oyster treatment received 20 juvenile oysters (shell height: 14–26 mm) that were randomly positioned within the central 250 × 250 mm area of each paver. This density was within the natural range observed on nearby rocky shores (Wilkie, Bishop, & O'Connor, 2012). Oysters were attached with non-toxic two-part marine epoxy (Vivacity Engineering) applied sparingly to the left valve to avoid thermal insulation of oysters from substrate temperatures.

In January 2014 (Austral summer), pavers were horizontally positioned on plastic commercial oyster trays (180 cm × 90 cm) at an elevation corresponding to the mean low water of neap tides. Deployment of pavers on oyster trays ensured that each was at exactly the same tidal elevation, and subject to the same climatic conditions. Each oyster tray was enclosed with 12 mm × 12 mm wire mesh which protected juvenile oysters from predation, while minimising shading. Protection of juvenile oysters in mesh bags is common in restoration projects (Taylor & Bushek, 2008). Oysters at Cromarty and Tilligerry remained in the field for 12 months, but a storm destroyed the NAC site 6 months after deployment.

To assess the influence of oysters on microclimate, iButton data loggers (DS1921G; Thermodata) waterproofed with Plastidip rubber coating (Performix Brand: McAfee, Cole, and Bishop, 2016) monitored the surface temperature on three of the six pavers per treatment every 20 min (accuracy: 0.5°C) for the 12-month duration of the experiment. The iButtons were positioned in the centre of pavers, in between oysters where present, and were positioned such that they measured the temperature of the substrate.

Oyster survivorship was assessed 1, 2, 3, 6, 9 and 12 months after deployment, and growth and habitat cover after 3, 6, 9 and 12 months. The number of live oysters remaining on pavers was assessed in situ; dead oysters were easily identified from damaged, gaping or missing valves. Growth rates (mm^2) were assessed from photos taken directly above each paver. The outline of each oyster in the plane of the paver was traced in ImageJ and used to estimate area. Each oysters' initial area, at time 0, was subtracted from subsequent measurements to calculate growth rate. Oyster habitat cover was the percentage of a paver's upper surface covered by living oysters. This was estimated by superimposing a grid of 100 evenly spaced points over a photo and recording the habitat beneath each.

Invertebrate recruitment to pavers was determined at Cromarty only, after 12 months, due to storm damage at the other two sites (NAC after 6 months, and Tilligerry in the final month of the experiment, affecting associated communities but not measurements of the oysters themselves). At Cromarty, oysters (where present) and recruiting invertebrate communities were scraped off each plate into a plastic bag and transported to the laboratory where associated communities were separated from oysters over a 500-µm sieve. Invertebrate fauna >500 µm were identified to mixed taxonomic resolution: barnacles, bivalves, crabs and gastropods to species, amphipods and polychaetes to family and isopods to suborder. This approach does not compromise detection of treatment effects (Chapman, 1998).

2.3 | Oyster thermal insulation

To assess whether any difference in thermal tolerance between the two oyster populations was due to differences in their thermal insulation, we measured the shell thickness of surviving oysters at Cromarty after 12 months and assessed internal tissue temperatures with biomimetic "robotic oysters" (hereafter robo-oysters) deployed on coloured pavers in September 2015. We hypothesised that due to the faster growth rate of fast-growing oysters, they would have a thinner shell than slow-growing oysters that renders them more susceptible to warming. Shell thickness was measured in the centre of the right valve, 15 mm in from the dorsal margin using outside dial callipers. Robo-oysters were produced by placing an iButton inside an emptied oyster shell and filling it with 3M Scotchcast 2121 resin, which displays similar thermal properties to the tissue of marine molluscs (Lima & Wethey, 2009). Robo-oysters were produced for a juvenile (10–12 months) and an adult (20 months) age class of each of the slow- and fast-growing oysters. The juvenile oysters were the smallest age class in which an iButton would fit, and had a mean (±1 *SE*) shell height of 51 ± 1.3 mm for each oyster type, matching the size of live oysters ~6 months after deployment. Due to the faster rate of growth of the fast- than slow-growing population, the size of the 20-month-old oysters differed between the two populations (slow-growing: 67.9 ± 2.4; fast-growing: 85.6 ± 1.9 mm). One 10- to 12-month and one 20-month robo-oyster from each population was attached to a paver (i.e. four per paver) of each colour treatment ($n = 4$) and deployed for 2 months.

2.4 | Shoreline recruitment experiment

In the first experiment, any difference in invertebrate recruitment among white, grey and black pavers with oysters may be a direct effect

of differences in the thermal environment of pavers or alternatively an indirect effect arising from differences in oyster habitat formation. To disentangle these two pathways, a fully orthogonal experiment manipulating the amount of habitat provided by slow-growing oysters (Habitat: zero, low, medium, high) and paver colour (Colour: white, grey and black; n = 6 replicates per treatment) was established on the natural rocky shore of Cromarty Bay in January 2015. Each colour treatment received habitat treatments that were based on the number and size of slow-growing oysters remaining on white (high: 18 oysters, 60–80 mm), grey (medium: 11 oysters, 50–70 mm) and black (low: 6 oysters of 50–60 mm shell height) pavers at the conclusion of the first experiment, with the zero habitat treatment receiving no oysters. The treatments were interspersed at a mid-intertidal elevation (0.6–0.9 m above Indian Spring Low Water), at which wild oysters naturally occur. Invertebrate colonisation was assessed 3 months later, in April 2015.

Throughout the experiment, temperature was monitored on three randomly selected pavers per treatment using waterproofed iButtons, as described above. At the end of the 3-month experiment, survivorship of oysters on each paver was assessed from damaged, gaping or disarticulated valves. The established community on each paver was scraped off and bagged, with invertebrates >500 µm assessed in the laboratory using the methods described above.

2.5 | Statistical analyses

Data were analysed using permutational analyses of variance (PERMANOVAs; Anderson, 2005). PERMANOVAs apply the traditional ANOVA partitioning procedure to a distance matrix, but use permutations to obtain p-values (Anderson, 2005). Although more commonly applied to multivariate data, PERMANOVAs can also analyse univariate data (Anderson, 2005) and were used here instead of ANOVAs because they do not assume data are normally distributed and allow interpretation of interaction terms within random factors (Anderson, 2005). Univariate analyses used Euclidean distance matrices produced from untransformed data while multivariate analyses used Bray Curtis dissimilarity matrices produced following square-root transformation of data to down-weight the influence of dominant taxa. PERMDISP analyses, conducted prior to each PERMANOVA, did not detect differences in the dispersion of data among treatments that influenced the outcome of analyses. PERMANOVAs detecting significant differences were followed by pairwise post hoc PERMANOVAs to determine the sources of the variation.

For the first experiment, comparing the capacity of the two oyster populations to provide a microhabitat refuge to invertebrates across an artificial temperature gradient, we determined the maximum temperature recorded from each paver over the duration of the experiment. Separate two-way univariate PERMANOVAs, with the factors oyster population (fixed: fast-growing, slow-growing, bare) and paver colour (fixed: white, grey, black), were run for each site due to their different experimental durations (12 months for Cromarty and Tilligerry and 6 months for NAC due to storm damage).

To assess how oyster population and paver colour affects oyster survival, growth, and habitat provision, separate three-way univariate PERMANOVAs were run for each variable, at each sampling time (1, 2, 3, 6, 9, 12 months for survival; 3, 6, 9, 12 months for growth and habitat formation). The analysis on oyster growth included oyster density as a covariate, as differential survivorship of oysters among treatments may influence resource availability for surviving individuals. Sampling times were analysed separately because (1) these were non-independent; and (2) the number of sites dropped from three to two after 6 months, due to damage to the NAC site. Two-way PERMANOVAs, with the factors oyster population (three levels) and paver colour (three levels) tested for sources of spatial variation in invertebrate: (1) community structure (multivariate); (2) abundance (univariate); and (3) taxon richness (univariate) at Cromarty after 12 months. SIMPER analysis (Primer 6) identified which taxa contributed most to multivariate differences in community structure among treatments.

Differences in shell thickness between fast-growing and slow-growing oysters were assessed using two-way univariate PERMANOVAs, with the factors population and paver colour. Three-way PERMANOVAs (factors: population, age class and paver colour) assessed variation in the maximum temperature recorded by of robo-oysters.

To assess how, in the shoreline experiment, oyster habitat and paver colour influenced environmental amelioration and invertebrate communities two-way PERMANOVAs, with the factors paver colour (white, grey, black) and oyster habitat (bare, low, medium, high) were run. Separate analyses were run on: (1) oyster survivorship (univariate); (2) maximum temperature (univariate); (3) invertebrate community structure (multivariate); (4) invertebrate abundance (univariate); and (5) taxon richness (univariate).

To test for relationships between maximum temperatures on pavers and univariate biological variables (oyster survivorship, habitat formation, and the abundance and richness of invertebrates), and relationships between habitat formation and each of oyster survivorship and the abundance of invertebrates recruiting, we ran Spearman's rank correlations for each variable.

3 | RESULTS

3.1 | Oyster growth, survivorship and habitat formation across a thermal gradient

At Cromarty, but not Tilligerry or NAC, oyster population mediated the relationship between paver colour and maximum temperature (sig. Population × Colour interaction; Table S1 in Appendix S1). At Cromarty, white pavers with fast-growing oysters displayed maximum temperatures that were on average 1.3 ± 0.7°C cooler than those with slow-growing oysters or no oysters, the latter two of which did not differ. On grey pavers, maximum temperatures were 5.7 ± 1.2°C cooler on pavers with than without oysters, irrespective of the oyster population. On black pavers, maximum temperatures were significantly cooler on pavers with slow- than fast-growing oysters, by 3 ± 1.2°C, and on pavers with slow-growing than no oysters, by 6.2 ± 1.5°C, with maximum temperatures on pavers with fast-growing and no oysters also differing (post hoc test, sig. Population × Colour; Figure 1a). At Tilligerry and NAC, maximum temperatures did not vary with oyster

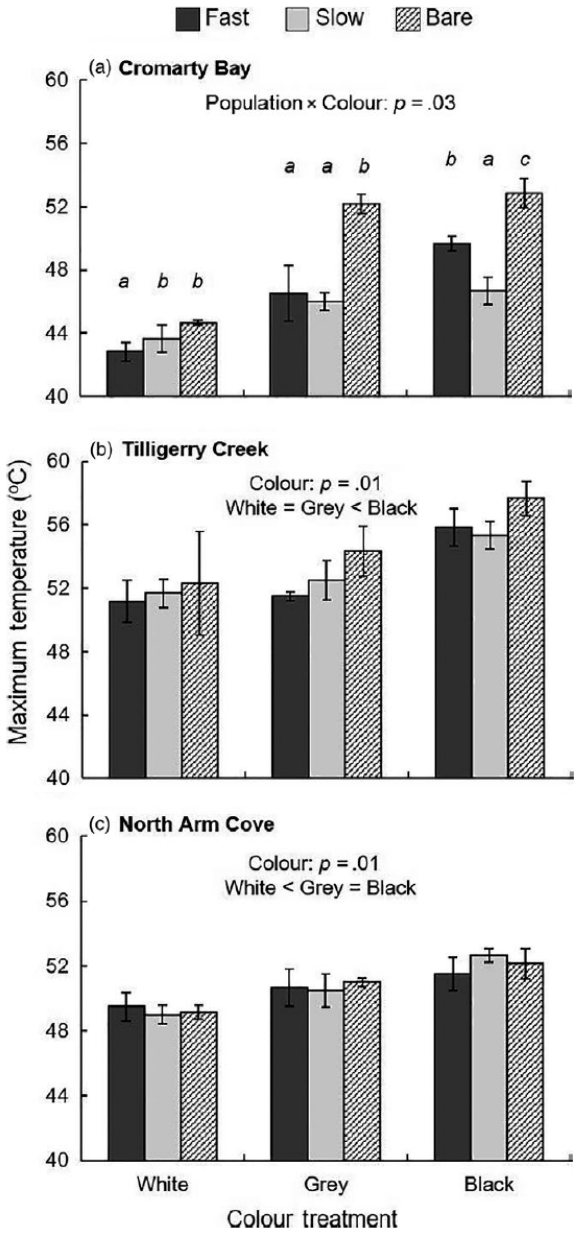

FIGURE 1 Mean (±1*SE*) maximum temperatures on pavers over 12 months at Cromarty Bay and Tilligerry Creek, and over 6 months at North Arm Cove, where the experiment was destroyed after 6 months. Pavers received one of three oyster population treatments (Fast, fast-growing oysters; Slow, slow-growing oysters; Bare) and were one of three colours (white, grey and black). Significant differences are described above bars, and with letters where a significant interaction was detected. $n = 6$ pavers per treatment

population (Table S1 in Appendix S1) but instead were always cooler on white than black pavers, with the grey pavers displaying intermediate temperatures that were not always statistically distinct from pavers of the other two colours (post hoc tests, sig. Colour, Table S1 in Appendix S1; Figure 1b,c).

The growth and survivorship of both fast- and slow-growing oyster populations was generally greatest on white, intermediate on grey, and lowest on black pavers (Figure 2a,b), although the magnitude of the colour effect varied among sites (Table S1 and S2 in Appendix S2), and differences between the grey and the white or black pavers were not always statistically significant (post hoc tests, sig. Colour × Site interaction; Figure 2a,b). Significantly greater survivorship of slow- than fast-growing oysters was apparent after 3 months (sig. Population, and Population × Colour interaction, Table S1 in Appendix S2) with the difference generally increasing from white, to grey, to black pavers (post hoc tests, sig. Population × Colour interaction, Figure 2a). In general, fast-growing oysters grew significantly more than slow-growing oysters on white pavers, whereas slow-growing oysters grew more than fast-growing oysters on black pavers, with no difference on grey pavers (sig. Population × Site × Colour interaction, Table S2 in Appendix S2; Figure 2b). Oysters on white pavers provided greater habitat cover than those on black pavers at all sampling times, while habitat cover was intermediate on grey pavers (Figure 2c). By the end of the experiment, slow-growing oysters provided greater habitat cover than fast-growing oysters on black pavers, whereas habitat cover was similar between populations on white and grey pavers (post hoc tests, sig. Population × Site × Colour interaction; Table S3 in Appendix S2; Figure 2c).

Significant negative correlations were detected between maximum temperature and each of habitat cover and oyster survival over 6 months (including all sites; cover: $r_s = .51$, $p = .032$; survival: $r_s = .47$, $p = .050$) and 12 months (including two of the three sites; cover: $r_s = .63$, $p = .029$; survival: $r_s = .62$, $p = .033$). Habitat cover was, in turn, positively correlated to oyster survival after 6 ($r_s = .91$, $p > .001$) and 12 ($r_s = .96$, $p > .001$) months.

Overall, 26 invertebrate taxa recruited to Cromarty pavers over the 12-month deployment period (Table S1 in Appendix S3), with barnacles, gastropods and polychaetes the most speciose and abundant taxa. Across all colour treatments, 69% of invertebrates were collected from pavers with slow-growing oysters, 27% from fast-growing oyster pavers, and 4% from bare pavers (Figure 3).

For each paver colour, invertebrate assemblages recruiting to fast-growing or slow-growing oysters did not significantly differ, but differed from those recruiting to bare pavers (post hoc test, sig. Habitat × Colour; Table S4 in Appendix S2). For each habitat, assemblages on white pavers differed significantly from those on grey or black, the latter two of which did not differ (Table S4 in Appendix S2). Dissimilarity in assemblages between the two oyster populations were primarily due to crustaceans (Barnacles 18.3%; Amphipods 18.2%; Isopods 14.8%; Crabs 4.8%) and gastropods (Species: *Patelloida mimula* 8.3%; *Bembicium auratum* 6.9%: SIMPER; Figure 3c,d,e).

On white pavers, invertebrate abundance was greater on pavers with slow- than fast-growing oysters, each of which supported more invertebrates than bare pavers (post hoc test, sig. Habitat × Colour, Table S4 in Appendix S2; Figure 3a). On grey and black pavers, the abundance of invertebrates did not differ between the two oyster populations, each of which supported significantly more invertebrates than bare pavers (Figure 3a). Within each habitat treatment, invertebrate abundance was

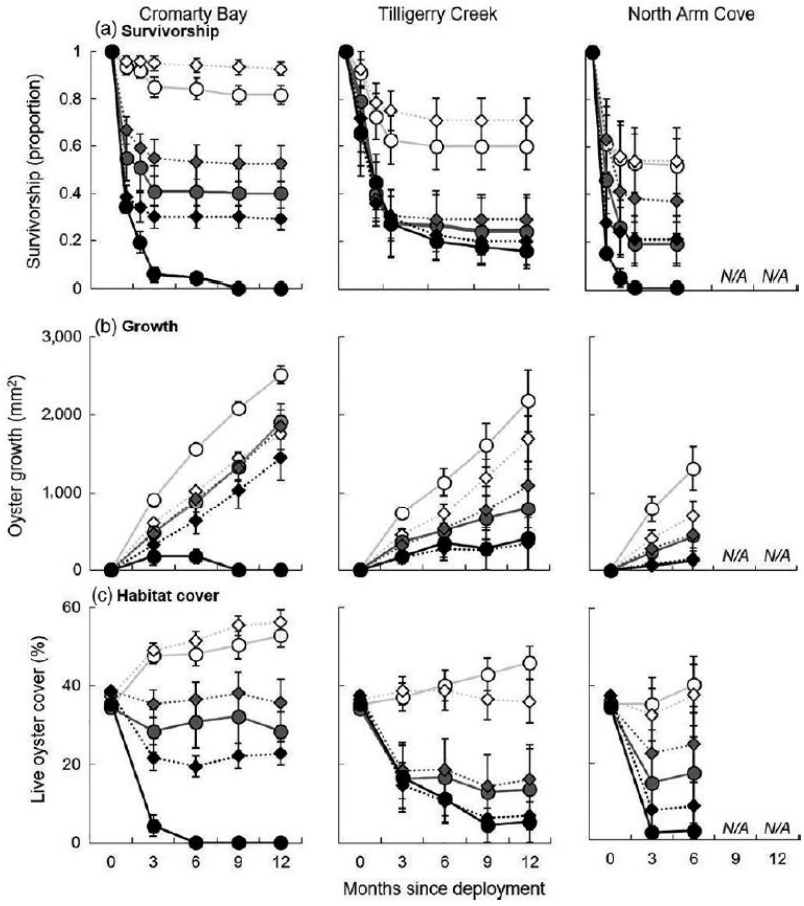

FIGURE 2 Mean (±1*SE*) (a) survivorship, (b) growth and (c) cover of fast-growing (circles with solid lines) and slow-growing (diamonds with dotted lines) oysters on white (white symbols), grey (grey symbols) and black (black symbols) pavers at Cromarty Bay, Tilligerry Creek and North Arm Cove. N/A = data not available at North Arm Cove as experiment was destroyed after 6 months. $n = 6$ pavers per treatment

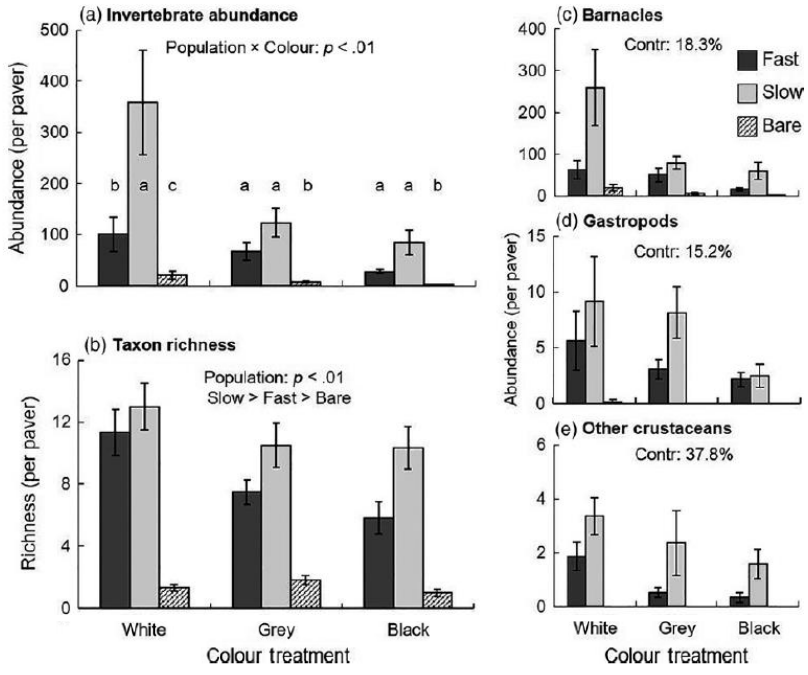

FIGURE 3 Mean (±1*SE*) (a) total abundance and (b) taxon richness of invertebrates and abundance of (c) barnacles, (d) gastropods and (e) crustaceans (other than barnacles) recruiting to white, grey and black pavers with fast-growing oysters (Fast; dark grey bars), slow-growing oysters (Slow; light grey bars), and bare habitat (Bare; striped bars) at Cromarty Bay. Letters denote treatments that significantly differed (at $\alpha = 0.05$); in (a) contrasts are provided for differences among habitat treatments within each level of colour. Contr. = the percent dissimilarity specific taxa contributed to multivariate differences in invertebrate communities among habitats. $n = 6$ pavers per treatment

typically greatest on white pavers and lowest on black pavers (Figure 3a). Invertebrate taxon richness was significantly greater on slow-growing (mean ± 1 *SE*: 11.3 ± 1.4) than fast-growing oysters (8.2 ± 1.1), but each supported greater richness than bare habitat (1.4 ± .3; sig. Habitat: post hoc test; Table S4 in Appendix S2; Figure 3b). Both invertebrate abundance and taxon richness were negatively correlated with maximum temperature (abundance: $r_s = -.92$, $p > .001$; richness: $r_s = -.89$, $p = .001$), but positively correlated with habitat cover (abundance: $r_s = .88$, $p = .019$; richness: $r_s = .15$, $p = .015$).

3.2 | Oyster thermal insulation

Comparisons between the shell thickness of the two oyster populations were limited to white and grey pavers, as there were insufficient oysters surviving on black pavers at the end of the experiment. There was no significant effect of paver colour on shell thickness (Table S1 in Appendix S4). Instead, slow-growing oysters had significantly thicker shells than fast-growing oysters by, on average, 0.25 ± 0.08 mm (post hoc test: sig. Population: Table S1 in Appendix S4).

Maximum temperatures recorded by robo-oysters did not vary between populations, but increased significantly from white (47.1 ± 0.4°C), to grey (50.4 ± 0.4°C), to black (51.7 ± 0.3°C) pavers (post hoc tests, sig. Colour: Table S2 in Appendix S4), and were warmer for 10- to 12-month-old (50.2 ± 0.5°C) than 20-month-old (49.2 ± 0.6°C) oysters (post hoc tests, sig. Age: Table S2 in Appendix S4).

3.3 | Shoreline recruitment experiment

The shoreline experiment confirmed that oyster cover modifies the effect of paver colour on temperature (sig. Colour × Habitat interaction; Table S1 in Appendix S5; Figure 4a). In the absence of oysters, and at low and medium levels of oyster habitat, black pavers attained significantly hotter maximum temperatures (by up to 9°C) than white pavers (post hoc test, sig. Colour × Habitat; Figure 4a), but at the high level of oyster habitat, there was no significant difference between white and black pavers (Figure 4a). Maximum temperatures on grey pavers did not significantly differ from those on black pavers at any density and were significantly hotter than white pavers at all oyster densities except the bare pavers (Figure 4a). On the black pavers, maximum temperatures increased significantly from pavers with high oyster cover, to low and medium cover (which did not differ), to no cover (i.e. bare, Figure 4a). In contrast, the density of oyster habitat had only weak effects on temperatures on white or grey pavers (Figure 4a).

Despite this, there was no significant interaction between paver colour and oyster habitat on oyster survivorship, invertebrate abundance or taxon richness (Table S1 in Appendix S5). Instead, the proportion of oysters surviving responded only to paver colour, being greater on white pavers (0.62 ± 0.09) than grey (0.31 ± 0.09) or black (0.23 ± 0.07) (sig. Colour effect: post hoc tests; Table S1 in Appendix S5). The abundance and richness of invertebrates, conversely, responded significantly to oyster habitat but not paver colour (sig. Habitat effect; Table S1 in Appendix S5, Figure 4c,d). Abundance increased with oyster habitat cover (post hoc tests: sig. Habitat, Figure 4c), and of the 41 sampled taxon in the shoreline experiment (Table S1 in Appendix S6), only *B. auratum* was recorded on bare habitat, increasing from an average (±1 *SE*) of 4 (±1.3) on bare pavers to 44 (±4.9), 87 (±3.7) and 128 (±6.8) on low, medium and high oyster habitat, respectively. Taxon richness increased from bare (0.72 ± 0.05), to low (5.6 ± 0.14) and medium densities (6.3 ± 0.2), to high density (8.7 ± 0.1: post hoc tests: sig. Density, Figure 4d).

Oyster survival was negatively correlated with maximum temperature ($r_s = -.85$, $p = .003$), as was the abundance ($r_s = -.62$, $p = .030$) and richness ($r_s = -.89$, $p < .001$) of recruiting invertebrates.

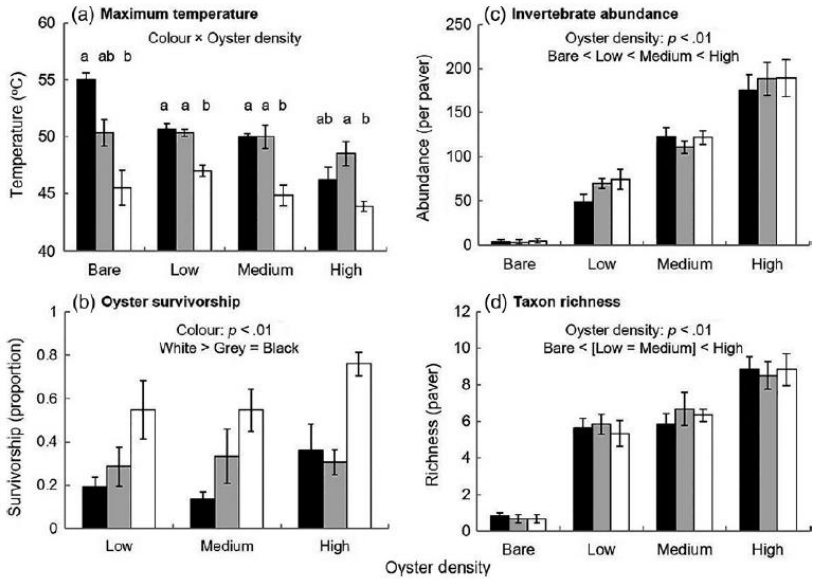

FIGURE 4 Mean (±1*SE*) (a) maximum temperature, (b) oyster survivorship, (c) invertebrate abundance and (d) invertebrate species on pavers varying in colour (white, grey, black) and amount of oyster habitat (bare, low, medium, high) positioned on a rocky shoreline at Cromarty Bay. Significant differences (at $\alpha = 0.05$) are described above bars, and with letters where appropriate. $n = 6$ pavers per treatment

4 | DISCUSSION

There is growing recognition that the persistence of ecosystem engineers may be critical to the climate change adaptation of associated species (Wright & Jones, 2006), but how this role varies among populations of ecosystem engineers is not well understood. Our study demonstrated significant intraspecific variation in the capacity of the Sydney rock oyster, *Saccostrea glomerata*, to persist, ameliorate temperature extremes and provide habitat to invertebrates under warmer conditions. A faster-growing population displayed reduced growth and survivorship as compared to a slower-growing population when exposed to elevated temperatures. The greater survivorship of slower-growing oysters, in turn, produced a cooler microclimate which fed back to further bolster oyster survivorship. The net effect was that the slower-growing oysters supported a greater density and richness of invertebrates at high temperatures than the faster-growing oysters. Overall, our results are suggestive of a trade-off between growth rate and thermal tolerance, with cascading effects on associated species.

4.1 | The growth–thermal tolerance trade-off

Based on the smaller body sizes of many organisms at lower than higher latitudes (Bergmann, 1847, revisited by Blackburn et al., 1999), it has been hypothesised that organismal growth rates may trade-off with thermal tolerance. In this study, increasing temperatures compromised the performance of the faster-growing, selectively bred oysters to a greater extent than the slower-growing, non-selected oysters. Although fast-growing oysters grew 34% faster than the slow-growing oysters on the coolest, white pavers, as the temperature on pavers increased, this pattern reversed. On the hot black pavers, not only did fast-growing oysters grow at half the rate of slow-growing oysters, but they also suffered greater rates of mortality than slow-growing oysters. Although our study compared only two populations of oysters, these results are consistent with a trade-off between rate of growth and thermal tolerance.

Both physiological and morphological differences may contribute to the reduced thermal tolerance of the fast-growing oysters as compared with the slower-growing oysters. First, the fast-growing oysters have a higher standard metabolic rate than the slower-growing oysters (Parker et al., 2012), such their population presumably exists close to its physiological limit, with diminished capacity to respond to increasing temperature. Among ectotherms, such as oysters, metabolic rate directly scales with temperature such that under climate change, reductions in organismal body size would be expected unless organisms can compensate with greater food intake or reallocation of resources (Sheridan & Bickford, 2011). Second, the thinner shells of the fast-growing oysters, which trade-off growth with shell thickening (see also Bishop & Peterson, 2006), than the slow-growing oysters may make them less thermally insulated against temperature extremes. However, the robo-oysters did not support a difference in maximum temperatures between populations. Third, it is possible that differences in colour between fast- and slow-growing populations could also contribute to differences in their thermal tolerance by influencing heat absorption. Nevertheless, although the shell colour of bivalves is under genetic, as well as environmental control (Brake, Evans, & Langdon, 2004), and in some species is correlated to performance traits such as growth (Wolff & Garrido, 1991), in this study there was no detectable difference in colour between the two oyster populations.

Limited introgression of fast-growing oyster genotypes, from oyster farms or seeded reefs, into wild populations are also suggestive of a trade-off between growth rate and environmental tolerance. Sydney rock oysters cultivated on aquaculture leases are reproductively capable and may spawn from 1 year of age, initially as males (Parker, O'Connor, Raftos, Pörtner, & Ross, 2015). Nevertheless, preliminary studies provide no evidence of introgression of Sydney rock oyster genotypes selectively bred for fast growth and disease resistance into wild oyster populations (Thompson et al., 2017). Similarly, in the United States, attempts to utilise selectively bred oysters in restoration have led to negligible introgression of these genotypes into wild populations (Carlsson et al., 2008), suggesting that these are mal-adaptive genotypes that do not persist in natural systems.

4.2 | Positive feedback between temperature amelioration and oyster density

Ecosystem engineers can reduce the thermal stress experienced by underlying or adjacent invertebrates by lowering the temperature of the substratum, its evaporative water loss, and by reducing solar radiation (Bertness & Grosholz, 1985; Lathlean, 2014; McAfee, Cole, and Bishop, 2016). In this study, the three-dimensional structure formed by oysters led to localised amelioration of maximum temperatures by up to 6°C on the hottest, black pavers—an effect that increased with oyster density and size. Although the scale at which we manipulated oysters was small (i.e. tens of centimetres), the magnitude by which temperatures were reduced was similar to in a field survey where temperatures were compared between patches of rocky shore habitat with and without oysters (McAfee, Cole, and Bishop, 2016). Nevertheless, as edge effects have the potential to weaken temperature amelioration by habitat-forming species (Murcia, 1995), it is possible that even larger effects of oysters may be seen in contiguous, as opposed to patchy, oyster habitat.

The positive relationship between oyster density and temperature amelioration, coupled with the greater survivorship of oysters at cooler temperatures, suggests a positive feedback whereby high densities of oysters facilitate greater survivorship by reducing temperatures. This phenomenon is also displayed by intertidal barnacles (Bertness, 1989), and this confirms previous findings that heat stress on mid-intertidal rocky shores decreases with increasing amounts of occupied primary substrata (Gedan, Bernhardt, Bertness, & Leslie, 2011; Lathlean, Ayre, & Minchinton, 2012). Our results suggest that in areas where radiant heat is the main source of temperature stress to organisms, conservation and restoration should prioritise achieving the densities of ecosystem engineers needed to trigger positive feedbacks (Jones et al., 2010).

4.3 | Facilitation of invertebrate communities

Undoubtedly, climate exerts a strong influence on intertidal communities (Helmuth et al., 2006). The amelioration of heat and desiccation stress by abiotic and biotic refugia enables species to persist on exposed intertidal rocky shores, where conditions are otherwise outside their fundamental niche (McAfee, Cole, and Bishop, 2016; Silliman, Bertness, Altieri, Griffin, Bazterrica, Hidalgo, and Reyna, 2011). Yet, besides abiotic amelioration, aggregated bivalves can provide associated fauna with a refuge from predation (Grabowski & Powers, 2004) and physical disturbance (i.e. wave action: Stephens & Bertness, 1991), and an enhanced surface area for grazing and attachment (Gutiérrez et al., 2003). Although oyster survivorship was driven by temperature, when oyster density and paver colour were manipulated independently, density had a much stronger effect on invertebrate assemblages.

As expected given the generally positive relationship between biodiversity and habitat complexity (St. Pierre & Kovalenko, 2014), within oyster populations, there was a positive relationship between the habitat cover of live oysters and invertebrate recruitment. Nevertheless, comparisons across oyster populations revealed more complex relationships. For example, on the white pavers, where the two oyster populations provided the same live cover, the slow-growing oyster habitat supported 250% the invertebrate abundance. This may be because few, larger individuals accounted for the cover provided by the faster-growing oysters, whereas more, smaller oysters accounted for the cover provided by the slower-growing oysters. These two configurations differ in the surface area they provide for invertebrate attachment and foraging, and their provision of interstitial refuges from predators and abiotic stress (Grabowski & Powers, 2004; Wilkie et al., 2012). On the black pavers where the slow-growing oysters had a much greater live cover, the two oyster populations supported similar abundances and richnesses of invertebrates. Although all fast-growing oysters died on black pavers, the structural legacy of bivalve shells can continue to provide habitat for settling organisms after death, and disarticulation of valves may even increase surface area for invertebrate attachment (Summerhayes et al., 2009).

Our experimental protocol included steps to ensure that surface chemistry did not differ between white, grey and black pavers. Nevertheless, it remains possible that differences in invertebrate recruitment among paver treatments represented a response to colour as well as to the differing thermal environment of these (Lathlean & Minchinton, 2012). Substratum colour can be an important factor influencing the settlement of benthic marine invertebrates (Pawlik, 1992). The general pattern observed in this study of greater recruitment of species to white than black pavers is, however, the reverse to studies examining the effect of substratum colour on barnacle and algal recruitment (Caffey, 1982; Swain, Herpe, Ralston, & Tribou, 2006), suggesting that here any effect of paver colour were driven by the thermal effect. The pavers manipulated radiant heat, but under a warming climate, thermal stress to intertidal organisms may result from interactive effects of increases in both air and water temperatures (Seabra, Wethey, Santos, Gomes, & Lima, 2016). Nevertheless, the results indicate that intertidal communities on dark substrata, such as basaltic rock, may be more susceptible to the effects of warming than those on lighter coloured substrata.

5 | CONCLUSIONS

Climate-adaptation strategies are increasingly necessary as rising temperatures push the physiological limits of species (Helmuth, Mieszkowska, Moore, and Hawkins, 2006). Provision of climate refugia will be particularly important in extreme physical environments such as the intertidal, where many species are highly vulnerable to warming because they already live at their physiological threshold (Somero, 2010). We have shown that ecosystem engineers, such as oysters, can provide refugia from radiant heat and facilitate biodiversity. This role for oysters, however, displayed considerable intraspecific variation, adding to growing evidence that ecosystem engineering is highly sensitive not only to interspecific but also intraspecific variation in ecosystem engineer traits (Harley & O'Riley, 2011; Irving & Bertness, 2009). Despite the ability of a faster-growing oyster population to more quickly form habitat under ambient conditions, we found that under elevated temperatures, the slower-growing population was the more effective ecosystem engineer due to its greater survivorship, likely reflecting a trade-off between growth rate and thermal tolerance. Hence, the success of climate-adaptation strategies aimed at conserving and restoring ecosystem engineers will be contingent on the identification of species and populations with high environmental resilience and associated biodiversity to ensure the persistence and value of such investments (Keppel & Wardell-Johnson, 2012).

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AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the experiment; D.M. collected the data; D.M. and M.B. analysed the data and W.O. contributed to its interpretation; D.M. and M.B. led the writing of the manuscript; all authors contributed to the drafts and gave approval of the publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.72nj2 (McAfee, O'Connor, & Bishop, 2017).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Appendix S1. Analyses of sources of variation in maximum temperature among pavers differing in colour, and in oyster population.

Table S1. Two-way univariate PERMANOVAs assessing sources of variation in the maximum temperature recorded on pavers that differed in oyster population (Pop: fast-growing oysters, slow-growing oysters, bare) and colour (Col: white, grey, black) over 12 months at Cromarty Bay and Tilligerry Creek, and over 6 months at North Arm Cove. Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold. $n = 6$.

		Cromarty Bay			Tilligerry Creek			North Arm Cove		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Pop	2	49.8	22.0	**0.002**	9.7	1.4	0.281	0.1	<0.1	**0.939**
Col	2	87.8	38.8	**0.001**	51.2	7.4	**0.003**	18.8	9.5	0.001
Pop x Col	4	8.2	3.6	**0.032**	1.0	0.1	0.966	0.6	0.3	0.864
Res	18									

Appendix S2. Analyses of sources of variation in the survivorship, growth and provision of habitat by fast-growing and slow-growing oysters on coloured pavers at three sites in Port Stephens, and the invertebrate communities that recruited to pavers at Cromarty Bay after 12 months.

Table S1. Three-way univariate PERMANOVAs assessing sources of variation in oyster survivorship between populations (Pop: fast-growing, slow-growing), among paver colours (Col: white, grey, black), and sites. Analyses for 1 - 6 months are for three sites (Cromarty Bay, Tilligerry Creek, North Arm Cove). Analyses for 9 - 12 months include data from two sites due to damage to the experiment at North Arm Cove after 6 months. Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold. $n = 6$.

	1 month				2 months			3 months			6 months			9 months			12 months		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Pop	1	330	0.7	0.500	1670	1.7	0.347	5627	4.2	**0.005**	5183	3.8	**0.003**	1	4733	2.1	0.242	5249	3.1	0.271
Col	2	9337	7.1	**0.028**	11890	3.9	0.051	15622	4.7	**0.016**	15911	4.9	**0.014**	2	14891	5.0	0.119	14939	5.7	0.088
Site	2	6461	11.1	**0.001**	4191	3.6	**0.001**	3966	2.4	**0.022**	4572	2.7	**0.012**	1	6657	5.3	**0.001**	4743	3.5	**0.002**
Pop x Col	2	346	0.8	0.566	1334	1.6	0.214	4580	3.7	**0.016**	4337	2.9	0.052	2	5085	2.0	0.293	7080	2.9	0.169
Pop x Site	2	477	0.8	0.497	1046	0.9	0.498	1328	0.8	0.598	1366	0.8	0.615	1	2292	1.8	0.072	1669	1.2	0.229
Col x Site	4	1313	2.3	**0.032**	3032	2.6	**0.001**	3295	1.9	**0.011**	3207	1.9	**0.020**	2	2956	2.3	**0.005**	2599	1.9	**0.011**
Pop x Col x Site	4	439	0.8	0.602	829	0.7	0.806	1225	0.7	0.797	1510	0.9	0.600	2	2512	2.0	**0.018**	2441	1.8	**0.025**
Res	84													60						

Table S2. Three-way univariate PERMANOVAs, with the co-variate, oyster density, assessing sources of variation in growth rates of oysters between populations (Pop: fast-growing, slow-growing), among paver colour treatments (Col: white, grey, black) and sites. Analyses for 3 - 6 months are for three sites (Cromarty Bay, Tilligerry Creek, North Arm Cove). Analyses for 9 - 12 months include data from two sites due to damage to the experiment at North Arm Cove after 6 months. Significant results (at $\alpha = 0.05$) are highlighted in bold. $n = 6$.

	3 months				6 months			9 months				12 months		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Density	1	51393	25.7	**0.001**	51292	23.8	**0.001**	1	37654	17.7	**0.001**	32888	15.3	**0.001**
Pop	1	6036	3.9	0.081	5664	2.5	0.181	1	3684	1.4	0.476	3565	1.3	0.385
Col	2	2489	0.8	0.582	2786	1.0	0.483	2	2619	0.8	**0**.594	3335	1.1	0.414
Site	2	3269	1.7	**0.017**	3598	1.7	**0.016**	1	9448	4.5	**0.001**	12853	6.1	**0.001**
Pop x Col	2	3160	1.5	0.226	3819	1.2	0.336	2	4083	1.1	0.458	4076	1.0	0.515
Pop x Site	2	1556	0.8	0.787	2254	1.1	0.300	1	2709	1.3	0.121	2713	1.3	0.091
Col x Site	4	3207	1.6	**0.005**	2962	1.4	**0.030**	2	3429	1.6	**0.006**	3115	1.5	**0.015**
Pop x Col x Site	4	2049	1.0	0.326	3140	1.5	**0.013**	2	3844	1.8	**0.002**	3968	1.9	**0.003**
Res	83							59						

Table S3. Three-way univariate PERMANOVAs assessing sources of variation in the habitat cover produced by live oysters between populations (Pop: fast-growing, slow-growing), among paver colour treatments (Col: white, grey, black) and sites. Analyses for 3 - 6 months are for three sites (Cromarty Bay, Tilligerry Creek, North Arm Cove). Analyses for 9 - 12 months include data from two sites due to damage to the experiment at North Arm Cove after 6 months. Significant results (at $\alpha = 0.05$) are highlighted in bold. $n = 6$.

	3 months				6 months				9 months				12 months			
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Pop	1	3878	2.1	0.157	3822	1.4	0.246	1	4694	1.6	0.495	4693	1.6	0.495
Col	2	18294	3.6	**0.039**	22216	5.3	**0.004**	2	18687	2.8	0.106	18687	2.8	0.106
Site	2	5043	2.0	**0.021**	4607	1.7	**0.042**	1	7507	3.0	**0.010**	7507	3.0	**0.010**
Pop x Col	2	2710	1.1	0.387	3173	0.9	0.560	2	3663	0.7	0.677	3663	0.7	0.677
Pop x Site	2	1889	0.7	0.703	2641	0.9	0.422	1	2993	1.2	0.226	2993	1.2	0.226
Col x Site	4	5116	2.0	**0.002**	4164	1.5	**0.044**	2	6747	2.7	**0.002**	6747	2.7	**0.002**
Pop x Col x Site	4	2498	1.0	0.490	3656	1.4	0.098	2	5271	2.1	**0.009**	5271	2.1	**0.009**
Res	84							60						

Table S4. Two-way multivariate (faunal assemblage) and univariate (invertebrate abundance, taxon richness) PERMANOVAs assessing sources of variation in invertebrate recruitment to pavers that differed in oyster population (Pop: fast-growing oysters, slow-growing oysters, bare habitat) and in colour (Col: white, grey, black). Pavers were deployed on a Cromarty Bay oyster lease for 12 months. Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold. *n* = 6.

		Faunal assemblage			Invertebrate abundance			Taxon richness		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Pop	2	19414	17.5	**0.001**	6567	22.2	**0.001**	11385	42.7	**0.001**
Col	2	3628	3.3	**0.003**	1360	4.6	**0.001**	363	1.4	0.175
Pop x Col	4	2174	2.0	**0.006**	602	2.0	**0.008**	367	1.4	0.099
Res	45									

Appendix S3. Invertebrate assemblages recruiting to experimental pavers following 12 months of experimental deployment on a Cromarty Bay oyster lease.

Table S1. Invertebrates that recruited to white (W), grey (G) or black (B) pavers with slow-growing oysters, fast-growing oysters, or bare habitat.

Taxon	Slow-growing oysters	Fast-growing oysters	Bare
ANNELIDA			
CLASS POLYCHAETA			
Capitellidae	W,G	W	-
Nephtyidae	-	B	-
Nereidae	W,G,B	W,G,B	-
Phyllodocidae	W	W	-
Sabellidae	-	W	-
Spionidae	W,G,B	W,G,B	-
Syllidae	W,G,B	W,G	-
Terebellidae	-	W	-
ARTHROPODA			
CLASS ARACHNIDA			
Halacaridae	-	W	-
CLASS MALOCOSTRACA			
Varunidae			
Paragrapsus laevis	W,G,B	W,G,B	-
Order Amphipoda			
Corophiidae	W,G,B	W,B	-
Gammaridae	W,G,B	W,G	G
Phoxocephalidae	W,G,B	-	-
Order Isopoda			
Suborder Cymothoida	W,G,B	W,G,B	-
Suborder Flabellifera	W,G,B	W,G	-
Suborder Sphaeromatidea	W,G,B	W	-
Order Tanaidacea	W,G,B	W,G,B	-
CLASS MAXILLOPODA			
Austrobalinidae			

Austrominius modestus	W,G,B	W,G,B	W,G,B
Balanidae			
Amphibalanus amphitrite	W,G,B	W,G,B	-
MOLLUSCA			
CLASS GASTROPODA			
Littorinidae			
Bembicium auratum	W,G,B	W,G,B	W
Lottidae			
Patelloida mimula	W,G,B	W,G,B	-
Trochidae			
Phasianotrochus eximius	-	W	-
CLASS BIVALVIA			
Lasaeidae			
Lasaea australis	W,G	-	-
Mytilidae			
Xenostrobus securis	W,G,B	W,G,B	-
Ostreidae			
Saccostrea glomerata	W,G,B	W,G,B	W,G,B
Veneridae			
Tapes conspersus	W,G	W,B	-

Appendix S4. Analyses of sources of variation in the thermal insulation of natural and robo-oysters.

Table S1. Two-way univariate PERMANOVA assessing sources of variation in the shell thickness of oysters between oyster populations (Pop: fast-growing, slow-growing) and paver colour (Col: white, grey). Pavers were deployed on a Cromarty Bay oyster lease for 12 months. Black pavers were excluded from the analysis as all selectively bred oysters on these had died by the end of the experiment. Res = residual. Significant results (at $\alpha = 0.05$) are highlighted in bold. $n = 6$.

Source	df	MS	p-*F*	P
Pop	1	209	9.1	**0.011**
Col	1	4	0.2	0.704
Pop x Col	1	33	1.4	0.269
Res	20			

Table S2. Three-way univariate PERMANOVA assessing sources of variation in the maximum temperature of robo-oysters produced using the shells of two oyster populations (Pop: fast-growing, slow-growing) and two age classes (Age: l0-12 months, 20 months), attached to colour pavers (Col: white, grey, black). Temperatures were recorded at Cromarty Bay during September 2015. Res = residual. Significant results (at $\alpha = 0.05$) are highlighted in bold. $n = 4$.

Source	df	MS	p-*F*	P
Pop	1	5.8	4.5	0.053
Col	2	66.1	50.6	**0.001**
Age	1	8.5	6.5	**0.018**
Pop x Col	2	2.4	1.8	0.180
Pop x Age	1	0.8	0.6	0.454
Col x Age	2	2.7	2.1	0.128
Pop x Col x Age	2	2.4	1.9	0.163
Res	24			

Appendix S5. Analyses of sources of variation in the maximum temperature, oyster survivorship, and recruitment of invertebrates on pavers which varied in colour and the density and size of oysters they received and were deployed on the Cromarty Bay shoreline for three months.

Table S1. Two-way univariate PERMANOVAs assessing sources of variation in maximum temperature, oyster survivorship, and the abundance and taxon richness of colonizing invertebrates on pavers differing in oyster habitat (Hab: bare, low, medium, high) and paver colour (Col: white, grey, black). Pavers were deployed along a natural rocky shoreline at Cromarty Bay for three months. Res = Residual. Significant differences (at $\alpha = 0.05$) are highlighted in bold. Maximum temperatures: $n = 3$; all other variables: $n = 6$.

	Maximum temperature				**Oyster survival**				**Invertebrate abundance**				**Taxon richness**		
Source	**df**	**MS**	**p-*F***	**P**	**df**	**MS**	**p-*F***	**P**	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Hab	3	198	12.8	**0.001**	2	1504	1.9	0.108	3	16852	22.0	**0.001**	9950	15.0	**0.001**
Col	2	607	39.2	**0.001**	2	7247	8.9	**0.001**	2	558	0.7	0.734	425	0.6	0.794
Hab x Col	6	46	2.9	**0.023**	4	1134	1.4	0.168	6	481	0.6	0.984	421	0.6	0.963
Res	24	15			41	808			60	747					

Appendix S6. Invertebrate assemblages recruiting to experimental pavers following 3 months of experimental deployment on the Cromarty Bay shoreline.

Table S1. Invertebrates that recruited to white (W), grey (G) or black (B) pavers with high, medium, low or no (bare) oyster habitat during a three month shoreline experiment at Cromarty Bay.

Taxon	High	Medium	Low	Bare
ANNELIDA				
CLASS POLYCHAETA				
Capitellidae	-	-	G	-
Nereidae	W,B	G,B	B	-
Oweniidae	W	-	-	-
Phyllodocidae	-	B	-	-
Polynoidae	-	-	G	-
Spionidae	G,B	-	-	-
Syllidae	W,G	-	-	-
Terebellidae	W	-	-	-
ARTHROPODA				
CLASS ARACHNIDA				
Halacaridae	-	-	G	-
Lepismatidae	W	-	-	-
CLASS MALOCOSTRACA				
Order Decapoda				
Cyclograpsus sp.	-	B	-	-
Helograpsus haswellianus	W,G	G	-	-
Paragrapsus laevis	W,G,B	W,G,B	W,B	-
Pilumnopus sp.	G	-	-	-
Sesarma erythrodactyla	W,G	W,G	W,G,B	-
Order Amphipoda				
Corophiidae	-	-	B	-
Gammaridae	B	-	-	-
Phoxocephalidae	W	-	-	-
Unknown Amphipoda A	W	-	-	-
Unknown Amphipoda B	G	-	-	-
Order Isopoda				
Suborder Flabellifera	W,B	-	W,G	-
Suborder Sphaeromatidea	G	-	-	-
CLASS MAXILLOPODA				
Austrobalinidae				

Austrominius modestus	W,G,B	W,G,B	W,G,B	-
MOLLUSCA				
CLASS GASTROPODA				
Calopiidae				
Calopia imitator	W,G	G	-	-
Hydrobiidae				
Ascorhis tasmanica	-	G	-	-
Littorinidae				
Bembicium auratum	W,G,B	W,G,B	W,G,B	W,G,B
Littoraria luteola	W,G,B	W,G	W,G,B	-
Nodilittorina pyramidalis	-	-	G	-
Lottidae				
Patelloida mimula	W,G,B	W,G,B	W,G,B	-
Neritidae				
Nerita atramentosa	-	B	-	-
Siphonariidae				
Siphonaria denticulata	G	-	-	-
Siphonaria diemenensis	G	W,G	-	-
Trochidae				
Austrocochlea porcata	W,G,B	W,G,B	-	-
Clanculus undatus	-	W	-	-
Phasianotrochus eximius	-	-	G	-
CLASS BIVALVIA				
Lasaeidae				
Lasaea australis	W,G,B	W,G,B	W,G,B	-
Laternulidae				
Laternula sp.	B	-	-	-
Mytilidae				
Trichomya hirsuta	-	W	-	-
Xenostrobus securis	W	-	-	-
Ostreidae				
Saccostrea glomerata	W,G,B	W,G,B	W,G,B	-
Veneridae				
Tapes conspersus	G,B	-	-	-

CHAPTER FOUR

INTRASPECIFIC DIFFERENCES IN THE TRANSCRIPTIONAL STRESS RESPONSE OF TWO POPULATIONS OF SYDNEY ROCK OYSTER INCREASE WITH RISING TEMPERATURES

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Intraspecific differences in the transcriptional stress response of two populations of Sydney rock oyster increase with rising temperatures

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ABSTRACT: The vulnerability of sessile organisms to warming temperatures may depend on their capacity to adaptively alter their expression of genes associated with stress mitigation. We compared the transcriptional profile of 2 populations of Sydney rock oysters *Saccostrea glomerata* (one that had been selectively bred over 7 generations for fast growth and disease resistance and one wild type that had not been subjected to selection) following exposure to an artificial temperature gradient in the field. Oysters were attached to white, grey or black stone pavers that experienced mean maximum substrate temperatures of approximately 34, 37 and 40°C, respectively, at low tide. Across all pavers, selectively bred oysters suffered 12% higher mortality than wild-type oysters, although this difference was not significant. Expression profiles did not differ between oyster populations on the coolest (white) pavers. However, divergent transcriptional profiles of genes associated with the key intracellular stress mechanisms of antioxidant defence, heat shock response, energy metabolism and the cytoskeleton were detected in oysters on the hottest (black) pavers. Expression of these genes was upregulated at high temperatures by the selectively bred oysters but displayed little change, or was suppressed at high temperature among the non-selected wild-type oysters. One potential explanation is that the selectively bred oysters have traded off rapid growth for a lower thermal maximum. Complementary physiological and ecological studies are needed to confirm this hypothesis.

KEY WORDS: Climate adaptation · Global warming · Molecular mechanisms · *Saccostrea glomerata* · Selective breeding · Thermal stress

INTRODUCTION

Rising temperature is the greatest challenge climate change poses to biodiversity, as temperature affects all levels of biological function (Rosenzweig et al. 2008, Tomanek 2014). For all organisms, cellular function is constrained to a limited range of body temperatures, with functionality compromised at the extremes of this range (Pörtner & Farrell 2008). Ectothermic organisms are particularly vulnerable to climate warming as their body temperatures typically conform to the surrounding ambient temperature (Huey et al. 2012). Hence, to stay within the temperature range across which they can function, ectotherms must either migrate with the shifting climate (Parmesan & Yohe 2003), behaviourally thermoregulate (Ng et al. 2017) or adapt their physiology (Somero 2010). For species limited in their capacity for behavioural or migratory response, the ability to adaptively alter their gene expression to rising temperatures may determine their vulnerability to climate change (Somero 2005).

Warmer ocean waters have already altered the phenology and metabolic function of marine species

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(Edwards & Richardson 2004, Pörtner & Knust 2007), with only modest amounts of ocean warming (~2°C) expected to breach the physiological limits of thermally sensitive ectotherms (Pörtner et al. 2007). As the early embryonic and larval stages of organisms are generally more vulnerable to changes in temperature than juveniles and adults (Pankhurst & Munday 2011), research on marine invertebrates has focused on the sensitivity of these early life history stages to warming (Parker et al. 2009, Sheppard Brennand et al. 2010). Nevertheless, invertebrates recruiting to intertidal rocky shores must not only tolerate warming air and water temperatures, but also increasing radiant heat from the substrate to which they are attached (Somero 2002, Helmuth et al. 2006). This may result in diurnal fluctuations in the body temperatures of juveniles and adults by as much as 10–20°C (see Ivanina et al. 2009). Many intertidal species already live close to their thermal maxima, and for these species, increases of just 1–2°C above present-day maximum temperatures may lead to osmotic and oxidative stress, culminating in heart failure (Stillman 2003). For organisms that cannot exploit spatial refuges, metabolic depression and intracellular stress responses that maintain cellular homeostasis and prevent macromolecular damage are key mechanisms for providing periodic tolerance to extreme temperatures (Pörtner & Farrell 2008, Evans & Hofmann 2012). Adaptation to changing environmental conditions is dependent on mutation rates and adequate genetic variation within the population on which selection can act, as well as the absence of deleterious mutations or trade-offs with other essential functions (Stearns 1989, Barrett & Schluter 2008). An understanding of how temperature-adapted gene expression determines an organism's stress resilience is important in identifying which species are most vulnerable to temperature increases and, within species, which populations might display the greatest capacity to adapt to a changing environment (Crain & Bertness 2006, Keppel & Wardell-Johnson 2012).

Many marine invertebrates exhibit a generic intracellular stress response to cope with exposure to temperature fluctuations, as well as to other stressors, which is characterised by the production of molecular chaperones, upregulation of antioxidant defence and cytoskeletal activity, and altered energy metabolism (Feder & Hofmann 1999, Tomanek 2014, Anderson et al. 2015, Groh & Suter 2015). Increased expression of heat shock proteins, which prevent damage to proteins by providing structural stabilisation, is the most documented thermal stress response in marine invertebrates (Iwama et al. 1998, Fabbri et al. 2008, Tomanek 2014), and may be critical in determining an organism's thermal plasticity (Shamseldin et al. 1997, Hamdoun et al. 2003, Fangue et al. 2006). Cellular homeostasis is further maintained via increased expression of antioxidant enzymes (i.e. catalase, peroxidase and superoxide dismutase) that detoxify damaging reactive oxygen species (ROS) (Heise et al. 2006, Jo et al. 2008, Zhang et al. 2016). As body temperatures approach their critical limits, dysfunction of the antioxidant enzymatic system can occur, potentially setting an organism's maximum temperature tolerance (Lang et al. 2009, Tomanek & Zuzow 2010). However, in contrast to metabolic maintenance of cellular homeostasis, some invertebrates may instead suppress their metabolic activity to minimise tissue energy demand and the mitochondrial production of damaging ROS (Storey & Storey 2004, Tomanek 2014).

The Sydney rock oyster *Saccostrea glomerata* (Gould 1850) is intensively farmed on the east Australian coastline (NSW DPI 2014), where it is also an important intertidal ecosystem engineer that supports biodiverse communities on rocky shorelines and in mangrove forests (McAfee et al. 2016). Laboratory studies have detected large intraspecific differences in the sub-cellular responses and individual fitness of *S. glomerata* when exposed to elevated temperatures and/or pCO_2 (Parker et al. 2011, 2012, Thompson et al. 2015, Goncalves et al. 2016). The larvae of *S. glomerata* selectively bred over 7 generations for fast growth and resistance to Queensland Unknown (QX) disease displayed greater survivorship and growth than wild-type oysters when exposed to elevated pCO_2 and/or temperature (Parker et al. 2012). Adults of the same generation of selectively bred oysters displayed differential expression of genes associated with antioxidant defence and energy metabolism (Goncalves et al. 2016) when exposed to elevated pCO_2. However, it remains unclear to what extent these differences represent generic responses, also evident following exposure of oysters to other stressors and that are applicable to field scenarios in which organisms may be simultaneously exposed to multiple stressors.

Here, we assessed transcriptional differences between selectively bred (for fast growth and disease resistance) and non-selected *S. glomerata* when exposed to an artificial temperature gradient in the field. We predicted that differences in the transcriptional profile of these 2 populations will increase with maximum temperatures, as will differences in mortality. Understanding the sub-cellular response of

oyster populations to rising temperatures will assist breeding programmes aiming to enhance the climate resilience of commercial aquaculture species, and may help identify populations with high environmental resilience that could benefit restoration projects targeting climate adaptation.

MATERIALS AND METHODS

Oysters were supplied by the New South Wales Department of Primary Industries (NSW DPI) and were 8.5 mo old (spawned mid-December 2014). Selectively bred oysters were from the B2 breeding line (mean shell length: 37.4 ± 4.5 [SD] mm; range: 30.7–45.5 mm), mass selected for fast growth and resistance to QX disease over 7 generations. Wild-type oysters (mean shell length: 34.9 ± 5.3 mm; range 26.7–43.7 mm) were hatchery spawned from wild oysters collected from Cromarty Bay. Both *Saccostrea glomerata* populations had overwintered on NSW DPI intertidal oyster farms in Cromarty Bay and were handled identically.

To test hypotheses about the transcriptomic response of these 2 populations of *S. glomerata* to different thermal regimes, a manipulative field experiment was conducted for 2 mo during the Austral spring (September and October) of 2015 at Cromarty Bay in Port Stephens estuary, New South Wales (NSW), Australia (32.71° S, 152.19° E). Port Stephens is a wave-dominated estuary, with semi-diurnal tides of ~1.5 m and mean salinities above 29.7 (Wolf & Collins 1979). Between September and October, sea surface temperatures range from 17.9–21.2°C (World Sea Temperatures 2017, Port Stephens: https://www.seatemperature.org/australia-pacific/australia/port-stephens.htm), and air temperature extremes range from 12.2–34.5°C (Bureau of Meteorology 2017).

Thermal gradient experiment

To expose oysters to different substrate temperatures, we produced an artificial thermal gradient by attaching oysters to white, grey or black stone pavers (300 × 300 × 17 mm; Fig. 1). When air-exposed in full sunlight for 30 min on a 27°C day, these white, grey and black pavers reached temperatures of 26, 30 and 39°C, respectively, when oysters were absent (infrared camera, Testo i80). Temperatures on white pavers were similar to those recorded from the neighbouring rocky shore (McAfee et al. 2017), while all temperatures were within the natural range that *S. glomerata*

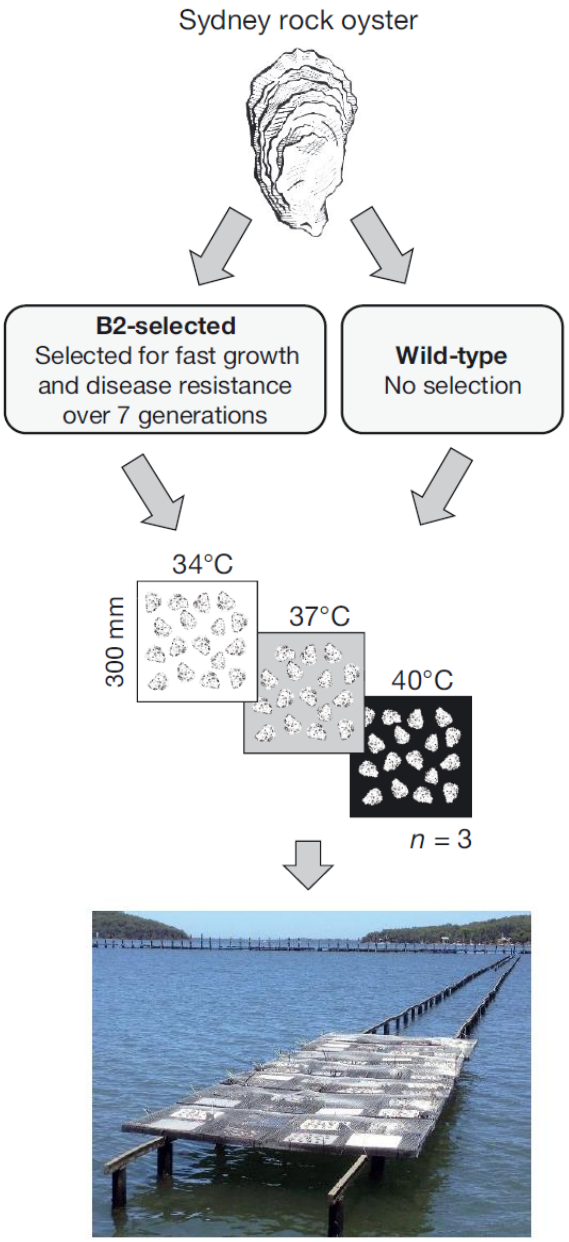

Fig. 1. Origin of the 2 hatchery-spawned Sydney rock oyster populations, and the experimental design separately exposing them to a passively warmed temperature gradient during aerial exposure. The average maximum temperature oysters experienced on each paver is provided above each treatment colour. During high tide, the oysters were covered in water

experiences during summer low tides (McAfee et al. 2016). We painted each paver with 2 coats of white, grey or black low-sheen paint (Dulux Weathershield). A homogenous surface chemistry among colours was

achieved by applying 3 coats of clear, non-toxic pond sealer (Crommelin Waterproofing) per paver.

We used 6 pavers of each colour treatment, 3 of which were randomly assigned to receive oysters of the wild-type and 3 received the B2 population. Each paver received 17 oysters of the assigned type, within the range of natural oyster densities observed on neighbouring rocky shorelines (Wilkie et al. 2012). Oysters were attached using non-toxic 2-part marine epoxy glue (Vivacity Engineering). Substrate temperatures on each paver were monitored using temperature dataloggers (DS1921G; Thermodata) programmed to record temperature every 20 min. Dataloggers were waterproofed using Plastidip rubber coating (Performix Brand: McAfee et al. 2016), and centrally positioned on each paver among the oysters. Pavers were deployed in Cromarty Bay on plastic commercial oyster trays (180 × 90 cm) that were secured to horizontal aquaculture racks at a height of mean low water of neap tides (Fig. 1). This ensured that all pavers were exposed to similar environmental conditions. Within each oyster tray, paver treatments were randomly arranged, and each tray was covered with a 12 × 12 mm wire mesh that excluded predators while minimising the shading of pavers. Every 2 wk, mud was gently washed from the pavers to maintain the colour treatments. After 2 mo, the pavers were returned to shore, and the number of oysters surviving was assessed by counting the gaping or missing valves.

RNA extraction and cDNA synthesis

Three surviving oysters from each of 3 replicate pavers (9 oysters treatment^{-1}) were randomly selected for gene expression analysis. Oysters were shucked and gill tissue (~2 × 2 mm), which is routinely used for transcriptomic analyses in *S. glomerata* because it is a high-quality source of RNA (Goncalves et al. 2016), was separately collected from each oyster and stored in 1 ml of RNA*later* (Sigma-Aldrich) at −20°C. All tissue samples were collected within 1 h of pavers emerging on the ebbing tide at the field site. Total RNA was isolated from approximately 100 mg of gill tissue, which was homogenised in 1 ml of Tri-reagent (Sigma-Aldrich) followed by phase separation with 1-bromo-3-chloropropane (Sigma-Aldrich). The upper clear phase was extracted and total RNA purified using an Illustra RNAspin Mini Isolation Kit (GE Healthcare Lifesciences) following the manufacturer's protocol (steps 2–8). We assessed the concentration and quality of each RNA sample using a NanoDrop spectrophotometer (Thermo Scientific NanoDrop 2000). Reverse transcription was performed for each oyster on 1 µg of total RNA using ImProm-II Reverse Transcription System (Promega) in 20 µl reactions with 0.5 µg Oligo(dT)$_{15}$, following the manufacturer's protocol.

qPCR and data analysis

Transcriptional responses of oysters to different thermal regimes were investigated using 12 genes associated with antioxidant defence, cellular stress, energy metabolism and the cytoskeleton (Table 1). These genes were chosen because their transcription is responsive to environmental stress (Zhang et al. 2012, Anderson et al. 2015) and varies between B2-selected and wild-type *S. glomerata* (Thompson et al. 2015, Goncalves et al. 2016).

qPCR assays were run on a LightCycler® 480 II (Roche). Each reaction (3 µl) consisted of 1.5 µl of KAPA SYBR® FAST qPCR Master Mix (Kapa Biosystems), 0.09 µl of forward and reverse primer, 0.32 µl PCR-grade water (ThermoFisher) and 1 µl of cDNA template (diluted 1:10). qPCR reactions (3 µl) were run in triplicate for each primer pair with the cDNA from individual oysters. qPCR cycling conditions consisted of 3 min at 95°C, followed by 45 cycles at 95°C for 10 s, 60°C for 20 s and 72°C for 30 s. At the end of the cycle, melting curve analysis was performed to collect fluorescence data between 65 and 95°C at 0.5°C increments. LightCycler® 480 II software analysed the amplification data to produce Cq values, with values averaged among the triplicate curves to provide a single value for each oyster, per gene.

Across all treatments, the most stable gene combination among our 4 reference and 12 target genes (Table 1), as determined by RefFinder (Xie et al. 2012), included 4 genes: β-actin, elongation factor 1 alpha, TATA-binding protein and ATP synthase alpha-subunit (combined geometric mean variance of 0.43 across all treatments). Changes in the relative expression of each target gene were calculated using the Livak & Schmittgen (2001) method, which normalises the Cq values of each gene relative to the combined geometric mean of the 4 reference genes.

To test for differences in the temperature of pavers and in the survivorship and the transcriptional response of oysters between populations and paver colours, 2-way permutational analyses of variance (PERMANOVAs; Anderson et al. 2008) were run. PERMANOVAs apply the traditional ANOVA parti-

Table 1. Genes and their primer sequences used for qPCR; Fw: forward, Rv: reverse

Functional group	Gene (abbreviation)	Sequence (Fw/Rv: 5′→ 3′)
Antioxidant defence	Catalase (Cat)	CGCTGACGTGGAGCAGATTG GGCGATGGGTGTCCGAATAA
	Superoxide dismutase (SOD)	AACTCTACCACGGCGAGCAT CCACGGTCGTCATCATGAAG
	Peroxiredoxin 6 (Perox)	GAAGGATGGAAGGACGGTGAT CACCTGTGGAAACACCTTCTC
	Glutathione peroxidase (Glut perox)	TGGTGGCCGAACTGGTTACA TCAGTACCACCAACTGAATGCA
	Glutathione S-transferase omega (Glut S-trans)	CGCTGGAGAAGGACGGAAAG TCCCGAGCTTGTTGGTATGG
Heat stress response	Heat shock protein70 (HSP70)	TGAATGGACACTCCTGGTTGG TGGGCATTGAAACTGCTGGA
	Heat shock protein 90 (HSP90)	CCCAGAGGATGAGGAGGAGA CAATACAGCAGGGCGATGTC
Energy metabolism	Cytochrome c oxidase I (COX1)	TTTCCTACCACGGGATGTG TGAGCTAATACCAGCCAAGTGA
	NADH dehydrogenase (NADH)	TCCTCCGGTACCCCAGTCAG TGCATCAAGGGGCTATTCCA
	ATP synthase alpha-subunit (used as reference)	CCTCCACTCTCGTCTGTTGG GAGATGACGTTGGTTGGGATG
Cytoskeleton	β-tubulin (TUB)	GCCATGACGAGGATCACAGG TGTCCCAGAACTCACACAGCAG
Protein folding	Peptidylprolyl isomerase A (PPIA)	CGGAGAAGACCACTTGGCTAGA ATCCATGCCCTCGACGACT
Reference genes	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	ACCGCGCCAGTCTTTGTTG GGCATTGTTGAGGGTCTGATG
	β-actin	GCACCTGAATCGCTCGTTG CAGCAGCATCGTCATCATCC
	Elongation factor 1 alpha	CCATAGCGGCATCTCCACTC CCTTGATTGCCACACTGCTC
	TATA-binding protein	GGACTTTGGCTCCTGTAAGCAC AGAATGGTGAAGCCTCGTATTG

tioning procedure to a distance matrix, but use permutations to obtain p-values (Anderson et al. 2008). Consequently, unlike ANOVAs, PERMANOVAs do not have explicit assumptions about the underlying distributions of data and can use any distance matrix that is appropriate to the data. A multivariate analysis was run on a Bray Curtis dissimilarity matrix calculated using the relative expression of all target genes. Multivariate differences in the expression of target genes between oyster populations and paver colours were visualised using non-metric multidimensional scaling (nMDS). Separate univariate analyses were run on Euclidean distance matrices calculated from each of maximum temperature, oyster survivorship and the individual target genes (Table 1). Analyses of relative gene expression used as replicates the averages calculated from the 3 oysters sampled per paver. Univariate analyses detecting significant differences were followed by pairwise post hoc PERMANOVAs to detect the source of variation. Differences in gene expression between populations were further visualised with a heat map (MeV 4.9).

RESULTS

Temperature and oyster survivorship in the field

The hottest temperature, 43°C, was observed on a black paver, with black pavers, on average, display-

ing maximum temperatures that were significantly hotter, by 3.8°C, than grey pavers and by 7.3°C than white pavers (PERMANOVA; Table A1 in the Appendix; Fig. 2a). There was no difference in paver temperature between those with B2-selected and wild-type oysters (Table A1, Fig. 2a).

Overall, 50% of the wild-type and 38% of the B2-selected oysters survived the 2 mo experiment. Nevertheless, there was no significant effect of oyster population on survivorship on any of the 3 paver colours (Table A1, Fig. 2b). Instead, survivorship differed with the main effect of colour treatment, with significantly more oysters on white pavers surviving than on grey or black pavers, which did not differ (PERMANOVA; Table A1, Fig. 2b).

Transcriptional profiles of all target genes

Multivariate differences in the transcriptional expression profiles of wild-type and B2-selected oysters depended on the colour treatment (PERMANOVA, significant Population × Colour interaction; Table A2). Expression profiles significantly differed between oyster populations on the hotter (black) pavers, where B2-selected oysters had greater expression than wild-type oysters for 10 of the 12 genes (post hoc test, Fig. 3). In contrast, the oyster populations did not differ significantly in gene expression on grey or white pavers (Fig. 3). Within populations, expression profiles for both the wild-type and the B2-selected oysters differed significantly between grey and black pavers, while oysters on white pavers did not differ from those on grey or black pavers (Fig. 3a).

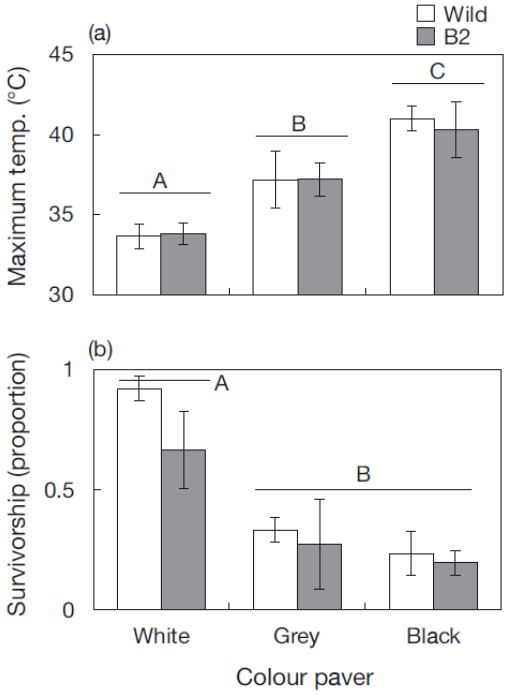

Fig. 2. Mean (±SE) (a) maximum temperature and (b) oyster survivorship recorded from white, grey and black stone pavers with wild-type (Wild; white bars) and B2-selected (B2; black bars) Sydney rock oysters attached. Significant differences (at $\alpha = 0.05$) between paver colours are denoted with letters above bars. n = 3 replicate pavers per colour treatment

Antioxidant defence genes

Separate analyses of the individual genes associated with antioxidant defence detected significant interactions between populations and colour treatments for catalase and peroxiredoxin 6, with no interaction detected for the other 3 genes (PERMANOVAs, significant Population × Colour interaction, Table A3, Fig. 4). For catalase, *a posteriori* tests revealed no difference between populations on any colour treatment, or among colour treatments for B2-selected oysters. However, for wild-type oysters, catalase expression was significantly greater on white (by 1.8-fold) and black (by 1.9-fold) than on grey pavers (post hoc test; Fig. 4a). *A posteriori* tests for peroxiredoxin 6 expression showed no difference between populations on pavers of any colour. The only significant difference occurred within the B2-selected oysters, where oysters on black pavers had greater expression (by 1.7-fold) than those on grey pavers (post hoc test; Fig. 4b). For superoxide dismutase (SOD), gene expression differed with the main effects of population, and among colours (PERMANOVA; Table A3, Fig. 4c). Between populations, SOD expression was greater in B2-selected oysters (by 1.3-fold) than in wild-types, while among colours, SOD expression was greater on black pavers than on white or grey pavers (by 1.5-fold for both), while white and grey did not differ (post hoc test; Fig. 4c). Glutathione S-transferase omega expression differed with the main effect of colour treatment, with significantly greater expression on white (by 1.4-fold) than on grey pavers, and greater on black (by 1.3-fold) than on grey pavers, with no difference between white and black pavers (PERMANOVA; Table A3). By contrast, no significant difference was detected in the expression of glutathione peroxidase (Table A3).

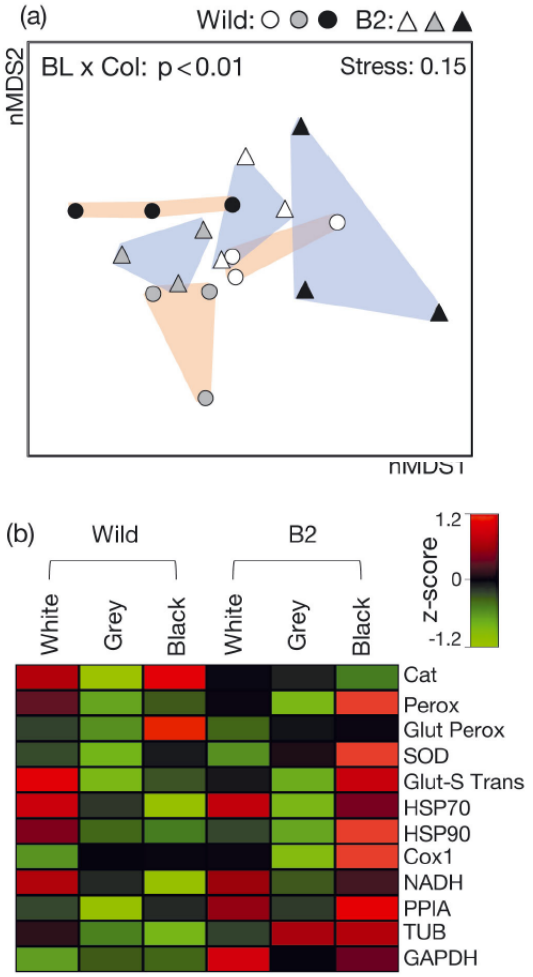

Fig. 3. Differences in the expression profiles for all target genes (a) between wild-type (circles and pink distribution markers) and B2-selected (triangles and blue distribution markers) Sydney rock oysters on white, grey and black colour treatments (depicted by the fill colour of data point symbols). Each point represents the mean expression of 3 oysters from 1 replicate paver. (b) Heat map of the mean expression of target genes (*z*-transformed). Scale reflects the *z*-score of individual gene expression: red indicates up-regulated expression; green indicates suppressed expression. Abbreviated gene names and their functional category provided in Table 1

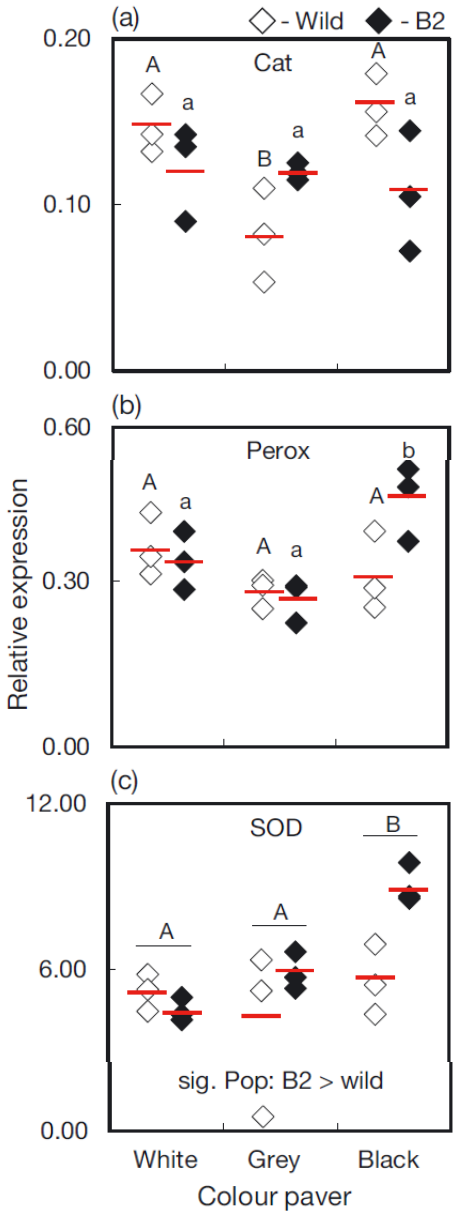

Fig. 4. Differences in the expression of 3 (catalase [cat], peroxiredoxin 6 [perox], superoxide dismutase [SOD]) of the 5 genes associated with antioxidant defence by wild-type (white diamonds; wild) and B2-selected (black diamonds; B2) oysters on white, grey and black pavers. Each point represents the mean expression of 3 oysters from 1 replicate paver. Horizontal red bars represent the treatment mean. For (a) cat and (b) perox 6, significant differences (at α = 0.05) within populations are denoted above treatments with upper-case letters for wild-type and lower-case letters for B2-selected oysters. For (c) SOD, significant differences (at α = 0.05) for the main effects of colour treatment are denoted with capital letters, and described for the main effects of population (Pop)

Heat stress response

Differences in the expression of HSP70 and HSP90 between populations were dependent on paver colour (PERMANOVAs, significant Population × Colour interaction, Table A4, Fig. 5). On black pavers, B2-selected oysters expressed significantly more HSP70 than wild-type oysters (by 1.5-fold;

Fig. 5a). However, on white and grey pavers, there was no difference in expression of HSP70 between the populations. Wild-type oysters displayed significantly greater expression of HSP70 on white than on black pavers (by 1.7-fold; Fig. 5a), with grey pavers not differing from either. B2-selected oysters expressed more HSP70 on white pavers than on grey pavers (by 1.5-fold; Fig. 5a), with black not differing from either. Expression of HSP90 did not differ between populations on pavers of any colour (Fig. 5b) and, for wild-type oysters, did not differ among paver colours (Fig. 5b). By contrast, HSP90 expression in B2-selected oysters was 1.4-fold greater on black than on grey pavers (Fig. 5b), with no other differences among treatments.

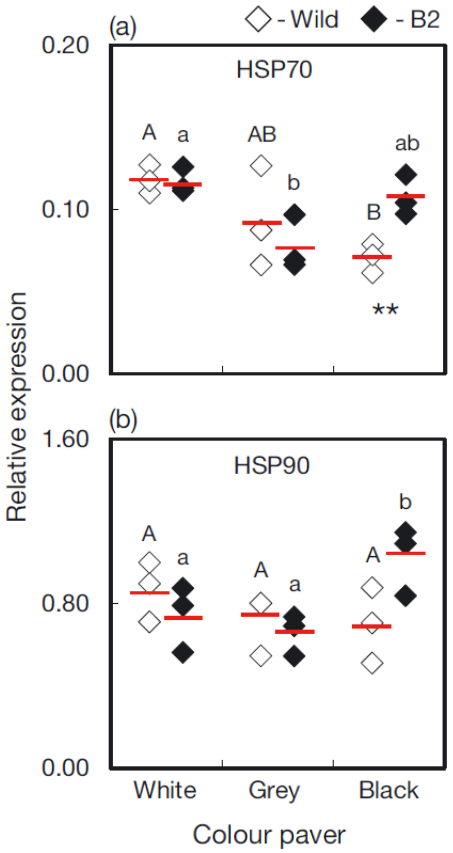

Fig. 5. Differences in the expression of heat-shock proteins (a) HSP70 and (b) HSP90 by wild-type (white diamonds) and B2-selected (black diamonds) oysters on white, grey and black colour treatments. Each point represents the mean expression of 3 oysters from 1 replicate paver. Horizontal red bars represent the treatment means. Significant post hoc differences (at $\alpha = 0.05$) within populations are denoted above treatments with upper-case letters for wild-type and lower-case letters for B2-selected oysters, and between populations are marked with asterisks (**) below the colour treatment

Energy metabolism

Expression of the genes associated with energy metabolism, cytochrome c oxidase 1 (COX1) and NADH dehydrogenase (NADH), did not display any significant difference between oyster populations, on pavers of any colour (Table A5). Instead, COX1 gene expression varied with the main effect of colour, with the expression of oysters significantly higher on black pavers than on grey (by 1.3-fold) or white (by 1.2-fold) pavers, while white and grey pavers did not differ (PERMANOVA; Table A5; Fig. 6a). In contrast, no significant differences among paver colours were detected for NADH expression (Fig. 6b).

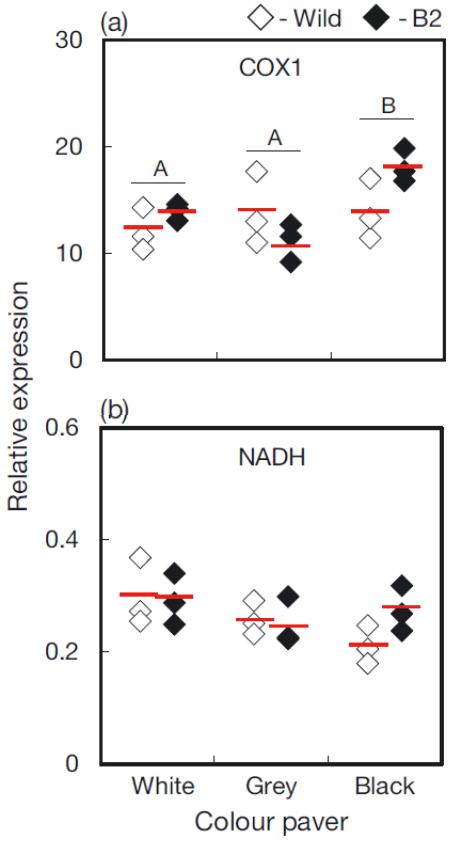

Fig. 6. Differences in the expression of the genes associated with energy metabolism, (a) cytochrome c oxidase I (COX1) and (b) NADH dehydrogenase (NADH), by wild-type (white diamonds) and B2-selected (black diamonds) oysters on white, grey and black pavers. Each point represents the mean expression of 3 oysters from 1 replicate paver. Horizontal red bars represent the treatment mean. For COX1, significant differences (at $\alpha = 0.05$) for the main effects of colour are denoted with letters above colour treatments. No significant differences were detected for NADH dehydrogenase

Expression of the gene associated with protein folding, peptidylproyl isomerase A (PPIA), did not show an interaction between population and colour (Table A6). Instead, PPIA expression differed with the main effect of population, with higher expression in B2-selected oysters (by 1.2-fold) than wild-type (PERMANOVA; Table A6). No significant differences were detected for the gene associated with the cytoskeleton (β-tubulin), or the anticipated reference gene, GAPDH (Table A6).

DISCUSSION

The capacity of marine ectotherms to adaptively shift their gene expression in response to rising temperature is key for their climate adaption, particularly for sessile intertidal organisms exposed to rising atmospheric, water and radiant temperatures (Somero 2010). Here, we found that intraspecific differences in the intracellular stress response between 2 populations of *Saccostrea glomerata* increased with maximum temperature. No difference in the expression profile of the 12 genes was detected between populations on the coolest (white) pavers, or on the grey pavers of intermediate temperature. However, on the hotter (black) pavers, expression profiles differed significantly between populations: B2-selected oysters displayed greater expression of genes associated with heat shock response, antioxidant defence and energy metabolism than on the cooler pavers, while wild-type oysters displayed similar or suppressed expression.

There are several potential explanations for the differential expression of stress response by the 2 oyster populations at high temperatures. On the one hand, upregulated expression may indicate that an organism is metabolically capable of mitigating extreme temperatures, while suppressed expression may indicate the compromised metabolism of a stressed organism (Feder & Hofmann 1999). For example, Zhang et al. (2012) attributed the ability of Pacific oysters to withstand extreme summer temperatures to their upregulation of HSP expression. On the other hand, upregulated expression may signify that an organism is experiencing temperatures approaching its thermal maxima, while suppressed expression may indicate that temperatures are not stressful enough to warrant metabolic thermoregulation (Fangue et al. 2006, Tomanek 2014). For example, *Collisella* limpets living at mid-intertidal elevations up-regulate HSP expression at lower temperatures than congenerics living in the upper intertidal that regularly experience maximum temperatures up to 5°C hotter (Sanders et al. 1991). Similarly, the highest temperatures at which intertidal *Tegula* sea snails synthesise HSPs closely relates to their upper temperature threshold (Tomanek & Somero 1999). Metabolic suppression is a common periodic stress response for intertidal ectotherms to avoid production of dangerous ROS, and may be followed by sudden metabolic upregulation if adverse conditions persist or strengthen (Hand & Hardewig 1996).

Here, 2 lines of evidence suggest that the second explanation – i.e. that the B2-selected oysters have a lower thermal maximum than the wild-type oysters — is the more likely cause of the differential stress response of the 2 oyster populations. (1) Although in the present study there was no difference in the survivorship of the 2 oyster populations across the thermal gradient, in a previous year-long study using the same 2 oyster populations, and in which much higher maximum temperatures of ~48°C were recorded on black pavers, we found much greater survivorship of the wild-type oysters (McAfee et al. 2017). This difference in survivorship between the 2 populations increased from white to grey to black pavers, so was interpreted as evidence for a greater thermal tolerance of the wild-type than the B2-selected oysters (McAfee et al. 2017). (2) The maximum temperature of 43°C recorded on the black pavers in the present study was just shy of the median lethal temperature (LT_{50}) of 45.4°C recorded for 2 yr old hatchery-reared *S. glomerata* exposed to elevated air temperatures for 6 h (Krassoi 2001). This suggests that the temperatures that the oysters experienced in this experiment are indeed approaching their thermal maximum. If the suppression of gene expression by the wild-type oysters was, alternatively, due to metabolic inhibition at high temperatures, greater mortality of the wild-type than B2-selected oysters would, to the contrary, be expected.

A lower thermal maximum of the B2-selected than the wild-type oysters would be consistent with the hypothesis of a trade-off between thermal tolerance and growth rate (Stearns 1989). Thermal tolerance comes at a high energetic cost to an organism (Pörtner et al. 2004), lowering the capacity for energetic investment in other fundamental metabolic activities (see Stearns 1989, Angeilletta et al. 2003). For example, increased resistance to stressful environmental temperatures is associated with a shorter life span and smaller body size among teleost fish (Martinez et al. 2016). Previous studies suggest that the faster rate of growth of the B2-selected than wild-type oysters is underpinned by a higher standard metabolic rate of the former (Parker et al. 2012), which may reduce

energy available for other stress responses. Additionally, contrary to the expectation that the mass-selection of the B2-selected oysters may reduce their genetic diversity (Zhong et al. 2016), and in turn reduce their capacity to adapt to environmental change (Barrett & Schluter 2008), B2-selected oysters have higher genetic diversity than wild *S. glomerata* populations (Thompson et al. 2017).

Other studies have also detected differential responses of the B2-selected and wild-type *S. glomerata* to other environmental stressors, but these have been variable in direction (e.g. Parker et al. 2011, Thompson et al. 2015, Goncalves et al. 2016). Larvae of the B2-selected oysters displayed greater fertilization success and decreased developmental abnormalities than wild-type oysters under ocean acidification scenarios (Parker et al. 2011). Among juveniles, similar to our findings, 50% of the genes upregulated by B2-selected oysters following exposure to high CO_2 conditions were downregulated in wild-type oysters (Goncalves et al. 2016). However, among adults (1.5–2 yr old), Thompson et al. (2015) found that the concentrations of proteins involved in antioxidant defence and energy metabolism were downregulated in B2-selected adults at elevated CO_2, and upregulated among wild-type adult oysters. The authors concluded that the higher standard metabolism emergent from the selection for fast growth and disease resistance (Parker et al. 2012) may enhance the developmental potential of B2-selected larvae at the cost of the adults' environmental resilience to additional stressors in natural settings (Thompson et al. 2015). However, in order for the physiological and ecological ramifications of these differences in sub-cellular stress responses to be interpreted, such transcriptional studies need to be coupled with measurements of key performance traits such as survivorship, growth and reproduction.

Studies investigating the transcriptional response of marine ectotherms to rising temperatures in the field are rare, and to our knowledge, this is the first study to investigate the sub-cellular stress response of oysters to an artificial temperature gradient in the wild. We found that intraspecific differences between *S. glomerata* populations increased with maximum temperatures, and that selectively bred oysters generally displayed greater gene expression in the hottest treatments. Knowledge of the sub-cellular mechanisms by which species respond to rising temperatures, and how these link to key performance traits, will assist breeding programmes focused on safeguarding the production of aquaculture species, and conservation projects aiming to build the resilience of ecologically important species.

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Appendix. Data output for PERMANOVA analyses

Table A1. Two-way univariate PERMANOVAs examining sources of variation in maximum temperature and oyster survivorship recorded from pavers that differed in the oyster population received (Pop: B2-selected, wild-type) and their colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	Max. temperature			Oyster survivorship		
		MS	p-*F*	p	MS	p-*F*	p
Pop	1	0.6	0.2	0.661	802.4	1.1	0.308
Col	2	66.9	23.1	**0.002**	4761.1	6.7	**0.004**
Pop × Col	2	0.7	0.2	0.761	741.2	1.1	0.408
Res	12						

Table A2. Two-way multivariate PERMANOVA examining sources of variation in the transcriptional expression of 12 genes by Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	MS	p-*F*	p
Pop	1	16.6	1.9	0.064
Col	2	22.3	2.5	**0.005**
Pop × Col	2	18.1	2.0	**0.015**
Res	12			

Table A3. Two-way univariate PERMANOVAs examining sources of variation in the transcriptional expression of genes associated with antioxidant defence (catalase, superoxide dismutase [SOD], peroxiredoxin 6 [Perox], glutathione peroxidase [Glut perox], glutathione S-transferase omega [Glut S-trans]) by 2 Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	Catalase			SOD			Perox		
		MS	p-*F*	p	MS	p-*F*	p	MS	p-*F*	p
Pop	1	0.7	1.2	0.265	2.5	5.0	**0.036**	0.9	1.8	0.185
Col	2	1.9	3.6	0.067	2.7	5.4	**0.010**	2.9	5.7	**0.017**
Pop × Col	2	2.9	5.3	**0.030**	1.5	3.0	0.070	2.1	4.0	**0.042**
Res	12									
		Glut S-trans			Glut perox					
Pop	1	<0.1	<0.1	0.921	0.3	0.3	0.621			
Col	2	3.0	5.1	**0.031**	1.6	1.7	0.241			
Pop × Col	2	1.9	3.3	0.080	1.0	1.1	0.399			
Res	12									

Table A4. Two-way univariate PERMANOVAs examining sources of variation in the transcriptional expression of genes associated with cellular stress (heat shock protein 70 [HSP70], heat shock protein 90 [HSP90]) by 2 Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	HSP70			HSP90		
		MS	p-*F*	p	MS	p-*F*	p
Pop	1	0.3	0.7	0.394	0.3	0.4	0.503
Col	2	3.8	6.9	**0.013**	1.4	2.1	0.192
Pop × Col	2	2.0	4.2	**0.044**	2.7	3.9	**0.048**
Res	12						

Table A5. Two-way univariate PERMANOVAs examining sources of variation in the transcriptional expression of genes associated with energy metabolism (cytochrome c oxidase I [COX1], NADH dehydrogenase [NADH]) by 2 Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	COX1			NADH		
		MS	p-*F*	p	MS	p-*F*	p
Pop	1	0.6	1.1	0.308	0.5	0.6	0.432
Col	2	2.5	4.3	**0.042**	2.1	2.4	0.099
Pop × Col	2	2.2	3.7	0.055	1.1	1.3	0.290
Res	12						

Table A6. Two-way univariate PERMANOVAs examining sources of variation in the transcriptional expression of genes associated with protein folding (peptidylprolyl isomerase A [PPIA]), cytoskeletal structure (β-tubulin [TUB]), and a potential reference gene (GAPDH) by 2 Sydney rock oyster populations (Pop: B2-selected, wild-type) that were attached to pavers that differed in colour (Col: white, grey, black) over 2 months. p-*F* = pseudo-*F*. Res = residual. Significant results (at α = 0.05) are highlighted in **bold**. n = 3

Source	df	PPIA			TUB			GAPDH		
		MS	p-*F*	p	MS	p-*F*	p	MS	p-*F*	p
Pop	1	4.5	6.7	**0.033**	2.9	3.1	0.089	3.6	3.6	0.079
Col	2	2.1	3.2	0.080	<0.1	<0.1	0.955	0.1	0.1	0.872
Pop × Col	2	<0.1	0.1	0.888	1.6	1.7	0.215	0.6	0.6	0.530
Res	12									

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CHAPTER FIVE

ARE ALL OYSTER BEDS CREATED EQUAL? TRAIT-MEDIATED CLIMATE AMELIORATION BY OYSTERS

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Under review at Functional Ecology

5.1 Abstract

1. Autogenic ecosystem engineers often provide cool microhabitats which associated organisms use to avoid thermal extremes. The value of such habitats is, however, dependant on key structural traits of the ecosystem engineer, and the intensity and duration of thermal exposure.
2. Using an artificial rocky shore environment, we assessed how the spatial configuration of habitat formed by an autogenic ecosystem engineer, the oyster, influences its capacity to mitigate heat stress experienced by intertidal invertebrates during simulated emersion periods on tropical, Hong Kong rocky shores.
3. At the average temperature experienced during summer-time low tides, oyster habitat ameliorated environmental and organismal temperatures, irrespective of the structural configuration of the oyster bed. As temperatures increased, however, vertically orientated oysters provided microclimates that facilitated cooler invertebrate body temperatures than horizontal beds, which no longer conferred any associational benefit as compared to bare rock surfaces.
4. In the absence of oysters, physiological indicators of stress to associated organisms increased with the intensity and duration of exposure to high temperatures. Such effects were, however, mitigated by association with vertical but not horizontal oyster configurations. In contrast, the osmolality of the habitat forming oyster haemolymph and mantle water was not related to temperature, suggesting that oysters remained in a state of metabolic quiescence throughout experimental emersion.
5. The spatial configuration of ecosystem engineers is, therefore, critical in influencing their effectiveness in environmental amelioration. Such variation in growth form of

oyster beds has important implications for ecological function and should be incorporated into projects aimed at building climate refugia through the conservation or restoration of ecosystem engineers.

5.2 Introduction

Warming temperatures and an increased frequency of extreme temperature events are among the most direct and predictable outcomes of climate change (Pachauri et al. 2014). High environmental temperatures directly influence the body temperatures of ectotherms, modifying their physiological performance and vulnerability to other stressors (Stevenson 1985; Huey et al. 2012). Although in some environments, warming may benefit ectotherms (e.g. basking in lizards, Angilletta, Niewiarowski & Navas 2002; Gunderson & Leal 2012), increased warming challenges the survival of many species, with species' persistence dependent on physiological or behavioural adaptations, especially in physically harsh environments such as the tropics (Somero 2010; Tewksbury et al. 2008; Ng et al. 2017).

In heterogeneous environments, one mechanism by which mobile ectotherms may buffer the impacts of warming is behavioural thermoregulation, with individuals seeking cooler microhabitats as refugia (Kearney et al. 2009; Cartwright & Williams 2014; Ng et al. 2017). Autogenic ecosystem engineers (*sensu* habitat-forming species) provide such microhabitat refuges from environmental stressors, especially extreme temperatures and desiccating conditions (Jones et al. 1994; Drezner 2006; Silliman et al. 2011), extending the realised niche of associated organisms (Bulleri et al. 2016). While the importance of autogenic ecosystem engineers in facilitating biodiversity is documented to increase with environmental stress (Bertness & Callaway 1994; He et al. 2013), few studies have investigated how such relationships depend upon intra- and inter-specific variation in the specific traits of the

ecosystem engineers (Bulleri et al. 2016). Within sites, variation in the size (Bruno & Kennedy 2000; Irving & Bertness 2009), shape (Bishop et al. 2009; 2013) and density (Bell & Westoby 1986; Bishop et al. 2012) of ecosystem engineers can, for example, influence the nature of species interactions, and feedback to influence the traits of the ecosystem engineer itself (Jones et al. 2010). The success of thermal amelioration by autogenic ecosystem engineers under warming scenarios may, therefore, display inter- or intra-specific variation according to variation in key structural traits of the ecosystem engineer.

In intertidal habitats, where many species already live at, or close to, their physiological temperature limits and are exposed to extreme heat and desiccation stresses at low tide (Somero 2010), provision of microhabitat refugia appears critical for maintaining biodiversity in a warming climate (Helmuth et al. 2006, McAfee et al. 2017). Intertidal ecosystem engineers (e.g. bivalves, barnacles, cordgrass, macroalgae: Seed 1996, Leonard 2000, Bruno & Kennedy 2000) can provide cool microclimates and, unlike topographic refuges (i.e. crevices, rock pools), can respond to the changing environment (Duarte et al. 2013; Ridge et al. 2015). Habitat-forming bivalves such as oysters and mussels can, for example, increase biodiversity by several orders of magnitude on rocky shores by increasing substrata for attachment and providing microhabitats that reduce temperature variability and enhance humidity (Seed 1996; McAfee et al. 2016). Protected by their hard shells and the ability to seal themselves from the external environment, oysters and mussels are also able to persist in hot and dry environments, and can be key to the persistence of less tolerant species (Silliman et al. 2011, McAfee et al. 2017). Habitat formed by aggregations of bivalves are commonly referred to as ‘beds’ or ‘reefs’ (hereafter beds), but whilst these terms acknowledge the abundance of bivalves in an area, they do not account for variation in the morphology and complexity of their structures. Such oyster beds, for example, can vary greatly from flat aggregations encrusting rock surfaces to three-dimensional agglomerations of individuals raised above the rock surface.

Given their importance, there is growing interest in the conservation and restoration of 'oyster beds' as autogenic ecosystem engineers to mitigate the effects of climate change on biodiversity (Crain & Bertness 2006; Duarte et al. 2013). The success of such projects will depend on the extent to which the key structural traits of the ecosystem engineers are rehabilitated. Here, we assess how the structural configuration of habitat produced by oysters influences their capacity to ameliorate the temperature and desiccation stress experienced by associated mobile invertebrates. Rock oysters, which encrust sheltered bays and estuaries, vary in orientation from being horizontal to vertically extending >10 cm above the rock surface (McAfee, D. personal observation). As such, there is likely to be great variation in the habitat provided by different configurations of oysters, which will have consequent impacts on their function as ecosystem engineers. We hypothesised that as the vertical relief of the oyster habitat increases, so will its capacity to reduce environmental temperature variability and desiccation, thereby reducing the body temperatures and physiological stress experienced by associated fauna and the oysters themselves; resulting in an increase in the survivorship of associated fauna. Further, we predicted that the magnitude of difference between the habitat types will increase with rising temperatures and duration of emersion; i.e., that the importance of habitat configuration will increase with environmental stress.

5.3 Materials and methods

To test hypotheses about how the structural configuration of oyster habitat may influence the abiotic environment, and hence the stress experienced by associated organisms, experiments were conducted in an intertidal mesocosm (see Cartwright & Williams 2014 for basic design) at the Swire Institute of Marine Science (SWIMS), Hong Kong (22^{o}N, 114^{o}E) in the hot and wet season (August 2016; see Kaehler & Williams 1996). Oyster habitat manipulations were

conducted with *Saccostrea cucullata* (Born 1778), which forms dominant beds on sheltered shores along the tropical Indo-Pacific coast and provides habitat for a variety of associated invertebrate species including, in Hong Kong, the chiton, *Liolophura japonica* (Lischke, 1873) and the gastropod, *Monodonta labio* (Linnaeus, 1758).

Animal collection and experiment protocol

Oysters were collected from sheltered rocky shores at Tai Tam, Hong Kong (22°14 N, 114°13 E), and immediately transported (~1 hour) to SWIMS where they were maintained in tanks (90 x 65 x 55 cm: l x w x h) supplied with unfiltered seawater (~27.5^{o}C) and with a simulated tidal-cycle in which oysters were emersed during midday low tides for four hours per day for a week prior to experimentation. *Liolophura* and *Monodonta* were collected on the ebbing tide at least a day prior to experimentation, and used within two days of collection. On return to SWIMS, *Liolophura* and *Monodonta* were placed on granite tiles (15 x 15 x 2 cm) under a seawater spray (~940 mOsmkg^{-1}) to allow replenishment of mantle water lost during transportation (see Williams et al. 2011).

To assess how the structural configuration of oysters influences their amelioration of environmental stress, four treatments were established on granite tiles. The first two treatments mimicked the variation in orientation and vertical relief provided by natural *Saccostrea* aggregations, which varied from horizontally orientated oysters with little vertical relief (~2.5 cm: "low" habitat), to vertically orientated aggregations that can extend ~10 cm ("high" habitat) above the substrate. These two treatments each utilised the same biomass of *Saccostrea* (wet weight: 192 ± 0.9 g; mean ± SE) but the low habitat treatment comprised oysters carefully chiselled from flat substrate so that the left valve could be placed flat to the tile surface; while the high habitat treatment was produced by stacking naturally clumped oysters to 10 cm height. The third, "solitary", oyster habitat treatment comprised three evenly spaced individual oysters

(combined wet weight: 47.5 ± 0.2 g) and was compared to low and high habitats to assess the climate amelioration benefits of oyster aggregations. The fourth, "bare", treatment was an oyster-free bare tile. Oyster habitats were established on tiles and held in tidal tanks (as described above) 24 hours prior to temperature manipulations.

Liolophura and *Monodonta* were placed in either bare, low, or high oyster habitat under seawater spray for 24 hours to allow them time to behaviourally respond to the habitat prior to temperature manipulations. Six *Monodonta* (total wet weight: 4.98 ± 0.1 g) and two *Liolophura* (wet weight: 11.4 ± 0.6 g) were randomly assigned to each tile to represent realistic densities on Hong Kong sheltered shores (McAfee, D. unpublished data). Solitary oyster habitats did not receive associated animals as this treatment's purpose was to determine the benefits of aggregation on the physiology of the oysters.

Simulating on-shore thermal and desiccation stress

To manipulate on-shore thermal conditions during emersion, tiles were placed in a large perspex tank (130 x 80 x 41 cm), above which halogen lamps (6 x 150 W; Philips) were fitted inside an isolated room to maintain stable conditions. The lamps heated bare tiles at an average rate similar to natural conditions on Hong Kong rocky shores during summer low tides (~0.2^{o}C per minute, Cartwright & Williams 2012). The tank was separated into two halves using a polystyrene board, with one side illuminated by two lamps and the other by four. Under two lamps, the mean temperature on bare tiles stabilised at ~32^{o}C ("ambient"), while mean temperatures under four lamps stabilised at ~42^{o}C ("elevated") after ~2 hrs. These temperatures are similar to average (ambient) and high (elevated) rock temperatures during the summer in the mid-shore in Hong Kong, although extreme rock temperatures in the mid-shore can exceed 55^{o}C (see Cartwright & Williams 2012). Potential interactive effects between habitat treatments, temperature regimes and the duration of exposure were investigated by randomly

assigning habitats to either two or four hour emersion durations, representing the aerial exposure experienced by oysters at mid-tide (~1.4 m above Chart Datum) during summer late afternoon (2 hours) and midday (4 hours) low tides (see Ng & Williams 2006). Six replicates of each habitat treatment were exposed to each of the four possible combinations of temperature and duration (Σn = 4 habitats x 2 temperatures x 2 emersion regimes x 6 replicates = 96). The six treatments were randomly assigned within six experimental runs.

The surface temperature of tiles was monitored with iButton dataloggers (DS1921G; Thermodata), placed in a central position on each tile, and programmed to record temperatures every two minutes. Additionally, infrared images (Testo, i80 camera) were taken from ~1 m above each tile within a minute of being exposed to lamps, and every 30 minutes thereafter to assess small-scale variation in maximum and minimum temperatures across tiles. Body temperatures of oysters were estimated using biomimetic oysters (hereafter 'robo-oysters') produced by encasing an iButton (programmed as above) inside an empty oyster shell (height: 44.9 ± 1.6 [mean ± SE], length: 30.2 ± 1.2 mm) with 3M Scotchcast 2121 (see McAfee et al. 2017). Due to the limited numbers of sufficiently large oysters, one robo-oyster per tile was placed on three of the tiles assigned to the four hour duration treatment (n=3). In each of the three oyster habitat treatments the robo-oyster replaced a living oyster of similar size. To assess desiccation, within each replicate, a water vial (clear, circular container lid: 20 x 3 mm: diameter x height) containing 2 ml of deionized water was placed at the tile center, among the oyster habitat where present, from which the evaporated water loss (EWL) was measured (± 0.1 ml, PSAW Pty Ltd) at the experiment's conclusion.

Heat lamps were turned on 1.5 hours prior to the transfer of tiles from the holding tanks to underneath the lamps, to allow temperatures to stabilise. Tiles were introduced using a random sequence to the experimental tank at staggered periods to allow adequate time to perform physiological measurements at the end of the experimental durations. Tiles were

returned to the holding tank following physiological measurements and held for an additional 24 hrs so that recovery of *Liolophura* and *Monodonta* could be assessed.

Monitoring organismal stress – biological responses

Liolophura foot temperatures and the internal body temperature of *Monodonta* were recorded using a digital K-type thermocouple following either two or four hours of heating. *Liolophura* foot temperatures were measured by inserting the thermocouple between the foot and the substrate, while body temperatures of three (of the six) randomly selected *Monodonta* were recorded by inserting the thermocouple ~5 mm past the operculum into the mantle body mass. Temperatures were averaged among individuals to provide a single value for each species per tile. *Liolophura* or *Monodonta* that did not respond to handling 24 hours after return of the tiles to the seawater spray tank were recorded as dead.

At the end of the two or four hours of heat exposure, the heart rate of *Monodonta*, osmolality of *Monodonta* and *Liolophura* mantle water, and the oyster mantle water and haemolymph were measured. Heart rates from three *Monodonta* per tile (those not used for body temperature measurements) were recorded using an oscilloscope (Fluke) and amplifier (Newshift, Portugal) with heart rate sensors (Vishay Semiconductors, CNY70, see Burnett et al. 2013 for details) attached to the dorsal surface of the shell's body whorl with Blu-Tack (Bostick). Heart beats (Hz beats sec^{-1}, mean of five readings over a 120 sec period) were averaged among individuals to provide a single value per tile.

The osmolality of *Monodonta* and *Liolophura* mantle water was measured by directly applying filter-paper discs (6 mm; Whatman Ashless, Grade 44) behind the operculum of *Monodonta* and within the mantle cavity of *Liolophura*. These saturated discs were immediately measured with a vapour pressure osmometer (VPO: Wescor 5520), which was regularly calibrated with 290 and 1000 mmol/kg NaCl standards (Wescor). Measurements of

Monodonta (those not used for heart rates) and *Liolophura* mantle osmolality were made on three and two individuals, respectively, per tile. From each oyster habitat (solitary, low, high) three oysters were randomly selected, carefully opened and the mantle water drained into an Eppendorf tube (0.6 ml). Abductor muscles were then cut to remove the right valve allowing haemolymph extraction from the pericardial cavity using a 1 ml syringe (Defer et al. 2013). Oyster mantle water and haemolymph were stored on ice until their osmolality could be measured from 10 µl samples pipetted onto filter-paper discs. On each tile, osmolality values were averaged to provide a mean value per tile. To establish the baseline osmolality of each species for reference to individuals exposed to temperature manipulations, the average osmolality among three individuals per species that were maintained in holding tanks and not exposed to heat lamps was recorded each day. Technical issues during two of the six experimental runs limited the collection of osmolality values and heart rates to four of the six replicates.

Statistical analysis

To assess variation in abiotic and biotic variables, univariate permutational analyses of variance (PERMANOVAs) were conducted on Euclidean distance matrices using untransformed data. Prior to each PERMANOVA analysis, homogeneity of variances were confirmed using the PERMDISP function in PRIMER (Anderson 2005). To assess variation in the maximum temperature of tile surfaces, as measured by iButton loggers, and the maximum and minimum temperatures from infrared images measured from each oyster habitat, three-way PERMANOVAs were used with the factors temperature (two levels: ambient, elevated; fixed), habitat (four levels: bare, solitary, low, high; fixed) and duration (two levels: two and four hours; fixed). The maximum temperature of robo-oysters over four hours was assessed with two-way PERMANOVA, with the factors habitat (three levels: solitary, low, high) and temperature (two levels).

Variation among treatments in evaporative water loss from tiles, the mortality and body temperatures of *Monodonta* and *Liolophura*, *Monodonta* heart rates and the osmolality of *Monodonta* and *Liolophura* mantle water, and oyster haemolymph and mantle water, were each separately assessed using three-way PERMANOVAs with the factors temperature (two levels), duration (two levels) and habitat (four levels for evaporative water loss, three levels [excluding solitary] for *Monodonta* and *Liolophura* measurements, and three levels [excluding bare] for oyster measurements). Where significant differences were detected, pairwise post-hoc PERMANOVAs were used to identify the source. The relationship between maximum tile and body temperatures, between body temperatures and mantle water osmolality, and between maximum temperatures and heart rates was separately assessed for *Monodonta* and *Liolophura* from each habitat using linear regressions (SPSS).

5.4 Results

Physical variables

Measurements of tile surface and habitat temperatures using iButtons and infrared imagery, respectively, revealed that the interaction between habitat and duration were significant for all measurements (iButton maximum, infrared maximum and minimum), whilst the interaction between temperature and duration was also important for infrared minimum temperatures (Table S1). In general, under ambient conditions, temperatures did not differ between high and low oyster habitats, each of which were significantly cooler than solitary and bare habitats which were similar (Table S1, Fig. 1a,b). At elevated temperatures, however, the high habitat had significantly cooler maximum and minimum temperatures than the low habitat, and both of these habitats were cooler than solitary and bare habitats (Table S1, Fig. 1a,b). Maximum temperatures also varied with duration, with tiles receiving two hours of temperature exposure

cooler than those receiving four (Table S1). Minimum temperatures did not differ between two and four hour durations at ambient temperatures, however at elevated temperatures, they increased with duration of exposure (Table S1).

The maximum temperatures recorded by robo-oysters were also dependent on the interaction between habitat type and temperature treatment (Table S2, Fig. 1c). Under ambient temperature there was no significant difference among oyster habitats, however, at elevated temperatures all habitats differed, with robo-oysters in high oyster habitat being 2.5ºC and 4.1ºC cooler than those in low and solitary oyster habitats, respectively (Table S2, Fig. 1c).

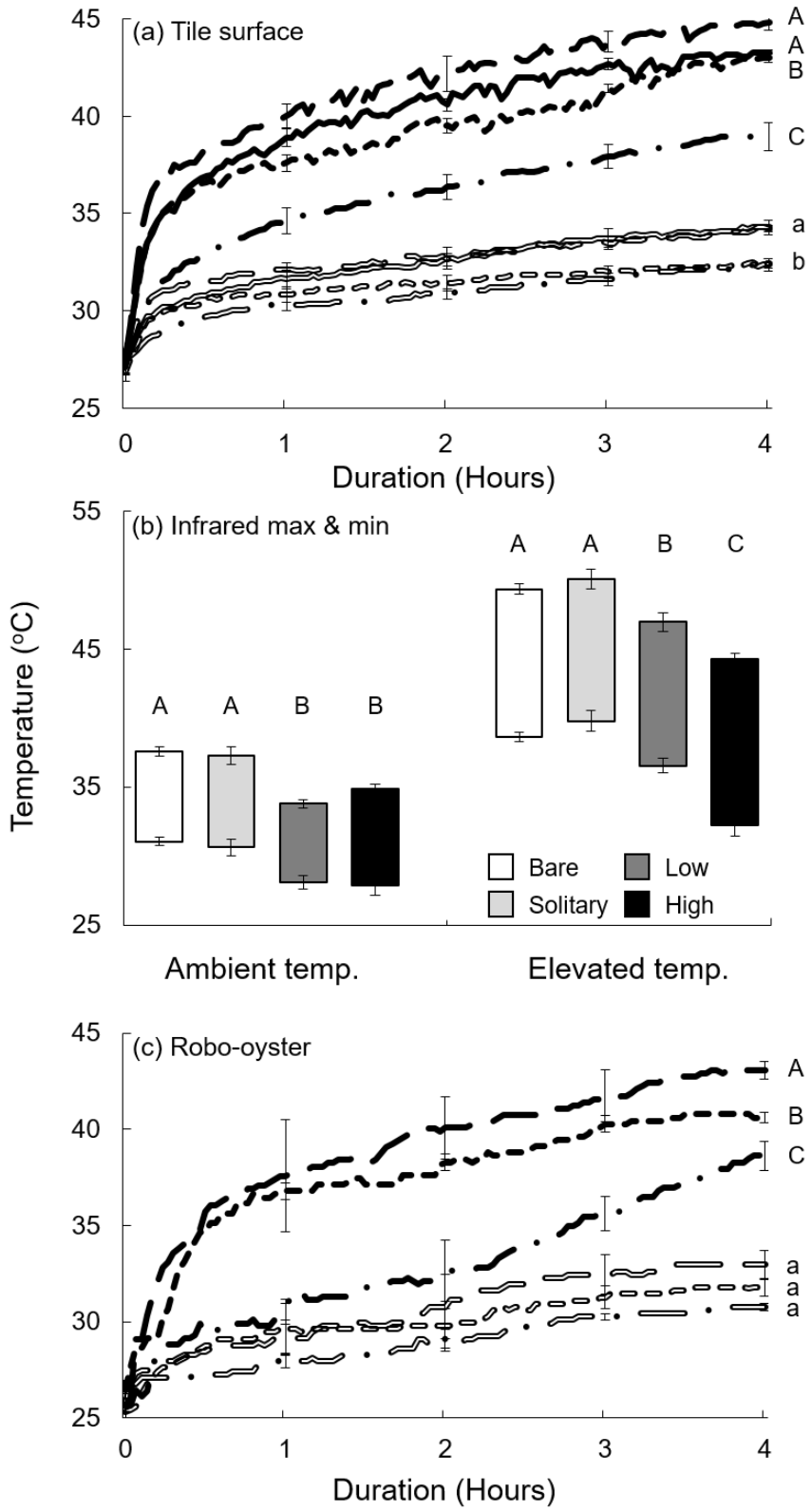

Fig. 4. Mean (±SE) (a) tile surface temperatures recorded using iButtons, (b) maximum and minimum temperatures recorded using infrared images, and (c) robo-oyster temperatures. Tiles were either devoid of oysters (Bare; solid lines in (a)), contained three solitary oysters (long-dashed lines in (a, c)), or had low (short-dashed

lines (a, c)) or high (dash-dot lines (a,c)) vertical relief. Each habitat was exposed to either ambient (white, a and c) or elevated (black, a and c) temperatures. (a) Only the 4 hour treatments are displayed as the 2 hour treatments displayed a similar trajectory in warming to the first 2 hours depicted here. Significant differences ($\alpha = 0.05$) among habitats in the maximum tile and robo-oyster temperatures, and the maximum and minimum temperatures recorded by infrared images, are denoted with different letters. n = 6 for (a) tiles and (b) habitats; n = 3 for (c) robo-oysters.

Evaporative water loss (EWL) displayed differences between durations that were dependent on its interaction with habitat and temperature (Table S3). For all habitats and both ambient and elevated temperature regimes, more (11-38%) EWL occurred in the four than the two hour duration (Fig. 2). For the two hour treatment, there was less EWL in low than solitary or bare habitats, while high habitats did not differ from any other habitat (Fig. 2). In the four hour treatments, high and low habitats did not differ but had significantly less EWL than solitary or bare habitats, which were similar (Fig. 2). For both durations there was less EWL at ambient than elevated temperatures.

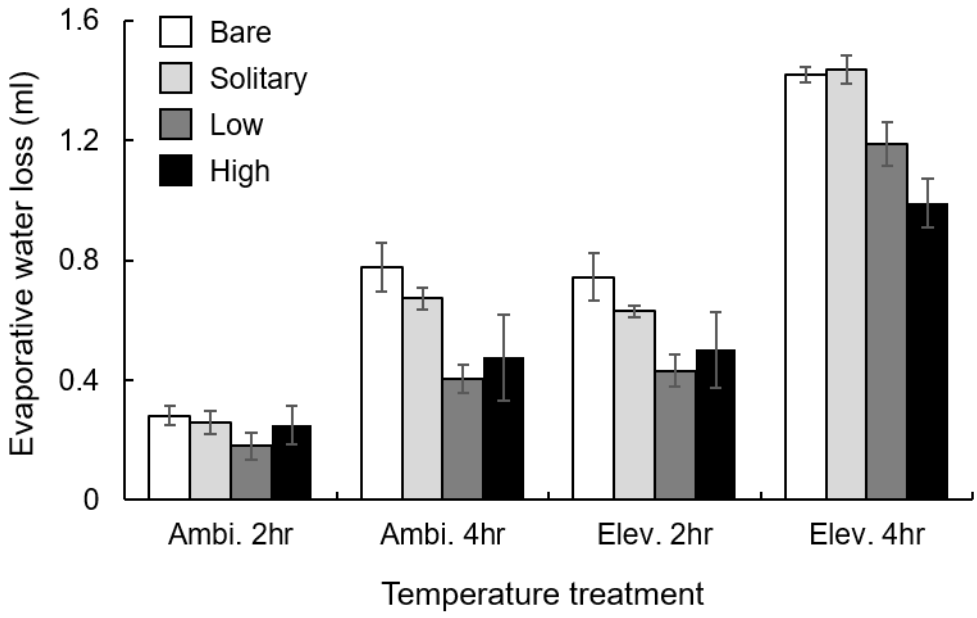

Fig. 2. Mean (±SE) evaporative water loss recorded from tiles with one of four oyster habitat treatments exposed to ambient (Ambi.) or elevated (Elev.) temperatures for experimental durations of either 2 or 4 hours. Significant post-hoc differences (at $\alpha = 0.05$) provided in Table S3. n = 6.

Biological responses

Across all treatments, mortality of *Monodonata* varied with temperature, with five individuals dying at elevated temperature, while none died at ambient temperature (Table S4; Table 1). The effect of temperature on *Liolophura* mortality, however, varied among treatments, with significant habitat by temperature, and temperature by duration interactions (Table S4; Table 1). For *Liolophura*, no mortality occurred in any of the habitats at the ambient temperature but at the elevated temperature, mortality was significantly higher in the bare than the high habitat, where no mortality occurred, and was intermediate in the low habitat, which did not differ significantly from the other treatments (Table S4; Table 1).

Table 1. Mortality of *Monodonta* and *Liolophura* exposed to elevated temperatures for 2 or 4 hours, in bare habitat or oyster beds of low or high vertical relief. No mortality was recorded at ambient temperature. n = 6.

Species	**Duration**	**Oyster habitat**		
		Bare	Low	High
Monodonta	2 hr	2	0	0
	4 hr	2	1	0
Liolophura	2 hr	1	0	0
	4hr	6	2	0

Monodonta body temperature was determined by the interaction between habitat, temperature and duration of exposure (Table S5). For each habitat and duration, *Monodonta* temperatures were significantly lower under ambient than elevated temperatures (Table S5; Fig. 3a). There was no effect of duration on *Monodonta* temperatures in bare habitats under elevated temperature, however, in all other habitats, *Monodonta* had lower temperatures after two than four hours (Fig. 3a). At ambient temperatures, *Monodonta* temperatures in low or

high habitats were similar and significantly cooler than those in bare habitats (Fig. 3a). Following two hours of elevated temperature, *Monodonta* temperatures increased from high, to low, to bare habitats; and after four hours, *Monodonta* were cooler in the high than the low or bare habitats, which were similar (Fig. 3a). *Liolophura* foot temperature displayed effects of habitat that were dependent on the temperature treatment they received (Table S5). For each habitat, *Liolophura* temperatures were cooler under ambient than elevated temperatures (Fig. 3b). At ambient temperatures, *Liolophura* in low habitats were significantly cooler than those in bare habitats, while temperatures in high habitats did not differ from either (Fig. 3b). At elevated temperatures, however, *Liolophura* in high habitats were significantly cooler than those in bare or low habitats, which were similar (Fig. 3b).

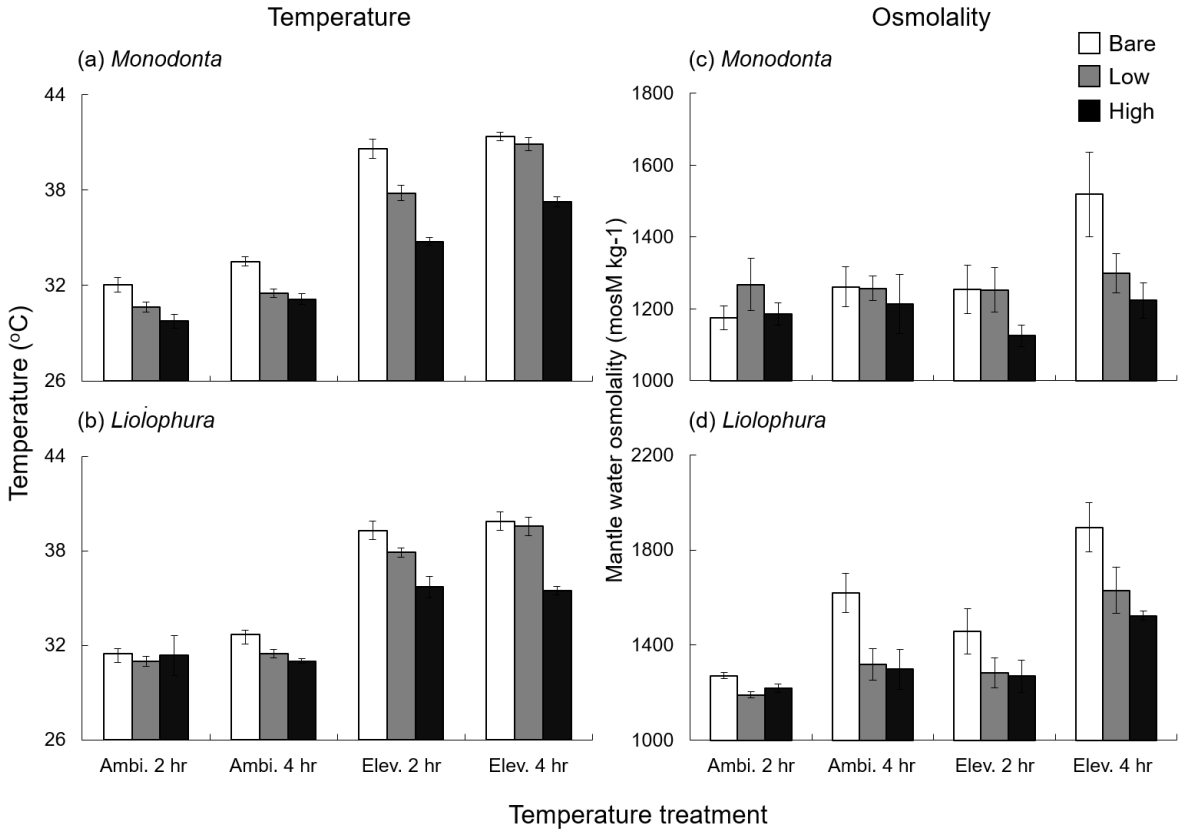

Fig. 3. Mean (±SE) (a) *Monodonta* body temperature, (b) *Liolophura* foot temperature, (c) *Monodonta* and (d) *Liolophura* mantle water osmolality recorded from tiles with either bare (white bars), low (grey bars) or high (black bars) oyster habitat that were exposed to either ambient (Ambi.) or elevated (Elev.) temperatures for either

2 or 4 hours. Significant post-hoc differences (at $\alpha = 0.05$) are provided in Table S5 and S6. n = 6 and 4 for temperatures and osmolality, respectively.

Variation in *Monodonta* mantle water osmolality displayed only the main effects of habitat and duration (Table S6), being significantly lower in high than bare habitats, with low habitats not differing from either, and following two than four hours of exposure (Fig. 3c). In contrast, *Liolophura* mantle osmolality was affected by the interaction of habitat and duration, and temperature and duration (Table S6). For each habitat, and for each temperature treatment, *Liolophura* osmolality was, as for *Monodonta*, lower after two than four hours duration (Fig. 3d). *Liolophura* osmolality was significantly lower in high and low habitats, which did not differ, than bare habitats after both two and four hours (Fig. 3d). Additionally for both durations, *Liolophura* osmolality was significant lower among ambient than elevated temperature treatments.

Monodonta heart rates displayed an interacting effect of habitat, temperature and duration (Fig. 4, Table S7). *Monodonta* exposed to elevated temperatures for two hours had significantly faster heart rates in bare than low or high habitats, which did not differ, but when exposed to elevated temperatures for four hours those in both bare and low habitats had faster heart rates than those in high habitats (Fig. 4). At ambient temperatures no significant differences were detected among habitats (Fig. 4). After two hours in bare habitats, heart rates were significantly faster in elevated than ambient temperatures, whereas after four hours, differences were detected in both bare and low oyster habitat (Fig. 4).

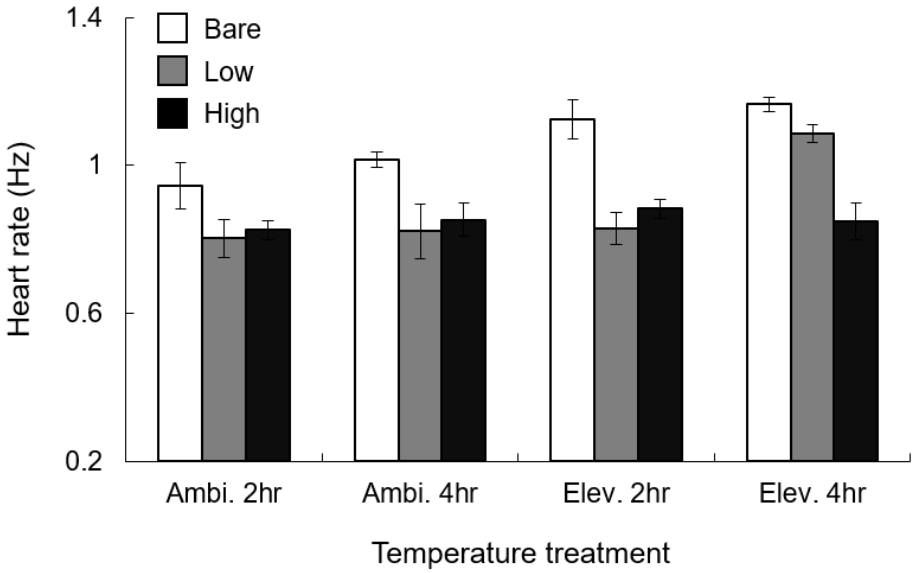

Fig. 4. Mean (±1SE) heart rate of *Monodonta* recorded from habitats where oysters were absent (white bars), or where oysters provided low (grey bars) or high (black bars) habitat when exposed to either ambient (Ambi.) or elevated (Elev.) temperatures for either 2 or 4 hours. Significant post-hoc differences (at $\alpha = 0.05$) provided in Table S7. $n = 4$.

The maximum temperature of the tiles predicted 95% of the variation in *Monodonta* body temperatures ($y = 3.15 + 0.87x$; df = 70, $p < 0.001$, $r^2 = 0.95$; Fig. 5a). The body temperature of *Monodonta* explained 22% of the variation in their mantle water osmolality ($y = 746.31 + 14.19x$, df = 58, $p < 0.001$, $r^2 = 0.22$; Fig. 5b), while *Monodonta* heart rates predicted 21% of the variation in their mantle water osmolality ($y = 858.50 + 6.93x$, df = 58, $p < 0.001$, $r^2 = 0.21$; Fig. 5c). For *Liolophura*, maximum tile temperatures predicted 86% of their variation in body temperature ($y = 8.01 + 0.73x$, df = 70, $p < 0.001$, $r^2 = 0.86$; Fig. 5d), while *Liolophura* body temperatures predicted 29% of the variation in their mantle water osmolality ($y = 235.82 + 33.82x$, df = 58, $p < 0.001$, $r^2 = 0.29$; Fig. 5e).

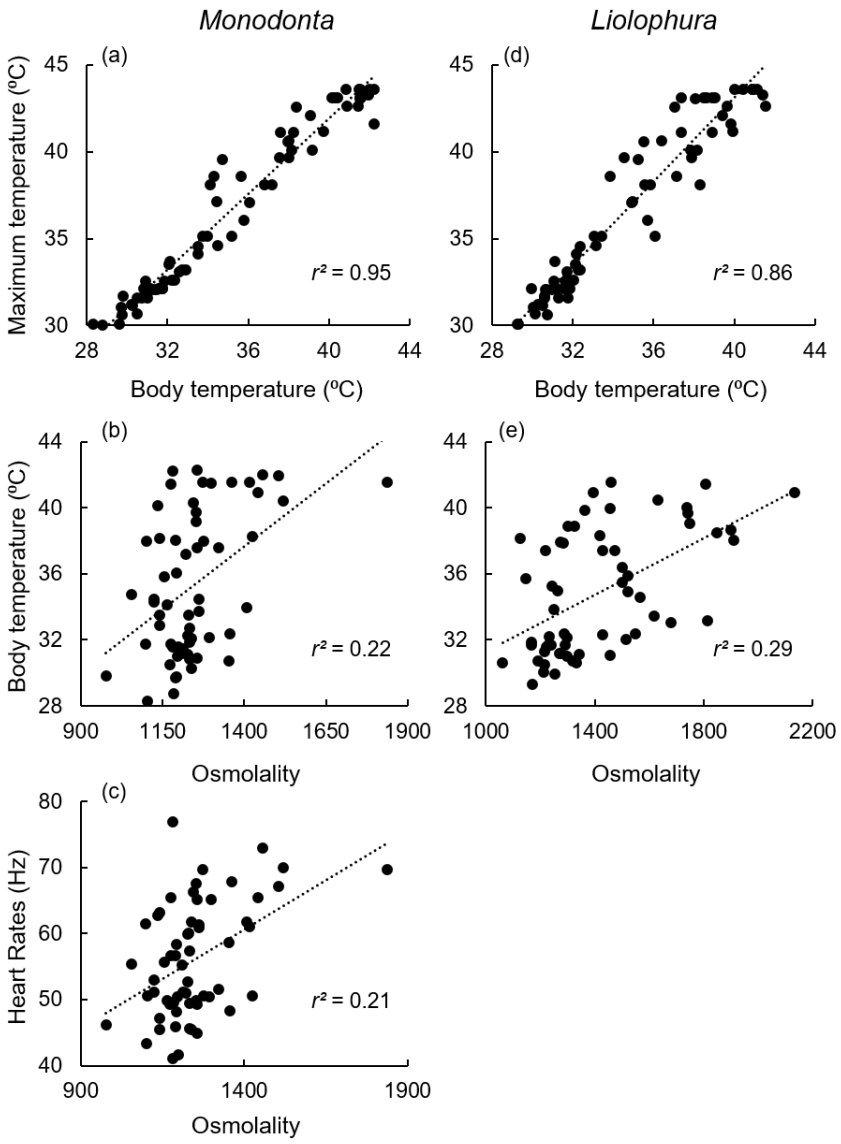

Fig. 5. Linear regressions between maximum tile temperatures and body temperatures, body temperatures and osmolality, and heat rates and osmolality for (a-c) *Monodonta* and (d-e) *Liolophura*.

The osmolality of oyster mantle water and haemolymph increased, on average across all treatments, by 5.5% and 2.3% from their baseline osmolality, respectively. There was, however, no statistically significant effect of habitat, temperature or duration on either osmolality of oyster mantle water or haemolymph (Table S8). Changes in maximum temperatures bore no relation to the osmolality of oyster mantle water ($r^2 = 2.4$, df = 18, $p = 0.515$) or haemolymph ($r^2 = 6.2$, df = 18, $p = 0.29$).

5.5 Discussion

We experimentally show that variation in the structural traits of oysters influences their capacity to ameliorate temperature and desiccation stress experienced by associated invertebrates. Whether provision of oyster microhabitat reduced body temperatures of *Liolophura* and *Monodonta* sheltering within them was dependent on the spatial configuration of the oyster habitat, and the magnitude of environmental warming. Whereas under average temperatures presently experienced during midday summer low tides, oysters reduced the body temperature of associated invertebrates irrespective of their shell orientation; at elevated temperatures, reduction of body temperature was dependent on oyster shell configuration. Vertically orientated oysters provided a microclimate that was up to 4.3^{o}C cooler than that of horizontally orientated oysters, and up to 17^{o}C cooler than solitary oysters after four hours of elevated temperature. Although the horizontally configured oysters were able to provide cooling over short durations of exposure to extreme temperatures, this capacity diminished with prolonged high temperature exposure, with invertebrate body temperatures being no cooler within this habitat than on bare substrate.

Heat stress within mid shore regions of rocky intertidal shores generally increases with the amount of primary substrata exposed to the sun (Lathlean 2014). The greater ameliorating function of vertically as compared to horizontally orientated oysters was, however, despite their occupation of less primary substrate, suggesting that this effect was, instead, caused by the greater influence of oyster orientation on shading. In contrast, previous studies with barnacles and mussels found no effect on amelioration of heat-stress as a result of variation in animal configuration (Cole 2010; Lathlean 2014). As compared to the oysters manipulated in our study, the body size of the barnacles and mussels manipulated by those studies was, however, small and the configurations tested were varying levels of aggregation. The importance of configuration may be expected to increase with animal body size as larger organisms have a

greater cross-sectional area that can influence shading (Falster & Westoby 2003), and also a greater thermal inertia (Angilletta et al. 2002).

Not only was the vertical orientation of oysters more effective at ameliorating temperature extremes experienced by associated organisms, but it also influenced the temperatures experienced by the oysters themselves. An organism's spatial orientation relative to the sun determines how much solar radiation they absorb, with body temperatures expected to increase more rapidly when broad lateral surfaces face the heat source (Muñoz et al. 2005; Denny & Harley 2006). Horizontally orientated robo-oysters displayed similar internal temperatures to solitary robo-oysters, whereas vertically orientated robo-oysters had internal temperatures 7^{o}C cooler than those of solitary oysters after even one hour. The similar body temperature of the horizontal and solitary oyster treatments contrasts with previous studies documenting lower body temperatures among aggregating organisms (Helmuth 1998; Chapperon, Le Bris & Seuront 2013). Although living in aggregations can reduce the projected area of an organism that is subject to solar radiation, it also substantially reduces convective heat exchange between organisms and the environment (Helmuth 1998). This greater "thermal inertia" may not only buffer animals against rapid spikes in temperature, but also slow the amount of heat that is lost through convection (Helmuth 1998).

Associational benefits among species are predicted to increase with physical stress (Bertness & Callaway 1994; Bruno, Stachowicz & Bertness 2003). In general, the lethal and sub-lethal effects of temperature on organisms are expected to increase as the thermal maximum for physiological processes is approached, and then exceeded (Pörtner & Farrell 2008). Here, we found little effect of oysters on the survivorship, heart rate and osmolality of associated invertebrates under ambient temperatures, but strong ameliorating effects of oysters under increased exposure to high temperatures. Previous studies have similarly found physiological benefits arising from association with intertidal ecosystem engineers (e.g.

barnacles, Cartwright &Williams 2012; algae, Burnaford 2004) to be greatest under conditions of extreme physical stress. This influence can vary seasonally, for example, where littorinid snails and limpets will seek climate-buffering habitat provided by barnacle tests during summer low tides, but in winter this association is weakened (Cartwright & Williams 2012).

Consistent with the greater effect of vertical than horizontal oyster configurations on *Liolophura* and *Monodonta* body temperature, vertical oyster cover also had a greater ameliorating effect on *Monodonta* heart rates and *Monodonta* and *Liolophura* osmolality. Interestingly, however, *Liolophura* osmolality also displayed a reduced response to high temperatures in horizontal oyster habitat as compared to bare habitat, despite no environmental or body temperature difference between these. This may be due to lower rates of evaporation in horizontal oyster than bare habitat, or the flexible body-plan of chitons allowing them to fully exploit the horizontal oyster habitat crevices (Harper & Williams 2001). Previously, *L. japonica* has been shown to lose water faster on horizontal surfaces than in crevices (Harper & Williams 2001). By contrast, the physiological measurements did not support any associational benefits between *Monodonta* and horizontal oyster habitat at high temperatures over four hours. The basal heart rate of *M. labio* (~0.9 Hz: Cartwright, pers. comm.) was maintained in horizontal habitats over two hours of high temperature, however, their heart rates increased with maximum temperature thereafter until they did not differ from those recorded in bare habitat.

Against expectation, the osmolality of oyster haemolymph and mantle water showed no relationship with temperature. Nevertheless, independent of temperature, oyster mantle water osmolality increased to more than twice the amount of the haemolymph osmolality during the experiment, suggesting that while mantle water may have been lost in response to aerial exposure (i.e. from clamping valves), there was no associated change in haemolymph, unlike patterns seen in other invertebrates (i.e. heat-stressed limpets: Williams et al. 2011). No

temperature associated change in haemolymph osmolality suggests that the metabolic activity of the oysters may remain suppressed throughout the experiment. Metabolic suppression is a common strategy for intertidal organisms enduring stressful thermal events, enabling them to avoid energetically costly metabolic thermoregulation until it is absolutely critical (Marshall et al. 2011, McAfee et al. 2018). Here, the lack of metabolic regulation of cellular homeostasis suggests that *S. cucullata* can endure the thermal extremes currently experienced on Hong Kong shorelines, with temperatures in solitary habitat not dissimilar to maximum rock temperatures recorded from local shores (max >60^{o}C: Lau, SLY, unpublished. data). Understanding the critical temperatures of such habitat-forming species is important, as mortality of these ecosystem engineers would have negative, cascading effects on associated biodiversity (Crain & Bertness 2006).

Clearly, in physically stressful intertidal habitats, vertical relief is an important habitat trait, and given the cooler microclimates and oyster temperatures recorded from vertical habitat, positive structural feedbacks are more likely to occur in vertically than horizontally orientated habitat (Jones et al. 2010). Examples of intra- and interspecific variation in the structural traits of ecosystem engineers that determine the distribution of associated biodiversity can be found in a diversity of habitats. In hot terrestrial environments, for example, increases in leaf density and the branching canopy of nurse plants can reduce spatial variability in sub-canopy microclimates, and subsequently, increase understory recruitment of desiccation-prone species (Drezner 2006). Successful adaption of terrestrial ectotherms to a warmer climate may depend on access to vegetation with a dense shading canopy, while open-canopy vegetation may not provide sufficient thermoregulatory benefits (Kearney et al. 2009). In saltmarsh environments, increasing height and density of *Spartina* cordgrass positively influences species interactions by reducing water flow and stabilising substrate (Irving & Bertness 2009). In areas of increased wave action, however, facilitation by *Spartina* is size

specific, with only large patches capable of stabilising substrate for associated infauna (Bruno & Kennedy 2000). Similarly, in our study, temperature amelioration was trait-dependent under prolonged high temperatures.

Conclusion

Globally, increasing temperatures are impacting biodiversity at such a rate that climate adaptation strategies appear increasingly essential to avoid catastrophic biodiversity loss (Urban 2015). Conservation of climate change refugia that maintain more favourable climates for retreating organisms is increasingly recognised as a key strategy for assisting species adaptation (Keppel et al. 2015). The conservation and/or restoration of ecosystem engineers may provide the most cost-effective strategy for providing climate refugia (Byers et al. 2006), especially where these habitats are self-perpetuating and can adapt with the changing environment (Jones et al. 2010, Duarte et al. 2013). Successful use of ecosystem engineers, however, is contingent on detailed knowledge of how the temperature amelioration capacity of the engineer changes with variation in their key structural traits (Irving & Bertness 2009). Where efforts to restore ecosystem engineers do not achieve the structural traits critical to environmental amelioration, the desired ecological outcomes may not be achieved (Irving & Bertness 2009). We have added to the growing evidence that oysters can protect intertidal biodiversity from climatic extremes (McAfee et al. 2016, 2017), and recommend that intertidal restoration projects targeting climate adaptation must achieve vertically structured configurations that provide substrate shading. The application of such knowledge will be important to ensure that costly restoration projects succeed in providing the greatest benefit to biodiversity.

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Table S1. Three-way PERMANOVA idenitifying sources of variation in the maximum tile surface temperature (iButton) and the maximum and minimum temperature recorded using infrared imagery from tiles that differed in oyster habitat (Hab: bare, solitary, low, high), temperature regime (Temp: ambient [Ambi], elevated [Elev]), and duration of exposure (Dur: 2 hours, 4 hours). p-*F* = pseudo *F*. Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold, and their post-hoc differences are listed below. $n = 6$ replicates.

		iButton Maximum			**Maximum**			**Minimum**		
Source	**df**	**MS**	**p -*F***	**P**	**MS**	**p -*F***	**P**	**MS**	**p -*F***	**P**
Hab	3	61.2	67.8	**0.001**	99.6	51.1	**0.001**	139.8	53.6	**0.001**
Temp	1	1934.1	2142.9	**0.001**	3271.8	1679.4	**0.001**	1303.6	499.8	**0.001**
Dur	1	77.7	86.1	**0.001**	80.2	41.2	**0.001**	92.7	35.5	**0.001**
Hab x Temp	3	14.9	16.5	**0.001**	20.7	10.6	**0.001**	26.9	10.3	**0.001**
Hab x Dur	3	1.2	1.3	0.305	3.8	1.9	0.139	6.6	2.5	0.066
Temp x Dur	1	1.7	1.9	0.176	3.6	1.8	0.186	14.2	5.4	**0.017**
Hab x Temp x Dur	3	1.9	2.1	0.097	1.9	0.9	0.422	5.6	2.1	0.101
Res	80									

		Post-hoc differences
iButton Maximum	Hab x Temp	Elev: [Bare = Sol] > Low > High Ambi: [Bare = Sol] > [Low = High] Elev > Ambi (all Hab)
	Dur	4hr > 2hr
Maximum	Hab x Temp	Elev: [Bare = Sol] > Low > High Ambi: [Bare = Sol] > [Low = High] Elev > Ambi (all Hab)
	Dur	4hr > 2hr
Minimum	Hab x Temp	Elev: [Bare = Sol] > Low > High Ambi: [Bare = Sol] > [Low = High] Elev > Ambi (all Hab)
	Temp x Dur	Elev: 4hr > 2hr Ambi: 4hr = 2hr Elev > Ambi (both Dur)

Table S2. Two-way PERMANOVA idenitifying sources of variation in robo-oyster temperatures recorded from tiles that differed in oyster habitat (Hab: solitary, low, high) and temperature regime (Temp: ambient [Ambi], elevated [Elev]). p-*F* = pseudo *F*. Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold, and their post-hoc differences are listed below. *n* = 3 replicates.

Source	**df**	**MS**	**p -*F***	**P**
Hab	2	12.6	19.1	**0.001**
Temp	1	376.5	572.9	**0.001**
Hab x Temp	2	2.5	3.8	**0.046**
Res	12			
	Post-hoc differences			
Hab x Temp	Hab:	Elev: Sol > Low > High Ambi: Sol = Low = High		
	Temp:	All Hab: Elev > Ambi		

Table S3. Three-way PERMANOVA idenitifying sources of variation in the amount of evaporated water loss from tiles that differed in oyster habitat (Hab: bare, solitary, low, high), temperature regime (Temp: ambient [Ambi], elevated [Elev]) and duration of exposure (Dur: 2 hours, 4 hours). p-*F* = pseudo *F*. Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold, and their post-hoc differences are listed below. *n* = 6 replicates.

Source	**df**	**MS**	**p -*F***	**P**
Hab	3	0.4	16.2	**0.001**
Temp	1	6.2	234.5	**0.001**
Dur	1	6.2	237	**0.001**
Hab x Temp	3	<0.1	1.4	0.263
Hab x Dur	3	<0.1	3.0	**0.035**
Temp x Dur	1	0.7	26.7	**0.001**
Hab x Temp x Dur	3	<0.1	1.3	0.253
Res	80			
	Post-hoc differences			
Hab x Dur	2hr:	[Bare = Sol] > Low; High = [Bare, Sol, Low]		
	4hr:	[Bare = Sol] > [Low = High]		
	Dur:	4hr > 2hr (all habitats)		
Temp x Dur	Temp:	Elev > Ambi (both Dur)		
	Dur:	4hr > 2hr (both Temp)		

Table S4. Three-way PERMANOVAs idenitifying sources of variation in the mortality of *Monodonta* and *Liolophura* from tiles that differed in oyster habitat (Hab: bare, solitary, low, high), temperature regime (Temp: ambient [Ambi], elevated [Elev]) and duration of exposure (Dur: 2 hours, 4 hours). p-*F* = pseudo *F*. Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold, and their post-hoc differences are listed below. *n* = 6 replicates.

		Monodonta			***Liolophura***		
Source	**df**	**MS**	**p -*F***	**P**	**MS**	**p -*F***	**P**
Hab	2	0.2	1.9	0.144	0.5	5.3	**0.008**
Temp	1	0.3	3.8	**0.042**	1.1	10.9	**0.001**
Dur	1	<0.1	0.1	0.719	0.7	6.6	**0.010**
Hab x Temp	2	0.2	1.9	0.142	0.5	5.3	**0.004**
Hab x Dur	2	<0.1	0.1	0.886	0.3	2.6	0.076
Temp x Dur	1	<0.1	0.1	0.741	0.7	6.6	**0.011**
Hab x Temp x Dur	2	<0.1	0.1	0.897	0.3	2.6	0.080
Res	60						

Post-hoc differences

Monodonta	Temp	Elev > Ambi	
Liolophura	Hab x Temp	Hab:	Elev: Bare > High; Low = [Bare, High]; Ambi: *ns*
		Temp:	Elev > Ambi (all habitats)
	Temp x Dur	Dur:	Elev > Ambi (both Dur)
		Temp:	4hr > 2hr (both Temp)

Table S5. Three-way PERMANOVAs idenitifying sources of variation in the body temperature of *Monodonta* and the foot temperature of *Liolophura* on tiles that differed in oyster habitat (Hab: bare, low, high), temperature regime (Temp: ambient [Ambi], elevated [Elev]) and duration of exposure (Dur: 2 hours, 4 hours). p-*F* = pseudo *F*. Res = Residual. Significant results (at $\alpha = 0.05$) are highlighted in bold, and their post-hoc differences are listed below. *n* = 6 replicates.

		Monodonta			***Liolophura***		
Source	**df**	**MS**	**p -*F***	**P**	**MS**	**p -*F***	**P**
Hab	2	4.4	89.8	**0.001**	2.7	21.8	**0.001**
Temp	1	54.0	1090.2	**0.001**	54.3	436.1	**0.001**
Dur	1	2.8	56.9	**0.001**	0.4	3.0	0.082
Hab x Temp	2	0.8	16.2	**0.001**	1.4	11.3	**0.001**
Hab x Dur	2	<0.1	1.6	0.191	0.2	1.9	0.171
Temp x Dur	1	0.2	3.9	**0.048**	<0.1	<0.1	0.756
Hab x Temp x Dur	2	0.2	3.5	**0.022**	<0.1	0.7	0.474
Res	60						

Post-hoc differences

Monodonta	Hab x Temp x Dur	Hab:	Elev, 2hr: Bare > Low > High; Elev, 4hr: [Bare = Low] > High; Ambi, 2hr & 4hr: Bare > [Low = High]
		Temp:	Elev > Ambi (all Hab & Dur)
		Dur:	Elev, Low & High: 4hr > 2hr; Elev, Bare: 2hr = 4hr Ambi: 2hr = 4hr (all Hab)
Liolophura	Hab x Temp	Hab:	Elev: [Bare = Low] > High; Ambi: Bare > Low; High = [Bare, Low]
		Temp:	Elev > Ambi (all Hab)

Table. S6. Three-way PERMANOVAs idenitifying sources of variation in the mantle water osmolality of *Monodonta* and *Liolophura* on tiles that differed in oyster habitat (Hab: bare, low, high), temperature regime (Temp: ambient [Ambi], elevated [Elev]) and their duration of exposure (Dur: 2 hours, 4 hours). p-*F* = pseudo *F*. Res = Residual. Significant results (at α = 0.05) are highlighted in bold, and their post-hoc differences are listed below. *n* = 4 replicates.

		Monodonta			***Liolophura***		
Source	**df**	**MS**	**p -*F***	**P**	**MS**	**p -*F***	**P**
Hab	2	53352	3.9	**0.02**	<0.1	15.2	**0.001**
Temp	1	49758	3.6	0.053	<0.1	24.9	**0.001**
Dur	1	<0.1	8.4	**0.006**	<0.1	49.4	**0.001**
Hab x Temp	2	38170	2.8	0.084	9916	0.6	0.564
Hab x Dur	2	18165	1.3	0.283	53106	3.1	**0.050**
Temp x Dur	1	17757	1.3	0.282	81387	4.7	**0.043**
Hab x Temp x Dur	2	9376	0.7	0.543	4072	0.2	0.808
Res	36						

Post-hoc differences

Monodonta	Hab	Bare > High; Low = [Bare, High]
	Dur	4hr > 2hr
Liolophura	Hab x Dur	Bare > [Low = High] (both Dur) 4hr > 2hr (all Hab)
	Temp x Dur	Elev > Ambi (both Dur) 4hr > 2hr (both Temp)

Table S7. Three-way PERMANOVAs idenitifying sources of variation in the heart beat rates of *Monodonta* on tiles that differed in oyster habitat (Hab: bare, low, high), temperature regime (Temp: ambient [Ambi], elevated [Elev]) and the duration of exposure (Dur: 2 hours, 4 hours). p-*F* = pseudo *F*. Res = Residual. Significant results (at α = 0.05) are highlighted in bold. *n* = 4 replicates, and their post-hoc differences are listed below.

Source	**df**	**MS**	**p -*F***	**P**
Hab	2	737.8	26.0	**0.022**
Temp	1	549.3	19.4	**0.001**
Dur	1	169.7	5.9	**0.001**
Hab x Temp	2	81.2	2.9	0.070
Hab x Dur	2	71.9	2.5	0.105
Temp x Dur	1	25.3	0.9	0.346
Hab x Temp x Dur	2	97.1	3.4	**0.044**
Res	36			

Post-hoc differences

Hab x Temp x Dur	Hab:	Elev, 2hr: Bare > [Low = High] Elev, 4hr: [Bare = Low] > High Ambi, 2hr & 4hr: Bare = Low = High
	Temp:	Bare: Elev > Ambi (2hr & 4hr) Low, 4hr: Elev > Ambi; Low, 2hr: Elev = Ambi High: Elev = Ambi (2hr & 4hr)
	Dur:	Low, Elev: 4hr > 2hr All other Hab & Temp: 2hr = 4hr

Table. S8. Three-way PERMANOVAs idenitifying sources of variation in the osmolality of the mantle water and haemolymph of oysters on tiles that differed in oyster habitat (Hab: solitary, low, high), temperature regime (Temp: ambient, elevated) and the duration of exposure (Dur: 2 hours, 4 hours). p-*F* = pseudo *F*. Res = Residual. Significant results (at α = 0.05) are highlighted in bold. *n* = 6 replicates.

		Oyster Mantle Water			**Oyster Haemolymph**		
Source	**df**	**MS**	**p -*F***	**P**	**MS**	**p -*F***	**P**
Hab	2	2.3	2.4	0.094	0.9	0.9	0.435
Temp	1	1.1	1.1	0.312	0.8	0.8	0.530
Dur	1	0.3	0.3	0.560	0.8	0.8	0.539
Hab x Temp	2	2.5	2.6	0.064	0.8	0.8	0.695
Hab x Dur	2	0.7	0.8	0.464	0.9	0.9	0.495
Temp x Dur	1	0.2	0.2	0.648	1.0	1.0	0.432
Hab x Temp x Dur	2	2.5	2.6	0.085	0.9	0.9	0.502
Res	60						

CHAPTER SIX

DISCUSSION

6.1 BUILDING RESILIENCE WITH POSITIVE INTERACTIONS

High levels of biodiversity are key to maintaining ecosystem function (Tilman et al. 1996), and are therefore a top priority for conservation (Balvanera et al. 2001). Incorporating positive species interactions into conservation provides the potential for cost-effective whole-ecosystem management (Byers et al. 2006, Halpern et al. 2007). The structure and function of many ecosystems is underpinned by either a solitary, or a functional unit of ecosystem engineers, such as the trees in a forest, corals in a tropical reef, or bivalves in a shellfish reef (see Jones et al. 1994, Bruno et al. 2003). Conservation of ecosystem engineers that support high levels of biodiversity by ameliorating environmental stress may provide the most feasible option for assisting the adaptation of biodiversity to environmental change (Crain and Bertness 2006, Byers et al. 2006).

This thesis has shown that the Sydney rock oyster, *Saccostrea glomerata*, by ameliorating environmental stressors and increasing the availability of hard substrate for attachment, facilitates diverse invertebrate communities in the intertidal. The strength of positive interactions between intertidal invertebrates and *S. glomerata* habitat varies spatially according to environmental stress, and intraspecific variation in the environmental resilience of oysters and their structural traits that influence ecosystem engineering. By determining where, when and how intertidal oyster habitat is most critical to supporting associated invertebrates, this research can improve our predicative capacity of where conservation of *S. glomerata* will be most effective in preserving and/or restoring biodiversity (Crain and Bertness 2006).

Consistent with the numerous studies that have documented high levels of biodiversity associated with habitat-forming bivalves (Seed 1996, Coen & Luckenbach 2000, Silliman et al 2011), in every environment into which we transplanted oysters, we detected substantial

recruitment of associated invertebrates, over periods of 3 months to a year. On the intertidal mudflats at the seaward fringe of mangrove forests, where pneumatophore roots provide the only other hard substrate, the presence of *S. glomerata* increased the abundance of invertebrates by up to 20 times, and increased species richness by 3 times that of oyster-free pneumatophore habitat (Chapter 2). In the rocky substrate experiment, we detected up to 17 times the abundance and 8 times the richness of recruited invertebrates to stone pavers with, than without, oysters following one year on intertidal aquaculture racks (Chapter 3). The complimentary rocky shoreline experiment detected only one species, *Bembicium auratum*, persisting outside of oyster habitat after 3 months, with the abundance of this species up to 32 times greater in the presence than the absence of oysters (Chapter 3). 78 other species were also detected amongst oysters (Chapter 2). Increasing surface complexity typically positively influences species richness (Johnson et al. 2003), and no doubt the altered structural state provided by *S. glomerata* habitat relative to oyster-free space increased the diversity of microhabitats and surface area available for recruiting organisms. The influence an ecosystem engineer has on biodiversity is predicted to increase with the extent to which the structural habitat it provides departs from the baseline, "unengineered" state (Jones et al. 2010). Therefore, particularly in sedimentary environments where the availability of hard substrate is limiting to many invertebrates (Peterson et al. 2003), oyster habitat can exert a strong influence on biodiversity (Grabowski et al. 2005, Borsje et al. 2011).

This study not only documented the high biodiversity value of bivalve habitat, but also partitioned the mechanisms by which *S. glomerata* facilitates associated invertebrates across environmental gradients (see also Silliman et al. 2011). Invertebrate facilitation resulted from both the direct effect of habitat provision and the indirect effect of environmental stress amelioration by *S. glomerata* habitat. When we manipulated the habitat structure provided by oysters and the abiotic and biotic pressure experienced by intertidal invertebrates with shading

and caging structures (Chapter 2), the provision of hard structure had the greatest effect on invertebrate assemblages irrespective of environmental context. The abundance and richness of recruiting invertebrates increased with both the density and amount of cover *S. glomerata* habitat provided (Chapter 3). This is consistent with the large number of studies that show that the density of an ecosystem engineer can be a fundamental determinant of the nature of species interactions (Bell and Westoby 1986, Harley and O'Riley 2011). In many instances, positive interactions increase with ecosystem engineer density. For example, the higher shoot density of seagrass increases the abundance of fish and decapods through increased habitat availability (Bell and Westoby 1986), branch density influences climate variability and seedling establishment beneath the canopy of desert nurse plants (Drezner 2006), and the indirect positive effect of predatory whelks on littorinids, that arises from the whelks creating empty barnacle tests which serve as habitat, increases with whelk density (Harley and O'Riley 2011). However, the relationship can be non-linear (e.g. Bishop et al. 2012) and where the ecosystem engineer competes with inhabitants for a limited resource, at very high densities interactions may shift from positive to negative (e.g. Bateman and Bishop 2017).

In contrast to the direct effect of habitat provision, the relative importance of amelioration of abiotic and biotic pressure varied spatially according to the type and magnitude of environmental stress. Consistent with Bertness and Callaway's (1994) theory on positive interactions, we found that as temperature increased so did the relative importance of environmental amelioration by shading, and conversely, as temperatures decreased the importance of providing a predation refuge increased (Chapter 2). McAfee et al. (2016) showed that environmental amelioration by *S. glomerata* was sufficient to disrupt biogeographic patterns in invertebrate assemblages that are presumably set by spatial variation in environmental stress. This thesis elaborated on this phenomenon, showing that facilitation of biodiversity by oysters across environmental gradients arises from their capacity to mitigate

multiple stressors, with the magnitude of mitigation increasing with the level of stress. There remain few empirical examinations of the predictions of the stress gradient hypothesis (Bertness and Callaway 1994) over broad spatial distributions from the marine realm (Bulleri 2009). This research addresses this research gap, and may assist in identifying where oyster-based conservation strategies are suitable for conserving biodiversity, and where they are not (Hobbs et al. 2009).

Concomitant with identifying the number and type of environmental conditions an ecosystem engineer may influence, knowledge of how other organisms respond to the altered environmental state is key to predicting an engineer's impact on biodiversity (Jones et al. 1997). Responses of key functional groups of invertebrates to oysters were dependent on the background environmental stress, and on specific attributes of the *S. glomerata* habitat (Chapter 2, 5). Similar to the interaction between intertidal fauna and ecosystem engineers habitat on other thermally stressful shores (Bertness et al 1999, Watt & Scrosati 2013, Cartwright and Williams 2014), sessile organisms (i.e. barnacles and oyster spat) and gastropods increasingly relied upon the provision of temperature ameliorating habitat as maximum temperatures increased. In contrast, at the coolest site the primary mechanism of facilitation of sessile organisms switched to provision of predator-free habitat, while shading had a negative effect on sessile organism recruitment. Mechanisms of facilitation are predicted to switch between abiotic and biologically stressed environments as these stressors are generally negatively correlated (Bertness and Callaway 1994), with positive species interactions increasing with stress a consistent and predictable trend of species interactions in nature (He et al. 2013). Leonard (2000) observed similar shifts from positive to negative engineering effects of *Ascophyllum nodosum* canopies on recruiting barnacles, with the positive interaction from the temperature buffering canopy on thermally stressful shorelines giving way to negative interactions at cooler sites where predators were abundant and *A.*

nodosum facilitated their access to the barnacles. As such, not all ecosystem engineers will sufficiently match the numerous environmental stressors associated organisms experience, and where these vary spatially and temporally, engineering effects may suddenly shift from positive to negative (Leonard 2000).

Following oyster death and the disarticulation of their valves, the surface area available for invertebrate colonisation typically increases relative to live oysters (Summerhayes et al. 2009). Indeed, we saw a greater abundance of invertebrates recruiting to dead oyster clumps than live oysters. Mobile arthropods (i.e. crabs, amphipods, springtails) showed a particularly positive response to dead oyster habitat, with the greater surface area of the disarticulated left valve (Gutierrez et al. 2003) potentially increasing foraging opportunities for mesopredators by increasing the habitat availability for their prey, and by reducing their exposure to apex predators and intraspecific competition (Grabowski 2004, Grabowski and Kimbro 2005). In contrast, infaunal polychaetes and bivalves responded more positively to live than dead oyster habitat, presumably in response to the biodeposition of faeces and pseudofaeces from oyster filter-feeding, the nutrients of which are exploited by polychaetes and suspension feeding bivalves (Dubois et al. 2007). By identifying the environmental stressors ecosystem engineers ameliorate, and how associated organisms response to these altered states (Jones et al. 1997), coastal managers can identify conservation targets and ecological goals based on the capacity of the engineer to deliver those outcomes.

6.2 ROCK OYSTERS AS CLIMATE REFUGIA

Intertidal ecosystems are highly sensitive to temperature increases because many intertidal organisms already live close to their thermal thresholds with limited capacity to physiologically "keep-pace" with the rate of warming (Somero 2010). Their persistence in extreme habitats may depend on access to spatial refuges that provide more favourable

microclimates than the surrounding environment, with the conservation of such refuges now being increasingly recognised as pivotal to the climate adaptation of ecological communities (see Morelli et al. 2016). The climate amelioration capacity of intertidal ecosystem engineers is well documented, with extreme temperature and/or desiccation stress amelioration observed from the habitats produced by macroalgae (Leonard 2000, Watt & Scrosati 2013), barnacles (Cartwright and Williams 2012, 2014), mussels (Bertness et al. 1999, Cole 2010, Silliman et al. 2011) and mangroves (McAfee et al. 2016), to name a few. With knowledge that oysters can ameliorate climate in stressful environments, this thesis explored when, and how oysters provide climate amelioration, to determine if oyster habitat can match the threat of predicted temperature rise.

Our results suggest that *S. glomerata* habitat has the capacity to provide climate refugia, that could increase the resilience of intertidal biodiversity to warming atmospheric temperatures. As we increased temperature stress beyond present day conditions, oyster habitat increasingly provided cooler microhabitats that maintained greater humidity than bare habitat (Chapter 3 & 5). In treatments mimicking present day conditions, oyster habitat provided microclimates that were typically ~2^{o}C cooler than bare habitat, whereas when exposed to extreme temperature stress (<12^{o}C above ambient treatments) oyster habitat maintained temperatures that were up to 8^{o}C cooler, and 21% more humid, than bare surfaces (Chapter 3 & 5). On the north Atlantic coast, intertidal macroalgae canopies buffer maximum temperatures by 1.2^{o}C at low tidal elevations, whereas in the upper intertidal of the same shore they can maintain microclimates 11.9^{o}C cooler than adjacent, algae-free habitat (Watt & Scrosati 2013). Similarly, in terrestrial environments, nurse plants are increasingly important for the survival of understory seedlings as temperature and desiccation increase (Gomez-Aparicio 2004). By maintaining climate refugia under increasingly stressful conditions, ecosystem engineers will

likely be even more critical to biodiversity as climates continue to warm (Crain and Bertness 2006).

The magnitude of temperature amelioration by oysters was dependent on both the intensity and duration of the heat stress, but was also determined by the structural traits and density of the oyster habitat. Whereas the structural traits of oysters had little influence on the amelioration of average summer temperatures, at extreme temperatures (up to 12^{o}C warmer than the average) structural configurations that provided vertical relief up to ~10 cm above the substrate provided cooler maximum and minimum temperatures, and greater thermal heterogeneity, than horizontally configured oyster habitat with low vertical relief (~2.5 cm: Chapter 5). Furthermore, the greater the density of oyster habitat, the more it disrupted the temperature gradient we produced by manipulating substrate colours (Chapter 3). Observations from a diversity of engineers and habitats suggest that the structural height and density of ecosystem engineered habitat are key population-level traits for maintaining positive interactions through stressful conditions (Bell & Westoby 1986, Fonseca et al. 1996, Lenihan 1999, Drezner 2006, Irving & Bertness 2009). As such, oyster restoration efforts targeting climate amelioration must achieve sufficient vertical relief above the substrate by restoring densities of oysters that encourage vertical growth patterns.

The efficacy of stress amelioration by ecosystem engineers not only depends on environmental context, but also how intraspecific variations in traits of the ecosystem engineer interact with the environment (Bruno and Kennedy 2000, Cartwright and Williams 2014). Here, the importance of a population-level trait - the spatial configuration of oysters - in determining climate amelioration was dependent on aerial exposure (Chapter 5). Whereas both vertically and horizontally orientated oysters ameliorated climatic extremes experienced by gastropods and chitons during short periods of aerial exposure, only vertically orientated

oysters continued to serve this role at longer periods of exposure. For the oysters themselves, the climate buffering benefits of aggregating was restricted to vertically orientated configurations under extreme temperature stress, presumable because their vertical orientation exposed less surface area that was available for thermal absorption (Helmuth 1998). Similarly, intraspecific crowding by barnacles provides group benefits at extreme temperatures (Bertness and Leonard 1999). Here, increasing oyster density resulted in greater temperature amelioration and supported generally higher oyster survivorship (Chapter 3). Where greater survivorship of an ecosystem engineer results in greater habitat formation, positive feedbacks are likely to result in habitat expansion (Jones et al. 2010). The collapse of positive interactions as stress levels surpass the habitat amelioration capacity of the engineer (Michalet et al. 2014) may lead to deviations in biogeographic patterns of ecosystem engineering from Bertness and Callaway's (1994) stress gradient hypothesis. Therefore, the inclusion of positive interactions into conservation management will require understanding not just where and when ecosystem engineers will facilitate, but how the individual and population-level traits determine this capacity.

6.3 RESILILENT FOUNDATIONS FOR THE FUTURE?

Restoration efforts have traditionally focused on returning degraded systems to a natural, pre-disturbed condition, without considering whether native assemblages are resilient to contemporary conditions (see Harris et al. 2006). Novel conservation strategies, such as implanting resilient genotypes into degraded systems (van Oppen et al. 2015), species translocations (Harris et al. 2006), and using invasive species to stabilise habitats (Hobbs et al. 2009) are increasingly being discussed in order to achieve restoration goals in the face of climate change. Restoration projects that establish environmentally resilient ecosystem engineers will continue to benefit from positive species interactions as the environment

changes (Halpern et al. 2007). Conversely, where ecosystem engineers struggle to adapt to environmental change, negative effects will occur as their habitat degrades (Coleman and Williams 2002). Novel restoration strategies may provide a more realistic solution to achieving relative environmental stability and valuable ecological outcomes than re-establishing historical assemblages (Hobbs et al. 2009, Jones and Monaco 2009).

To build environmental resilience, the selective breeding of habitat-forming species for tolerance to climate stressors is increasingly being discussed (Jones and Monaco 2009, van Oppen et al. 2015). Selectively breeding climate-proof genotypes of commercially important species is a key management strategy for numerous aquaculture industries (Doubleday et al. 2013), and may be appropriate for ecological application where contemporary environmental stressors are altering environmental conditions at a faster rate than that at which wild genotypes can adapt (Jones and Monaco 2009). However, increased environmental resilience may trade-off against other important life-history traits (Stearn 1989, Pörtner et al. 2004).

Selective breeding of *S. glomerata* by the New South Wales aquaculture industry for the fast growth and disease resistance has also inadvertently enhanced the resilience of early developmental processes and stages to ocean acidification (Parker et al. 2011, Anderson et al. 2015, Goncalves et al. 2016,). Oysters respond to a broad diversity of environmental stressors with a generic, inducible stress response involving antioxidant enzymes, molecular chaperons and the cytoskeleton, in order to maintain cellular homeostasis during stressful conditions (Anderson et al. 2015). Under scenarios of ocean acidification, the larvae of *S. glomerata* mass-selected for fast growth and disease resistance were found to be more resilient than non-selected oyster larvae in their development and growth rates (Parker et al. 2011, 2012), and had a greater adaptive cellular stress response to elevated CO_2 (Goncalves et al. 2016). Contrary to these studies on oyster larvae, we found that although juvenile oysters (~6 months old) from

the mass-selected line grew faster in the coolest treatments, as temperatures increased they suffered greater mortality and slower growth rates, resulting in less habitat production and, consequently, providing an inferior climate refuge, that facilitated fewer invertebrates than wild-type oysters (Chapter 3). These findings agree with those of the proteomic study by Thompson et al. (2015), who found that adults from this same mass-selected line showed cellular dysfunction under elevated CO_2 conditions. Thompson et al. (2015) suggested that the increased metabolic potential of mass-selected oysters during their larvae stage may come at a cost to the adult's adaptive capacity when exposed to elevated CO_2.

Selection to enhance desirable traits may result in trade-offs whereby resources are diverted away from other processes that are fundamental to maintaining cellular function and resilience to unselected pressures (Stearns 1989, van Oppen et al. 2015). Ontogenetic resource reallocation is metabolically costly, and may trade-off against the organism's potential for growth and reproduction (Pörtner et al. 2004), with such trade-offs potentially varying with life-history stage (Thompson et al. 2015). The greater metabolic investment of mass-selected oyster larvae relative to the wild-type oysters during early developmental stages (Parker et al. 2012) appears to have diminished the environmental resilience of thermally stressed juveniles in the field, reducing their capacity to facilitate biodiversity (Chapter 3). As the selection of traits that benefit aquaculture produced an oyster genotype with inferior temperature tolerance in the field, breeding programs specifically selecting for climate resilience may be required to produce appropriate genotypes for restoration projects.

Similar to the increased cellular response of mass-selected oysters to ocean acidification (Goncalves et al. 2016), we found that the transcriptional response of mass-selected oysters was greater than wild-type oysters in the hottest treatments, with generally greater expression of antioxidant enzymes and molecular chaperons (Chapter 4). In contrast, wild-type oysters

generally depressed their gene expression with rising temperatures, potentially in favour of metabolic suppression over physiological mitigation. Similarly, the lack of relationship between temperatures experienced and the osmolality of oyster haemolymph and mantle water in our artificial rocky shore experiment (Chapter 5), suggested that these oysters remained in a state of metabolic quiescence throughout the experiment. Upregulated gene expression during times of stress is typically interpreted as an indication of adaptive resilience to stress (i.e. Goncalves et al. 2016), however, a greater cellular stress response by mass-selected oysters somewhat contrasts their poor ecological performance over in the field (Chapter 3). Surprisingly, mass-selected oysters have a greater genetic diversity then wild-type breeding lines (Thompson 2015), which may explain both the greater cellular response and higher mortality during thermal extremes, with a greater range of phenotypes displayed relative to wild-type oysters.

Recent studies suggest that early life stages of wild *S. glomerata* populations may be poorly equipped to deal with future environmental change, particularly those oysters in exposed intertidal habitats (Goncalves et al. 2016, Scanes et al. 2017). However, we found that some wild-type oysters in this study were incredibly resilient, surviving extreme maximum temperatures on black pavers of up to 58^{o}C (at Tilligerry Creek: Chapter 3). The high phenotypic plasticity of wild *S. glomerata* populations (Scanes et al. 2017) suggests they possess the genetic material from which greater climate resilience could be selected for. Restoration projects must prioritise resilient genotypes and genetic diversity when establishing new projects (Jones and Monaco 2009, Prober et al. 2015). Additionally, populations of *S. glomerata* that have low genetic diversity and are vulnerable to climate change could receive genetic enhancement from distant populations within their broad distribution (van Oppen et al. 2015), particularly from populations adapted to warmer climates.

Gene expression that influences the traits with which an individual interacts with other species may extend an organism's phenotype from the individual- to the ecosystem-level (Whitham et al. 2003). Some genetic processes can produce predictable ecosystem-level effects, however scaling up molecular processes to predict organismal and ecosystem-level interactions risks neglecting environmental influences occurring at each organisational level, and should therefore be investigated in compliment (Doney et al. 2004, Whitham et al. 2006). Our investigation from sub-cellular to community-level processes demonstrated a capacity of *S. glomerata* to adapt with climate, and recognised there is sufficient phenotypic diversity for selective breeding programs to specifically target climate resilience. As is the case here, this genes-to-ecosystem approach has particular relevance when selection for environmental traits alters an organism's sub-cellular stress response, and may allow us to pin-point which genotypes are most suitable for use in restoration projects (Whitham et al. 2006).

Considering that over 99% of oyster populations have been extirpated from the Australian coastline over the past two centuries (Beck et al. 2011), the restoration potential for these foundation species is enormous (Gillies et al. 2015). Although oyster reefs have not recovered since their mass exploitation ceased, Australia's flourishing oyster aquaculture industry suggests that coastal conditions are still suitable to support large bivalve populations (O'Connor and Dove 2009). Incorporating resilient and diverse genetic stock will be important to ensure restored populations are equipped to response to future climate change (Prober et al. 2015).

6.4 FINAL THOUGHTS

Anthropogenic greenhouse gas emissions have rapidly increased atmospheric concentrations to levels not seen in the past 800,000 years, forcing a rate of global environmental change that will result in high levels of species extinctions (Pachauri et al. 2014). The severity and

frequency of extreme temperature events are fast increasing, with mean global surface temperatures predicted to increase by up to 4.8^{o}C by end of this century (Pachauri et al. 2014). Although intertidal habitats are dynamic, constantly-changing systems, this rate of warming will exceed the capacity of many intertidal species to adapt (Somero 2010). A single extreme heat event can result in the mass mortality and removal of entire intertidal communities (Tsuchiya 1983), therefore large biogeographic shifts and regional extinctions are expected, but where these occur may be difficult to predict (Helmuth et al. 2002, 2006).

As atmospheric temperature rises, the upper intertidal distribution of organisms is anticipated to shift downward (see Helmuth et al. 2006), potentially exposing organisms to primary predators leading to extirpation (Harley 2003). Furthermore, rising sea-levels, an increasingly acidified ocean, and human encroachment all hinder the capacity of intertidal fauna to response to change. Combined, these stressors are reducing the amount of habitable space available to intertidal organisms, and hence, refuge habitats that maintain more favourable conditions and increase habitat availability will be increasingly important in future (Crain and Bertness 2006). Conservation strategies that can maintain the refuge function of ecosystem engineers in the face of climate change appear essential to the functionality of intertidal communities over the coming decades.

Observations of positive interactions underpinning species distributions, community richness, and individual- and population-level fitness come from virtually every ecosystem on Earth, suggesting that facilitation is a near universal community-level process (see Bruno et al. 2003). The inclusion of facilitative interactions into ecological theory and conservation planning is in its infancy for marine habitats (Bulleri 2009), yet examples from projects restoring terrestrial plant communities demonstrate the benefits of habitat amelioration to local biodiversity (Gómez-Aparicio et al. 2004, He et al. 2013). For intertidal communities, physiological stress

is typically greatest during aerial exposure, and therefore facilitative processes may be analogous to those occurring in terrestrial habitats, such that the lessons learnt there can be applied (Bulleri 2009). Furthermore, investigating how positive interactions change over broad environmental gradients (Bertness and Callaway 1994) will increase our understanding of the robustness of facilitation as the climate changes. However, few marine examples over broad biogeographic scales exist (but see Silliman et al. 2011), reducing our understanding on how community-level processes operate at the landscape scale and our capacity to plan conservation strategies (Hobbs and Cramer 2008).

This thesis has addressed this knowledge gap, demonstrating that oyster habitat can reduce environmental pressures on intertidal communities over broad spatial and temporal scales. Consistent with the predictions of the stress gradient hypothesis (Bertness and Callaway 1994), the mechanisms of facilitation by oyster habitat varied with the magnitude and type of environmental stress, with positive interactions generally increasing with stress. Similar to species interactions in terrestrial plant communities (Drezner 2006, He et al. 2012, 2013), positive interactions were not just a function of the background environmental stress, but also fundamentally varied with the environmental resilience and structural traits of the oysters. Access to oyster habitat greatly increased the individual fitness of inhabiting organisms during periods of temperature stress, and may therefore provide an important spatial refuge to transient species during extreme climate (Cartwright and Williams 2012). Restoration projects using intertidal oysters must identify the trait-dependent thresholds to facilitation at extreme temperature stress, and aim to establish oyster populations at densities where positive structural-feedbacks result in the growth of the habitat (Jones et al. 2010).

The outcomes of this thesis provide knowledge of where, when and how oysters can support ecological communities on Australia's east coast. Ecosystem engineers with broad spatial

distributions across which positive interactions are maintained, such as *S. glomerata*, provide appropriate conservation targets as they can maximise the spatial and temporal capacity of conservation efforts (Hastings et al. 2007). Conservation and/or restoration of *S. glomerata* habitat at strategic locations of biological importance could provide localised conservation of vulnerable communities, habitat from which retreating organisms can recolonise degraded habitats when conditions are favourable, and increased landscape connectivity to assist the climate adaption of dispersing species (Hobbs and Cramer 2008, Keppel and Wardell-Johnson 2012). This thesis provides key information that can assist the development of a management strategy for *S. glomerata* habitat to assist the adaptation of intertidal species to climate change.

6.5 LITERATURE CITED

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