Sources of spatio-temporal variation in habitat provisioning by the Sydney rock oyster, *Saccostrea glomerata*

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For my grandparents.

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SUMMARY

Habitat-forming species provide structure that facilitates colonisation and survival of associated species. Along the east coast of Australia, there is growing interest in restoring reefs once formed by the Sydney rock oyster, *Saccostrea glomerata*, to enhance biodiversity, fisheries productivity and water quality. To provide information on when and where restoration efforts might be most successful, I investigated sources of spatial and temporal variation in the colonisation of oysters and their facilitation of invertebrates.

In many areas oysters are substrate limited and live or dead shell is added to facilitate reef growth. I assessed how the timing of substrate deployment, and its status as live or dead shell, in loose or consolidated arrangements influences oyster recruitment and the development of associated communities. I found that the timing of substrate deployment influenced oyster recruitment and community assembly by determining whether oysters were able to colonise prior to competitors such as barnacles and algae. Dense and diverse communities of invertebrates, however, colonised irrespective of whether substrate was live oysters or dead shell, or was consolidated or loose. Hence, the timing of substrate deployment will be critical to the success of restoration projects.

Although many restoration projects have focused on the rehabilitation of a single habitat-forming species, in most instances biodiversity is underpinned by interactions between multiple co-occurring habitat-forming species. I assessed how the presence of a second habitat-forming species influences habitat provisioning by *S. glomerata*. I found that in mangrove forests, *S. glomerata* and the co-occurring, habitat-forming alga *Hormosira banksii* had distinct, and additive effects on invertebrate recruitment and on mitigating predator-prey interactions. Hence, there may be benefits of restoring multiple habitat-forming species at a site.
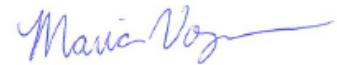
Finally, I investigated variation in facilitation of invertebrates by oysters across a

wave exposure gradient. Using a survey and a manipulative field experiment, I partitioned effects of wave energy on oyster communities into direct effects and indirect effects arising from responses of oyster morphology and density to wave energy. Across a wave exposure gradient, invertebrate abundance and richness, and oyster size and density were negatively correlated with wave energy. Indirect effects of wave energy on invertebrate communities, arising from effects on oyster density and morphology, were more important than direct effects.

Overall, my results support the important role of habitat-forming species morphology and environmental context in shaping positive interactions. To maximise the success of restoration, programs should target environments in which the growth forms of habitat-forming species have the greatest positive effect on biodiversity.

DECLARATION

I declare that this thesis entitled "Sources of spatio-temporal variation in habitat provisioning by the Sydney rock oyster, *Saccostrea glomerata*" is my own work and has not been submitted in any form for another degree or at any other University or institution. This thesis contains original material. Any additional help received during the preparation of this work has been indicated in the 'Contributions' section.

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CONTRIBUTIONS

Chapter 1: General Introduction

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

Chapter 2: The timing and type of substrate deployment influences recruitment of Sydney rock oysters, *Saccostrea glomerata*, and associated communities

Authors: Maria Vozzo and Melanie Bishop

My contribution to this research: Concept = 70%, Data collection = 100%, Data analysis = 100%, Writing = 80%, Total = 87.5 %. I received constructive feedback and suggestions from my supervisor, Melanie Bishop.

Preliminary results from research were presented at the Shellfish Reef Restoration Network (SRRN) in Sydney, Australia, May 2016 and at the Estuarine Coastal Science Association (ECSA) Annual Conference 56 in Bremen, Germany. September 2016.

Chapter 3: Co-occurring secondary foundation species have distinct effects on community assembly

Authors: Maria Vozzo and Melanie Bishop

This chapter has been prepared for submission to *Oecologia*. My contribution to this research: Concept = 70%, Data collection = 100%, Data analysis = 100%, Writing = 70%, Total = 85%. I received constructive feedback and suggestions from my supervisor, Melanie Bishop.

This research was presented at three conferences: 1) The Australian Marine Science Association Annual Conference (AMSA), Geelong, Victoria, Australia. July 2015; 2) The 5th International EcoSummit, Montpellier, France. August 2016; and 3) The Estuarine Coastal Science Association (ECSA) Annual Conference 56 in Bremen, Germany. September 2016.

I was awarded "Highly Commended Oral Presentation" for my presentation of this work at the ECSA conference in Bremen, Germany.

Chapter 4: Wave energy alters biodiversity by shaping intraspecific traits of a habitat-forming species

Authors: Maria Vozzo, Vivian Cumbo, Joseph Crosswell and Melanie Bishop

This chapter has been prepared for submission to *Ecology*. My contribution to this research: Concept: 60%, Data collection = 90%, Data analysis = 90%, Writing = 60%, Total = 75%. I received constructive feedback and suggestions from my two supervisors for this project, Vivian Cumbo and Melanie Bishop, and from my co-author, Joseph Crosswell. I also received help in invertebrate identification from Jade Jang.

This research was presented at The Australian Marine Science Association (AMSA) annual conference, Darwin, Northern Territory, Australia. July 2017. This chapter was also presented at the 2017 Macquarie University Department of Biological Sciences Annual HDR Conference for which I won the award "Best Presentation of Field Based Research, Runner Up".

Chapter 5: General Discussion

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

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I. GENERAL INTRODUCTION

PROCESSES THAT SHAPE COMMUNITY ASSEMBLY

The question of how species co-exist is fundamental to ecology. The composition of ecological communities is of critical importance in determining the ecosystem services they provide, their stability and resilience (Worm et al. 2006, Cadotte et al. 2011). Communities that contain a diversity of functional groups generally support a wider range of ecosystem services than those with few functional groups (Duarte 2000, Díaz and Cabido 2001). Additionally, those that are species rich are more likely to contain functionally important species, and functional redundancy among species that buffers the functional consequences of species loss (Tilman et al. 1997, Hooper et al. 2005). Consequently, processes that shape community assembly have long been the focus of ecological research (MacArthur 1955, Levin and Paine 1974, Connell 1978, Chesson and Case 1986, DeAngelis and Waterhouse 1987, McCann 2000).

Early ecological theories for coexistence comprised equilibrium models, formulated on the assumption that over time the number and type of species comprising a community stabilises (MacArthur 1955, Elton 1958). Other early ecological models argued that this stability was maintained by ecosystem complexity (MacArthur 1955, Elton 1958, Hutchinson 1959, Margalef 1968). For example, MacArthur (1955) proposed that a system with diverse predator and prey species would be less subject to fluctuations in overall population abundance caused by interspecific interactions that led to changes in abundance of individual species. Greater species diversity of species should result in a variety of alternative resources (i.e. food or space) that maintain the system. Such ecological models are based on the idea that each species can be modelled on the basis of its fundamental niche – the set of environmental conditions under which a species can survive (Hutchinson 1957, 1959). Where there is niche overlap, species will compete for resources, and coexistence

may be dependent on niche partitioning, reductions in niche breadth, or an abundant supply of resources (MacArthur and Levins 1967, Pianka 1969, Colwell and Futuyma 1971, Sale 1974, Schoener 1974). According to equilibrium models, species interactions such as competition and predation are critical to the assembly of communities. For example, keystone predators play a critical role in maintaining community assembly (Paine 1969). Keystone predators (e.g. starfish, Paine 1966; sea otters, Estes and Palmisano 1974) consume dominant competitors within a system which allows less-competitive species to persist. If removed, superior competitors will dominate communities resulting in an altered, less diverse community structure (Paine 1966, Estes and Palmisano 1974).

Non-equilibrium models, by contrast, recognise that the structure of communities varies in time and space due to the influence of stochastic processes such as disturbance (Connell 1978, Huston 1979, Sousa 1984, Reice 1994). Disturbances can be frequent and predictable (i.e. frost or freezing), or rare and unpredictable (i.e. fires), with the disturbance regime determining community assembly (Levin and Paine 1974, Pickett 1980, Reice 1994). For example, the Intermediate Disturbance Hypothesis posits that intermediate levels or frequencies of disturbance within a system maintain maximum species diversity (Connell 1978). Disturbance maintains nonequilibrium states where frequent successional processes determine community structure (Connell 1978, Sousa 1979). Sousa (1979) studied algae community assemblages within intertidal boulder fields and found that boulders with intermediate levels of wave-induced disturbance supported the most diverse algal communities. Although disturbance can maintain diversity, the successional process may be shaped other factors.

Stochasticity in the timing of reproductive events or processes (e.g. wind, currents, waves) that influence propagule dispersal can also influence community assembly (Tokeshi 1994). For example, the lottery hypothesis was proposed to describe how reef fish

community development is shaped by factors such as larval supply, habitat availability, and stochastic variation in current movement (Sale 1977). In the lottery hypothesis, Sale (1977) proposed species colonisation habitat was dependent upon the chance order of propagule arrival of propagules to a site rather than interspecific interactions after settlement. Thus, community assembly may be shaped by broader processes that alter dispersal patterns rather than abiotic or biotic interactions, alone.

THE ROLE OF POSITIVE INTERACTIONS IN PROMOTING BIODIVERSITY

Whereas the importance of negative interactions, such as predation and competition, in influencing biodiversity has been recognised in both equilibrium and non-equilibrium models of community assembly, (e.g. Hutchinson 1961, Paine 1966, Menge and Sutherland 1976, Connell 1978), the role of positive interactions was, historically, largely overlooked. However, increasing evidence suggests that positive interactions such as facilitation or mutualism may be just as important as negative interactions in supporting diverse communities and range from obligate to facultative (Vance 1978, Callaway 1995, Stachowicz and Hay 1996, Bertness and Leonard 1997, Stachowicz 2001). For example, the mutualistic interaction between corals and their associated zooxanthellae algae create complex habitats that facilitate fish and invertebrates, and ultimately maintain some of the most biodiverse hotspots in the world (Roberts et al. 2002). Habitat-forming species facilitate dense and diverse communities in a variety of terrestrial and aquatic environments by creating structure that ameliorates abiotic and biotic stressors (Stachowicz 2001, Bruno et al. 2003). For example, in physically stressful environments such as deserts or intertidal rocky shores on which organisms may experience significant temperature and desiccation stress during midday low tides, habitat-forming species support high biodiversity by providing shade or retaining moisture (Holzapfel and Mahall 1999, McAfee et al. 2016). Further, where biotic interactions are prevalent, the complex structures created by habitat-

forming species can reduce negative biotic interactions such as predation (Coull and Wells 1983, Finke and Denno 2002, Grabowski 2004). For example, predation of copepods and meiofauna on intertidal rocky shores was mitigated by the complex coralline algae substrate, but not by other macroalgae with less complex structures (Coull and Wells 1983).

Early studies investigated the effects of facilitation by single habitat forming species in isolation (e.g. Orth et al. 1984 and references therein, Allen and Allen 1988, Seed 1996 and references therein, Bruno 2000). However, there is growing recognition that habitat-forming species can co-occur in space and time (e.g. Altieri et al. 2007, Bishop et al. 2012), in either adjacent or nested assemblages (Angelini et al. 2011). Environmental factors and the traits of habitat forming-species may influence their spatial configuration within landscapes, and the way in which they interact to influence biodiversity.

FACILITATION ACROSS ENVIRONMENTAL GRADIENTS

Although at landscape scales, habitat forming species positively affect biodiversity by adding habitat heterogeneity and, hence, niches, at smaller scales habitat-forming species may have positive, negative or neutral effects on the abundance of individual species (Jones et al. 1997). For example, habitat that supports prey survival by providing protection from predators could also be interpreted as reducing predator fitness (Gotceitas and Colgan 1989, Grabowski and Powers 2004). Or, complex habitat structure that alters water flow and enables larval settlement (Hata et al. 2017), might simultaneously diminish food availability for species that feed effectively in high water flow environments (Bertness et al. 1991). The number and types of resource flows or characteristics that are modified by habitat-forming species (i.e. light availability and temperature, Lebrija-Trejos et al. 2010; or snowfall and soil chemistry, Kreyling et al. 2012 within forest understories) and the species assemblages that depend on these resource flows will determine whether effects on biodiversity are positive or negative.

The Stress Gradient Hypothesis predicts that positive species interactions will be more prevalent in physically or biologically stressful environments, than in benign environments in which negative interactions, such as competition, instead dominate (Bertness and Callaway 1994). In physically stressful environments, habitat-forming species ameliorate 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, biological stresses, such as predation, tend to increase, and habitat-forming species may facilitate biodiversity by providing refuges from predation and by providing structures that weaken interference competition (Bertness and Callaway 1994, Grabowski and Powers 2004). Further, where biological stress such as consumer pressure is high, positive interactions such as associational defences help maintain survival (Bertness and Callaway 1994, Hay 1986). In the absence of physical or abiotic stress, facilitative interactions may become competitive (Bertness and Callaway 1994).

Support for the stress gradient hypothesis has come from a variety of habitats including alpine (Callaway et al. 2002) and subalpine (Callaway 1998) plant communities, and intertidal marine environments where numerous studies have demonstrated the importance of facilitation along stress gradients (e.g. mussels and barnacles, Kawai and Tokeshi 2007; oysters, McAfee et al. 2016; saltmarsh, Bruno 2000). In the aforementioned studies, habitat-forming species facilitate communities notwithstanding the abiotically stressful conditions. Thus, the capacity of habitat-forming species to ameliorate environmental extremes may be contingent on their ability to survive and continue to form habitat under stressful conditions (McAfee et al. 2017). Nevertheless, some habitat-forming species, such as bivalves, are able to continue to facilitate biodiversity even following death due to the persistence of hard structures, such as shells (Lenihan 1999, Gutiérrez et al. 2003).

TRAIT-DEPENDENCE OF FACILITATION

Whether net effects of habitat forming species on biodiversity are positive or negative may also be determined by their density (Curtis and Vincent 2005, Chu et al. 2008, Bishop et al. 2012), morphology (Bishop et al. 2009, 2013) or the longevity of the habitat modifications they create (Lenihan 1999) – traits that may vary both among and within species according to genetic and environmental influences. The density and morphology of habitat-forming species may influence both the amount of habitat they provide to associated species (Finke and Denno 2002, Fahrig 2003, Grabowski 2004) and also their ability to ameliorate environmental stressors, for example high water flow (Peterson et al. 2004). Large- and small-scale density-dependent effects of habitat forming species on biodiversity have been described for a variety of species and ecosystems (i.e. habitat fragmentation of forests, Laurance et al. 2006; or seagrass, Hovel and Lipcius 2001).

Most studies have focused on how interspecific variation in traits influences facilitation (e.g. Altieri et al. 2007, Dijkstra et al. 2012, Bishop et al. 2013, Angelini and Silliman 2014). Species with divergent traits tend to facilitate different communities, even within the same system (Bruno et al. 2003, Bishop et al. 2013, Angelini et al. 2015). Variation in the morphological traits of habitat-forming species enhances the number of different microhabitats present within a habitat, and the resources or conditions that are modified (Angelini and Silliman 2014). By contrast, co-occurring habitat-forming species that have similar traits tend to be functionally redundant and may, instead, compete for resources (i.e. Wilkie et al. 2012). Thus, the benefits to biodiversity are generally greatest when multiple habitat-forming species with divergent morphological traits co-occur in nested or adjacent configurations (Angelini et al. 2011, Bishop et al. 2012, Spasojevic and Suding 2012, Angelini et al. 2015). Indeed, there is growing interest in how secondary habitat-forming species, which are part of the species assemblage that is obligately or

facultatively facilitated by a primary habitat-forming species, enhance biodiversity over that sustained by the primary habitat forming species alone (Bishop et al. 2012, 2013, Angelini and Silliman 2014, Gedan et al. 2014).

Just as interspecific variation in traits can influence community assembly, so too can intraspecific variation (e.g. Bishop et al. 2009, 2012, 2013). Although intraspecific trait variation is often assumed to be small as compared to interspecific variation, studies have demonstrated that intraspecific trait variation is in some instances sufficient to influence whether habitat forming species are able to sufficiently protect associated species from physical stressor such as temperature and desiccation stress (McAfee et al. 2017), reduce competition (Bertness 1989, Schutte and Byers 2017,), or alter predator-prey interactions (Grabowski 2004). In some instances, the impacts on biodiversity of such intraspecific trait variation may be equally or of greater importance than interspecific variation in the traits of habitat-forming species (e.g. van Hulzen et al. 2007, Harley and O'Riley 2011, Bishop et al. 2009, 2012, 2013).

INCORPORATING FACILITATION THEORY INTO RESTORATION ECOLOGY

Ecological restoration is often described as the "acid test" of how well ecologists understand an ecosystem, and has been an important test ground for ecological theories about succession and community assembly (Bradshaw 1987, Young et al. 2001, Young et al. 2005). Restoration projects often involve the reintroduction or rehabilitation of populations of habitat-forming species and/or ecosystem engineers – species that create, maintain or destroy habitats – due to the important role they play in facilitating and maintaining biodiversity (Byers et al. 2006, Crain & Bertness 2006, Marzinelli et al. 2016). Such species may be used to convert unfavourable abiotic or biotic conditions to those that support the characteristic biodiversity of an ecosystem (Jones et al. 1994, Crain & Bertness 2006, Byers et al. 2006). They may also add habitat structure to substrate limited environments (Dame

1979, Buhl-Mortensen et al. 2010, Bishop et al. 2012). Successful use of habitat-forming species and ecosystem engineers in restoration, however, requires knowledge of the range of environmental conditions under which such species will survive and form habitat, as well as knowledge of how their individual- (e.g. morphology) and population-level (e.g. density) traits interact with the environment to modify conditions (Hammond and Griffths 2004, Bishop et al. 2009, 2012, 2013, Hughes et al. 2009).

Additionally, successful use of habitat-forming species in restoration requires knowledge of how their biological interactions vary in time and space, as a function of their size and developmental stage (Hastings et al. 2007). For example, what may later become a competitive interaction between trees and shrubs, may begin as facilitative interactions whereby shrubs shade threatened tree seedlings from harsh climatic conditions (Gomez-Aparicio et al. 2004). Further, spatial variation in the size or density of reef-building ecosystem engineers such as corals or shellfish will alter their facilitative ability to provide habitat (Lenihan 1999, Lenihan et al. 2011, Graham and Nash 2013). Identifying the mechanisms by which habitat-forming species facilitate biodiverse communities, and how these vary in time and space, is crucial to successfully restoring habitat-forming species and their associated communities.

OYSTER REEF RESTORATION

Globally, >85% of native oyster reefs have been lost, largely due to historic overharvest for food and lime using destructive harvesting practices, such as dredging that remove not only spawning stock biomass from the ecosystem, but also shell substrate (Beck et al. 2011). More recently, declining water quality, that has both directly and indirectly through resultant disease outbreaks caused oyster mortality, as well as habitat modification through coastal development, have hampered recovery (Beck et al. 2011). Oyster reefs provide numerous ecosystem services such as improvement of water quality through

filtration (Newell and Koch 2004, Piehler and Smyth 2011), shoreline stabilisation (Scyphers et al. 2011) and the production of juvenile oysters that are an important food supply and augment reef production (Lenihan and Peterson 1998). Further, the provision of hard substrate and complex habitat by oysters protects juvenile fish and invertebrates from predation or stress, enhancing native biodiversity and underpinning fisheries productivity (Posey et al. 1999, Peterson et al. 2003, Grabowski et al. 2005, Tolley and Volety 2005, Cole et al. 2007).

With increasing recognition of the magnitude of the loss of native oyster populations, and of associated ecosystem services, there has been growing global interest in restoring oyster reefs (Coen and Luckenbach 2000, Ruesink et al. 2005, Grabowski and Peterson 2007, Beck et al. 2011, La Peyre et al. 2014b). Oyster reef restoration has been practiced for over two decades in the United States, and has been accompanied by a solid program of scientific monitoring and evaluation to inform restoration efforts (Powers et al. 2009, Schulte et al. 2009, La Peyre et al. 2014a). By contrast, there have been relatively few attempts to restore oyster reefs in areas with huge native species loss, such as Australia (e.g. Gillies et al. 2015b, 2017). Although lessons from United States oyster reef restoration projects may be instructive for fledgling projects elsewhere, for restoration to be successful the local factors that are limiting recovery need to be well-understood.

The successful restoration of oyster reefs requires there to be suitable local environmental conditions (e.g. water quality, salinity) to support oyster growth, survival and reproduction (Lenihan and Peterson 1998, Coen and Luckenbach 2000), a source of larvae to recruit to restored habitats (Brumbaugh and Coen 2009, and references therein), adequate substrate for oyster settlement (Brown et al. 2014, Dunn et al. 2014) and predator and competitor populations that are insufficiently large to hamper recovery (Underwood and Anderson 1994). Hence, after it has been established that a site possesses suitable

environmental conditions to support oyster reefs, assessment is needed of whether recovery is limited by the availability of larvae or substrate (Geraldi et al. 2013). If the availability of larvae is limiting, spawning stock biomass may be added through transplants of oysters from remnant reefs or from aquaculture facilities. These transplants should be certified as disease free. If substrate needs to be added, consideration should be given as to the optimal timing and type of substrate to be added, so as not to facilitate competitors that pre-empt space (Underwood and Anderson 1994) or non-native species (Wilkie et al. 2012), and the spatial configuration of substrate that will best support healthy, structurally resilient reefs (Dunn et al. 2014, La Peyre et al. 2014a). The sites that should be prioritised for restoration are ideally those that will produce the greatest benefits, in terms of oyster population growth and restoration of associated ecosystem services. Considerations may include the proximity of the restored oyster reef to other habitat-forming species with which additive or synergistic effects on biodiversity might be seen (Peterson and Lipcius 2003).

TEMPERATE AUSTRALIAN OYSTER REEFS

Along the temperate Australian coastline, oysters once formed extensive reefs but are now considered functionally extinct (Beck et al. 2011). Temperate systems of Australia supported two main reef-forming oysters with largely distinct distributions: the flat oyster, *Ostrea angasi*, which is found in low intertidal to subtidal habitats from southern New South Wales (37.322°S, 149.978°E) and south around Tasmania (43.641°S, 146.720°E), to Western Australia (34.370°S, 115.132°E); and the Sydney rock oyster, *Saccostrea glomerata*, which is found from southern Queensland (21.068°S, 149.244°E) to northern Victoria (37.550°S, 149.824°E), in the mid to low-intertidal range (Gillies et al. 2015a).

This thesis focuses on the provision of habitat to invertebrates by *S. glomerata* populations. Although *S. glomerata* once formed extensive reefs, it is now predominantly limited to small patches within mangrove forests, where it is a secondary habitat-forming

species, attached to mangrove roots, and on the rocky shore, where it is a primary habitat-forming species directly attached to rock (McAfee et al. 2016). In each habitat, oysters facilitate dense and diverse invertebrate communities, by adding hard substrate, and by mitigating abiotic and biotic stressors (Bishop et al. 2012, McAfee et al. 2016). In addition, the Sydney rock oyster supports the largest aquaculture industry in New South Wales, now based largely on grow-out of hatchery produced oysters (NSW DPI 2017). The aquaculture industry provides a potential source of spawning stock biomass for oyster reef restoration projects, both through spill-over from farms and transplant of hatchery produced oysters to sites limited by a source of recruits.

THIS THESIS

The overall aim of this thesis is to investigate the mechanisms by which the habitat-forming Sydney rock oyster, *S. glomerata*, maintains invertebrate biodiversity along the east Australian coast, when and where its effects on invertebrate biodiversity are greatest and factors limiting its habitat provision. Over 80 per cent of Australia's plants and animals are endemic and, in the marine environment, we have one of the most species-rich and diverse invertebrate faunas on earth (Wilson and Allen 1987). Marine invertebrates underpin fisheries productivity, providing the vital link between primary producers and higher trophic levels, are critically important in nutrient cycling, and filter feeders are important in maintaining water quality (Peterson and Heck 2001, Newell et al. 2002, Peterson et al. 2003, Newell and Koch 2004, Coen et al. 2007, Piehler and Smyth 2011). Increasingly, however, Australia's biodiversity and the enormous economic value that it represents is under threat from human impacts such as land use change, regulation of streams, invasive species, over-harvesting of commercially valuable species and climate change (e.g. Saintilan and Williams 1999, Hughes 2003, Ogburn et al. 2007, Kroon et al. 2012). Hence, understanding those processes that support and maintain invertebrate biodiversity is of critical importance.

In **chapter 2**, I first describe monthly substrate deployments at 5 estuarine sites of Sydney, New South Wales, Australia to assess: (1) whether there is a supply of oyster recruits to each; (2) the timing of oyster recruitment; and (3) the timing of oyster recruitment as compared to recruitment of other invertebrates (barnacles and mussels) and algae that may compete with oysters for space and other resources. Additionally, I examine how the type of substrate provided (i.e. live oysters, dead oysters, or a mix; of loose or consolidated form) influences oyster recruitment, as well as associate community development. Previous studies suggest that the timing and type of substrate deployed in estuarine and coastal settlings can greatly influence the trajectory of fouling community development (e.g. Anderson and Underwood 1994, Underwood and Anderson 1994, Nell et al. 2000), and I expect strong interacting effects of the type of substrate deployed, and the timing of its deployment on community assembly. Given the increasing interest in restoring native oyster reefs in Australia, it is important to understand factors limiting establishment of oyster reefs, and methods of restoration (i.e. type of substrate deployed; timing of substrate deployment) that might maximise the success of projects. While international oyster restoration projects provide case studies on approaches to oyster reef restoration that have led to successful outcomes (i.e. Peterson et al. 2003, Schulte et al. 2009, Geraldi et al. 2013, La Peyre et al. 2014a), factors limiting oyster establishment and community development are likely to vary spatially.

Chapters 3 and **4** assess how the morphology and spatial arrangement of habitat-forming species affects biodiversity of associated communities. In **chapter 3**, I compare the role of oysters and another habitat-forming species, the alga *Hormosira banksii*, in promoting invertebrate recruitment and mediating predator-prey interactions, and examine the ways in which the two species interact to influence biodiversity, where they co-occur. In mangroves of southeast Australia, the free-floating fucalean algae, *H. banksii*, and Sydney

rock oysters are each secondary habitat-forming species, each partially or wholly dependent on the root structure of the primary habitat-forming species, the mangrove, and may overlap in space. There has been increasing recognition of the important role secondary habitat-forming species have on associated communities (Thomsen et al. 2010, Angelini et al. 2011, Bishop et al. 2012). Interactive effects of *H. banksii* and *S. glomerata* on community assembly have been identified in temperate Australian mangroves (Bishop et al. 2012, Hughes et al. 2014), but the differential mechanisms by which they influence predator-prey interactions, as well as the recruitment and survival of invertebrates has not been examined, nor how the two species interact to influence these processes. I expect that the two secondary habitat-forming species will differ in their effects on predator-prey interactions and on invertebrate recruitment, but that effects will be additive as opposed to interactive where the two habitat-formers co-occur.

Finally, **chapter 4** investigates how facilitation of invertebrates by oysters varies across a wave exposure gradient, both as a direct consequence of spatial variation in the wave stressor, and as an indirect effect of intraspecific variation in oyster morphology across the gradient. On rocky shores of New South Wales, *S. glomerata* is one of the dominant habitat-forming species that facilitates biodiverse communities (Hedge et al. 2013, McAfee et al. 2016). The effect of wave energy on the morphology of rocky shore inhabitants has been described for a variety of habitat-forming species (Taylor and Schiel 2003, Steffani and Branch 2003, Wernberg and Thomsen 2005). However, there are a paucity of studies examining how effects of wave energy on the morphology of habitat-forming species cascades to influence associated invertebrates (but see Hammond and Griffiths 2004, Lunt et al. 2017). A field survey of 9 sites is coupled with a manipulative field experiment to disentangle direct versus indirect effects of wave energy on invertebrate communities supported by oysters. I predict that oyster habitat complexity will be negatively correlated

with wave energy, and that associate invertebrate abundance and richness will be positively correlated with habitat complexity, such that invertebrate abundance and richness decrease across the gradient of increasing wave exposure. Further, I expect that manipulative experiments will reveal indirect effects of wave energy on invertebrate communities, arising from changes in morphology will be just as great, if not greater, than direct effects.

Understanding when and where *S. glomerata* will form habitat structure of greatest value to invertebrates is critical to prioritising and planning restoration projects that maximise benefits to coastal ecosystems. In particular, understanding how anthropogenic activities modify habitat provision is imperative for developing strategies for maintaining biodiversity in urban settings.

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II.   THE TIMING AND TYPE OF SUBSTRATE DEPLOYMENT INFLUENCES RECRUITMENT OF SYDNEY ROCK OYSTERS, *SACCOSTREA GLOMERATA,* AND ASSOCIATED COMMUNITIES

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SAMPLING CONDUCTED FOR CHAPTER 2

Due to the time constraint of a field-based, three-year PhD, the number of sampling periods were reduced for the body of work of this thesis. However, I recognise that longer temporal studies are necessary to draw conclusions about seasonal variation of community development. Thus, additional sampling times were conducted and will be processed for the final manuscript version of this data chapter.

Table i. Sampling periods for each study component of Chapter 2 that are included in this thesis and sampling periods that will be included in the final manuscript of this study.

		Sampling Periods	
Study Component		*This Thesis*	*Final Manuscript*
Timing of sessile species	*Monthly sampling*	December 2015 to August 2017	December 2015 to December 2017
	Testing time of deployment and duration in the field	January to August 2017	January to August 2017
Influence of substrate	Colonisation of sessile and mobile community	Four sampling periods (one full year): November 2015 to December 2016	Eight sampling periods (two full years): November 2015 to December 2017
	Colonisation within loose or attached substrate	One sampling period: January to April 2017	Four sampling periods (one full year): January to December 2017

ABSTRACT

Community development is dependent upon propagule supply, environmental factors, and interspecific interactions. Restoration of shellfish reefs typically involves the deployment of substrate, and/or live oysters that provide spawning stock biomass and recruitment cues for conspecifics. Substrate deployed just prior to oyster recruitment may favour oyster community development, while substrate deployed too early or late may favour recruitment of competitors of oysters, thereby altering the trajectory of community development. This project assessed how the type and the timing of substrate deployment influences recruitment of Sydney rock oysters, and associated invertebrates, in south eastern Australia. At five estuarine sites in Sydney, New South Wales live oysters, dead oyster shells, or a mix of the two were deployed and retrieved during four sampling times, in one year. In the second year, additional substrate was deployed in the summer when oysters and key competitors recruit to 1) disentangle effects of the timing and duration of substrate deployment, and 2) assess how the deployment of shell in a loose or attached form affects community development. Recruitment of oysters and barnacles was greatest during the summer months, with barnacle recruitment peaking just prior to oyster recruitment. Recruitment of each was greater when at least some dead oyster shell was present, than when live oysters were deployed alone, and was not influenced by whether shells were loose or attached in clumps. Effects of the timing of substrate deployment on the structure of developing communities were apparent after one and three months, but had weakened by six months. While sessile invertebrate recruitment peaked during warm summer months, the diversity of mobile invertebrates was generally greatest in the winter. Our results suggest that oyster reef recovery in the Sydney region may be limited by the availability of substrate rather than larvae. Projects seeking to restore reefs through the addition of substrate should consider the

type and timing of substrate that is deployed in order to facilitate establishment of oyster reefs as opposed to alternative communities.

Keywords: colonisation; disturbance; restoration; oysters; shell; habitat; oyster reef; barnacles; invertebrates

INTRODUCTION

Mechanisms determining community assembly have long been of interest to ecologists, both from the fundamental perspective of explaining species co-existence (Pianka 1966, Connell 1978, Chesson 2000), and the applied perspective of restoring or rehabilitating degraded ecosystems (Hulvey and Aigner 2014). Early equilibrium models focused on the role of negative species interactions such as predation and competition in shaping community assembly (Connell 1961, Paine 1966, Paine 1974, Menge and Sutherland 1976). However, later models recognised that communities are not necessarily at equilibrium, with composition in some instances determined by the order in which species colonise (e.g. lottery hypothesis, Sale 1977). Ecological filter models integrate equilibrium and non-equilibrium models by proposing that three broad filters determine the final composition of communities: (1) the dispersal filter, whereby dispersal barriers and vectors influence the frequency and number of arriving propagules that arrive at a site (Zobel 1997, Baums et al. 2006); (2) the abiotic filter, whereby environmental conditions determine the establishment and survival of arriving species (Belyea and Lancaster 1999, Chase 2007); and (3) the biotic filter, whereby biotic interactions, including both positive and negative, at the site influence the likelihood of establishment and survival (Roughgarden and Diamond 1986, Belyea and Lancaster 1999, Lebrija-Trejos 2010). In degraded ecosystems, understanding which of

these processes is limiting community recovery can help to identify whether species transplants, environmental remediation and/or species eradications are required for rehabilitation (Hulvey and Aigner 2014).

On marine hard substrata, space is often a limited resource for which competition is intense (Connell 1961, Dayton 1971, Wahl 1989). Consequently, the assembly of communities fouling these surfaces is often explained by the hierarchy of their competitive abilities (Connell 1972, Paine 1974, Jackson 1977). Assembly of fouling communities may, however, also be determined by the order in which species colonise (Underwood and Anderson 1994), whereby early colonisers pre-empt space that inhibits establishment of later colonists (Dean and Hurd 1980). When space is occupied, later colonizers must settle elsewhere or have the capacity to outcompete early colonizers (Connell and Slatyer 1977). Alternatively, early colonisers can facilitate development of diverse communities through successional processes (Bertness and Leonard 1997). For example, fouling organisms such as algae and bivalves can provide complex three-dimensional structure that protects associated species from environmental stressors, such as high temperatures and desiccation, or biotic stressors such as predation (Menge 1978, Coull and Wells 1983, Grabowski 2004, McAfee et al. 2016) and may also provide a source of food (Grabowski 2004). Generally, greater structural complexity of fouling communities supports more abundant and diverse mobile communities (Dean 1981, Norling et al. 2015, Lavender et al. 2017), with the composition of mobile communities dependent on the identity of the fouling community (Russ 1980). The relative importance of colonisation versus post-recruitment processes in shaping marine community assembly is still a topic of much debate (Odum 1969, Connell and Slatyer 1977, Greene et al. 1983, Underwood and Chapman 2006, Lebrija-Trejos et al. 2010), and, given rates at which coastal urbanisation is modifying coastal habitats (Bulleri and Chapman 2010, Dafforn et al. 2015), is a debate that is important to reconcile, if

appropriate conservation and restoration strategies are to be applied.

Oysters are an ecologically and economically significant habitat-forming species of high restoration potential. Globally, oysters have been reduced to less than 15% of their historic population densities due to overharvest using destructive fishing practices that not only remove spawning stock biomass, but also the shell substrate on which growth of oyster reefs depends (Beck et al. 2011). Oysters provide many ecosystem services such as water quality improvement (e.g., Nelson et al. 2004, Newell & Koch 2004, Piehler & Smyth 2011), shoreline stabilisation (e.g., Meyer et al. 1997, Piazza et al. 2005, Scyphers et al. 2011) and augmentation of reef production through the production of juvenile oysters that serve as food for higher trophic levels (Lenihan and Peterson 1998, Posey et al. 1999). Further, oysters facilitate diverse communities by forming complex structures that protect juvenile invertebrates and fish from predation, and also buffer abiotic stressors such as temperature (Peterson et al. 2003, Grabowski 2004, Tolley and Volety 2005, Cole et al. 2007, McAfee et al. 2016). Along the east coast of Australia, reefs formed by the native Sydney rock oyster*, Saccostrea glomerata*, are considered functionally extinct (Beck et al. 2011). Despite loss of reefs, *S. glomerata,* supports the largest aquaculture industry in New South Wales (NSW DPI 2017) and remnant wild populations of *S. glomerata* can be found on rocky shores, attached to mangroves or on artificial hard substrate (Gillies et al. 2015a, McAfee et al. 2016, Scanes et al. 2016). Thus, it appears that environmental conditions remain suitable for *S. glomerata* growth and survival at many locations, and that spawning stock biomass is present within estuarine systems. Consequently, initial attempts to restore *S. glomerata* reefs have been focused around providing the appropriate substrate for natural colonisation and reef development.

Key considerations for the provision of new substrate are the timing of its introduction and its material type, with significant risks associated with poor planning.

Propagules that are present at the time of substrate introduction may be the first to colonise, and if these are not the target species, may pre-empt space, preventing or impeding target species settlement (Underwood and Anderson 1994). As an outcome, restoration attempts might inadvertently facilitate non-native species that will compete with native species for resources such as space (Ruesink et al. 2005, Wilkie et al. 2013). Additionally, substrate type can influence community development (Anderson and Underwood 1994). Oyster restoration projects typically use live oysters, dead oyster shell, or concrete or maerl substrate for colonisation (Brumbaugh and Coen 2009, Geraldi et al. 2013, Dunn et al. 2014). Whether oysters are live or dead influences settlement cues (Tamburri et al. 2007), the surface area of shell substrate available for attachment (Summerhayes et al. 2009), and determines if processes such as larval predation, the generation of feeding currents and the production of organic waste can influence community development (Lenihan 1999, Tamburri et al. 2007, Wilkie et al. 2013). Whereas natural oyster reefs consist of a mixture of live and dead oysters that are naturally cemented to one another, many restoration projects introduce substrate as loose shell within bags that could move around with water movement. The frequency of disturbances, such as substrate movement are known to alter sessile community development (Sousa 1979). Successful restoration not only requires our understanding of the current state, but also the factors that led to the reduced state so those can be avoided.

In this study, we determine how the timing and type of substrate introduced to estuarine habitats influences the colonisation of oysters, and the subsequent development of associate communities. First, we assess how the timing of *S. glomerata* recruitment events compares to those of key competitors such as barnacles, mussels and algae. Second, we assess how substrate type (live oysters, dead oysters, a mix) interacts with timing of deployment to influence recruitment of fouling organisms, and subsequent colonisation of mobile taxa. Third, we assess how sessile community development varies when oyster shell

substrate is loose or fixed. Determining the mechanisms that shape colonisation by sessile and mobile communities within *S. glomerata* habitat will inform future restoration projects in order to successful restore complex, biodiverse oyster reefs.

METHODS

Study Sites

We compared recruitment of oysters and invertebrates among substrata at five intertidal *Avicennia marina* mangrove forests of Port Jackson, Sydney, Australia: Sugarloaf Point (SP; 33°49'01.2"S, 151°08'35.9"E), Looking Glass Bay (LGB; 33°50'19.7"S, 151°07'35.1"E), Murray Prior Reserve (MP; 33°50'08.0"S, 151°08'34.8"E), Tambourine Bay (TB; 33°49'38.9"S, 151°09'39.2"E) and Woodford Bay (WB; 33°49'40.5"S, 151°10'25.4"E). At each site, naturally occurring oysters were found attached to mangrove pneumatophores with populations dominated by the native oyster, *S. glomerata*, which contributed >85% of oysters, with smaller numbers of the non-native oyster *Crassostrea gigas* (Scanes et al. 2016). Substrata were deployed under the mangrove canopy, 0.6-0.8 m above Indian Spring Low Water. All sites experienced semidiurnal tides, with a spring tidal range of approximately 1.5 m. During the study, which extended from November 2015 to August 2017, surface water temperature at the sites ranged from 12-29 °C and salinity, 8-38 (Table 1).

Table 1. Salinity and temperature (°C) ranges at each site from November 2015 – September 2017 for sites SP, LGB, MP, TB and from April 2016-September 2017 for WB. Sites are arranged from locations upstream to downstream.

Site	Salinity	Temperature °C
Sugarloaf Point (SP)	8 - 34	13 - 26
Looking Glass Bay (LGB)	18 - 37	13 - 29
Murray Prior Reserve (MP)	26 - 37	14 - 27
Tambourine Bay (TB)	8 - 37	12 - 29
Woodford Bay (WB)	13 - 38	13 - 29

Sampling units

At each site, oyster and invertebrate recruitment was assessed on sampling units, comprising: 100% live oysters (hereafter 'live'); 50% live oysters and 50% dead oyster shell (hereafter '50/50'); or 100% dead oyster shell (hereafter 'dead'). Each sampling unit contained a total of 350 cm^3 of live oysters and/or shell, enclosed within 20 mm clear plastic mesh that was attached to a 270 mm long and 12.5 mm wide wooden dowel rod using a metal eye-hook and cable ties (Fig. 1). The wooden dowel rods were designed to mimic the mangrove pneumatophores to which oysters naturally attach (Bishop et al. 2012), and were inserted 150 mm into the sediment such that 120 mm extended above the sediment surface, to match the range of pneumatophore heights at sampling locations.

The live oysters (mean ± SE shell height: 52.83 ± 0.56 mm) used in sampling units were obtained from oyster farms in Port Stephens and Nowra, New South Wales and were *S. glomerata* mass selected for fast growth and resistance to QX disease. Oyster shell was disarticulated valves of *S. glomerata* (mean ± SE shell height: 69.14 ± 0.92 mm) obtained from oyster farmers in Port Stephens and Botany Bay, NSW. Live oysters and oyster shells were inspected for invertebrates prior to deployment, and any associated organisms removed.

Fig. 1. Examples of sampling units colonised by sessile and mobile invertebrates. The units contained either: (a) only live oysters (live); (b) half live oysters and half dead oyster shell (50/50); or (c) only dead oyster shells (dead), with the total volume of material standardised to 350 cm^3.

Sampling

Timing of sessile species recruitment and its influence on community assembly

To determine how the timing of oyster recruitment compares to that of key competitors, barnacles, mussels and algae, sampling units containing dead oyster shell were deployed monthly from November 2015 to August 2017 at four of the five sites (SP, LGB, MP, and TB) and from April 2016 to August 2017 at the fifth site, WB. An alternate site to WB was initially sampled from November 2015, but due to repeated loss of samples from that site, it was replaced with WB in April 2016. During the first four months, 6 replicate units were deployed at each site but this was reduced to 5 per site for subsequent months. Occasionally, a sampling unit was lost, in which case analyses were run on the 4 remaining

units. Sampling units remained in the field for one month prior to collection, at which time we quantified the abundance per shell of recruiting oysters, barnacles and mussels, and the presence or absence of algae on each shell under a magnifying lamp. The total abundance of oysters, barnacles and mussels, and the total proportion of shells with algae present were determined for each sampling unit.

Preliminary results indicated barnacle recruitment primarily occurred in January whereas oyster and mussel recruitment occurred later, in February. To determine how timing substrate deployment to occur immediately before or after barnacle recruitment influences sessile community development, we deployed an additional 10 sampling units of dead oyster shell at each site in January and February 2017. Five of these were sampled after 3 months, and the other five, 6 months after deployment. Combined with the month-long deployments, this gave a fully orthogonal design with January or February deployment, and 1, 3 and 6 month soak-times. To confirm that any difference in sessile community development between treatments deployed in January versus February was due to the difference in the timing of deployment as opposed to the timing of collection, we deployed an additional 12 sampling units in January 2017 that were collected ($n = 4$) at the same three times as the deployments made in February 2017. The number of oyster spat, barnacles and mussels, and presence of algae were determined as described above.

Influence of substrate on community development

To test hypotheses about how the composition of substrate interacts with its time of deployment to determine oyster recruitment and associated community development, sampling units with live, 50/50 and dead oyster shell were deployed at each of the five sites every three months from November 2015 to October 2016, with the exception of WB where sampling began in June 2016. Initially, 6 replicate units of each substrate type were deployed at each site but this was reduced to 5 following the first of the quarterly sampling and

occasionally 4 units per substrate type, where a unit was lost. Sampling units remained at sites for three months, after which they were collected during low tide and placed into individual plastic bags. The contents of the mesh bags enclosing oysters and shell was washed over a 500 µm sieve, with mobile invertebrates retained on the sieve stored in 70% ethanol. Mobile invertebrates were identified and enumerated to species except for soft-bodied worms which were identified to family, and small crustaceans (isopods, amphipods and tanneids) which were enumerated by morphospecies under a dissecting microscope. The abundance of recruiting oysters, barnacles and mussels, and the presence or absence of algae was also scored for each shell individually, and the total abundance of each invertebrate and total proportion of shells with algae determined for each sampling unit.

Oysters and shell used in sampling units were loose, however wild oysters form clumps in which individual oysters are cemented to one another. To assess how attachment of oysters to one another influences the communities that colonise, for each of the three substrate treatments - live, 50/50 and dead - we created a second set of treatments in which oysters and/or shell were glued to one another. Glued oyster treatments consisted of three to four clumps comprised of four to five oysters each, to give an identical total volume of oysters/shell to the loose treatments. Epoxy (Megapoxy PM®, Vivacity Engineering Pty Ltd, Sydney, Australia) was placed on the rounded left valve of one oyster, with other oysters and shells attached to this area. This approach prevented epoxy from adhering to the opening of oyster valves, which could result in oyster death. Glue control treatments in which epoxy was added to the left valve of 3-4 oysters per sampling unit but left to dry without attaching other oysters or shell, assessed whether any difference between loose and glued (hereafter 'attached') treatments were due to differences in substrate structure and not the presence of epoxy. Loose ($n = 5$), attached ($n = 5$) and glue control ($n = 3$) sampling units of live, 50/50 and dead oyster treatments were deployed in early January 2017, just prior to barnacle and

oyster recruitment, and collected after three months. Sampling units were processed as for the quarterly sampling, but the total number of oysters and barnacles on oysters and shells, and the proportion of shells on which algae was present was estimated by randomly sub-sampling 1/3 to 1/2 of shells and oysters from each sampling unit, and multiplying up to give totals.

Statistical Analyses

We used univariate permutational analyses of variance (PERMANOVAs, Anderson et al. 2008) to assess treatment effects on abundance of oysters, barnacles and mussels, algal presence, and for experiments examining effects of substratum type, invertebrate taxon richness, total abundance, and Shannon-Weiner diversity. PERMANOVAs apply the traditional ANOVA partitioning 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. Analyses used Euclidean distance matrices calculated from square root transformed data.

Three-way fully-orthogonal PERMANOVAs with the factors sampling time (22 levels: December 2015 – September 2017), and site (4 levels, random) tested for differences among months in oyster, barnacle, mussel and algal recruitment. A separate analysis was done for WB for the factor, sampling time (18 levels: April 2016-September 2017). Three-way analyses with the factors deployment time (3 levels: January, February or Control), soak time (3 levels: one-, three- or six-months) and site (5 levels) tested for differences in sessile invertebrate community development among deployment and soak times. To test hypotheses about temporal differences in sessile and mobile invertebrate community development among different substrates, three-way analyses with the factors treatment (3 levels: live, 50/50, or dead), time (4 levels) and site (4 levels) were run. A separate analysis was run for

WB with the factors treatment (3 levels) and time (2 levels). A separate three-way analysis with the factors substrate treatment (3 levels: live, 50/50, or dead), substrate structure (3 levels: loose, attached, or glue control) and site (5 levels, random) tested for differences in oyster and barnacle recruitment due to habitat structure. Where PERMANOVAs detected significant treatment effects, they were followed by pairwise post-hoc PERMANOVAs to identify sources of differences. All analyses were run in PRIMER 6.

RESULTS

Sessile fouling community

In addition to oysters, multiple species of barnacles, mussels and algae recruited to sampling units. Barnacles were predominately *Amphibalanus amphitrite*, with *A. variegatus* and *Austrominius covertus* occasionally present. Mussels were predominately *Trichomya hirsuta* with *Xenostrobus pulex* occasionally present. Algae were *Dictyota* sp., *Porphyra columbina* and *Ulva intestinalis.*

Timing of sessile species recruitment and its influence on community assembly

The timing of each of oyster, barnacle, mussel and algal recruitment varied among study sites and sampling times (PERMANOVA, sig. Time x Site interaction; Table 2). In 2016, oyster recruitment peaked at all sites sampled during February (except WB where sampling began in April 2016), although there were differences among sites in the magnitude of this peak (post-hoc tests, $p \leq 0.02$). In 2017, oyster recruitment at three of the sites (SP, TB, WB) peaked in January (post-hoc tests, $p \leq 0.04$) and at two sites (MP, LGB) peaked in February (post-hoc tests, $p \leq 0.05$). At one site (SP), a second oyster recruitment peak was observed in April (post-hoc tests, $p \leq 0.03$; Fig. 2A).

Barnacle recruitment in 2016 occurred in January at three of the four sites (SP, LGB, TB) and February at the remaining site (MP). A second recruitment period began in

September 2016 and lasted until February 2017. Recruitment was greatest in September 2016 at one site (SP; post-hoc tests, $p \leq 0.02$) followed by a maximum recruitment period at another site extending from October 2016 to January 2017 (WB; post-hoc tests, $p \leq 0.05$). Barnacle recruitment also occurred at one additional site in November 2016 (MP), but did not significantly differ from other sampling times at this site. During 2017, peak barnacle recruitment occurred at three sites in January (WB, LGB, SP; post-hoc tests, $p < 0.03$). On average, barnacle recruitment densities were lower in February than the previous month at the same three sites (WB, LGB, SP). By March, barnacle recruitment was only observed at one site (WB; Fig. 2B).

Mussel recruitment was only observed at one of the five sites (SP) over the two years of sampling except for the first month of sampling, where mussel recruitment was also observed at MP. At the site where mussel recruitment was repeatedly observed, recruitment occurred between February and April of 2016 and December 2016 to February 2017 (SP; post-hoc tests, $p < 0.05$; Fig. 2C).

Generally, algal recruitment occurred during the summer (November - February). In 2016 the presence of algae was greatest in November, followed by December for one site (SP). In 2017, the presence of algae was greatest at one site in February (SP; post-hoc tests, $p \leq 0.02$). Across all other sampling times, the proportion of shells with algae was relatively low, maintaining mean values between 0 - 0.3. At one site, algal presence peaked in April (MP; post-hoc tests $p < 0.05$), but there were no differences among sampling times for any of the other sites (Fig. 2D).

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Table 2. Three-way PERMANOVAs testing for sources of variation in the recruitment of oysters, barnacles, mussels and algae to oyster shell deployed monthly at (A) four sites: SP, LGB, MP, TB and (B) one site: WB. Ti = Time (A: 22 levels, monthly December 2015 – September 2017; B: 18 levels, monthly April 2016-September 2017); Si = Site (5 levels: random). Significant results (at $\alpha = 0.05$) are highlighted in bold. The results of *a posteriori* tests for significant treatment effects are given within the text and illustrated in Fig. 2.

A)			Oyster Density			Barnacle Density			Mussel Density			Algal Presence		
	Source	df	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P
	Ti	21	16.79	3.10	**0.002**	11.05	1.95	**0.029**	3.53	1.02	0.436	0.10	2.08	**0.019**
	Si	3	4.42	9.74	**0.001**	9.79	11.98	**0.001**	27.12	56.38	**0.001**	0.33	43.35	**0.001**
	Ti x Si	63	5.45	12.00	**0.001**	5.69	6.97	**0.001**	3.50	7.27	**0.001**	0.05	6.38	**0.001**
	Res	359	0.45			0.82			0.48			0.01		
B)	Source	df	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P
	Ti	17	3.58	9.22	**0.001**	30.70	11.38	**0.001**	0.06	0.88	0.419	0.01	1.68	0.071
	Res	72	0.39			2.70			0.07			0.01		
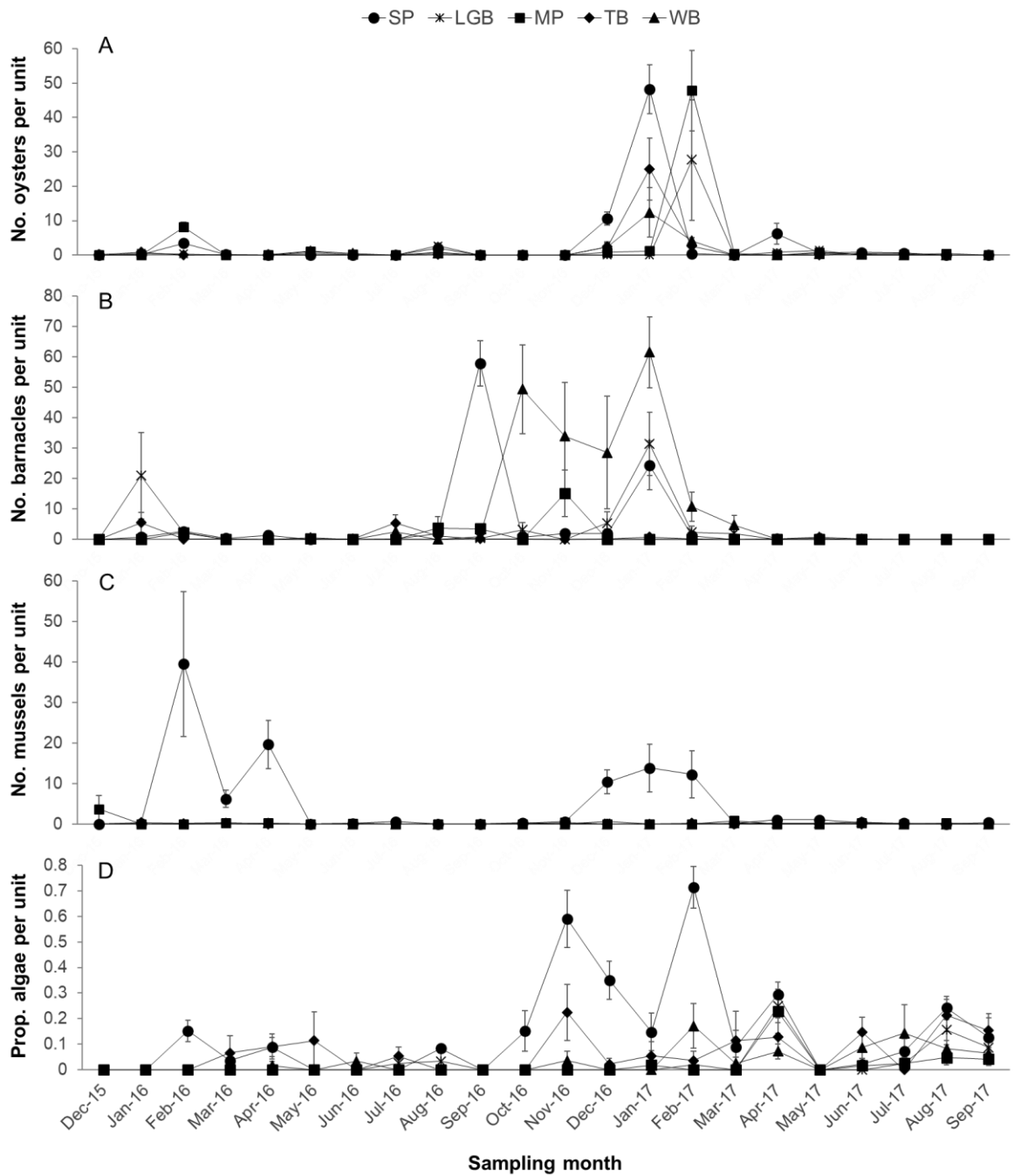
Fig. 2. Mean (± SE) abundance per sampling unit of (A) oysters, (B) barnacles, (C) mussels and (D) proportion of shells colonised by algae at each site across 22 months. The mean values were calculated from $n = 4$ to 6 replicate units per site.

There were site-specific effects of the timing and duration of shell deployment on the recruitment of oysters, mussels and algae (PERMANOVA, sig. Deploy x Soak x Site interaction; Table 3). Whereas the timing of deployment influenced oyster recruitment at all five sites after 1 month, and three of the five sites after 3 months, by 6 months there was only one site at which oyster recruitment was still influenced by the timing of deployment (post-hoc tests, $p \leq 0.04$). At three of the five sites (SP, TB, WB), the pattern after 1 month was of greater oyster density on sampling units deployed in January than February, with the reverse pattern observed at the other two (LGB, MP; post-hoc tests; $p \leq 0.04$; Fig. 3A). After 3 months, at two of the sites (MP, TB) recruitment of oysters was greater on the January than the February deployed sampling units, whereas at one site (SP) the reverse pattern of greater recruitment to the February-deployed units was apparent (post-hoc tests, $p \leq 0.04$; Fig. 3B). At TB, the only site to display a persistent difference between treatments through time, the greater recruitment of oysters to January than February deployed units remained apparent at 6 months (post-hoc tests, $p \leq 0.03$; Fig. 3C). In general, the Control units, deployed with the January units, but retrieved with the February units, had more similar densities of oysters to the January units, confirming patterns were indeed driven by deployment not retrieval time.

Barnacle recruitment patterns displayed site-specific effects of substrate deployment time, that were independent of soak time, as well as site-specific effects of soak time (PERMANOVA, sig. Deploy x Site interaction and Soak x Site interaction; non sig. Deploy x Soak x Site interaction; Table 3). At three of the sites (WB, TB, LGB) barnacle densities were greater in units deployed in January than February across all soak times (post-hoc tests, $p < 0.05$), with no significant differences at the other two. The more similar barnacle recruitment between Control and January-deployed than February-deployed units confirmed that differences in recruitment between January and February treatments were driven by differences in deployment, not retrieval, time. Barnacle recruitment densities at two of the

five sites (LGB, WB) were smaller after one-month of soak time (post-hoc tests, $p < 0.05$) than after three- or six-months of soak time, which in turn did not differ (post-hoc tests, $p > 0.2$). Additionally, barnacle densities were lower at one-than at three-months at MP (post-hoc tests, $p \leq 0.02$) with no differences between the other pairs of treatments (post-hoc tests, $p > 0.1$). No differences in barnacle densities among soak times were apparent at the remaining two sites (SP, TB; post-hoc tests, $p \geq 0.4$; Fig. 3 panels D-F).

Mussel densities only differed among deployment times at one site, SP, and only after six months soak time. The density of mussels was more than two times greater in January-deployed (72 ± 14) than in February-deployed units (28 ± 6; post-hoc tests, $p \leq 0.03$). Again, the Controls, deployed with the January treatment, but retrieved with the February treatment, did not differ in mussel density compared to the January treatment (post-hoc test, $p = 0.89$), indicating that deployment, not retrieval time, drove the pattern here.

Algal recruitment displayed idiosyncratic differences between January and February deployment times that varied among sites and soak times. After one month, algal presence at only one of the sites (SP) displayed a difference between January and February deployments. More shells were fouled when collected in March than February (post-hoc tests, $p = 0.01$) - a pattern that appeared to be driven by retrieval, not deployment time, as the Controls had similar algal abundance to the February-deployed units (Fig. 3G). After three months in the field, the pattern at this site had reversed, with more shells with algae among the January than February deployed units (post-hoc test, $p = 0.03$). A comparison of the experimental to control treatments again indicated that this difference was related to retrieval time (post-hoc test, $p \geq 0.07$, Fig. 3H). After six months, January-deployed units had higher algal presence than February-deployed units and that matched the controls at two sites, TB and MP, indicating an effect of deployment time (post-hoc tests, $p = 0.02$; Fig. 3I).

Table 3. Three-way PERMANOVAs testing for variation in the recruitment of oysters, barnacles, mussels, and algae to oyster shell deployed at each of five sites in January or February of 2017 and collected after 1, 3 or 6 months. A control treatment was also included in the design, that was deployed in January, but retrieved at the same time as the February-deployed sampling units. De = Deployment (3 levels: January, February, Control); So = Soak time (3 levels: 1, 3 and 6 months); Si = Site (5 levels: random). Significant results (at $\alpha = 0.05$) are highlighted in bold. The results of *a posteriori* tests for significant treatment effects are given within the text and illustrated in Figures 3 and 4.

Source	df	Oyster Density			Barnacle Density			Mussel Density			Algal Presence		
		MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P
De	2	334.71	3.63	**0.013**	237.42	5.02	**0.036**	7.39	1.44	0.281	0.12	1.04	0.400
So	2	338.31	5.70	**0.002**	105.58	3.64	0.076	35.38	9.14	**0.010**	0.53	1.81	0.213
Si	4	1304.50	64.75	**0.001**	1056.10	73.81	**0.001**	200.15	129.88	**0.001**	0.86	22.67	**0.001**
De x So	4	76.30	1.67	**0.124**	9.50	0.55	0.686	2.66	0.91	0.513	0.35	2.53	0.069
De x Si	8	92.24	4.58	**0.001**	47.33	3.31	**0.003**	5.15	3.34	**0.001**	0.11	2.99	**0.002**
So x Si	8	59.44	2.95	**0.001**	29.02	2.03	**0.043**	3.87	2.51	**0.018**	0.29	7.68	**0.001**
De x So x Si	16	45.70	2.27	**0.001**	17.18	1.20	0.264	2.93	1.90	**0.023**	0.14	3.67	**0.001**
Res	163	20.15			14.31			1.54			0.04		
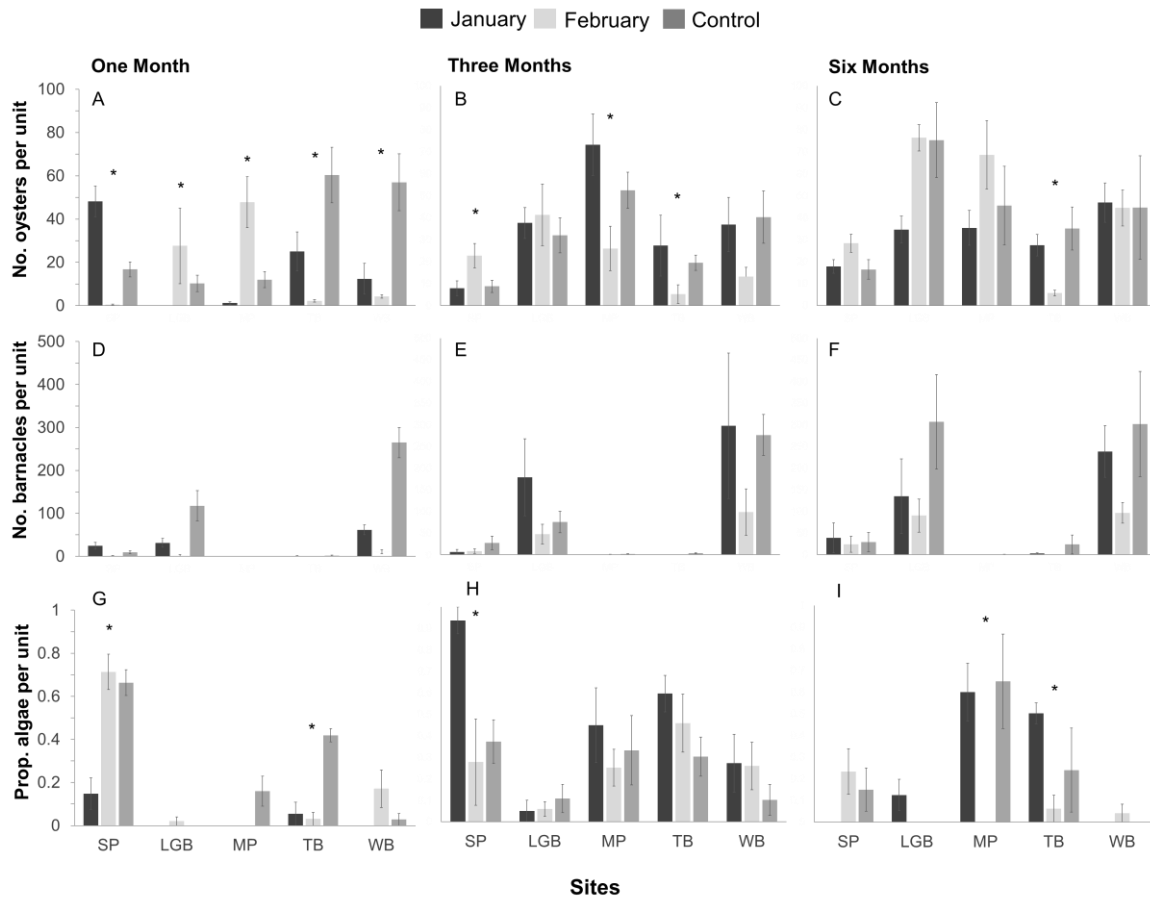
Fig. 3. Mean (± SE) density of oyster (panels A-C) and barnacle (panels D-F) recruits, and proportion of shells with algae present (panels G-I) on experimental units deployed in January or February, and retrieved one, three or six months later, at each of five sites. Control treatments were deployed with the January units but retrieved with the February units. Means are calculated from $n = 4$ to 5 replicate sampling units for each site, deployment and sampling time. Asterisks indicate significant differences among deployment treatments (at $\alpha = 0.05$) within each site.

Influence of substrate on community development

Fouling organisms

Analyses revealed significant two-way interactions between time and substrate treatment on oyster recruitment (PERMANOVAs: sig. Time x Treatment interaction; Table 4). In autumn, oyster recruitment to live substrate units (0.8 ± 0.3, mean density $\pm$ SE) was greater than to dead substrate units (0.05 ± 0.05; post-hoc test, $p = 0.03$) with no significant difference among the other treatments (post-hoc tests, $p \geq 0.1$; Fig. 4A). At all other times, substrate type had no influence on oyster recruitment.

Barnacle recruitment displayed a significant three-way interaction among time, substrate and site (PERMANOVA: sig. Time x Treatment x Site interaction; Table 4). In autumn and winter at MP and TB, in spring at SP and summer at LGB, substrate units with at least some live oysters (i.e. live, 50/50) supported more barnacle recruits than those with only dead shell (post-hoc tests, $p \leq 0.03$). Interestingly, live substrate units had more than 15 times fewer barnacles than 50/50 and dead substrate units during the spring at MP (post-hoc tests, $p = 0.003$; Fig. 4B). There were no pairwise differences detected among treatment levels at WB.

Mussel recruitment displayed a significant three-way interaction among time, substrate and site (PERMANOVA: sig. Time x Treatment x Site interaction; Table 4). However, mussel densities only differed among substrate treatments during one time (spring) at three of the five sites (LGB, MP, SP). At one site (SP), mussel densities were more than two times greater in substrates with dead shells (i.e. 50/50, dead) than within live substrate units, which had 29 ± 11 mussels (mean density $\pm$ SE; post-hoc tests, $p \leq 0.03$). However, the opposite pattern occurred at another site (LGB) wherein live substrate units supported more than seven times the number of mussels (7 ± 2) than substrates with dead shells (50/50, dead; post-hoc tests, $p < 0.01$). At the third site (MP), 50/50 substrate units had more mussels than live substrate units (post-hoc tests, $p = 0.01$) with no difference

between live and dead or 50/50 and dead substrate units (post-hoc tests, $p \geq 0.07$).

The proportion of shells with algae varied among substrate treatments within each sampling time at all sites (PERMANOVA: sig. Time x Treatment x Site interaction; Table 4; Fig. 4C). Dead and 50/50 substrate units had a higher proportion of shells with algae present than live substrate units at all sites, during autumn, winter and spring sampling times (post-hoc tests, $p \leq 0.02$).

Whether substrate was loose or attached had no influence on oyster or barnacle recruitment at any of the sites (Table 5). Rather, during this second summer sampling time, habitat treatments again influenced oyster recruitment wherein, dead substrate supported more oysters than live substrate units at two sites (LGB, TB; Fig. 5A). Algal presence on live substrates did, however, vary between loose and attached treatments at one site (TB) and, on substrates with at least some dead shell, varied between control and loose, or control and attached treatments in another four instances (PERMANOVA: sig. Treatment x Structure x Site interaction; Table 5). At TB, the presence of algae was lower on live oysters that were loose (0.38 ± 0.19, mean $\pm$ SE proportion) than attached (0.82 ± 0.37) or received the glue control (0.88 ± 0.51; post-hoc tests $p \leq 0.02$). At three sites (LGB, WB and TB) dead substrate receiving the glue control treatment (0.39 ± 0.22, 0.60 ± 0.35 and 1.00 ± 0.60, respectively) had more algae than the attached treatment (0 ± 0 at LGB and WB, 0.20 ± 0.09 at TB; post-hoc tests $p \leq 0.02$). Among 50/50 substrate units, one site (SP) displayed differences among treatments, with the control treatment (0.49 ± 0.28) supporting less algae than the attached treatment (1.00 ± 0.45; post-hoc tests, $p = 0.02$), with no other differences among substrate or treatments. Overall, epoxy had a slight tendency to increase algal presence in some cases.

Table 4. Three-way PERMANOVAs testing for variation in the recruitment of oysters, barnacles, mussels and algae among live, dead and mixed oyster shell substrates, sites and sampling times at (A) four sites: SP, LGB, MP, TB and (B) one site: WB. Sa = Sampling time (4 levels: autumn, winter, spring, summer (A) or 2 levels: winter, spring (B)); Tr = Treatment (3 levels: live, 50/50, dead); Si = Site (5 levels: random). Significant results (at α = 0.05) are highlighted in bold. The results of *a posteriori* tests for significant treatment effects are given within the text and illustrated in Fig. 5.

A)		Oyster Density			Barnacle Density			Mussel Density			Algal Presence		
Source	df	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P
Sa	3	20.22	1.07	0.441	289.35	4.71	**0.031**	18.02	0.81	0.495	3.95	11.77	**0.006**
Tr	2	0.10	0.11	0.919	26.26	3.02	0.143	0.03	0.01	0.995	4.37	75.60	**0.001**
Si	3	21.63	27.75	**0.001**	40.18	9.92	**0.001**	293.10	190.32	**0.001**	0.65	23.91	**0.001**
Sa x Tr	6	2.66	2.69	**0.039**	14.87	0.80	0.571	2.89	0.78	0.604	0.70	7.30	**0.001**
Sa x Si	9	18.88	24.22	**0.001**	61.52	15.20	**0.001**	22.34	14.51	**0.001**	0.34	12.36	**0.001**
Tr x Si	6	0.96	1.24	0.284	8.69	2.15	**0.048**	2.50	1.63	0.146	0.06	2.13	**0.045**
Sa x Tr x Si	18	0.99	1.27	0.21	18.67	4.61	**0.001**	3.71	2.41	**0.002**	0.10	3.53	**0.001**
Res	223	0.78			4.05			1.54			0.03		
B) Source	**df**	**MS**	***p*-F**	**P**	**MS**	***p*-F**	**P**	**MS**	***p*-F**	**P**	**MS**	***p*-F**	**P**
Sa	1	0.58	1.54	0.234	1001.6	89.62	**0.001**	19.6	7.12	0.013	0.06	1.32	0.249
Tr	2	1.6	4.22	**0.029**	17.64	1.58	0.226	2.86	1.04	0.367	1.57	35.71	**0.001**
Sa x Tr	2	2.93	7.72	**0.002**	19.89	1.78	0.188	2.19	0.8	0.449	0.01	0.34	0.729
Res	29	0.38			11.18			2.75			0.04		
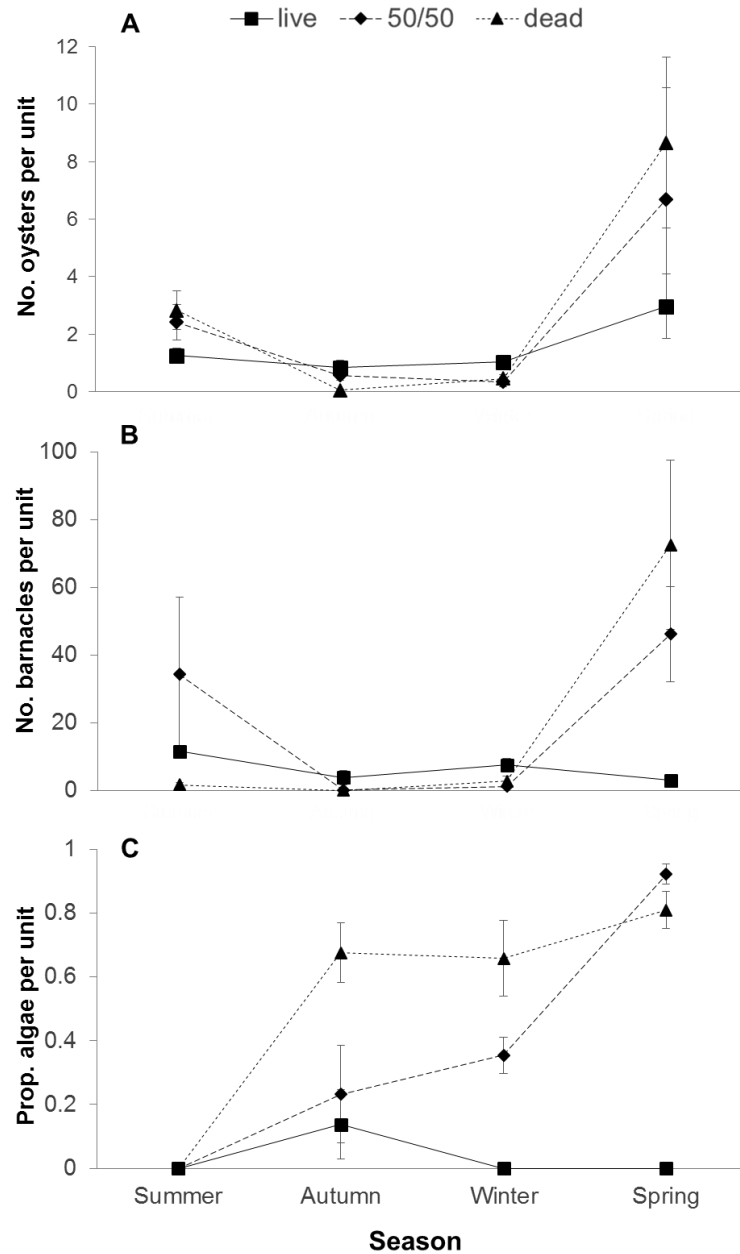
Fig. 4. Mean (± SE) number of (A) oysters and (B) barnacles at MP recruiting to each three oyster substrates (live, 50/50, dead), at each of four sampling times, and (C) proportion of shells to which algae recruited at SP. Oyster density values are averaged across four sites in summer and autumn and five sites in winter and spring, which did not statistically differ. Means are calculated from $n = 20$ to 30 replicate sampling units for oysters and $n = 4$ to 6 replicate sampling units for barnacles and algae. Pairwise differences among treatment levels are given within the text.

Table 5. Three-way PERMANOVAs examining sources of variation in the recruitment of oysters, barnacles and algae to oyster shell substrates that were loose or attached to one another with glue. A control treatment was also included in the design, whereby oysters were loose but with glue applied. Tr = Treatment (3 levels; live, 50/50, dead); St = Structure (3 levels: loose, attached, glue control); Si = Site (5 levels: random). Significant results (at $\alpha = 0.05$) are highlighted in bold. The results of *a posteriori* tests for significant treatment effects are given within the text and illustrated in Fig. 5C.

		Spat Density			Barnacle Density			Algae Presence		
Source	**df**	**MS**	***p*-F**	**P**	**MS**	***p*-F**	**P**	**MS**	***p*-F**	**P**
Tr	2	18.22	2.10	0.183	25.67	1.18	0.354	0.33	1.88	0.229
St	2	3.11	0.53	0.598	45.42	1.82	0.239	0.54	2.27	0.174
Si	4	112.18	29.93	**0.001**	1909.60	98.63	**0.001**	2.22	29.68	**0.001**
Tr x St	4	2.66	0.74	0.602	31.39	1.55	0.229	0.59	8.96	**0.001**
Tr x Si	8	8.69	2.32	**0.026**	21.73	1.12	0.329	0.17	2.32	**0.020**
St x Si	8	5.92	1.58	0.140	24.95	1.29	0.271	0.24	3.17	**0.002**
Tr x St x Si	16	3.58	0.96	0.530	20.26	1.05	0.413	0.07	0.88	0.590
Res	148	3.75			19.36			0.07		
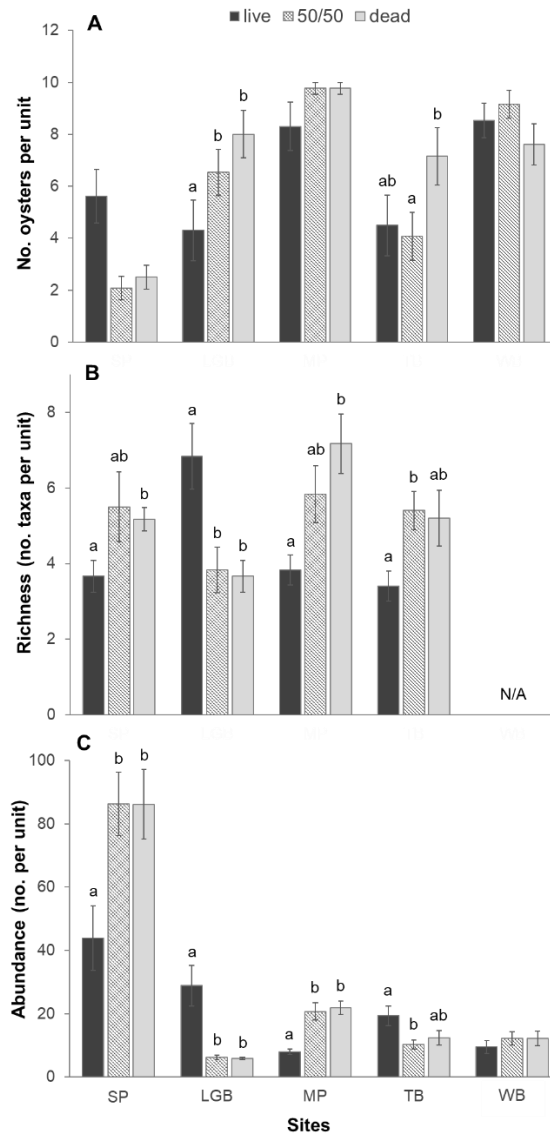
Fig. 5. Mean (± SE) (A) number of oysters, (B) taxon richness for specific sampling times, and (C) abundance of invertebrates in the spring, within each of three oyster substrate treatments (live, 50/50, dead) deployed at each of five study sites. Oyster density values are averaged across three structure treatments (loose, attached, control), which did not statistically differ. Taxon richness values are for single sampling times per site in which significant differences among treatments were detected: summer at SP, spring at LGB and MP, and autumn at TB. No differences among treatments were detected during sampling times at WB. Means are calculated from $n = 13$ replicate units for oyster densities, $n = 5$ or 6 replicate units for taxon richness and $n = 5$ replicate sampling units for invertebrate abundance. Different letters indicate significant differences among substrate treatments (at $\alpha = 0.05$) within each site.

Associate invertebrates

Across the four sampling times, 50 mobile invertebrate taxa colonised sampling units. The community consisted of: twelve families of soft-bodied worms; four species of crabs; six species of bivalves, excluding mussels and oysters, which were separately enumerated as the fouling community; fifteen gastropods; and thirteen morphospecies of small crustaceans of which six were amphipods, five were isopods, one was a tanneid and one an unidentified decapod. The most abundant of the taxa were gastropods, amphipods, and crabs, accounting for 28, 25, and 23% of total abundance, respectively.

The richness and abundance of mobile species associated with sampling units displayed effects of substrate type that varied among time and site (PERMANOVA: sig. Time x Treatment x Site interaction; Table 6). At four of the sites (SP, TB, MP, LGB), differences in taxon richness among substrate treatments were apparent in one of the sampling times a piece, but at the fifth (WB) no differences among treatments were detected at any sampling time (Table 6). In three out of the four of the instances in which differences among substrate treatments were apparent, live substrate supported fewer taxa than 50/50 or dead substrate. However, taxon richness was greater in live than 50/50 or dead substrate units at the fourth site (LGB; Fig. 5B).

The total abundance of invertebrates displayed differences among substrate treatments at four sites in spring, two sites in summer, one site in autumn and no sites in winter (PERMANOVA: sig. Time x Treatment x Site interaction; Table 6). One site (again WB) displayed no effects of substrate treatment in each of the two times in which it was sampled. In the spring, 50/50 and dead substrate units supported a greater abundance of mobile invertebrates than live substrate units at two sites (SP, MP; post-hoc tests, $p \leq 0.02$), but the opposite pattern was observed at the remaining two sites (LGB, TB). At both of these sites, live substrates had more invertebrates than 50/50 substrates (post-hoc tests, $p \leq 0.03$), but invertebrate abundance was greater in live than dead substrates at only one site (LGB;

post-hoc tests, $p < 0.01$) with no difference at the other (TB; post-hoc tests, $p \geq 0.1$; Fig. 5C). In the summer, 50/50 substrate units supported greater abundances of invertebrates than the other treatments at TB (post-hoc tests, $p \leq 0.02$) and a similar effect that continued into autumn at TB where substrate units with dead shell (i.e. 50/50, dead) supported more invertebrates than live substrate (post-hoc tests, $p < 0.01$).

Invertebrate diversity (Shannon-Weiner index) varied among sampling time and sites (PERMANOVA: sig. Time x Site interaction; Table 6). On average, diversity was greater in the winter or spring than in the summer. At three out of the five sites (LGB, MP, TB), diversity was lower in the summer than the winter and at two out of five sites (MP, TB), diversity was lower in the summer than the spring (post-hoc tests, $p < 0.05$, Fig. 6), with no differences among other sampling times (post-hoc tests, $p > 0.06$).

Table 6. Three-way PERMANOVAs testing for variation in the species richness, total abundance and diversity of invertebrates among live, dead and mixed oyster shell substrates, sites and sampling times at (A) four sites: SP, LGB, MP, TB and (B) one site: WB. Sa = Sampling time (4 levels, autumn, winter, spring, summer (A) or 2 levels: winter, spring (B)); Tr = Treatment (3 levels: live, 50/50, dead); Si = Site (5 levels, random). Significant results (at α = 0.05) are highlighted in bold. The results of *a posteriori* tests for significant treatment effects are given within the text and Table 5, and illustrated in Figures 5 and 6.

A)			Species richness			Total abundance			Shannon-Weiner diversity index		
Source	df	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P
Sa	3	40.26	4.04	**0.049**	10.58	1.56	0.300	1.76	4.73	**0.018**
Tr	2	2.52	1.69	0.274	2.13	3.53	0.118	0.04	0.75	0.488
Si	3	45.74	17.74	**0.001**	15.89	15.55	**0.001**	1.78	11.01	**0.001**
Sa x Tr	6	2.98	0.87	0.518	3.04	1.47	0.261	0.08	0.54	0.796
Sa x Si	9	9.98	3.87	**0.001**	6.78	6.63	**0.001**	0.37	2.30	**0.018**
Tr x Si	6	1.49	0.58	0.717	0.60	0.59	0.753	0.06	0.35	0.896
Sa x Tr x Si	18	3.42	1.33	0.189	2.07	2.03	**0.010**	0.14	0.87	0.615
Res	221	2.58			1.02			0.16		
B) Source	df	MS	*p*-F	P	MS	*p*-F	P	MS	*p*-F	P
Sa	1	0.15	0.05	0.823	0.31	0.25	0.617	0.04	0.13	0.708
Tr	2	0.67	0.21	0.802	1.69	1.36	0.313	0.22	0.77	0.494
Sa x Tr	2	1.22	0.37	0.701	0.36	0.29	0.744	0.05	0.19	0.839
Res	29	3.27			1.25			0.29		
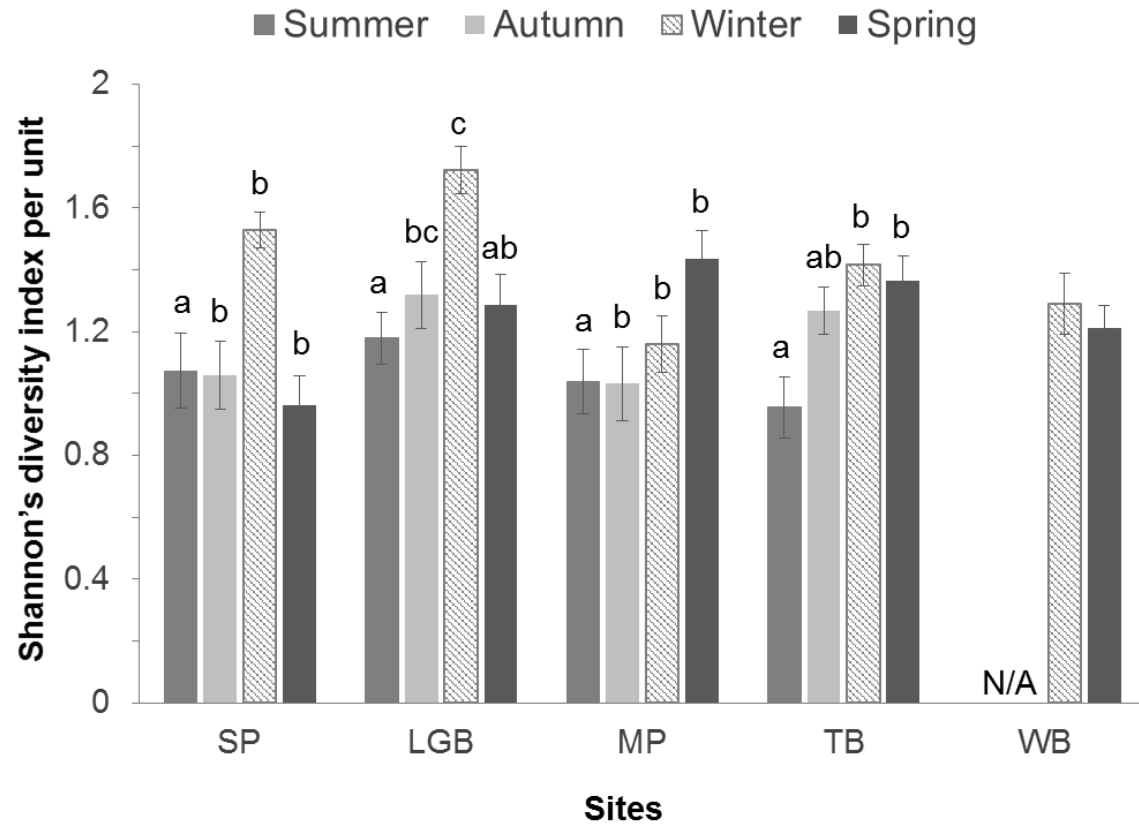
Fig. 6. Mean (± SE) diversity of invertebrates within each substrate unit at each of four sampling times, deployed at each of five study sites. Values are averaged across three substrate types (live, 50/50, dead), which did not statistically differ. Means are calculated from $n = 12$ to 18 replicate sampling units. Different letters indicate significant differences among substrate treatments (at $\alpha = 0.05$) within each site.

DISCUSSION

The success of restoration projects is contingent on understanding the biotic and abiotic processes that limit recovery, and how stochastic processes influence community assembly (Hulvey and Aigner 2014). While studies done in other parts of the world suggest that the availability of larvae, substrate and/or poor water quality can limit recovery of degraded oyster reefs (Brumbaugh and Coen 2009, and references therein; Geraldi et al. 2013), in Australia, where oyster reef restoration is a fledgling industry (Gillies et al. 2015b, 2017), there are a paucity of studies investigating processes that affect recovery of oyster reefs and their associate communities. At all five of our study sites in Sydney Harbour, deployment of substrate units led to oyster recruitment, indicating the presence of larvae in the water, and suitable environmental conditions for establishment. What type of substrate was deployed and when did, however, influence community development, with effects most pronounced shortly after substrate introduction, and diminishing over time.

In influencing chemical cues for settlement, surface microtopography, surface area and complexity, substrate type can have large influences on the composition of fouling assemblages (Karlson 1978, Anderson and Underwood 1994, Tyrrell and Byers 2007, Barnes et al. 2010). Oyster reef restoration projects often spend millions of dollars "seeding" substrate with juvenile oysters or deploying adult oysters that provide spawning stock biomass (Brumbaugh and Coen 2009) and chemical cues for settlement of conspecifics (Tamburri et al. 2007). Biofilms on dead oyster shell are, however, sufficient to attract recruiting oyster larvae (Tamburri et al. 2008, Carroll et al. 2015) and the greater surface area of dead, disarticulated, oyster shells, than live, articulated, animals may provide greater substrate for settlement (Summerhayes et al. 2009). Hence, where there is natural larval supply, except where hydrodynamic processes transport larvae away from the reef, or environmental conditions are unfavourable for reproduction, fertilisation and/or settlement,

seeding dead shell with live oysters does not necessarily lead to increased reef productivity (Geraldi et al. 2013). Here, including live oysters in substrate units positively influenced recruitment during only one of four deployment periods, which was outside of the time of peak oyster recruitment. Nevertheless, as in other studies (e.g. Summerhayes et al. 2009), live oysters reduced the abundance of algae – a competitor for space of oysters (Anderson and Underwood 1994) - presumably by filtering propagules (Tamburri and Zimmer-Faust 1996). Although this reduction in algal colonisation was of little consequence for oyster recruitment in the present study, this effect may become more important in environments that more strongly favour algal growth. Whether substrate was live, dead or a mix, had variable effects on the abundance and richness of mobile invertebrates. Nevertheless, consistent with the greater surface area and interstices provided by dead, disarticulated, than live, articulated, substrate (Gutiérrez et al. 2003, Summerhayes et al. 2009), at the sites of greatest invertebrate colonisation, the abundance and taxon richness of mobile species was greater in substrate with some dead shell than substrate with live shell alone.

Whether substrate is fixed or free to move can influence community assembly via effects on disturbance regime (Sousa 1979). Whereas natural oyster beds constitute a matrix of live and dead oysters cemented to one another to form a complex three-dimensional structure, oyster reef restoration projects often introduce substrate as loose shell in bags (Brumbaugh and Coen 2009). Here, however, whether oysters in deployment bags were glued to one another or free to move had little influence on oyster, barnacle or mussel recruitment but had a slight tendency to increase algal recruitment, which could potentially compete with target species (i.e. oysters) for space. However, this effect appeared to be due to the glue that had a unique microtopography and thus we cannot extend this conclusion to natural oyster clumps. The tight packing of shell in bags may limit shell movement below threshold levels of disturbance that are required to produce major changes in community

structure. Our results suggest that at locations with larval supply, the provision of loose, dead oyster shell as reef building material, rather than the transplantation of live oysters will best promote the formation of healthy reefs that facilitate sessile- and mobile-invertebrate communities.

In addition to the type of substrate deployed, the time at which it was introduced influenced the development of sessile and mobile invertebrate assemblages. The lottery hypothesis (Sale 1977) posits that the priority arrival of recruits, rather than subtle differences in their requirements or competitive abilities determines which will dominate. In line with this, a previous study in a proximate New South Wales estuary found that the development and composition of estuarine communities was strongly influenced by the time of year at which substrate was provided, reflecting which of the primary space occupiers had propagules in the water column at the time of space provision (Underwood and Anderson 1994). Barnacles, oysters and algae, three of the key primary space occupants on hard substrates at our study site, displayed differences in the timing of their peak recruitment, with barnacle recruitment typically peaking earlier in summer than oyster or algal recruitment, the latter two of which extended into autumn. Consequently, whether substrate was deployed in January, during peak barnacle recruitment but prior to peak oyster and algal recruitment, or February, when oyster and algal recruitment peaked, but barnacle recruitment petered, initially influenced which of these groups was the primary space occupant. Mobile species, like sessile species, displayed temporal variation in their colonisation, with diversity generally greater in winter or spring.

However, as time progressed, and subsequent recruitment events occurred, community structure converged on substrate units that had been deployed at different times. By 6 months, very few effects of the timing of deployment remained, with the exceptions being for algal and mussel abundance. Consequently, in this instance, and at odds with the

lottery hypothesis (Sale 1977), community assembly appeared to converge towards one equilibrium point (Greene and Schoener 1982), determined by the competitive hierarchy of dominant space occupants. Sites with barnacle recruitment maintained high barnacle densities after six months, with oysters attached to available space around the barnacles. At sites where oyster recruitment was high, there was generally lower algal presence. A study of longer temporal duration would confirm whether this is indeed the case, or whether priority effects occur. Additionally, deploying substrata at a different time of year, for example winter when only algae recruit, may lead to a different community structure.

Our results suggest that in much of the Sydney region recovery of oyster reefs may be limited by substrate rather than larval supply, such that there is great potential for oyster reef restoration through the addition of substrate. However, the type of substrate and the timing of its placement should be considered in order to maximise establishment of native oysters, and their associated communities, over potential competitors for space. Oysters have the potential to form complex reefs that will facilitate native biodiversity. Our study assists in the understanding of the ways in which the environment, stochastic processes and interspecific interactions combine to influence the functioning of *Saccostrea glomerata* oyster reef community assemblages.

ACKNOWLEDGEMENTS

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III. CO-OCCURRING SECONDARY FOUNDATION SPECIES HAVE DISTINCT EFFECTS ON COMMUNITY ASSEMBLY

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ABSTRACT

There is growing realisation that foundation species often co-occur in nested or adjacent assemblages. Primary foundation species may support multiple secondary foundation species, which have distinct effects on community assembly from the primary foundation species. Nevertheless, it is unclear the extent to which multiple secondary foundation species are unique to one another in the communities they facilitate and what the processes are that underpin any differences. We compared how two secondary foundation species, the Sydney rock oyster, *Saccostrea glomerata*, and the free-floating fucalean algae, Neptune's necklace, *Hormosira banksii,* each facilitated by the peg roots of the grey mangrove, *Avicennia marina,* influence the recruitment and survival of associate invertebrates. Field experiments revealed that effects of the two species on recruitment processes were generally distinct and additive. Whereas *S. glomerata* recruitment responded positively to the presence of oysters, it was unaffected by algal biomass. Barnacle (*Amphibalanus* spp. and *Hexaminius* spp.) recruitment, however, decreased with the biomass of oyster or algal habitat. By contrast, there appeared greater redundancy between the two species in their mediation of predator-prey interactions. The efficacy of the secondary foundation species in ameliorating predator-prey interactions was dependent on the body size of the predator and prey species relative to the refuge space provided by the foundation species. The naticid gastropod, *Conuber sordidum*, was sufficiently small to penetrate habitats, such that neither foundation species influenced its predation on the gastropod *Batillaria australis*. By contrast, each foundation species reduced predation of the toadfish *Tetractenos hamiltoni* on small crabs, *Paragrapsus laevis,* which were able to seek refuge in the interstitial space provided by either habitat. Overall this study demonstrates that multiple co-occurring secondary foundation species have distinct effects on biodiversity where their structure and function has differential effects on resource availability. Hence,

models of community assembly need to consider interactions among co-occurring secondary as well as primary foundation species, which may occur in complex networks.

Keywords: oysters; recruitment; foundation species; *Saccostrea glomerata*; algae; *Hormosira banksia;* mangroves; predator; prey; facilitation; additive

INTRODUCTION

Foundation species (*sensu* Dayton 1972) are critical to the maintenance of biodiversity (Bertness and Callaway 1994, Stachowicz 2001, Bruno et al. 2003). They maintain complex habitat, and in doing so may ameliorate abiotic stressors such as temperature or desiccation stress, and biotic stressors such as competition and predation (e.g. Hay 1986, Jones et al. 1997, Cole et al. 2007, Jackson et al. 2008, McAfee et al. 2016). Most studies have considered the effects of foundation species independently of one another, but many overlap in time and space (e.g. Altieri et al. 2007, Angelini et al. 2011, Bishop et al. 2012). In some instances, primary foundation species simultaneously facilitate multiple secondary foundation species that may form nested or adjacent configurations (Bishop et al. 2012, Hughes et al. 2014). There is growing evidence that habitat cascades – nested interactions whereby primary foundation species provide habitat for secondary foundation species that in turn provide habitat for a focal community (Thomsen et al. 2010, Angelini et al. 2011) – are common in a wide range of terrestrial and aquatic ecosystems (e.g. Martin-Smith 1993, Altieri et al. 2007, Gribben et al. 2009, Angelini and Silliman 2014, Bell et al. 2014, Watson et al. 2011).

The way in which spatially overlapping foundation species interact to facilitate biodiversity is determined by interspecific differences in their functional traits (Angelini et al. 2011) and by intraspecific variation in traits at the population- (e.g. density) and

individual-level (e.g. morphology; Bishop et al. 2013). At a species level, foundation species that are functionally similar are more likely to compete (Krassoi et al. 2008, Angelini et al. 2011) and be functionally redundant in terms of the biodiversity that they support (e.g. Wilkie et al. 2012). By contrast, species that are functionally distinct and fill different niches can coexist (Angelini et al. 2011) and may have large additive or synergistic effects on biodiversity (e.g. Bishop et al. 2012, Hughes et al. 2014). Within species, intraspecific variation in density, morphology and key functions can lead to variation in the biological communities they support (e.g. Bruno and Kennedy 2000, Bishop et al. 2009, Nicastro and Bishop 2013, Hughes et al. 2014) and determine how foundation species interact (Bishop et al. 2012, 2013). For example, in nested assemblages of foundation species a critical density or particular morphology of the primary foundation species might be required to support the secondary foundation species, and particular densities or morphologies of the secondary foundation species might be required to facilitate a focal community (Bishop et al. 2013). How intraspecific variation in traits influences the way secondary foundation species interact to facilitate biodiversity has, however, received little attention (but see Hughes et al. 2014). Furthermore, the mechanisms by which secondary habitat formers enhance biodiversity remains poorly understood (Thomsen et al. 2018).

In estuarine and coastal environments of eastern Australia, the grey mangrove, *Avicennia marina*, is a primary foundation species that creates structure and shading in the otherwise sedimentary environment (McAfee et al. 2016). Among the species facilitated by *A. marina* are the secondary foundation species, the Sydney rock oyster, *Saccostrea glomerata*, and the fucalean algae, *Hormosira banksii* (Bishop et al. 2012, 2013, Hughes et al. 2014). *Saccostrea glomerata* use the pneumatophores (peg-roots) and trunks of *A. marina* as a substrate for attachment, on which they build dense aggregations (Bishop et al. 2012, McAfee et al. 2016). Mangrove pneumatophores facilitate free-living *H. banksii* by

providing a structure around which the alga's fronds - bead-like chains of spherical receptacles - become entangled and trapped (Bishop et al. 2012, 2013). The net effect is mosaics in which the two secondary foundation species, *S. glomerata* and *A. marina* are found in overlapping and adjacent configurations (Bishop et al. 2012). The indirect effect of mangroves on invertebrate biodiversity, arising from their facilitation of oysters and algae, overwhelms their direct effect (Bishop et al. 2012). In previous studies the two secondary foundation species have been demonstrated to have additive effects on associated communities of invertebrates (Hughes et al. 2014). Nevertheless, the mechanisms by which their distinct effects arise have not been investigated.

The mechanisms by which *S. glomerata* and *H. banksii* facilitate invertebrates in temperate mangroves may include provision of substrate for attachment and grazing, and provision of a microhabitat refuge from predation (Hughes et al. 2014, McAfee et al. 2016). In mangrove forests, hard substrate is otherwise limited to mangrove trunks and pneumatophores, with competition for space and resources intense (Branch and Branch 1980, Minchinton and Ross 1999). Each of *H. banksii* and *S. glomerata* offer a potential substrate for recruitment of organisms, but their functional roles may differ as a result of the hardness of their surfaces, the biofilms they support and the chemical cues they release that influence settlement (Anderson 1996, Minchinton and Ross 1999). Mangrove invertebrate communities can be subject to high rates of predation by marine fishes and invertebrates that feed in mangrove forests at high tide, shore and wading birds that forage at low tide, and invertebrate predators, such as crabs and muricid and naticid gastropods that are resident within the mangrove benthos (Warren 1990, Miranda and Collazo 1997, Bishop et al. 2008, Nagelkerken et al. 2008). The match between invertebrate body size and habitat architecture can influence habitat selection (Hacker and Steneck 1990) and influence susceptibility of fauna to predation (Pennings 1990, Eggleston and Lipcius 1992). Foundation species also

provide critical protection for juvenile species which rely on complex habitat, often formed by secondary foundation species, for protection from predation but also abiotic stress such as desiccation (Altieri et al. 2007). Hence differences in the habitat architecture of *S. glomerata* and *H. banksii* may lead to functional differences in the protection they offer prey from predators.

Here we utilise a combination of field and aquarium experiments to assess the independent and interactive effects of *H. banksii* and *S. glomerata* on invertebrate recruitment and survivorship in a temperate Australian mangrove forest, and their independent and interactive effects in modifying predator-prey interactions. We hypothesise that due to morphological and functional differences between the two foundation species, they will differ in the invertebrate species they offer habitat to and the types of predator-prey interactions they ameliorate. We hypothesise that not only will there be interspecific differences in such functions of the two foundation species, but that these functions will also vary according to intraspecific variation in the density and habitat configuration of the foundation species. We expect that with increasing biomass and density of *H. banksii* and *S. glomerata*, invertebrate recruitment and survival will increase.

METHODS

Field Experiments

Experimental Design

Densities of the two secondary foundation species, *Saccostrea glomerata* and *Hormosira banksii*, were manipulated in the *Avicennia marina* mangrove forest of Quibray Bay (-34.025051, 151.180300), within the Towra point Aquatic Reserve, Botany Bay, New South Wales (NSW), Australia. During March 2015, six sites, each separated by at least 4m, were established in the seaward pneumatophore fringe, at a tidal elevation of mean low water

springs + 0.7 m and along a ~80 m length of shoreline. Sites had a similar pneumatophore density of 586 ± 26/m^2 (mean ± SE).

Within each site, twelve 0.5 x 0.5 m^2 experimental plots, at least 1.5 m apart, were cleared of all oysters and algae. A 0.5 m area around each plot was also cleared to ensure that adjacent habitat structure did not dominate the effects of experimental interventions. Within each site, a single plot was randomly assigned to each of 12 habitat treatments arising from every possible combination of each of four oyster and three algal treatments. Oyster treatments contained naturally occurring clumps of oysters varying in number and size: no (0 clumps), low (2 small clumps), high (4 small clumps) or large (1 large clump). Small oyster clumps contained 9 ± 1 (mean ± SE) oysters, while large clumps contained 31 ± 2 oysters. Oyster treatments were based on the range of naturally occurring densities within this system (Hughes et al. 2014). The large oyster clumps contained a similar number of oysters to four small clumps, with the comparison between high and large oyster treatments assessing whether the configuration rather than just density of oyster habitat influences community structure. The positioning of small clumps of oysters within low or high treatments was random whereas large clumps were placed in the center of their assigned experimental plots. Algal treatments were based on the range of naturally occurring densities within this system (Bishop et al. 2012, Hughes et al. 2014) and were no (0 kg), low (1.25 kg), or high (2.5 kg) biomass (wet weight) which was placed evenly throughout the 0.25 m^2 plot. All plots were checked every two weeks to maintain habitat treatments and the cleared area around each plot.

Oyster and Barnacle Recruitment

To compare how the two secondary foundation species influence oyster and barnacle recruitment, and to assess the extent to which their varying effects on predation drives differences between the two, caged and uncaged roughened pieces of polyvinyl chloride

(PVC) were introduced into experimental plots as recruitment sticks. Each plot received six randomly positioned 25 cm-long and 1.9 cm-diameter PVC posts that were pushed 15 cm into the sediment so that approximately 10 cm of PVC was exposed. Cylindrical cages that were 15 cm in length and 8 cm in diameter and constructed of 25 x 25 mm galvanised steel mesh enclosed the top section of three of the PVC posts per plot (caged treatment). The coarse mesh size of cages was designed to exclude predators such as fish and crabs that forage on oyster recruits at high tide, whilst minimising shading artifacts. Recruitment sticks were checked every two weeks until recruitment of oysters and barnacles was observed. Once oyster recruitment was observed (September 2015), one randomly selected caged and one uncaged PVC stake was collected from each plot two weeks later, and again after four and six weeks, and the number of oyster spat and barnacles on each was quantified. Because sediment accretion in some plots affected the length of each stake that was exposed above the sediment, densities of barnacles and oysters were expressed as the number per unit area of surface exposed. Any difference in the density of recruits between pairs of caged and uncaged stakes was interpreted as an effect of predation.

Juvenile Invertebrate Survival

The interacting effect of oysters and algae on the survival of juvenile *Bembicium aurtaum* and of juvenile *S. glomerata* was monitored over three to four months. The gastropod, *B. auratum*, is common within the mangrove forest and is found living on oysters, *Hormosira*, sediment and pneumatophores (Reid 1988, Bishop et al. 2009, 2012, Hughes et al. 2014). Juvenile *S. glomerata* recruit to pneumatophores, the shells of conspecifics and other molluscs, as well as any other hard substrates that may be present (Bishop et al. 2012, Hughes et al. 2014).

In May 2015, eight *B. auratum* snails (mean ± SE width: 7.12 ± 0.17; height: 5.63 ± 0.19) were tethered within each plot. Tethers consisted of 25 cm piece of fishing line secured

at one end to a single snail using SikaBond super glue gel and at the other end to a galvanized steel mesh stake (approximately 2.5 cm in width and 6 cm in height) that was anchored beneath the sediment surface. The spire of each snail was marked with a small dot of red nail polish so that in the event that snails were missing from tethers, plots could be searched for marked individuals to determine if this was due to glue failure or predation. Pilot studies indicated that the nail polish did not influence snail survival.

In August 2015, nine juvenile *Saccostrea glomerata* oyster spat (17.5 ± 0.24 mm, mean shell height ± SE) were marked with red nail polish and placed in each plot. In treatments with oysters, spat were attached using Sikaflex-291 marine sealant and evenly distributed among three clumps of dead oysters, comprising 4 ± 1 (mean ± SE) pieces of shell, that were in turn attached with marine sealant to the end of a wooden chopstick (23.0 cm length). In plots with no oysters, spat were evenly distributed among three bare chopsticks, with oysters attached to one end of each using the marine sealant. Chopsticks were used to mimic pneumatophores, onto which oysters recruit within this system (Bishop et al. 2012) and were secured in plots by depressing the end without oysters ~12 cm into the sediment.

Snails and spat were checked every month and classified as alive, dead or missing. Among dead molluscs it was noted whether they had drill-holes (indicative of predation by naticid or muricid gastropods), were cracked (likely from crab or fin-fish predators) or had entire shells (indicative of non-predatory mortality; Bishop et al. 2008). Where snails or spat were missing, the surrounding area was checked for painted individuals. On the rare occasion (6 occurrences) a marked individual was found, it was recorded as alive and reattached. Monitoring of snail survival was terminated after three months because simultaneous monitoring of tethered snails in bare 0.5 x 0.5 m plots (*n* = 3) caged with 25 x 25 mm galvanised steel mesh to exclude predators revealed that glue failure occurred over longer

time intervals. Although oyster spat remained attached to wooden pegs over longer time intervals, their monitoring was terminated at four months, because almost all spat had been consumed by this time.

Aquarium Experiments

To assess the interactive and independent effect of algae and oysters on predator-prey interactions, we conducted two aquarium experiments. Each followed the same fully orthogonal design as the field experiments, with four oyster treatments (no clumps, low density of small clumps, high density of small clumps, single large clump) and three *H. banksii* treatments (no algae, low biomass and high biomass). The first experiment, conducted in October-December 2015 (i.e. the Austral spring and summer), considered how the two foundation species influence predation by common toadfish, *Tetractenos hamiltoni*, on shore crabs, *Paragrapsus laevis.* The second experiment, run in March-April 2016 (i.e. the Austral fall), considered how the foundation species affect moon snail, *Conuber sordidum*, predation on the mud whelk, *Batillaria australis.* Both toadfish and moon snails are generalist predators of invertebrates in temperate Australian mangrove forests, that in tethering and meoscosm experiments have been demonstrated to account for a significant proportion of predatory mortality (Warren 1990, Bishop et al. 2008). Toadfish forage in mangrove forests at high tide (Warren 1990). Moon snails are resident on and in mangrove sediments (Bishop et al. 2008), sometimes living in association with *H. banksii* (Bishop et al. 2009). Shore crabs are ubiquitous across intertidal oyster habitat (Hughes et al. 2014) and are often found hiding in and beneath oyster shell (M. Vozzo pers. obs.). *Batillaria australis* is an epibenthic species that displays enhanced abundances under *H. banksii* (Bishop et al. 2009, Bishop et al. 2012, Hughes et al. 2014).

Experiments examining toadfish predation on shore crabs were conducted in the Macquarie University seawater facility, a recirculating system utilising seawater trucked

from Sydney Harbour, while experiments examining moon snail predation on mud whelks were run in the Sydney Institute of Marine Science (SIMS) aquarium, a flow-through system which directly sources water from the Harbour. *Paragrapsus laevis* (10.81 ± 0.35 mm, mean carapace width ± SE), *B. australis* (18.71 ± 0.30 mm, mean shell height ± SE) and *C. sordidum* (18.65 ± 0.24 mm) for use in experiments were collected by hand from the Quibray Bay mangrove forest at low tide, the day before commencement of each experiment. Toadfish (10.1 ± 0.2 cm, mean total length ± SE) could not be collected from Quibray Bay due to the status of this site as an Aquatic Reserve and were instead collected by seine net from Tambourine Bay, Lane Cove River, Sydney, NSW 5-7 days prior to experiments. Until the start of experiments, toadfish were housed in 55L tanks supplied with ~18°C recirculating seawater, and that were exposed to a natural lighting regime and cleaned daily. They were fed a varied diet of prawns, oysters, and crabs daily, but were starved 36 hours prior to use in the experiment. Predatory snails were kept in individual 0.5L containers and prey snails were kept in two 10L containers of aerated seawater (22-24°C) supplied by the SIMS flow-through water system.

Experiments at Macquarie University utilising toadfish and crabs were conducted in 27L tanks (47 x 35 x 25 cm, length x height x width). The tanks were each closed systems, filled with seawater, and individually aerated. The air temperature in the seawater facility was set to match water temperatures recorded in Sydney Harbour, Australia to mimic natural conditions (18.5-20.5°C) in the housing and trial tanks. A total of 72 tanks were established, and randomly assigned to each of four oyster treatments, to give 18 tanks of each. The percentage covers of oysters in treatments (0 (mean ± SE; no), 8.5 ± 0.53 (low), 17.33 ± 1.05 (high), or 15.33 ± 0.56 (large)) and number of oyster clumps matched those of oyster clumps within plots of the field experiment with small clumps positioned randomly and large clumps positioned in the center of the aquarium. Clumps were smaller due to the aquarium set up

and contained 5 ± 1 oysters (mean $\pm$ SE) in small clumps and 22 ± 4 oysters (mean $\pm$ SE) in large clumps. For each oyster treatment, six tanks were randomly assigned to each of three algal treatments - no algae, 0.82 kg (towel-dried wet weight) or 1.65 kg to match the densities (0, 0.5 or 1 kg per 0.25 m^2) utilised in field experiments. Once the habitats were constructed, an individual toadfish and 10 shore crabs were added to each tank. The number of shore crabs added to tanks was based on pilot studies that indicated that even in the absence of structured habitat, toadfish would consume no more than 9 crabs over the experimental duration, of nine hours. Fish were added 30 min prior to crabs to give them time to acclimate, but were gently kept to the side of the tank when crabs were added, allowing the crabs time to hide within the habitat. Trials ($n = 6$ for each of the 12 habitat treatments) were run during daylight hours, as toadfish are omnivorous scavengers that are active during the day and night (Miller & Skilleter 2006). After 9 hours, fish were removed from tanks, tanks were thoroughly searched for crabs and the number of crabs remaining was quantified. Fish were only used once in the experiment to eliminate any learned foraging behavior and were returned to their collection site at the end of the experiment.

Experiments at SIMS utilising *C. sordidum* and *B. australis* were run in 4L plastic ice cream tubs (19 x 19 x 12 cm, length x height x width), that were fully submerged in one of two 20 cm deep water tables. Seawater from Sydney Harbour, ranging from 22-24°C during February-April 2016, was supplied to the water tables via a flow-through system at a constant flow rate (1 litre/min). As with the first predator-prey experiment, oyster treatments were established so that they had the same percent cover of oysters as the four treatments used in the field experiment (0 % (mean $\pm$ SE; no), 8.5 ± 0.53 % (low), 17.33 ± 1.05 % (high), or 15.33 ± 0.56 % (large)) and the same number and positioning of oyster clumps. Small oyster clumps contained 3 ± 1 (mean $\pm$ SE) and large oyster clumps contained 12 ± 1 (mean $\pm$ SE) oysters. There were 18 tubs of each oyster treatment, that were randomly

assigned to each of three algal treatments (no, low or high biomass of *H. banksii*) to give n=6 for each of the 12 treatments. The low biomass treatment received 0.06 kg of algae per tub, and the high biomass treatment, 0.11 kg per tub, to match the biomass per unit area of algae in the field experiment.

Once the habitats were constructed, an individual moon snail and 10 mud whelks were added to each tank. The number of mud whelks added to tanks was based on a pilot study that indicated that in unstructured habitat, moon snails consumed no more than 10 snails within the experimental period of 12 days. The mud whelks were added to the tanks first to allow them time to explore the habitat before adding the predatory snail, approximately 30 minutes later. Tubs were covered and sealed with wire screen mesh, to prevent escape of snails, and were aerated via an individual airline fed through a small hole in the mesh for each tub. After 12 days, the moon snails were removed from tubs and the number of mud whelks with drill holes (indicative of moon snail predation) was quantified. Moon snails and mud whelks were only used once and released to Quibray Bay after use in the experiment.

Statistical Analyses

Four-way ANOVAs, with the factors oyster habitat (4 levels: zero oyster clumps [no], low density of small clumps [low], high density of small clumps [high] and large [large] clump), algal habitat (3 levels: no, low, and high biomass), caging (2 levels: caged, uncaged) and time (3 levels: two, four and six weeks) examined sources of variation in the recruitment of barnacles and oysters to experimental plots and their subsequent survival. Time was considered an independent factor because different PVC stakes were sampled each time. Site was not considered as a factor because these were used solely for the purposes of ensuring interspersion of plots, with the distance between sites the same order or magnitude

as the distance among plots. Separate two-way fully-orthogonal ANOVAs tested for effects of oyster and algal habitat on the survivorship of juvenile *B. auratum* snails after 1, 2 and 3 months, and *S. glomerata* oysters after 1, 2, 3 and 4 months in field plots, and on *P. laevis* and *B. australis* over the duration of the laboratory predator-prey experiments. For these, sampling times were analysed separately as the same invertebrates were sampled through time and times were, hence, non-independent. Prior to each analysis, Cochran's *C*-test was performed to confirm homogeneity of variance, and where necessary, data were square root (recruitment counts) or arcsine (surviving invertebrate percentages) transformed (Underwood 1997) to achieve homogeneity of variance. After transformation, the three and four-month *S. glomerata* survival data still did not meet homogeneity of variance requirements for ANOVAs; therefore, significant differences were determined at $p = 0.01$ (Underwood 1997). Where ANOVAs found significant treatment effects, Tukey HSD tests were conducted *a posteriori* to determine significant differences among means ($\alpha = 0.05$). A Welch's t-test was done to test whether there was any difference in the mean percentage of damaged *B. auratum* snails that had been drilled and cracked across all treatments. Statistical tests were conducted in R version 3.0.2.

RESULTS

Field Experiment

Oyster and Barnacle recruitment

Neither the density of oysters nor barnacles on recruitment sticks displayed interacting effects of any combination of algal habitat, oyster habitat, time and caging (ANOVA, $p > 0.05$ for interaction terms), allowing for the interpretation of main effects. Densities of oysters (ANOVA: cage, $F_{1,360} = 11.54$, $p = 0.001$) and barnacles ($F_{1,360} = 15.47$, $p = 0.001$) were greater on average on caged than uncaged recruitment sticks (62.8cm^2

surface area; oysters: 3.29 ± 0.21 [±SE] caged vs 2.36 ± 0.18 uncaged; barnacles: 30.45 ± 3.32 caged vs 15.78 ± 2.07 uncaged). Oyster habitat had differing effects on each of oyster and barnacle recruitment. Whereas greater oyster recruitment occurred in plots with oyster habitat of any type than in plots that received no oyster habitat (ANOVA: oysters, $F_{3,360} = 7.84$, $p < 0.001$; Fig. 1A), less barnacle recruitment occurred in plots with than without oyster habitat, although this trend was not significant (Fig. 1C). Among treatments with oyster habitat, there was no significant difference in oyster recruitment between plots with a low or high density of small oyster clumps, or a single large oyster clump (Tukey: $p > 0.05$; Fig. 1A). While the algal habitat had no effect on the density of oyster recruits (ANOVA: algae, $F_{2,360} = 2.5$, $p = 0.084$; Fig. 1B), barnacle density varied with algal biomass (ANOVA: algae, $F_{2,360} = 9.94$, $p < 0.001$), with lower barnacle recruitment occurring in the high algal biomass treatment than the no or low algal biomass treatments which, in turn did not significantly differ (Tukey: $p \leq 0.02$, Fig. 1D).
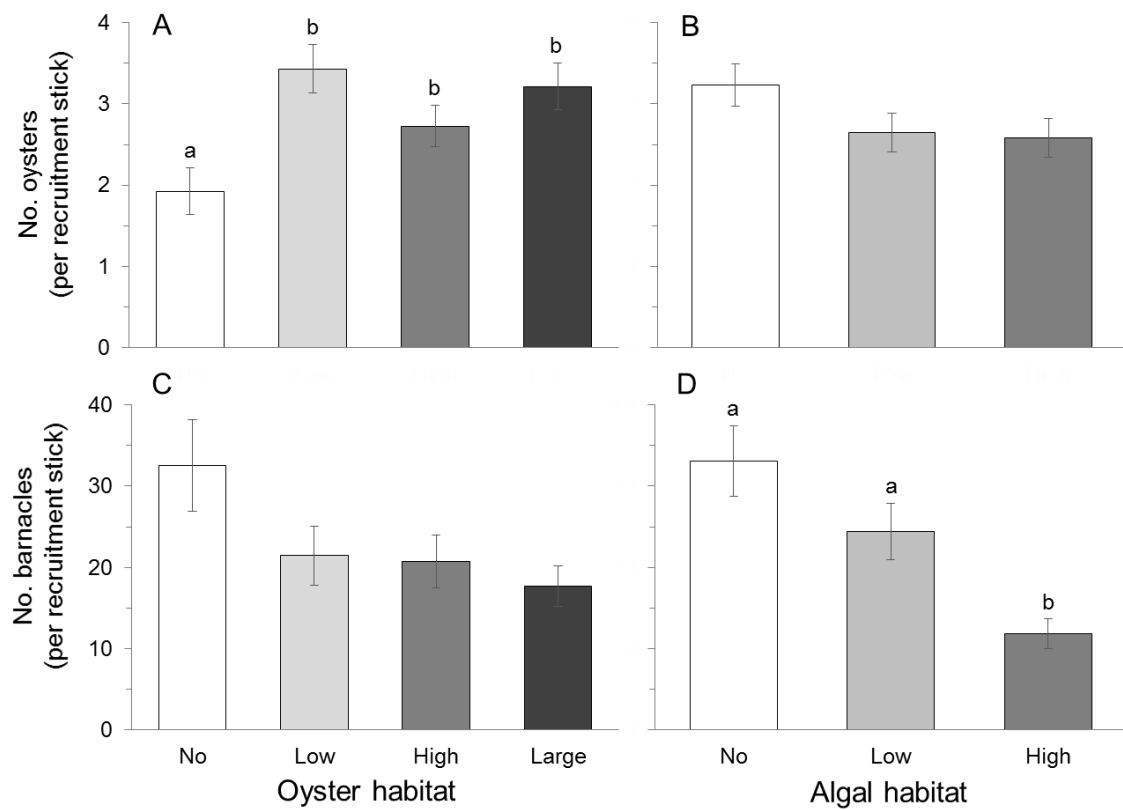
Fig. 1. The mean (± SE) density of (A) oysters and (B) barnacles recruiting to PVC stakes in plots with no, low, high or large oyster biomass and of (C) oysters and (D) barnacles recruiting to PVC stakes (62.8 cm^2) in plots with no, low or high algal biomass. Barnacle recruitment to PVC stakes in plots with oysters showed the opposite trend to oyster recruitment, but was not significant. Oyster recruitment to PVC stakes in plots with algae showed the same trend as barnacle recruitment, but was not significant. Letters above bars indicate significant differences (ANOVA, Tukey: $p < 0.05$).

Juvenile Invertebrate survival

One and three months after tethering, survival of the snail *B. auratum* was not significantly affected by oyster habitat, algal habitat or the interaction (ANOVA: $p > 0.05$). By contrast, two months after tethering, snail survival displayed an effect of algal (ANOVA: $F_{2,60} = 3.49$, $p = 0.037$) but not oyster habitat (ANOVA: $F_{3,60} = 1.29$, $p = 0.288$) or the interaction of the two (ANOVA: $F_{6,60} = 1.10$, $p = 0.372$). Snail survival was greater in plots with low than no algal biomass (Tukey test: $p = 0.031$), but there were no significant differences among other pairwise comparisons of no, low and high algal biomass treatments (Tukey tests: $p > 0.05$, Fig. 2A). Among damaged snails, a significantly greater ($t_{87} = 3.302$, $p = 0.001$) percentage were drilled (7.61 ± 1.6 %, mean SE) than cracked (1.62 ± 0.55 %) but there were no effects of habitat (oysters, algae, or the interaction) on the percentages of drilled and cracked snails ($p > 0.05$, see Table S1 in the Supplement for full ANOVA results).

Oyster survival was not influenced by the interaction between oyster and algal habitat at any of the four sampling times (ANOVAs: $p > 0.05$) allowing for the interpretation of main effects. At the one month time interval, algae but not oysters, had a significant effect on oyster survival (ANOVA: algae, $F_{2,60} = 5.46$, $p = 0.007$; oysters, $F_{3,60} = 1.72$, $p = 0.173$), with survival greater in low than no or high algal biomass treatments (Tukey tests: $p \leq 0.02$; Fig. 2B). There were no differences in oyster survival due to main effects of oysters or algae after two months (ANOVA: oysters, $F_{3,60} = 0.51$, $p = 0.677$; algae, $F_{2,60} = 2.02$, $p = 0.142$), but at each of the three and four month time intervals, the main effect of oyster treatment but not algal biomass had a significant effect on oyster survival (ANOVA, 3 months: oysters, $F_{3,60} = 16.34$, $p < 0.001$; algae $F_{2,60} = 0.1$, $p = 0.906$; 4 months: oysters, $F_{3,60} = 23.81$, $p < 0.001$; algae $F_{2,60} = 0.02$, $p = 0.984$). During the third and fourth month, oyster survival was greater in plots with any density of oyster habitat than plots without oysters (Tukey: $p < 0.001$; Fig. 3).
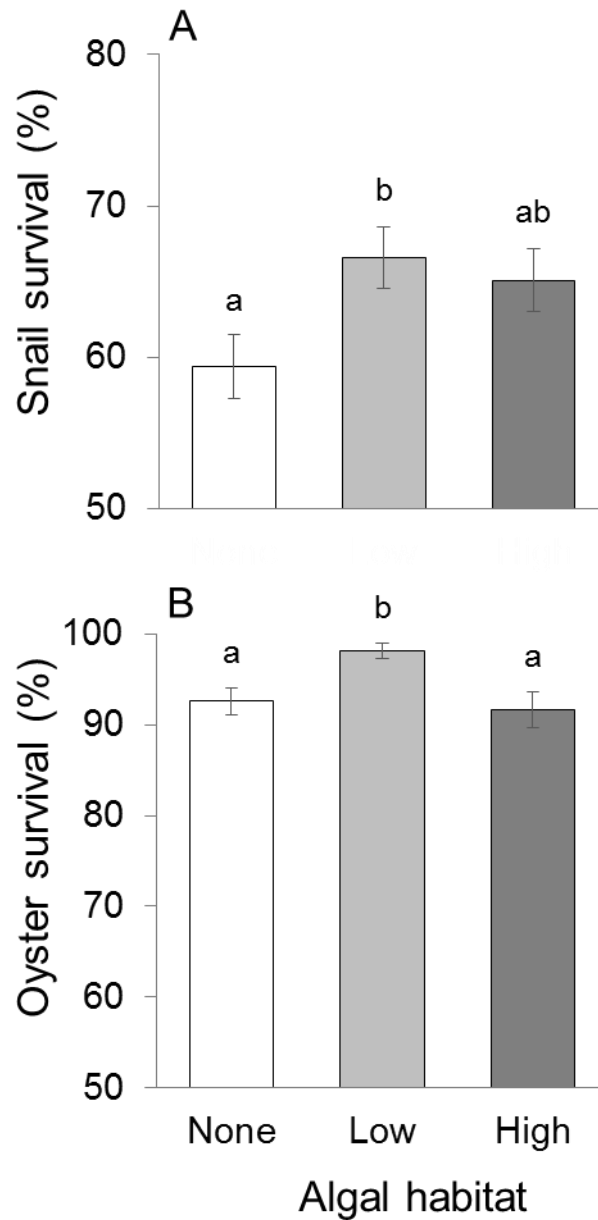
Fig. 2. The mean (± SE) percentage of (A) *Bembicium auratum* snails surviving after two months and (B) oysters surviving after one month in each algal habitat treatment (no, low or high algal biomass). Letters above bars indicate significant differences (ANOVA, Tukey: $p < 0.05$).
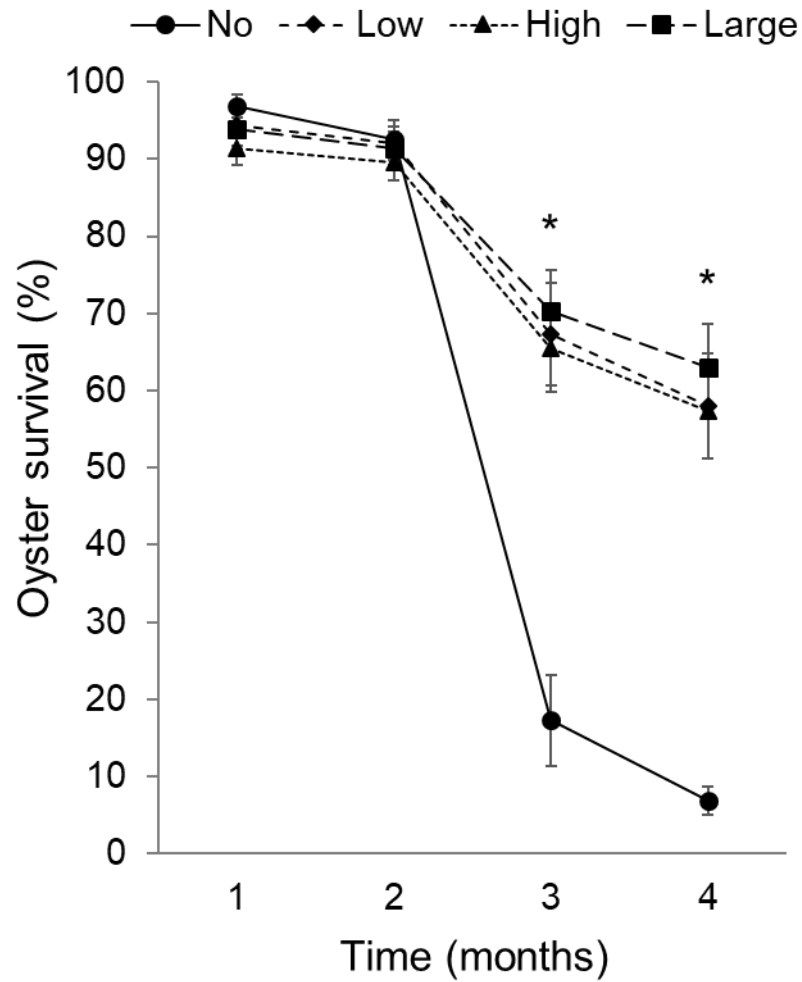
Fig. 3. The mean (± SE) percentage of oysters surviving in each oyster habitat treatment across the four months of monitoring. Plots received no oyster habitat (no), two small clumps (low), four small clumps (high) or a single large clump (large). After three and four months, survival was greater in plots that contained any oyster habitat than plots without oyster habitat (ANOVA, Tukey: $p < 0.001$).

Aquarium Experiments

Predation by toadfish on crabs was determined by the interacting effect of oyster habitat and algal habitat (ANOVA: $F_{6,60} = 4.99$, $p < 0.001$). In the absence of oysters, survivorship was significantly greater at low or high biomasses of algae than in the absence of algae (Tukey: $p \leq 0.004$), but in the presence of oysters, of any habitat configuration, there was no effect of algae on crab survivorship (Tukey: $p > 0.05$, Fig. 4). Similarly, in the absence of algae, survivorship was greater in tanks with oysters than those without oysters (Tukey: $p < 0.001$), which in turn did not significantly differ, and at the low biomass of algae, there was lower survivorship in tanks without oysters than those with a large oyster clump (Tukey: $p = 0.05$). At the high algal biomass, there was little effect of oysters on survivorship (Tukey: $p > 0.05$, Fig. 4).

In trials examining *C. sordidum* predation on *B. australis*, there was no significant effect of oysters, algae or the interaction on snail survivorship (ANOVA: main effect of oysters, $F_{3,60} = 1.34$, $p = 0.266$; main effect of algae, $F_{2,60} = 0.46$, $p = 0.633$; oyster by algae interaction, $F_{6,60} = 0.67$, $p = 0.671$). Across all habitat treatments, the predatory snails consumed an average ($\pm$ SE) of 5 ± 1 snails per 12 day trial.
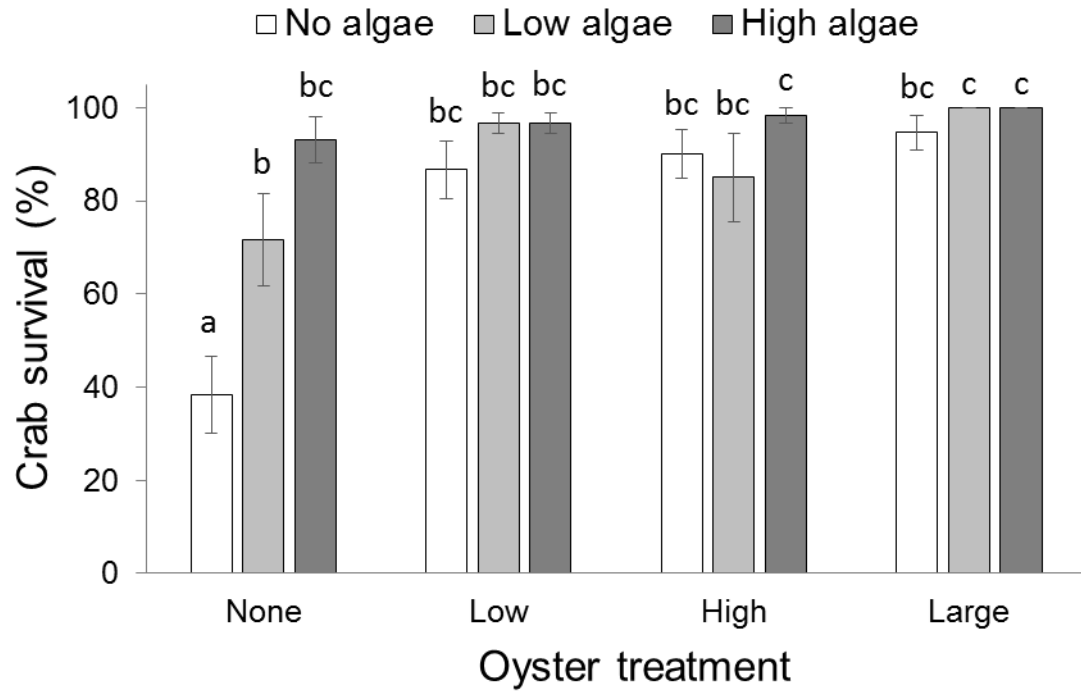
Fig. 4. The mean (± SE) percentage of shore crabs surviving in each of 12 habitats after the 9 hour feeding trial with toadfish. Tanks received either no oyster habitat (no), two small clumps (low), four small clumps (high) or a single large clump (large), and either no (white bars), low (light grey bars) or high algal biomass (dark grey bars) in a fully factorial design. Letters above bars indicate significant differences (ANOVA, Tukey: $p < 0.05$).

DISCUSSION

This study investigated how two secondary foundation species, the Sydney rock oyster, *Saccostrea glomerata,* and the free-floating fucalean algae, *Hormosira banksii*, interact to influence two key biological processes critical to community assembly – recruitment and predator-prey interactions. It was hypothesised that due to structural and functional differences between the two foundation species, each would produce a different effect on these processes. As expected, effects of the two species on recruitment and survival of invertebrates were generally distinct and independent. Nevertheless, some redundancy between the two species in their mediation of predator-prey interactions was apparent.

Barnacles and oysters displayed divergent patterns of recruitment to experimental field plots, dependent on the identity of the two secondary foundation species present. Whereas *S. glomerata* recruitment responded positively to the presence of oysters, it was unaffected by algal biomass. Barnacle (*Amphibalanus* spp. and *Hexaminius* spp.) recruitment, however, decreased with the biomass of oyster or algal habitat. Recruitment of sessile invertebrates is the net effect of larval supply, settlement and post-settlement mortality (Pawlik1992). The divergent response of oyster recruitment to the two foundation species appeared to primarily be due to differences in settlement, with the absence of an interaction between the caging and habitat treatments indicating no differential effect of the two foundation species on post-settlement predation. Oysters are well known to be gregarious settlers, responding positively to the chemical cues of conspecifics (Tamburri et al. 2007, 2008). It is unclear the mechanism by which the algae and oysters diminished barnacle recruitment. It has been hypothesised that algal canopies may reduce barnacle recruitment by reducing larval supply to substrates below (Hatton 1938, Southward 1956, Connell 1961). The whip-lash effects of algae on barnacle recruits that have been observed on rocky shores (Leonard 1999, Beermann et al. 2013) are unlikely to have occurred here

due to the sheltered environment of the mangrove forest. The smothering effect of algae on barnacle recruits observed on rocky shores (Denley & Underwood 1979), although plausible in sheltered environments, is also unlikely within this study system. At low tide the vertical distributions of *H. banksii* and barnacles did not overlap, because <u>*H. banksii*</u> rested on the muddy substrate, sitting below the band on recruitment sticks at which barnacles were found. The negative influence of oysters on barnacle settlement is to the benefit of oyster recruits, which can compete with barnacles for space and food resources (Luckens 1975, Anderson & Underwood 1997).

The survival of oysters in the field displayed similar responses to the two habitat forming species as recruitment, with greater survival of oysters in the presence than absence of conspecifics over periods of 3 months or longer, irrespective of oyster biomass or habitat configuration. By contrast, effects of algae on oyster recruitment were seen only after 1 month, and were generally non-linear, with survivorship of oysters greater at the low biomass of algae than the high or no algae treatments. Whereas low densities of algae may protect oysters from finfish predators, high densities may disrupt feeding by inhibiting water flow, or facilitate predatory naticid gastropod which, unlike fish, are able to penetrate algal habitat and benefit from its structure (Bishop et al. 2009). Nevertheless, there was no difference in the percentage of oysters that were drilled across treatments. These results highlight the importance of examining effects of foundation species across a range of biomasses and patch configurations, as their interactions with associate species are not necessarily linear.

The effects of the secondary foundation species on survival of the two snail species were also generally independent. The two snail species used in this study, *Bembicium auratum* and *Batillaria australis*, were numerically dominant species in our mangrove study system with *B. auratum* more common on oysters and *B. australis* often found under *H.*

banksii (Reid 1988, Bishop et al. 2009, Hughes et al. 2014). Despite the stronger association of *B. auratum* with oysters than with algae in the field (Bishop et al. 2009, Hughes et al. 2014), in the tethering study, oysters had no influence on the snail's survivorship, as compared to weak positive effects of low densities of the alga. This result suggests that small-scale variation in *B. auratum* abundance is not driven by predation, but by an alternate factor. For example, in mangrove forests where the availability of hard substrate is limited, *B. auratum* may use hard surface provided by oyster shell as a substrate for grazing (Reid 1988, Hughes et al. 2014). The weak positive effect of *Hormosira* but not oysters on *B. auratum* survival may reflect differences in the fit between the body size of the snail and the predator refuges provided by each of the habitats: whereas the body size of adult *B. auratum* is too large to fit in many of the interstices between oyster shells, the snail can move amongst the *H. banksii* habitat.

In laboratory experiments, predation by *C. sordidum* on *B. australis* was influenced by neither the presence nor density of oysters or algae. This may be because *B. australis* was too large to shelter in the interstices provided by either habitat, and *C. sordidum* was sufficiently small to move freely into each habitat to forage. Prey handling, which can take anywhere from 36 to 60 hours, rather than prey detection and capture limited the rate of prey consumption. Although the laboratory experiment only considered effects of secondary habitat forming species on predation by a single species on *B. australis*, this and a previous study (Bishop et al. 2008) indicate that this species, *C. sordidum*, is the dominant predator of shelled gastropods at our study site. Over four times more *B. auratum* were drilled than cracked in the field tethering study, indicating the greater significance of naticid gastropod than crab or fish predation on its survival. Hence, this study does not support the hypothesis that the aggregation of *B. australis* underneath *H. banksii* is a predator avoidance strategy. Instead, this behavior may be driven by the enhancement of organic matter concentrations

beneath the algal mats, upon which *B. australis* feeds (Bishop et al. 2009, 2012).

In contrast to the differential effects of the two habitats on snail predation, both algae and oysters reduced predation by toadfish on small crabs and appeared largely redundant in their effects. In the absence of the alga, the presence of the oyster enhanced crab survivorship. Conversely, in the absence of the oyster, increasing biomasses of *H. banksii* enhanced crab survivorship. However, if one foundation species was already present, adding a second had little or no effect. We hypothesise that in this case the two foundation species were functionally redundant in their effect on this predator-prey interaction because the structure of each was largely impenetrable by toadfish, but each provided interstices in which crabs could seek refuge. Nevertheless, whereas crab survivorship responded only to the presence or absence of oysters, the alga had a density-dependent effect on the crabs. Theory (Bruno and Bertness 2001) and evidence (Bishop et al. 2012, 2013) suggest that above a certain threshold, the biomass of a foundation species can be less important in influencing associated communities than just its presence. Here, the threshold above which further increases in foundation species biomass produced no further enhancement of crab survivorship may have been lower for oysters than the alga. Previous studies suggest that in the intertidal zone, oysters, which provide a rigid three dimensional structure, with persistent interstices between shells, are a higher value anti-predator refuge for small crabs, that algae, which has a more malleable form that collapses at low tide, when the alga is immersed (Bishop and Byers 2015).

Facilitative interactions can vary with foundation species abundance or biomass (Bracken et al. 2007, Irving and Bertness 2009, Stier et al. 2012, Hughes et al. 2014), with variation in these population-level traits in some instances influencing community assembly more than foundation species identity (Hughes et al. 2014). Effects of the biomass and spatial arrangement of individual secondary foundation species on recruitment and survivorship of

colonists were apparent in this study. Overall, however, these effects were secondary to interspecific differences between the alga and oysters. The Foundation Species-Biodiversity model (Angelini and Silliman 2014) predicts that benefits to biodiversity will be greatest where the structure of secondary foundation species provides novel habitat compared to the primary foundation species. This study extends this model by showing that multiple co-occurring secondary foundation species have distinct effects on biodiversity where their structure and function has differential effects on resource availability. This study did not attempt to disentangle structural versus functional effects of the two foundation species through the inclusion of structural mimics in experimental designs. However, we suspect that effects on prey survival were predominantly structural (see Heck and Thoman 1981, Crowder and Cooper 1982, Grabowski 2004), with functional effects potentially also influencing recruitment. Irrespective, our study provides evidence that the pathways by which two secondary foundation species influence associate communities include the provision of refuge from predators, and providing habitat for recruitment. Previous studies on habitat cascades have focused on the role of secondary foundation species in boosting the biodiversity facilitated by the primary foundation species (e.g. Altieri et al. 2007, Bishop et al. 2012, Angelini and Silliman 2014). Here we have shown that where primary foundation species facilitate multiple secondary foundation species, these can each have distinct effects on associate community structure. Hence, models of community assembly need to consider interactions among co-occurring foundation species, which may occur in complex networks.

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Ethics Approval

All applicable institutional and/or national guidelines for the care and use of animals were followed (MQ AEC ARA 2015/002).

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IV. WAVE ENERGY ALTERS BIODIVERSITY BY SHAPING INTRASPECIFIC TRAITS OF A HABITAT-FORMING SPECIES

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ABSTRACT

The role of habitat-forming species in facilitating biodiversity is widely acknowledged to vary across environmental gradients according to the extent to which they modify resources and environmental conditions. Across such gradients, spatial variation in the population- and individual-level traits of habitat-forming species may also influence species interactions, but the importance of this indirect effect of environmental context is seldom considered. Here, we conducted surveys and field experiments to partition the direct and indirect effects, arising from changes to the morphology of habitat-forming species, of wave exposure on facilitation of invertebrates by oysters. A survey of nine sites, in Port Jackson, New South Wales, Australia, varying in wave exposure, revealed that as wave energy increased, the density and surface area of oysters decreased. The richness and abundance of associated invertebrates, which were each positively correlated with oyster surface area, similarly decreased across the gradient. Taxon diversity, by contrast, displayed a positive relationship with wave energy. Experimental deployments of oysters from a common source at high and low wave-energy sites confirmed that the variation in oyster morphology among sites was a phenotypically plastic response to environmental conditions. Oyster recruitment was also much greater at low than high wave-energy sites, contributing to the variation in oyster habitat between these. A colonisation experiment in which oyster habitats representative of those at low and high wave-energy sites were deployed at high- and low-wave energy sites in a fully orthogonal design found that invertebrate communities were influenced not only by the wave energy of sites, but also by habitat structure. Overall our study suggests that in some instances the indirect effects of environment on facilitation, arising from changes in habitat-forming species density and morphology, may be just as or of greater importance than the direct effects. Hence, understanding how traits of habitat-forming species respond to environmental conditions, and how intraspecific trait-variation

cascades to influence associated community structure is critical to predicting when and where positive species interactions will be greatest.

Key words: habitat complexity; facilitation; wave exposure; environmental stressor; rocky shore; oyster; amelioration; biodiversity; growth form; *Saccostrea glomerata*; invertebrate community

INTRODUCTION

Habitat-forming species, that create or modify habitat, facilitate dense and diverse biological communities (Callaway and Walker 1997, Bruno et al. 2003). At landscape scales, they do so by enhancing habitat heterogeneity and niche space (Jones et al. 1994, 1997). At smaller scales, they alter environmental conditions and resource flows (Jones et al. 1997, 2010). Consequently, at small scales, the role of habitat-forming species in facilitating biodiversity varies spatially, according to environmental conditions and resource availability, as well as the species assemblage that depends on these (Jones et al. 1997, 2010). This context-dependency has been recognised by ecological theories, such as the stress-gradient hypothesis, which posits that as stress increases in an ecosystem, positive interactions become more prevalent and negative interactions, such as competition, decrease (Bertness and Callaway 1994).

Additionally, in determining how a species interacts with its environment, species traits can also dictate the influence of habitat-forming species on community assembly (Gutiérrez et al. 2003). Although it is often assumed that interspecific variation in traits is of greater ecological significance than intraspecific variation, a growing number of studies demonstrate that intraspecific variation in traits at the population-level (e.g. density) and species-level (e.g. morphology) can also influence the outcome of species interactions (e.g.

van Hulzen et al. 2007, Irving and Bertness 2009, Harley and O'Riley 2011, Bishop et al. 2009, 2012, 2013). Such intraspecific trait variation may be genetic, or a phenotypcially plastic response to the prevailing abiotic and biotic conditions (Bertness 1989, Bruno 2000, Bishop et al. 2009). For example, in high winds the rigidity of plant branches is reduced (Ennos 1997), and where predation is high bivalves produce thicker shells to reduce the likelihood of shell penetration (Leonard et al. 1999). Although the environment may influence species interactions both directly, by determining resource flows and environmental conditions, and indirectly, by influencing species traits, these two pathways of effect are, however, seldom disentangled.

Wave action is a key environmental factor influencing community assembly and the population- and individual-level traits of species in coastal habitats (Koehl and Wainwright 1977, Denny et al. 1985, Denny 2014). High wave action represents a disturbance to coastal communities, influencing community structure by periodically freeing space through damage and loss of organisms (Sousa 1979, Underwood 1998). Algae and invertebrates on wave-swept shores are typically smaller than those on sheltered shorelines, with a lower aspect ratio to minimise drag, and a thicker structure to resist breakage and dislodgement (i.e. limpets, Denny 2000; mussels, Akester and Martel 2000, Steffani and Branch 2003, McQuaid and Lindsay 2007; algae, Wernberg and Thomsen 2005). Wave action can also influence the density of organisms by causing dislodgement, damage and death (Taylor and Schiel 2003). Although the effects of wave energy on species morphology are well established, there are a paucity of studies examining how these effects on individual species cascade to influence other species with which they interact (but see Hammond and Griffiths 2004, Lunt et al. 2017).

In coastal marine environments, oysters are key habitat-forming species that facilitate biodiverse, native invertebrate communities across a range of environmental settings that

include those exposed to anthropogenic boat wake, as well as natural wind-driven waves (e.g. Wells 1961, Dame 1979, Coates 1998, Lenihan and Peterson 1998, Jackson et al. 2008, Lunt et al. 2017). The structure provided by oysters can enhance the availability of hard substrate for attachment of organisms (Bateman and Bishop 2017), protect invertebrates from predators (Grabowski 2004), and alleviate environmental stressors, such as temperature and desiccation (McAfee et al. 2016). The structure formed by oysters can also dissipate wave energy (Manis et al. 2015), potentially reducing the forces experienced by associated organisms. These functions of oysters are dependent on the complexity of habitat they form (e.g. Grabowski 2004; McAfee et al. 2017). Hence, where wave energy influences the morphology of oysters, changes in dependent faunal communities may be seen, that occur independently of or interact with direct effects of wave energy on these communities.

Here, we conduct surveys and field experiments to partition the direct and indirect effects, arising from changes in morphology and density, of wave exposure on facilitation of invertebrates by oysters. First, using a field survey, we examine how oyster habitat morphology and associated invertebrate community structure vary across a gradient of wave exposure. Second, we deploy oysters of a common source across high and low wave-energy sites to assess whether growth and condition of oysters differs between these. Third, we compare colonisation of invertebrates to oyster habitats representative of high and low wave-energy sites, deployed in both high and low wave-energy environments to disentangle direct and indirect pathways by which wave action influences oyster communities. We expect that at high wave-energy sites, oysters will grow more slowly than at low wave-energy sites, and display reduced recruitment, such that they form habitat of reduced surface area and density. Consequently, as a result of this differing habitat formation, we expect that oysters at high wave-energy sites will support fewer invertebrates of fewer species than at low wave-energy sites – an effect that will outweigh any direct effects of wave action on oyster communities.

METHODS

Study Sites

This study was conducted in Port Jackson (Sydney Harbour), New South Wales, Australia (33°51'08.5"S 151°13'57.7"E). Port Jackson is a highly urbanised, drowned river-valley (Roy et al. 2001), with a spring tidal range of ~1.5 m. Intertidal rocky shoreline, comprising Hawkesbury Sandstone, is a dominant habitat type along the estuary. The rocky shoreline varies in wave exposure according to its distance to the estuarine mouth, through which oceanic waves enter and rapidly dissipate, and its proximity to boating channels that are used by Sydney's ferry network, cruise ships and other commercial and recreational vessels. Nine rocky shores were selected across the gradient of wave energy within Port Jackson (Fig. 1). Each contained existing populations of oysters dominated by the native Sydney rock oyster *Saccostrea glomerata,* and with smaller numbers (<15%) of the non-native Pacific oyster, *Crassostrea gigas* (Scanes et al. 2016) at a mid-intertidal elevation.

The wave environment of each site was characterised using a custom pressure logger, mounted using a bracket and dynabolts, to each rocky shoreline at the mid-intertidal elevation (Indian Spring Low Water + 0.5-0.8 m) at which oysters were found. A MS5803 digital pressure sensor (resolution = 0.024 cmH20), set to a sampling frequency of 8Hz, and connected to an ARM Cortex M0 processor recorded the wave environment at each site for 24-hr periods (i.e. full semidiurnal tidal cycles), the first in November and the second in December of 2016. Within each period, sites were sampled in random order, on weekdays. Pilot studies indicated that the sampling frequency was able to resolve wave heights <1cm and there were no artifacts on wave measurements of mounting. Pressure data were converted to wave energy (J) by removing the tidal signal using a high-pass filter, after which signal attenuation with depth was corrected based on linear wave theory, and a Fast Fourier Transform was applied to estimate the total spectral energy over four frequency intervals (Möller et a. 1999). The total spectral energy data at each site were aggregated to 10-minute

averages and the maximum value from each site, hereafter referred to as wave energy (J), was used in analyses.

Survey

To assess how the morphology of oysters and their facilitation of invertebrate communities varies across the wave exposure gradient, a survey of the nine sites was conducted during low tides in July-August 2016. During the sampling period, sites had surface water temperatures of 15.9-17.7°C, and salinities of 31-35 ppt. Two spatially interspersed microhabitats - oyster and bare - were sampled at a mid-intertidal elevation (Indian Spring Low Water + 0.5-0.8 m) of each site using six 25 x 25 cm quadrats randomly positioned within each. The oyster microhabitat contained >60% cover of oysters while the bare microhabitat lacked oysters, or other habitat forming species, but was otherwise similar the oyster microhabitat.

Within the oyster microhabitat, the surface area of habitat provided by oysters was estimated by taking 60-80 photos of 8 MP resolution in a 180° dome around each quadrat, importing these into Agisoft Photoscan (Agisoft LLC), and using these to create a three-dimensional model (see Raoult et al. 2016) of 500,000-900,000 polygons from which the photogrammetry software could estimate surface area. All oysters and invertebrates within each quadrat were then removed from the rocky substrate using hammer and chisel, placed in plastic bags, and transported to the laboratory for further processing. Mobile and sessile invertebrates in the bare habitat plots were identified *in situ*, due to their sparse number. Invertebrates were enumerated by species, except for small crustaceans (isopods and amphipods), which were enumerated by genera and barnacles, which were pooled and enumerated by morphological traits (stalked or acorn).

In the laboratory, oysters and their associated communities were separated and washed over a 500 µm sieve. Invertebrates retained on the sieve were stored in 70% ethanol

until time permitted processing. The number of oysters per quadrat with a shell height of > 10 mm shell height (umbo to valve opening) was counted, and the shell height of up to 30 randomly selected oysters per plot (fewer if the total number of oysters was less than this) were measured to the nearest mm using Vernier calipers. Invertebrates were enumerated as described for the bare plots except polychaetes, which were not observed in the bare plots, were enumerated by family. The effect size of oyster habitat was determined by calculating the log response ratio for each invertebrate community metric between bare and oyster plots.

Direct versus indirect effects of wave energy on associated communities

To assess how oyster growth and condition is influenced by wave exposure, cultivated oysters (54 ± 1 mm, mean shell length ± SE) that were approximately fifteen months old that had been wild-caught and grown out on a Port Stephens, NSW oyster farm were deployed at four sites, two with high wave-energy (sites 4, 5; Fig. 1) and two with low wave-energy (sites 2, 6; Fig. 1), in April 2017. At each site, 33 oysters were attached to the rocky shore at low tide in six groups of 5 or 6 separated by at least 1.5 m along the shoreline. Individual oysters within each group were placed approximately 1cm apart to prevent epoxy on individual oysters from spreading to others before curing, but also to mimic gregarious spatial configurations of natural oysters. Two-part waterproof epoxy resin (FIS EM 390S, Fischer Fixing Systems) affixed the left valve of each oyster to the rocky shore. Oysters were monitored for 45 minutes following attachment to ensure none were dislodged prior to the epoxy curing. In addition to the oysters deployed, 33 were retained for calculation of condition index (CI) at the time of deployment:

$$CI = \frac{Dry\ oyster\ meat\ (g)}{Whole\ oyster\ weight - oyster\ shell\ weight\ (g)} * 100 \quad \text{(Crosby and Gale 1990)}$$

After four months, the shell height (to the nearest mm) and CI of each oyster was determined after removing any fouling organisms from oyster shells. Differences in condition index among sites provided indication of how investment in tissue versus shell

growth varies with wave energy.

To disentangle direct effects of wave action on the facilitation of invertebrate communities by oysters from any indirect effect arising from changes in oyster morphology, a colonisation experiment was performed at each of the same four sites as the oyster deployments. At each site, three habitat treatments were established on 20 x 20 x 4 cm sandstone pavers: bare (0 oysters); low complexity (25 ± 8 oysters, mean ± SE, arranged as individuals or as small clusters of 2-3 flat against the rock); and high complexity (228 ± 28 oysters, arranged in clusters of greater vertical projection). Oyster densities and configurations on the low complexity tiles matched the high wave-energy sites and on the high complexity tiles matched the low wave-energy sites (see Results, Survey). The oysters attached to pavers were hand collected from the two high and two low wave-energy sites, taking care not to damage their shells and to preserve natural clusters, where present. Following collection, oysters were defaunated through immersion in freshwater for 2-3 days, followed by hand-picking of any remaining invertebrates. 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. Oysters collected at high wave-energy sites were attached to low complexity pavers, and clusters of oysters collected at low wave-energy sites were attached to high complexity sandstone pavers at the required density using a non-toxic two-part epoxy resin (Vivacity Engineering, Sydney, Australia) previously used to attach oysters to habitat pavers in similar studies (McAfee et al. 2017, Strain et al., *in press*).

In February 2017, six replicate pavers of each habitat type were attached to each of the four rocky shores using metal brackets and dynabolts in a fully crossed design. Pavers were checked monthly for any glue or dynabolt failure. In only two instances – one high complexity oyster paver from each of the high wave-energy sites – was damage detected. To

determine if wave energy influences oyster recruitment, the total number of oysters on habitat pavers were quantified *in situ* each month from March-June 2017. After four months, the number of mobile invertebrates on each paver were also identified and counted *in situ*. A four-month study period was chosen because recolonisation of experimental plots by invertebrates begins to reach undisturbed levels after three months (Bishop et al. 2009), and any longer time period would have increased the risk of loss or damage to experimental plots.

Statistical Analyses

Linear regressions using site averages tested for relationships between wave energy and each of: the surface area of oyster habitat; oyster shell height and density; the total abundance, taxon richness and Shannon's diversity of invertebrates inhabiting oysters; and the log response ratios of invertebrate total abundance, taxon richness and Shannon's diversity between oyster and bare habitat patches. To examine the relationship between oyster habitat metrics and invertebrate communities, separate linear regressions were run between the surface area of oyster habitat, and each of invertebrate total abundance, taxon richness and Shannon's diversity. Prior to each analysis, a Shapiro test assessed normality and histograms of residuals were inspected for homogeneity of variance. Dependent variables were log- or square-root transformed as necessary to achieve assumptions of normality and homogeneity of variance.

A Spearman's rank correlation, run using the RELATE procedure of PRIMER (Clarke and Gorley 2006), tested for a relationship between the Bray Curtis dissimilarity matrix produced from multivariate invertebrate community data and the Euclidean distance matrix produced from univariate wave energy data. To identify the individual taxa that responded most strongly to the wave exposure gradient, we ran a similarity percentage (SIMPER) analysis to determine which taxa contributed most to multivariate differences in

communities among sites (Clarke 1993). For those taxa with dissimilarity to standard deviation ratios greater than 1.75, separate linear regression analyses tested for relationships between their abundance and wave exposure. Abundance data were square root transformed to achieve assumptions of normality and homogeneity of variance.

Two-way ANOVAs with the factors wave energy (2 levels: low, high) and site nested within wave energy tested for effects of environment on the growth (i.e. final size) and condition of oysters after 4 months in the field. Three-way ANOVAs with the factors habitat (3 levels: bare; low complexity; high complexity), wave energy (2 levels: low; high) and site nested within wave energy (4 levels, random: L1, L2, H1, H2) tested for direct and indirect effects of wave energy on the 1) community assemblage metrics of total abundance, taxon richness and Shannon's diversity of colonising invertebrates at the end of the study; 2) taxa identified from the survey as displaying the strongest variation across the wave energy gradient; and 3) recruitment of oysters (with each month analysed separately, due to temporal non-independence of data). Prior to each test, a Shapiro test checked for normality, and a Levene's test (R package: car) confirmed homogeneity of variances among treatments. In several instances (oyster shell height, invertebrate total abundance, taxon richness and individual taxa abundance), data failed to meet assumptions of normality but were not transformed because transformations are not necessary for sufficient sample sizes when assumptions of homogeneity of variance are met (Underwood 1997). Condition index values and counts of oyster recruits were square root transformed to achieve homogeneity of variance (Underwood 1997). Homogeneity of variances in oyster conditions and counts of oyster recruits at one- and four-months and individual taxa abundance could not be achieved even after square-root transformation, so data were analysed untransformed and significant differences were assessed at $a = 0.01$ to account for the inflated probability of Type I error (Underwood 1997). Where significant treatment effects were found, Tukey's HSD tests were

conducted *a posteriori* to determine significant differences among means ($\alpha = 0.05$). All linear regressions and ANOVAs were done in R version 3.4.0 using RStudio version 1.0.143 and all RELATE and SIMPER analyses were run in PRIMER 6.

RESULTS

Survey

Across the nine sites, maximum wave energy varied from 13.10 to 114.81 J over four tidal cycles (Fig. 1). The surface area and density of oysters were each negatively correlated with wave energy but there was no relationship between wave energy and the mean shell height of oysters (Table 1). The surface area of oysters was approximately 50% less in the sites with the highest as compared to lowest wave-energy (Fig. 2). The total abundance of invertebrates was also negatively correlated with wave energy, with the low wave-energy sites supporting at least 200% more invertebrates than high wave-energy sites (Fig. 3A). Conversely, Shannon's diversity increased with increasing wave energy (Fig. 3B) and there was no relationship between wave energy and taxon richness (Table 2a). Invertebrate taxon richness and abundance both increased with the surface area of oysters (Fig. 3C). However, Shannon's diversity decreased as the surface area of oysters increased (Fig. 3D; Table 2b). Despite the relationships between wave energy and each of total invertebrate abundance and Shannon's diversity in the oyster habitat, the effect size by which oysters increased these metrics over those found in bare habitat did not display any relationship with wave energy (Table 1).
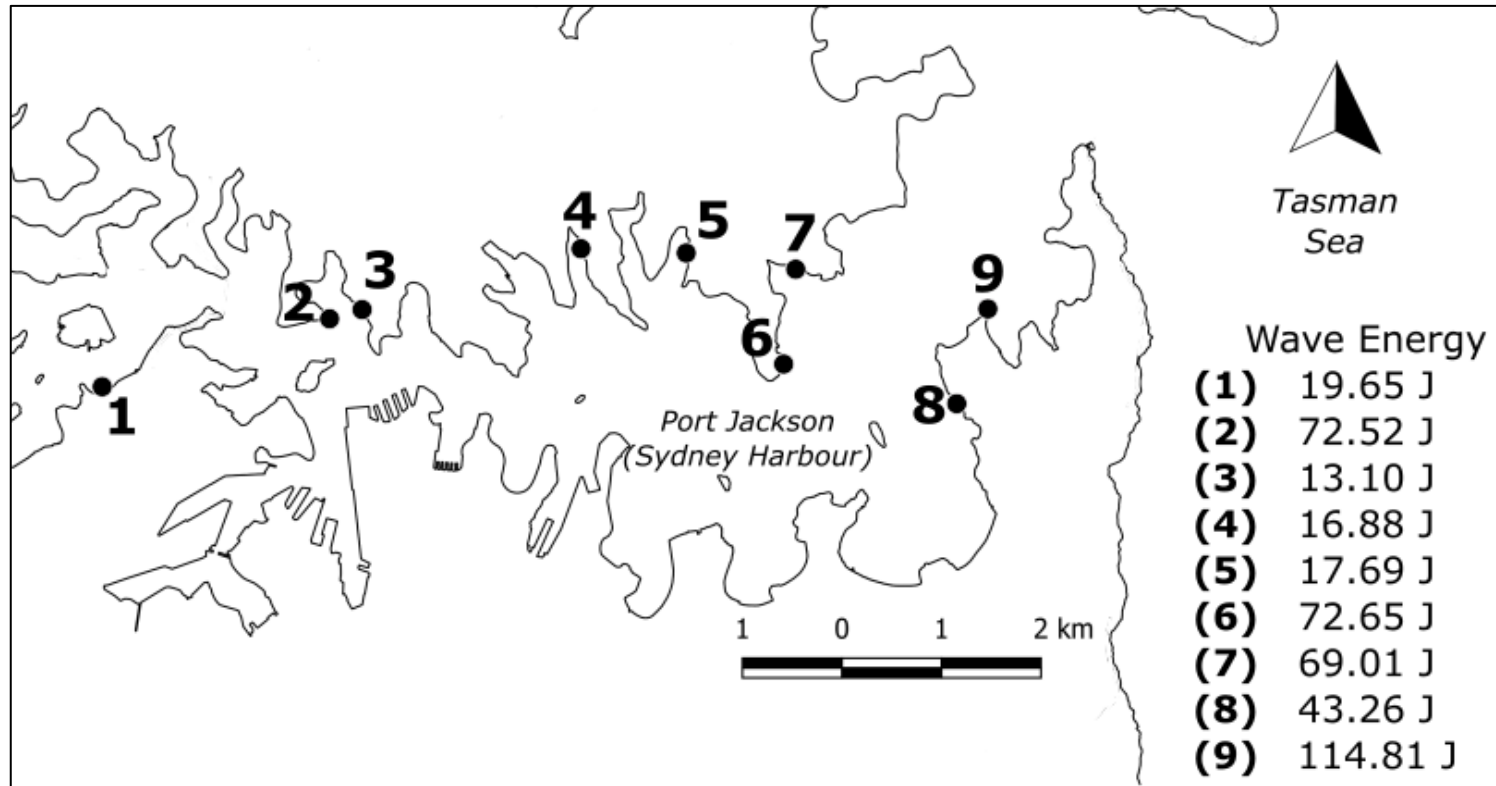
Fig. 1. Map of study sites in Port Jackson (Sydney Harbour): (1) Dawn Fraser (33°51'10.7"S, 151°10'18.7"E); (2) Balls Head Reserve (33°50'49.8"S, 151°11'53.8"E); (3) Sawmillers Reserve (33°50'41.4"S, 151°12'00.1"E); (4) Cremorne Point, Mosman Bay (33°50'26.3"S, 151°13'32.2"E); (5) Sirius Cove Reserve (33°50'31.0"S, 151°14'15.3"E); (6) Bradley's Head (33°51'05.2"S, 151°14'52.0"E); (7) Neutral Bay (33°50'34.9"S, 151°14'49.9"E); (8) Milk Beach (33°51'25.9"S, 151°16'02.8"E); and (9) Bottle and Glass Point (33°50'51.3"S, 151°16'11.5"E). Sites are numbered upstream to downstream and correspond to numbers listed within the text, figures and tables. The maximum wave energy (J) recorded across all sensor deployment times is given for each site.

Table 1. Parameter estimates (± SE) from linear modelling of oyster morphology metrics and log response ratios of fauna in oyster as compared to bare habitat, versus wave energy. Oyster surface area was log transformed prior to analysis. Asterisks indicate *p* value significance levels: (*) < 0.05, (**) < 0.01, (***) < 0.001. Significant results are indicated in bold.

Source	df	Oyster Habitat Morphology			Log Response Ratios		
		Surface area	No. Oysters	Mean shell height	Taxon richness	Total abundance	Shannon's diversity
Intercept	7	-1.493***	16.900***	36.003***	0.819***	1.159**	0.424**
		(0.098)	(1.639)	(2.680)	(0.064)	(0.242)	(0.085)
Wave Energy	7	**-0.010****	**-0.126****	-0.006	-0.001	-0.004	0.001
		(<0.002)	**(0.028)**	(0.045)	(0.001)	(0.004)	(0.001)
Residual error	7	0.166	2.776	4.538	0.108	0.410	0.143
$F_{1,7}$ statistic		32.89	20.740	0.020	0.906	1.096	0.691
R^2		0.825	0.748	0.003	0.115	0.135	0.090
Adjusted R^2		0.800	0.712	-0.140	-0.012	0.012	-0.040
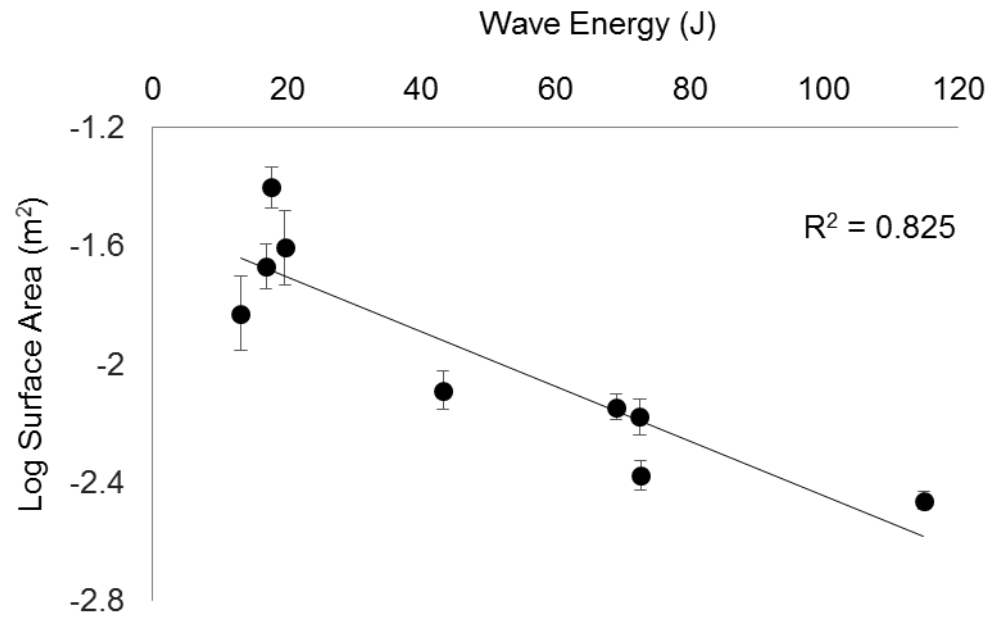
Fig. 2. Relationship between maximum wave energy and the log-transformed surface area of oyster habitat (mean ± SE, $n = 6$) at each of nine sites, as measured using Agisoft Photoscan software.

Table 2. Parameter estimates (± SE) from linear modelling of invertebrate community metrics: taxon richness, total abundance and Shannon's diversity index versus wave energy (J) and mean oyster habitat surface area (m^2) for each site. Total abundance was log transformed prior to analysis. Asterisks indicate *p* value significance levels: (*) < 0.05, (**) < 0.01, (***) < 0.001. Significant results are indicated in bold.

a) Wave Energy					**b) Surface Area**				
Source	*df*	*Taxon richness*	*Total abundance*	*Shannon's diversity index*	*Source*	*df*	*Taxon richness*	*Total abundance*	*Shannon's diversity index*
Intercept	7	23.02*** (2.098)	6.67*** (0.274)	1.714*** (0.114)	Intercept	50	17.446*** (1.815)	4.637*** (0.185)	1.538*** (0.036)
Wave Energy	7	-0.028 (0.035)	**-0.013*** **(0.005)**	**0.005*** **(0.002)**	Surface Area	50	**27.748*** **(11.379)**	**8.54*** **(1.163)**	**-0.976*** **(0.227)**
Residual error	7	3.553	0.464	0.193	Residual error	50	4.979	0.509	0.099
$F_{1,7}$ statistic		0.627	8.421	5.947	$F_{1,50}$ statistic		5.946	53.93	18.54
R^2		0.082	0.546	0.459	R^2		0.106	0.519	0.271
Adjusted R^2		-0.049	0.481	0.382	Adjusted R^2		0.088	0.509	0.256
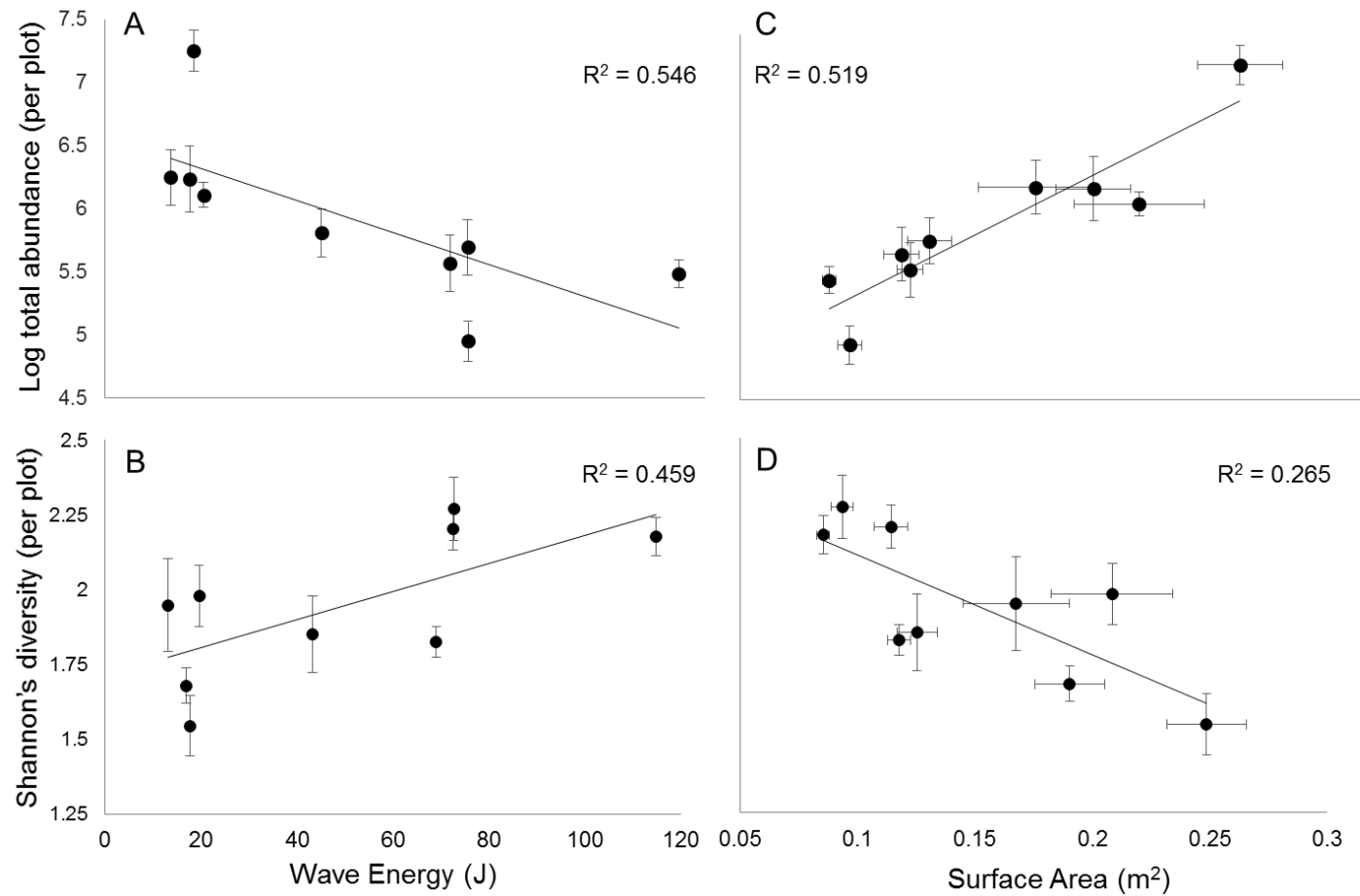
Fig. 3. Relationships between wave energy and (A) the log-transformed total abundance and (B) Shannon's diversity of invertebrates inhabiting the oyster microhabitat and between the surface area (m^2) of oyster habitat and (C) the log-transformed total abundance and (D) Shannon's diversity of these invertebrates. Oyster habitat surface area, total abundance and diversity are means ($\pm$ SE) calculated from $n = 6$ replicate quadrats sampled at each site.

Across the nine sites, a total of 93 different taxa were identified from samples. Gastropods were the most taxa-rich group accounting for 47% of taxa and 24% of total invertebrate abundance, followed by soft-bodied worms (polychaetes, annelids and sipunculas), which were 19% of the taxa and 2% of the total species abundance. By contrast, bivalves were the most abundant group at 52% of the total abundance but only accounted for 13% of taxa. Mobile crustaceans (crabs, isopods, amphipods and tanneids) also represented 13% of taxa but only 4% of total abundance, and sessile crustaceans (stalked and acorn barnacles) accounted for 2% of taxa and 12% of total abundance. Polyplacophorans represented 3% of taxa and 5% of the total abundance while cnidarians and echinoderms each represented 1% of taxa but less than 1% of the total abundance (Supplementary Material: Table S2).

Differences in invertebrate assemblages among sites were correlated to differences in their wave energy (RELATE: $r_s = 0.61$, $p = 0.04$). Twenty-four taxa had dissimilarity to standard deviation ratios for pairwise comparisons between sites that were greater than 1.75 but separate linear regressions were run only for the top ten contributors: five gastropods, *Bembicium auratum, B. nanum*, *Montfortula rugosa*, *Patelloida mimula* and *Onchidella nigricans;* three crustaceans*, stalked barnacles, acorn barnacles,* and *Paragrapsus laevis;* and two bivalves*, Lasaea australis and Trichomya hirsuta.* Stalked barnacles were *Ibla quadrivalvis*, and acorn barnacles were *Amphibalanus amphitrite*, *Balanus trigonus*, *Chthamalus antennatus*, *Epopella simplex*, *Tesseropora rosea* and *Tetraclitella purpuascens*. Of the ten taxa, four displayed a significant linear relationship with wave energy (Table 3). The limpet *M. rugosa* was positively correlated to wave energy, while the crab *P. laevis*, snail *B. auratum,* and limpet *P. mimula* were each negatively correlated to wave energy. The other six taxa did not display significant linear relationships with wave energy (Table 3; Fig. 4).

Table 3. Parameter estimates (± SE) from linear modelling of the top ten invertebrates identified as contributing most to differences in communities among sites. Asterisks indicate *p* value significance levels: (*) < 0.05, (**) < 0.01, (***) < 0.001. Significant results are indicated in bold.

Source	df	Bivalves		Crustaceans			Gastropods				
		Lasaea australis	*Trichomya hirsuta*	Acorn barnacles	Stalked barnacles	*Paragrapsus laevis*	*Bembicium auratum*	*Bembicium nanum*	*Monfortula rugosa*	*Patelloida mimula*	*Onchidella nigricans*
Intercept	7	16.657** (4.186)	4.509* (1.387)	5.939** (1.510)	5.638* (1.717)	4.434** (0.878)	9.758*** (1.374)	1.950 (0.839)	-2.361 (1.942)	7.700** (1.541)	0.875 (0.861)
Wave Energy	7	-0.091 (0.071)	-0.037 (0.023)	-0.037 (0.026)	-0.033 (0.029)	**-0.045* (0.015)**	**-0.109** (0.023)**	-0.002 (0.014)	**0.094* (0.034)**	**-0.082* (0.026)**	0.020 (0.015)
Residual error	7	7.088	2.349	2.557	2.908	1.486	2.326	1.420	3.289	2.610	1.458
$F_{1,7}$ statistic		1.643	2.498	2.046	1.271	9.318	22.19	0.029	8.121	9.851	1.962
R^2		0.190	0.263	0.226	0.154	0.571	0.760	0.004	0.537	0.585	0.219
Adjusted R^2		0.074	0.158	0.116	0.033	0.510	0.726	-0.138	0.471	0.525	0.107
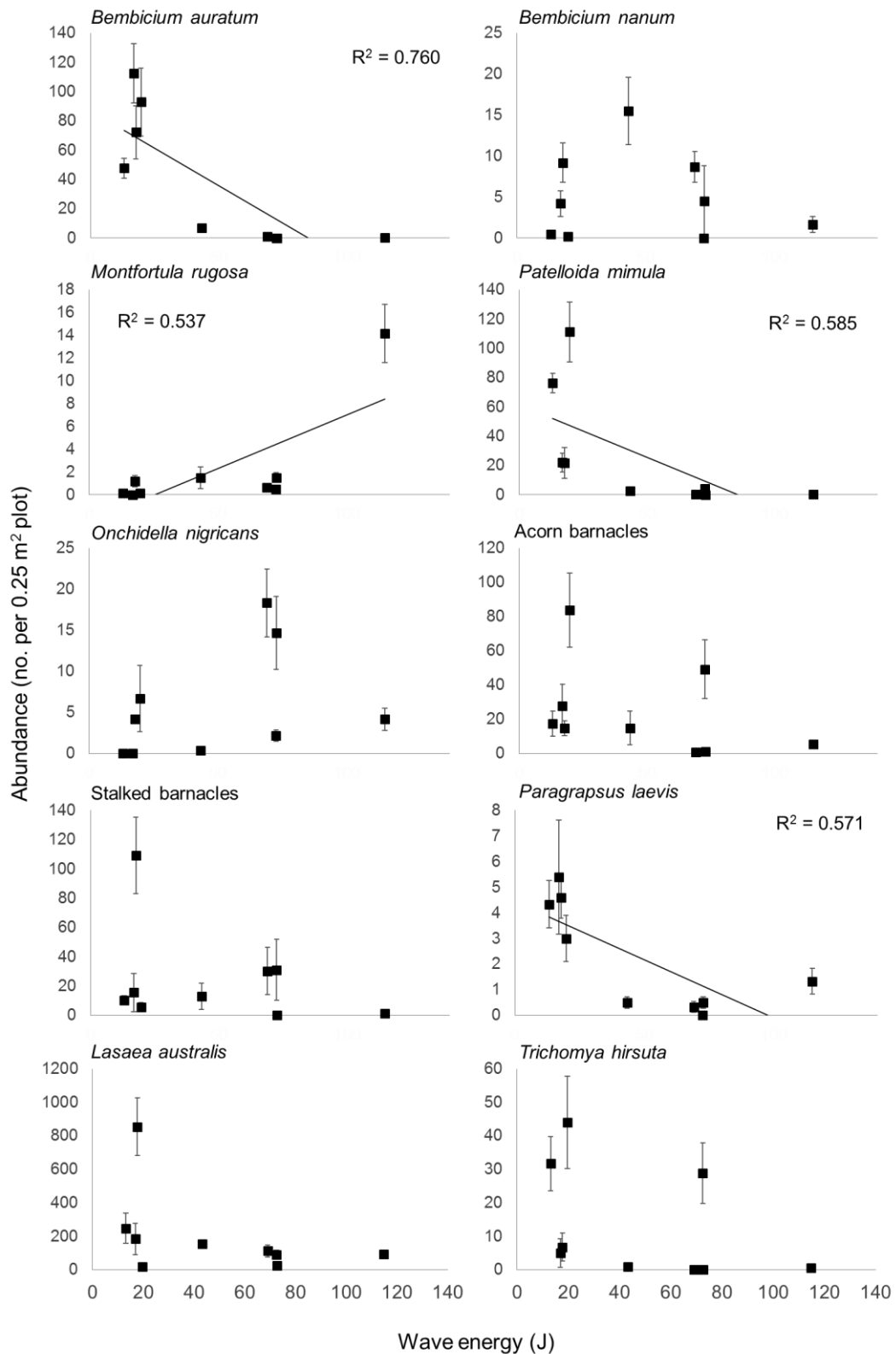
Fig. 4. Relationships between maximum wave energy and the top ten taxa identified as contributing most to the differences in communities among sites. Abundances (means ± SE) are calculated from *n* = 6 replicate quadrats sampled at each site.

Direct versus indirect effects of wave energy on associated communities

Over and above significant site effects, differences in oyster growth and condition between high- and low wave-energy sites were apparent (Table 4). After four months, oysters were larger at low- (56 ± 1 mm, mean shell height $\pm$ SE) than high wave-energy sites (53 ± 1 mm; Tukey: $p = 0.044$), as compared to a starting size of 54 ± 1 mm. After four months, the condition of oysters at low wave-energy sites was 14% smaller than condition of the oysters at the high wave-energy site (Tukey: $p < 0.001$), the latter of which had increased from a starting condition of 10.43 ± 0.22 to 11.61 ± 0.38.

During the 4 month study, 24 taxa recruited to experimental pavers. Gastropods accounted for 16 of the 24 taxa, followed by crustaceans which were 4 of the 24 taxa. Bivalves and polyplacophorans each represented 2 taxa and echinoderms represented just 1 of the 24 taxa. Habitat complexity influenced invertebrate richness regardless of wave energy or site, with 52-55% more taxa occurring on low- or high-complexity pavers, which did not differ, than on bare pavers (Tukey: $p < 0.01$; Fig. 5A; Table 4). Taxon diversity, by contrast, was influenced by the main effects of both habitat complexity and wave energy (Table 4). High-complexity pavers had a more diverse community of invertebrates than bare pavers (Tukey: $p < 0.01$, Fig. 5B), however there was no difference in diversity between high- and low-complexity pavers, or low-complexity and bare pavers. Diversity at high wave-energy sites (1.00 ± 0.08, mean $\pm$ SE) was greater than low wave-energy sites (0.75 ± 0.06; Tukey: $p = 0.02$). Total invertebrate abundance did not vary according to wave energy, site or habitat (ANOVA, $p \geq 0.1$; Table 4).

Table 4. Two-way ANOVAs compared oyster shell height and condition index among Energy (2 levels: low or high energy) and Site nested within Energy (4 levels: L1, L2, H1, H2). Three-way ANOVAs examined effects of Habitat (3 levels: bare, low or high complexity); Energy (levels as described above); and Site nested within Energy (levels as described above) on invertebrate community richness, abundance and diversity. Significant results at $\alpha = 0.05$ are indicated in bold.

			Shell height		
	Source	*DF*	*Mean Sq*	*F-value*	*p*
	Energy	1	139.48	5.51	**0.021**
	Site (Energy)	2	3.65	0.14	0.866
	Residuals	94	25.34		
			Condition index		
	Source	*DF*	*Mean Sq*	*F-value*	*p*
	Energy	1	46.75	19.48	**<0.001**
	Site (Energy)	2	5.98	2.49	0.088
	Residuals	94	2.40		
			Taxon richness		
	Source	*DF*	*Mean Sq*	*F-value*	*p*
	Habitat	2	17.06	10.15	**<0.001**
	Energy	1	0.68	0.41	0.527
	Site (Energy)	2	1.51	0.90	0.412
	Habitat*Energy	2	1.06	0.63	0.537
	Habitat*Site (Energy)	4	3.72	2.22	0.078
	Residuals	60	1.68		
			Total abundance		
	Source	*DF*	*Mean Sq*	*F-value*	*p*
	Habitat	2	3240	1.80	0.175
	Energy	1	741	0.41	0.524
	Site (Energy)	2	3653	2.02	0.141
	Habitat*Energy	2	2418	1.34	0.270
	Habitat*Site (Energy)	4	688	0.37	0.829
	Residuals	60	1805		
			Shannon's diversity index		
	Source	*DF*	*Mean Sq*	*F-value*	*p*
	Habitat	2	0.54	3.49	**0.037**
	Energy	1	0.86	5.50	**0.022**
	Site (Energy)	2	0.08	0.54	0.588
	Habitat*Energy	2	0.19	1.22	0.302
	Habitat*Site (Energy)	4	0.02	0.10	0.983
	Residuals	60	0.16		

Individual Oyster Responses spans the first two sections (Shell height, Condition index). *Invertebrate Communities* spans the remaining sections (Taxon richness, Total abundance, Shannon's diversity index).
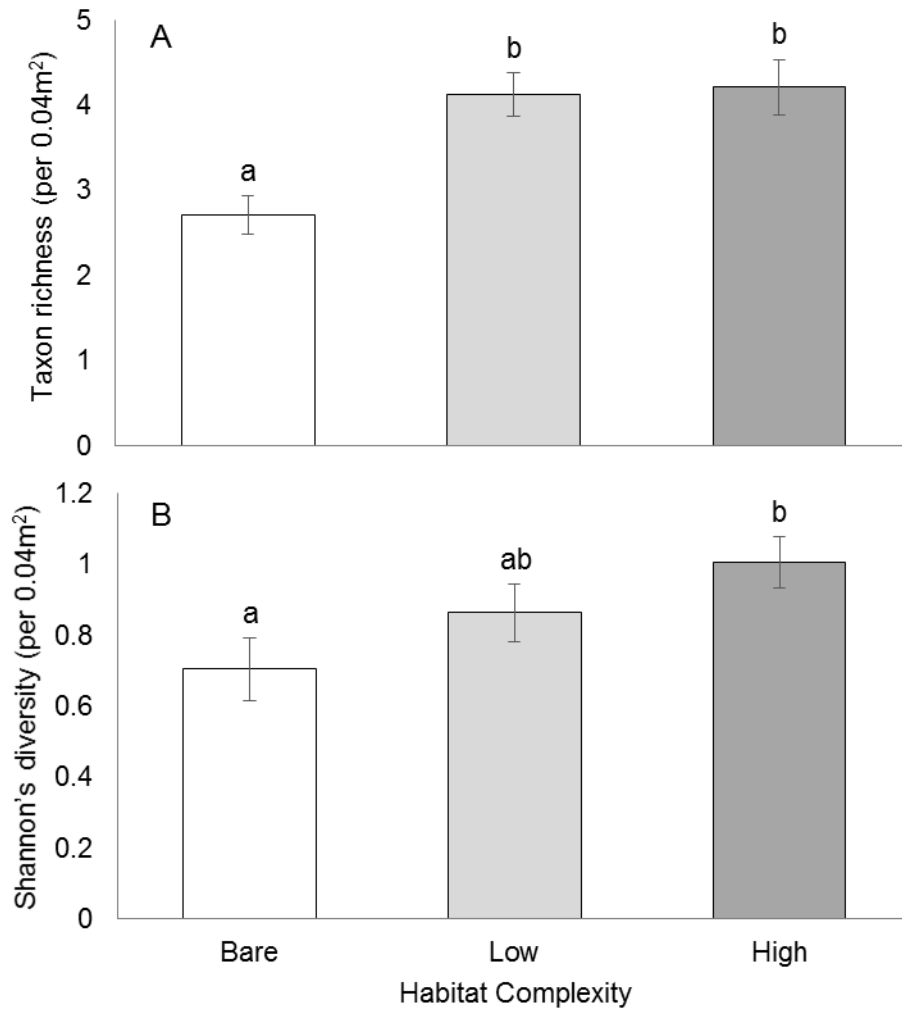
Fig. 5. Differences in invertebrate (A) taxon richness and (B) Shannon's diversity index (mean ± SE, *n* = 24) among habitat complexity treatments at the end of the habitat manipulation study. Different letters above the bars indicate significant differences between treatments (Tukey: Taxon richness: $p \leq 0.004$; Shannon's diversity: $p \leq 0.002$).

Seven of the ten taxa identified as contributing most to the dissimilarity to standard deviation ratios in the field survey were observed in the habitat manipulation study. Four of these (*B. auratum, P. mimula, P. laevis, B. nanum*) displayed responses to habitat complexity and/or wave energy while the other three (*O. nigricans, M. rugosa* and acorn barnacles) did not respond to either factor (Table 5). *B. auratum* abundance displayed effects of habitat complexity that varied between wave energy treatments (Table 5). On average, *B. auratum*

abundances were greater at low than high wave-energy sites. At low wave-energy sites, low-complexity habitat supported more *B. auratum* than high-complexity habitat both of which supported more snails than bare pavers at low wave-energy sites and all habitat treatments at high wave-energy sites. There was no difference in abundance among habitat treatments at high wave-energy sites or bare pavers at low wave-energy sites (Fig. 6A). *B. nanum* displayed effects of habitat complexity that varied among sites (Table 5). At one of the high wave-energy locations (site 6, H2), high-complexity pavers supported over 3 times more *B. nanum* density than bare paver habitats, but neither significantly differed from low-complexity pavers (Tukey: $p \geq 0.2$). There was no difference in *B. nanum* abundance among habitat treatments at the other three sites (Tukey: $p \geq 0.2$; Fig. 6B). Irrespective of the wave energy of sites, high-complexity pavers supported more *P. mimula* than bare pavers (Tukey: $p = 0.005$), but neither significantly differed from low-complexity pavers (Tukey: $p \geq 0.2$; Fig. 6C). *P. laevis* density responded to the main effect of wave energy wherein more crabs were counted at low- (1 ± 0) than high wave-energy locations (0 ± 0; Table 5; Tukey: $p = 0.002$).

Table 5. Three-way ANOVAs examined effects of Habitat (3 levels: bare, low or high complexity); Energy (2 levels: low or high energy); and Site nested within Energy (4 levels, L1, L2, H1, H2) in the habitat manipulation study on key taxa that were identified as contributing most to multivariate differences in communities among sites in the field survey. Individual taxa failed to meet assumptions of normality and homogeneity of variance so significant results at $\alpha = 0.01$ are indicated in bold.

		Gastropods								
		Bembicium auratum			*Bembicium nanum*			*Onchidella nigricans*		
Source	DF	Mean Sq	F-value	p	Mean Sq	F-value	p	Mean Sq	F-value	p
Habitat	2	2116	18.38	**<0.001**	66.68	5.35	**0.007**	0.01	1.00	0.374
Energy	1	9777	84.94	**<0.001**	62.35	5.00	0.029	0.01	1.00	0.321
Site (Energy)	2	27	0.24	0.790	228.12	18.3	**<0.001**	0.01	1.00	0.374
Habitat*Energy	2	1792	15.64	**<0.001**	39.18	3.14	0.050	0.01	1.00	0.374
Habitat*Site (Energy)	4	166	1.45	0.230	77.29	6.20	**<0.001**	0.01	1.00	0.415
Residuals	60	115			12.46			0.01		

		Gastropods					
		Montfortula rugosa			*Patelloida mimula*		
Source	DF	Mean Sq	F-value	p	Mean Sq	F-value	p
Habitat	2	0.06	0.16	0.849	59.72	9.97	**<0.001**
Energy	1	0.89	2.62	0.111	5.01	0.84	0.364
Site (Energy)	2	0.69	2.05	0.138	126.62	21.13	**<0.001**
Habitat*Energy	2	0.22	0.66	0.523	11.06	1.85	0.167
Habitat*Site (Energy)	4	0.36	1.07	0.381	22.33	3.73	0.009
Residuals	60	0.34			5.99		

		Crustaceans					
		Paragrapsus laevis			*Acorn barnacles*		
Source	DF	Mean Sq	F-value	p	Mean Sq	F-value	p
Habitat	2	0.26	3.80	0.028	28314	1.22	0.302
Energy	1	0.68	9.80	**0.003**	21806	0.94	0.336
Site (Energy)	2	0.13	1.80	0.174	351448	15.17	**<0.001**
Habitat*Energy	2	0.26	3.80	0.028	12741	0.55	0.580
Habitat*Site (Energy)	4	0.04	0.60	0.664	20338	0.88	0.482
Residuals	60	0.07			23161		
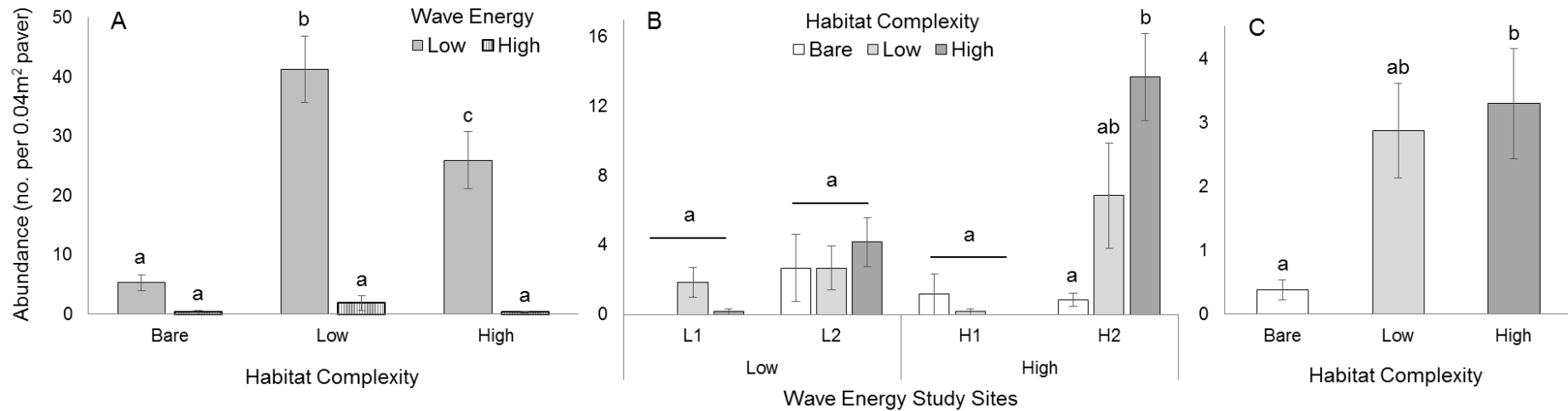
Fig. 6. Differences in abundance of (A) *Bembicium auratum*, (B) *Bembicium nanum* and (C) *Patelloida mimula* among habitat complexity treatments and wave energy at the end of the habitat manipulation study. Abundances (mean ± SE) are calculated from n = *12*, *n = 6* and *n = 24* replicate habitat pavers for *B. auratum*, *B. nanum* and *P. mimula*, respectively. Different letters above the bars indicate significant differences between treatments (Tukey tests: $p \leq 0.01$).

Table 6. Three-way ANOVAs with the factors Habitat (3 levels: bare, low or high complexity); Energy (2 levels: low or high energy); and Site nested within Energy (4 levels, L1, L2, H1, H2), examining sources of spatial variation in oyster recruitment for each month of the habitat-manipulation study. Data for month 2 and 3 were square root transformed prior to analysis and significant differences were interpreted at $\alpha = 0.05$. Significant differences were interpreted at $\alpha = 0.01$ on untransformed data for months 1 and 4 because data failed to meet assumptions of homogeneity of variance and normality. Significant results are indicated in bold.

a) Month 1

Source	DF	Mean Sq	F-value	p
Habitat	2	2.04	1.13	0.330
Energy	1	7.35	4.06	0.048
Site (Energy)	2	16.18	8.95	**< 0.001**
Habitat*Energy	2	1.01	0.56	0.574
Habitat*Site (Energy)	4	1.14	0.63	0.643
Residuals	60	1.81		

b) Month 2

Source	DF	Mean Sq	F-value	p
Habitat	2	0.40	0.18	0.835
Energy	1	41.94	18.89	**< 0.001**
Site (Energy)	2	75.59	34.05	**< 0.001**
Habitat*Energy	2	0.02	0.01	0.992
Habitat*Site (Energy)	4	2.17	0.98	0.427
Residuals	60	2.22		

c) Month 3

Source	DF	Mean Sq	F-value	p
Habitat	2	1.54	0.76	0.473
Energy	1	42.35	20.86	**< 0.001**
Site (Energy)	2	31.62	15.57	**< 0.001**
Habitat*Energy	2	0.95	0.47	0.629
Habitat*Site (Energy)	4	10.10	4.98	**0.002**
Residuals	60	2.03		

d) Month 4

Source	DF	Mean Sq	F-value	p
Habitat	2	167	0.84	0.437
Energy	1	11704	58.77	**< 0.001**
Site (Energy)	2	7771	39.02	**< 0.001**
Habitat*Energy	2	129	0.65	0.528
Habitat*Site (Energy)	4	167	0.84	0.505
Residuals	60	199		

From month two onwards, oyster recruitment differed between high- and low wave-energy treatments (Table 6). This effect of wave energy was apparent over and above significant site variation that occurred at all sampling times. By month four, pavers deployed at low wave-energy sites supported on average 30 ± 5 (SE) oyster recruits as compared to only 4 ± 1 at high wave-energy locations (Tukey: $p < 0.001$; Table 6; Fig. 7). Effects of habitat complexity were only apparent at the three month sampling time but varied among sites (Table 6). Driving this result was the difference in recruitment densities on low- and high-complexity habitat pavers between one low wave-energy site (site 4, L1) and one high wave-energy site (site 6, H2). Recruitment densities on low- and high-complexity habitat pavers at H2 were 88 and 99% lower than recruitment densities on low- and high-complexity habitat pavers at L1, respectively (Tukey: $p < 0.05$).
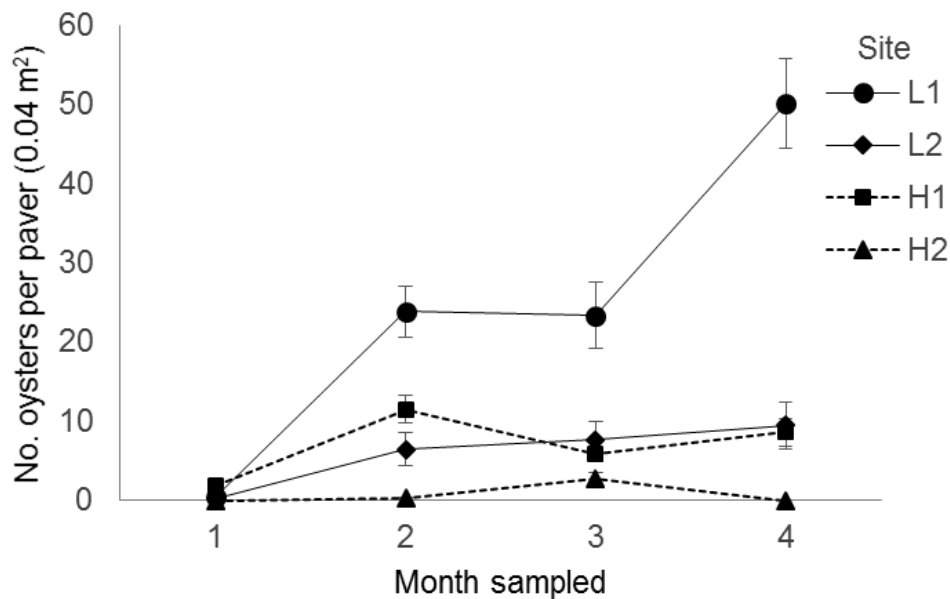
Fig. 14. Oyster recruitment density per habitat paver (mean $\pm$ SE, $n = 18$) during each month at each of the low- (L1, L2, solid lines) and high (H1, H2, dashed lines) wave energy sites utilised in the manipulative study. During each sampling period, there was a significant effect of site nested within wave energy. For month 3, there was a significant interaction between habitat and site nested within wave energy.

DISCUSSION

Our study provides evidence for both direct effects of wave energy on the communities supported by intertidal oysters as well as indirect effects, arising from effects of wave energy on oyster morphology and density. At high wave-energy sites, oysters displayed reduced rates of recruitment and growth as compared to low wave-energy sites. The net effect was lower densities of oysters, comprised of smaller individuals, at the high wave-energy sites, that provided a reduced surface area of substrate for attachment, and supported smaller densities of invertebrates, of fewer taxa as compared to low wave-energy sites. The manipulative field experiment indicated that both the indirect effect of waves, arising from their effect on habitat structure, and direct effects were important determinants of invertebrate community structure.

Several previous studies also report a smaller size and density of bivalves at high than low wave-energy sites (Seed 1969, Jørgensen 1976, Alvarado and Castilla 1996), although others report the opposite pattern (Jones and Demetropoulos 1968, McQuaid and Lindsay 2000). A smaller mean body size of bivalves at more exposed sites may be attributed to greater predation of small individuals on sheltered shores (Menge 1976) or greater dislodgement of larger individuals on exposed shores (Harger and Landenberger 1971, Griffiths 1981, Paine and Levin 1981, Denny 1987). Here, however, it appears that a lower growth-rate at high than low wave-energy sites was at least partially responsible. Although several studies report the opposite pattern of greater growth rates of bivalves at wave exposed than sheltered sites, attributing this to a greater supply of food (e.g. McQuaid and Lindsay 2000, Steffani and Branch 2003), growth rate may be reduced where the time available for feeding is shortened (Brown and Quinn 1988). Oysters may shut their valves during extreme wave events so as to avoid damage to gills. Alternatively, the reduced growth rate of oysters at high wave-energy sites, as measured by shell height, may reflect a greater susceptibility

of the thin shell at the growing margin to erosion and breakage. Although oysters at high wave-energy sites grew more slowly than those at low wave-energy sites, high wave-energy oysters had higher condition indices, indicating a proportionately greater biomass of tissue versus shell. Whereas previous studies have found that the size and density of oysters is often negatively correlated due to crowding effects (Krassoi et al. 2008), here the two were uncoupled indicating that the small size of oysters at wave-exposed sites did not reflect a limited availability of space. As with other species (Taylor and Schiel 2003), we found that wave energy reduces recruitment and survival of oysters thereby altering the ability of oysters to form complex morphologies at high wave-energy sites.

The invertebrate communities found at low wave-energy sites were dominated by two gastropod species, *Bembicium auratum* and *Patelloida mimula*, each of which displayed a negative relationship with wave energy. Despite the similar patterns displayed by the two species in the survey, the results of the manipulative experiment suggested that different processes produced them. Whereas *B. auratum* responded to the interacting effect of habitat complexity and wave energy, *P. mimula*, responded to habitat complexity alone. However, the effect of habitat complexity on *B. auratum* was only detected at low wave-energy sites as very few *B. auratum* were found at high wave-energy sites. In contrast to sessile species cemented to the substratum, many mobile species are highly susceptible to dislodgement by waves, such that their densities are negatively correlated with wave exposure (Denny et al. 1985). Limpets, such as *P. mimula*, are able to strongly adhere to rock (Denny 2000), but are instead highly responsive to the availability of substrate for grazing (Anderson and Underwood 1994). Despite the abundance of hard rocky substrate on rocky shores, *P. mimula* was almost exclusively found on oysters, the surface of which it grazes (Minchinton and Ross 1999). Its density is often limited to one limpet per oyster (Minchinton and Ross 1999), such that its abundance can be constrained by the availability of oyster habitat

(Hughes et al. 2014). *B. auratum* abundance also benefited from enhancement of oyster density at low-wave energy sites, perhaps because of the protective role of oyster structure, or because the enhancement by oysters of the surface area of hard substrate alleviated competitive interactions (Branch and Branch 1980). However, enhancement of oyster habitat complexity failed to increase *B. auratum* abundances at high wave-energy sites where, unlike limpets, wave energy is perhaps too high for attachment and grazing (Denny et al. 1985). The limpet *M. rugosa* was, by contrast, the only species for which density increased with wave energy. Their density did not vary with habitat complexity, so at high wave-energy sites where abundances of other gastropods are low, they may have benefited from reduced competition for grazing space. The abundance of the shore crab, *Paragrapsus laevis*, while relatively low compared to other taxa, displayed consistent, negative responses to high wave-energy. Crabs seek shelter in microhabitat refuges on wave-swept shores (Wieters et al. 2009). Unlike molluscs, which can strongly adhere to the substratum to avoid dislodgement by waves, perhaps even the high complexity habitat was insufficient in sheltering crabs from waves.

Additionally, abundance of one gastropod species – the grazer *Bembicium nanum* – had a slight tendency to peak at low to intermediate wave energies. At sites of intermediate wave energy, grazers may benefit from intermediate cover of oysters which provide protective spaces alongside bare rock for grazing (Range et al. 2008). However, in the manipulative experiment, the abundance of *B. nanum* generally did not vary with habitat or wave energy except for one high wave-energy site where the abundance was greater in high-complexity habitat than bare habitat. Optimal densities of macroalgae for *B. nanum* growth (Underwood 1984) were perhaps found on oysters at this site, where the greater surface area of high-complexity oysters provided the most grazing space. As we cannot fully disentangle the habitat- or wave-driven mechanism behind differences in *B. nanum* abundance, density

differences for this snail are perhaps more site-dependent.

Despite variation in the communities of oyster habitat across the wave exposure gradient, there was no relationship between wave energy and the magnitude by which oysters enhanced the abundance, richness or diversity of invertebrates over bare spaces. This is contrary to the prediction of the stress gradient hypothesis that facilitation should increase across stress gradients (Bertness and Callaway 1994) and the observation by other studies that the magnitude of the positive influence of oysters increase across gradients of temperature and desiccation stress (e.g. McAfee et al. 2016, Bateman and Bishop 2017). One potential explanation is that in intertidal harbour environments wave exposure may be of secondary importance in structuring invertebrate communities to desiccation and heat stress, and/or biotic interactions such as predation and competition, against which oysters also offer protection. Alternatively, the relatively low profile of the oyster habitat growing on rock may be insufficient to buffer associated organisms from wave forces. The results instead support a universally positive influence of oysters on invertebrate abundance and richness (see also McAfee et al. 2016, Bateman and Bishop 2017).

While wave energy can be a natural disturbance, its effects can be induced or enhanced by human activity (see Bishop and Chapman 2004, Bishop 2005). In large, highly urbanised estuaries such as Port Jackson, boat wake can be the main source of wave energy, influencing rocky shore habitats where it can dislodge or alter growth forms of species (Taylor and Schiel 2003, Hammond and Griffiths 2004, Transport for NSW 2015). Hence, understanding the mechanisms by which waves influence community assembly is of critical importance given growing coastal populations, increasing boat ownership, and the increasing horsepower requirements of larger vessels (Maritime NSW 2010, Neumann et al. 2015). Our study has indicated that in addition to the direct effects of waves on organisms and the communities to which they contribute, indirect effects, arising from changes to

habitat-forming species morphology and density, can influence community assembly. Hence, understanding how traits of habitat-forming species respond to environmental conditions, and how intraspecific trait-variation cascades to influence associated community structure is critical to understanding spatial variation in community assembly.

ACKNOWLEDGEMENTS

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V. GENERAL DISCUSSION

The goal of this thesis was to address sources of spatial and temporal variation in the facilitation of invertebrates by Sydney rock oysters, *Saccostrea glomerata.* Ecological theory regarding the effects of environment, biotic interactions, and stochastic processes on community assembly were tested with small-scale manipulations of habitat structure across a range of environmental contexts, experimental deployments of shell substrate through time, and surveys of natural oysters across environmental gradients. Like previous studies (e.g. Wells 1961, Dame 1979, Lenihan and Peterson 1998, Gutiérrez et al. 2003, McAfee et al. 2016), I found that at a patch scale, the effect of *S. glomerata* on invertebrate abundance and richness was universally positive. However, the magnitude of this effect varied according to the contribution of live versus dead oysters to habitat, the orientation and density of oyster shells, the wave climate in which oysters were present, and the timing of oyster shell deployment. Additionally, I found that:

a. Patterns of oyster colonisation and associate community development varied temporally, reflecting temporal variation in recruitment of oysters and competitors.

b. Where oysters co-occurred with other habitat-forming species, interspecific differences in the morphological traits of the habitat-formers lead to generally distinct effects of these on recruitment and provision of refuge from predators.

c. In some instances, the indirect effects of environment on facilitation, arising from changes in habitat-forming species morphology, were greater than direct effects.

The results of this study will assist in identifying how, when and where to restore native oyster reefs, presently considered functionally extinct.

RESTORATION OF HABITAT-FORMING OYSTERS AND ASSOCIATED BIODIVERSITY

Due to the high rates of habitat modification and loss, restoration of marine habitat-forming species has been implemented within a variety of settings (e.g. corals, Rinkevich 1995; macroalgae, Marzinelli et al. 2016; mussels, Wilcox et al. *in press*; seagrass, Orth et al. 2006). Globally, >85% of native oyster species have been lost and for these habitat-forming species, restoration is imperative (Beck et al. 2011). Oyster restoration has been practiced in the United States for decades (Luckenbach et al. 1999), but is still in its infancy in Australia (e.g. Gillies et al. 2015, 2017) where native oyster reefs are considered functionally extinct (Beck et al. 2011). The presence of *S. glomerata* on rocky shores, artificial substrates and in mangroves (McAfee et al. 2016, Scanes et al. 2016) suggests that propagule supply is present along much of the east coast of Australia. However, pilot studies that address the extent to which previous restoration methods (i.e. deploying shell substrate or transplanting adults, Brumbaugh and Coen 2009) will be successful at restoring oyster reefs and associated communities within Australian systems, are needed prior to implementation of large-scale restoration projects.

This thesis examined (**chapter 2**) how the development of communities associated with *S. glomerata* varies with the type and timing of substrate deployment at 5 sites of Sydney Harbour. All substrate types (live oysters, dead oysters or a mix; that were loose or attached to one another) were colonised by sessile and mobile invertebrates as well as algae, with the timing of substrate deployment the most important factor in determining community composition. Fouling assemblages initially displayed patterns consistent with the lottery hypothesis (Sale 1977) wherein dominant primary space occupants were species with propagules in the water at the time of substrate deployment. However, as time progressed, community assembly appeared to converge towards one equilibrium point (Greene and Schoener 1982). Hence, short term studies are inadequate to test for effects of the timing of

substrate provision on community assembly at time scales of ecological significance.

Despite temporal variation in community development, all types of oyster substrate (irrespective of whether it was comprised of live or dead shell, that was loose or attached) were colonised by diverse, mobile invertebrate communities. Natural oyster beds constitute a matrix of live and dead oysters, and consistent with the greater surface area provided by dead, disarticulated shells than live, articulated oysters, substrate that contained some dead shell tended to support slightly higher abundances of mobile invertebrates. These results reinforce that one of the benefits of successful restoration of native oyster reefs is the provision of habitat to fouling and mobile organisms (Posey et al. 1999, Luckenbach et al. 2005, Rodney and Paynter 2006, Humphries et al. 2011).

Collectively these results suggest that at locations with a natural supply of oyster larvae, deployment of dead oyster shell will be adequate to facilitate colonisation of oysters, and will provide a greater surface area and complexity of habitat for colonisation of associate communities than live oysters until the live oyster matrix is established. The results also suggest in influencing whether oysters or competitors for space first colonise substrate, the timing of substrate deployment may be critical to the success of restoration projects and should be considered in their planning. Habitat size can alter species-specific colonisation rates (Eggleston et al. 1999) and thus, future studies should expand the scale of substrate deployment. Testing recruitment and colonisation of large, contiguous patches of restored reefs would help ascertain if the community assemblage processes observed in this study occur within habitat patches more representative of the scale at which restoration will likely occur.

INTER- AND INTRASPECIFIC VARIATION IN MORPHOLOGY OF HABITAT-FORMING SPECIES

The communities facilitated by habitat-forming species are a function of their specific traits (Gutiérrez et al. 2003). The presence of multiple morphotypes of habitat-forming species might be expected to enhance biodiversity by increasing the number of different microhabitats present within a habitat, and the resources or conditions that are modified (van Hulzen et al. 2007, Bishop et al. 2009, 2012, Irving and Bertness 2009, Angelini & Silliman 2014). In **chapters 3** and **4**, I investigated the mechanisms by which morphological variation between and within habitat-forming species influences community assembly. In both chapters I hypothesised that habitats of greater complexity – formed either by two habitat-forming species or the morphological variation of one – would have an overall positive effect on associated communities.

This thesis found that two co-occurring secondary habitat-forming species (or foundation species, *sensu* Dayon 1972), of differing morphology, had generally distinct effects on associated communities. In mangrove forests, where pneumatophores of *Avicennia marina* facilitate the secondary habitat-forming species, *S. glomerata* and the alga, *Hormosira banksii*, the two secondary habitat-forming species differed in their effect on species recruitment and predator-prey interactions. For example, whereas the presence of *S. glomerata* positively influenced recruitment and survival of oysters, the alga had no influence. Conversely, the alga positively influenced survivorship of the snail, *Bembicum auratum*, but the oyster had no influence. These differences likely stemmed from both differences in functional traits between the two species (e.g. emission of chemical cues) as well as differences in structural traits, which influenced the size, number and geometry of anti-predator refuges they provide. This study did not attempt to disentangle structural from functional effects using structural mimics. Nevertheless, some redundancy between the two species was also apparent. Both *S. glomerata* and *H. banksii* reduced fish predation on crabs

when the other was absent. Additionally, each negatively impacted barnacle recruitment, demonstrating that although habitat-forming species have net positive effects on biodiversity, they can negatively impact abundances of individual species.

Whether or not structural or functional differences between the oyster and alga habitat explain variation in recruitment and predator-prey interactions between habitat types could be identified in future studies using structural mimics. For some marine habitat-forming species, structural differences are less important than functional differences on the development of associated communities (i.e. seagrass, Lee et al. 2001; or seagrass epiphytes, Bologna and Heck 1999). However, structural variation in a single habitat-forming bivalve species can result in different recruitment densities and abundances of associated sessile and mobile communities that use the habitat (Summerhayes et al. 2009, Wilkie et al. 2013).

A broad scope of interactions among primary and secondary foundation species have been well documented within this study system (e.g. Bishop et al. 2012, 2013, Hughes et al. 2014), and this thesis adds to the understanding of mechanisms that maintain recruitment and prey survival within intertidal mangrove habitat. The effects of co-occurring habitat-forming species on associated species were monitored across multiple periods throughout one year. However, expanding this study to broader geographic and temporal scales would indicate whether recruitment processes and predator-prey interactions within habitat-forming species, are site- or time-dependent, or if they are representative of interactions that are consistent through time and space.

Although interspecific variation has been the focus of studies examining effects of trait variation in habitat-forming species morphology on associate communities (i.e. Bishop et al. 2012, Angelini and Silliman 2014), a growing number of studies have recognised that intraspecific variation in traits can also influence the outcome of species interactions (e.g. van Hulzen et al. 2007, Irving and Bertness 2009, Bishop et al. 2009, 2013). Intraspecific

variation in morphology is often shaped by environmental conditions (e.g. Ennos 1997, Steffani and Branch 2003, Bishop et al. 2009). Thus, I hypothesised that intraspecific variation in oyster morphology would vary along a wave gradient. I also hypothesised that the indirect effect of wave energy, arising from intraspecific variation in oyster morphology, would have a greater influence on biodiversity than direct effects of wave energy.

Consistent with the rich literature documenting the effects of wave energy on the growth forms and recruitment of invertebrates (Akester and Martel 2000, Denny 2000, Steffani and Branch 2003, Taylor and Schiel 2003, McQuaid and Lindsay 2007, Lunt et al. 2017), wave energy negatively affected oyster recruitment and growth, resulting in smaller densities of oysters, of reduced surface area at exposed as compared to sheltered locations. A combination of a field survey across a wave exposure gradient, and a manipulative field experiment indicated that these differences in oyster morphology were, in turn, more important than direct effects of the physical environment in influencing the abundance and richness of invertebrates, each of which increased with oyster density and surface area. Hence, environmental conditions not only influence intraspecific trait-variation, but can also cascade to influence the associated community, resulting in intraspecific variation in facilitation.

This thesis did not attempt to disentangle the effects of natural versus boat-induced wave energy on oyster communities. However, a future study that spans broader geographic scales by testing the effect of wave energy on communities in natural and urbanised rocky shores would better indicate the magnitude by which anthropogenic boating activity alters growth forms and thus the habitat-provisioning by oysters. Additionally, colonisation of different oyster habitat complexities should be monitored over longer temporal-scales to identify if observed patterns are consistent through time and space (Underwood 2000).

My thesis adds to growing literature that suggests that both inter- and intraspecific

variation in the traits of habitat-forming species are important in determining the communities they support (van Hulzen et al. 2007, Bishop et al. 2012, Angelini et al. 2014). Thus, restoration or rehabilitation projects need to be cognisant of how the morphology of the habitat-forming species on which they may be based influences community assembly, and how environmental conditions, genetic provenance and restoration methods influences growth-form. Additionally, because habitat-forming species differ in the communities they facilitate, there may be benefits of targeting the rehabilitation of multiple habitat-forming species in restoration projects.

DISTURBANCE THEORY WITHIN HABITAT RESTORATION

Disturbances influence community assembly (Levin & Paine 1974, Pickett 1980, Reice 1994) by disrupting interspecific interactions or clearing space for habitat colonisation to occur. Disturbances occur on scales ranging from entire ecosystems (i.e. forest fires) to small habitat patches (i.e. tree fall), with effects on biodiversity that depend on their spatial scale, intensity and frequency (e.g. Intermediate Disturbance Hypothesis, Connell 1978). Restoration is often approached in the context of habitat-creation or remediation of environmental conditions following a disturbance, but at its foundation, many restoration methods (i.e. substrate deployment, site remediation, transplantation) are analogous to disturbances in that they lead to freeing of resources, providing opportunities to test ecological theory. For example, the addition of new substrate provides unoccupied space for colonisation, providing the opportunity to assess through sequential deployments how the timing of 'disturbance' influences community assembly. Community assembly was dependent on the timing of substrate introduction (**chapter 2**), indicating the role of priority effects of early colonists in shaping community development.

EFFECTS OF THE ECOLOGICAL FILTER ON COMMUNITY ASSEMBLAGE

Results from this thesis are consistent with ecological filter models that posit that dispersal, biotic and abiotic processes (Belyea and Lancaster 1999) interact to influence community assembly (e.g. Kraft et al. 2015). In **chapter 2** I found that propagule availability at the time of substrate provision influenced short-term outcomes of oyster community development, although temporal effects lessened with time. In **chapters 3** and **4**, biotic interactions between habitat-forming species and the associated community influenced community assembly by influencing patterns of colonisation and survivorship. Finally, the abiotic factor, wave energy, both directly and indirectly altered community composition (**chapter 4**). Although ecologists have focused on components of the ecological filter in isolation (e.g. Nobel and Slatyer 1977, Baums et al. 2006), results from this thesis point to the growing recognition that community assemblages are maintained by a combination of environmental, biotic and stochastic processes (Roughgarden et al. 1988, Poff 1997, Houseman and Gross 2006, Chase 2007, Kraft et al. 2015).

POSITIVE INTERACTIONS: FACILITATION BY OYSTER HABITAT

The role of positive interactions in maintaining community assembly were initially over looked by ecologists, with early studies focusing on the role of competition or predation in maintaining marine community assembly (Paine 1966, Menge and Sutherland 1976). However, there is growing recognition of the role positive interactions play in maintaining community structure (Hacker and Gaines 1997, Stachowicz 2001, Bruno et al. 2003). I hypothesised that facilitation by habitat-forming oysters would maintain community assembly within a variety of biotic and environmental settings. This thesis has shown that although the magnitude of invertebrate facilitation by oysters may vary in time and space according to the magnitude of biotic and abiotic stressors, or their morphology, in all instances effects of oysters on biodiversity were positive.

The stress gradient hypothesis predicts that with increasing abiotic or biotic stress, the frequency of positive interactions will increase, as will the benefits of facilitation (Bertness and Callaway 1994). Here, the positive effect of oysters on invertebrates was apparent in all sites and habitats examined. Biotic stressors like competition and predation occur within all habitats, but intertidal systems, and especially rocky shores, are stressful habitats for marine species in that inhabitants also face extreme as physiological stressors such as temperature, desiccation and wave energy (Connell 1972, Bustamante and Branch 1996, Emery et al. 2001). Counter to the predictions of the stress gradient hypothesis, the magnitude by which oysters enhanced invertebrate abundance and richness did not vary across a wave exposure gradient. One potential explanation is that marine organisms are adapted to life in a wave-swept environment, and this stressor was less important in shaping biodiversity as compared to others, such as desiccation and temperature stress. Alternatively, the range in wave energies across the gradient may have been insufficient to influence the magnitude of interspecific interactions.

Habitat-forming species and/or ecosystem engineers such as oysters create, maintain or alter habitats and play an important role in facilitating and maintaining biodiversity (Gutiérrez et al. 2003, Byers et al. 2006, Crain and Bertness 2006, Marzinelli et al. 2016). Australia's marine ecosystems support some of the most species-rich and diverse invertebrate assemblages in the world (Wilson and Allen 1987) and are underpinned by habitat-forming marine species which globally are being reduced to less complex habitats by human activity (Airoldi et al. 2008). Thus, understanding where positive interactions among species are strongest will help us to harness these in conservation and restoration efforts seeking to curb biodiversity loss.

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SUPPLEMENTAL MATERIAL

Chapter III Supplement: Effects of oysters and algal habitat treatments on predation of *Bembicium auratum* snails.

Table S1: ANOVA results for the effects of oysters and algae on the types of *B. auratum* snail predation. *Evaluation of Tukey test results revealed no significant pairwise interactions.

Month	Factor	df	Cracked MS	Cracked F	Cracked p	Drilled MS	Drilled F	Drilled p
1	Oyster	3	0.002	1.00	0.399	0.002	1.00	0.399
	Algae	2	0.002	1.00	0.374	0.002	1.00	0.374
	Oyster x Algae	6	0.002	1.00	0.434	0.002	1.00	0.434
	Residuals	60	0.002			0.002		
2	Oyster	3	0.003	0.24	0.866	0.009	0.32	0.812
	Algae	2	0.006	0.50	0.610	0.018	0.63	0.536
	Oyster x Algae	6	0.027	2.38	0.040*	0.042	1.52	0.188
	Residuals	60	0.012			0.028		
3	Oyster	3	0.008	0.58	0.630	0.078	1.31	0.278
	Algae	2	0.014	1.01	0.371	0.055	0.92	0.403
	Oyster x Algae	6	0.022	1.59	0.165	0.032	0.55	0.769
	Residuals	60	0.014			0.059		

Chapter IV Supplement: Summary of invertebrates sampled during the oyster and bare habitat survey.

Table S2. Mean (± SE) abundance of taxa present within oyster and bare habitat plots in the habitat survey. $n = 6$ replicate quadrats (25 x 25 cm) were sampled per site.

Taxon	1) Dawn Fraser		2) Balls Head Reserve		3) Sawmillers Reserve		4) Cremorne Point, Mosman Bay		5) Sirius Cove Reserve		6) Bradley's Head		7) Neutral Bay		8) Milk Beach		9) Bottle and Glass Point	
	Oyster	Bare	Oyster	Bare	Oyster	Bare	Oyster	Bare	Oyster	Bare	Oyster	Bare	Oyster	Bare	Oyster	Bare	Oyster	Bare
ANNELIDA																		
CLASS POLYCHAETA																		
Nereididae	1.8 (0.9)	0 (0)	0.7 (0.3)	0 (0)	0.2 (0.2)	0 (0)	0.2 (0.2)	0 (0)	1.4 (0.7)	0 (0)	2.3 (0.7)	0 (0)	2.5 (0.8)	0 (0)	0.5 (0.2)	0 (0)	2.2 (0.7)	0 (0)
Onuphidae	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)
Orbiniidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)
Phyllodocidae	0.7 (0.3)	0 (0)	0.7 (0.5)	0 (0)	0.8 (0.5)	0 (0)	1.6 (0.7)	0 (0)	0.8 (0.4)	0 (0)	3.0 (0.8)	0 (0)	0.8 (0.4)	0 (0)	0.5 (0.3)	0 (0)	0 (0)	0 (0)
Polynoidae	2.2 (0.7)	0 (0)	2.7 (1.1)	0 (0)	4.0 (2.5)	0 (0)	2.2 (1.1)	0 (0)	8.0 (0.9)	0 (0)	2.8 (1.2)	0 (0)	0.8 (0.5)	0 (0)	3.8 (2.0)	0 (0)	4.0 (1.4)	0 (0)
Sabellariidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Serpulidae																		
Galeolaria caespitosa	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0.7 (0.5)	0 (0)
Spionidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)
Syllidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0.3 (0.3)	0 (0)	1.0 (0.6)	0 (0)
Unidentified Polychaete 1	0 (0)	0 (0)	0.3 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unidentified Polychaete 2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.4 (0.4)	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Unidentified Polychaete 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.6 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.7 (0.7)	0 (0)	0 (0)	0 (0)
Unidentified Polychaete 4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0.3 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Unidentified Polychaete 5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)

ARTHROPODA

CLASS INSECTA

Chathamiidae

Philanisus plebeius	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0.7 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.5 (1.5)	0 (0)

Chironomidae

***Pontomyia* spp.**	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Subphylum: CRUSTACEA

CLASS: HEXANAUPLIA

Iblidae

Ibla quadrivalvis	5.7 (2.9)	0 (0)	31.0 (20.8)	0 (0)	10.3 (3.2)	0 (0)	15.6 (13.2)	0 (0)	109.2 (26.0)	0 (0)	0.3 (0.2)	0 (0)	30.3 (16.1)	0 (0)	13.2 (8.9)	0 (0)	1.2 (0.6)	0 (0)

CLASS MALACOSTRACA

Order: AMPHIPODA

Unidentified Amphipod	0.3 (0.3)	0 (0)	0.3 (0.3)	0.2 (0.2)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.3 (0.2)	0 (0)	0.3 (0.3)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)

Order: DECAPODA

Oziidae

Ozius truncatus	0.8 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0.2 (0.2)	0 (0)	0.2 (0.2)	0 (0)	0.3 (0.2)	0 (0)	0 (0)	0 (0)

Varunidae

Helograpsus haswellianus	2.7 (1.0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Paragrapsus laevis	3.0 (0.9)	0 (0)	0 (0)	0 (0)	4.3 (0.9)	0 (0)	5.4 (2.2)	0 (0)	4.6 (0.8)	0 (0)	0.5 (0.2)	0 (0)	0.3 (0.2)	0 (0)	0 (0)	0 (0)	0.7 (0.7)	0 (0)
Unidentified Decapod	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0.8 (0.6)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Order: ISOPODA

Cirolanidae

Cirolana harfordi	0 (0)	0 (0)	10.7 (6.6)	0 (0)	24.0 (13.2)	0 (0)	5.4 (3.7)	0 (0)	24.6 (7.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3.3 (1.6)	0 (0)	0 (0)	0 (0)
Isopod morphospecies 1	0.7 (0.2)	0 (0)	5.7 (1.1)	0 (0)	2.2 (0.5)	0 (0)	1.0 (1.0)	0 (0)	5.6 (3.9)	0 (0)	7.0 (2.0)	0 (0)	0.7 (0.2)	0 (0)	9.2 (4.0)	0 (0)	3.8 (2.1)	0 (0)
Isopod morphospecies 2	0 (0)	0 (0)	0.5 (0.3)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.3 (1.1)	0 (0)	0.2 (0.2)	0 (0)	0.2 (0.2)	0 (0)	0.3 (0.2)	0 (0)
Isopod morphospecies 3	0 (0)	0 (0)	1.2 (0.8)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	2.0 (1.6)	0 (0)	0 (0)	0 (0)	0.7 (0.3)	0 (0)	0 (0)	0 (0)
Isopod morphospecies 4	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Isopod morphospecies 5	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Isopod morphospecies 6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

CNIDARIA

CLASS ANTHOZOA

Actiniidae

Actinia tenebrosa	0.3 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	25.2 (6.6)	0 (0)

ECHINODERMATA

CLASS ASTEROIDEA

Asterinidae

Parvulastra exigua	0 (0)	0 (0)	1.3 (0.5)	0 (0)	0 (0)	0 (0)	0.8 (0.4)	0 (0)	2.0 (1.1)	0 (0)	0.5 (0.2)	1.5 (0.7)	0 (0)	0 (0)	2.5 (1.9)	0.2 (0.2)	7.5 (2.3)	0.2 (0.2)

MOLLUSCA

CLASS BIVALVIA

Arcidae

Anadara trapezia	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Lasaeidae

Lasaea australis	19.7 (8.6)	0 (0)	90.0 (28.2)	0 (0)	247.3 (90.4)	0 (0)	183.8 (95.0)	0 (0)	854.6 (172.1)	0 (0)	24.0 (5.7)	0 (0)	112.7 (36.9)	0 (0)	154.0 (23.8)	0 (0)	93.8 (7.3)	0 (0)

Mytilidae

Arcuatula senhousia	2.3 (2.3)	0 (0)	0 (0)	0 (0)	1.8 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Trichomya hirsuta	46.5 (14.2)	0 (0)	28.8 (9.0)	0 (0)	31.7 (8.1)	0 (0)	5.0 (4.3)	0 (0)	6.8 (4.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.0 (0.6)	0 (0)	1.3 (0.8)	0 (0)
Xenostrobus pulex	4.7 (1.7)	0 (0)	6.3 (3.8)	0 (0)	2.8 (1.4)	0 (0)	0.6 (0.6)	0 (0)	0.8 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Veneridae

Dosinia sculpta	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.8 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Irus crenatus	8.2 (2.6)	0 (0)	0.3 (0.2)	0 (0)	6.2 (2.6)	0 (0)	5.6 (4.2)	0 (0)	4.2 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Venerupis anomala	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Venerupis galactites	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unidentified Veneridae 1	0.5 (0.5)	0 (0)	0 (0)	0 (0)	0.7 (0.7)	0 (0)	0 (0)	0 (0)	2.0 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unidentified Veneridae 2	2.5 (1.1)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	1.8 (1.1)	0 (0)	0.4 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

CLASS GASTROPODA

Anabathridae

Amphithalamus incidatus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Batillariidae

Batillaria australis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.7 (1.5)	0 (0)	0 (0)	0 (0)
Zeacumantus subcarinatus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)

Cingulopsidae

Eatonina fulvicolumella	0 (0)	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudopisinna gregaria	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.7 (0.5)	0 (0)	0.3 (0.2)	0 (0)

Cocculinidae

Coccopigya barbatula	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Eatoniellidae

Eatoniella atropurpurea	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)

Fissurellidae

Montfortula rugosa	0.2 (0.2)	0 (0)	0.5 (0.2)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	1.2 (0.5)	0 (0)	1.5 (0.4)	0 (0)	0.7 (0.3)	0 (0)	1.5 (1.0)	0 (0)	14.2 (2.6)	0 (0)
Tugali parmophoidea	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)

Littorinidae

Afrolittorina	1.2	0 (0)	8.7	0 (0)	8.0	0 (0)	1.2	0.3	29.0	0 (0)	0 (0)	0 (0)	0.2	0 (0)	0.7	0.2	0 (0)	1.0

acutispira	(0.7)		(3.3)		(2.6)		(0.5)	(0.3)	(8.6)				(0.2)		(0.4)	(0.2)		(0.5)
Bembicium auratum	92.8 (23.2)	8.7 (4.6)	0 (0)	0.2 (0.2)	47.7 (6.8)	5.5 (2.4)	112.6 (20.3)	39.0 (13.2)	72.2 (18.2)	3.3 (1.9)	0 (0)	0 (0)	1.2 (0.3)	0 (0)	6.8 (2.5)	0 (0)	0.2 (0.2)	0 (0)
Bembicium nanum	0.2 (0.2)	0 (0)	0 (0)	2.3 (1.5)	0.5 (0.3)	2.0 (1.4)	4.2 (1.6)	2.5 (1.0)	9.2 (2.4)	2.0 (0.7)	4.5 (4.3)	0 (0)	8.7 (1.9)	0.2 (0.2)	15.5 (4.1)	1.0 (0.7)	1.7 (1.0)	0 (0)
Macquariella kingensis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Lottiidae																		
Asteracmea illibrata	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Notoacmea flammea	0.7 (0.7)	0 (0)	0.3 (0.3)	0 (0)	0.5 (0.3)	0 (0)	0.6 (0.4)	0 (0)	2.2 (1.1)	0 (0)	0 (0)	0 (0)	0.3 (0.2)	0 (0)	0.5 (0.5)	0 (0)	0.3 (0.3)	0 (0)
Notoacmea petterdi	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Patelloida alticostata	0 (0)	0 (0)	0.2 (0.2)	0.7 (0.7)									0.2 (0.2)		0.2 (0.2)			4.7 (1.3)
Patelloida latistrigata	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0.5 (0.5)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)
Patelloida mimula	111.2 (20.4)	0 (0)	4.2 (1.6)	0 (0)	76.2 (6.6)	0.7 (0.3)	22.0 (6.5)	0.7 (0.4)	21.8 (10.4)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	2.5 (0.7)	0 (0)	0.5 (0.3)	0 (0)
Patelloida mufria	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0.2 (0.2)	0 (0)	0.3 (0.3)	0 (0)	2.2 (1.2)	0 (0)
Patelloida saccharina stella	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.2)	0 (0)
Muricidae																		
Bedeva paivae	0.3 (0.3)	0 (0)	0 (0)	0 (0)	1.2 (0.8)	0 (0)	0.2 (0.2)	0 (0)	4.2 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)
Tenguella marginalba	0.8 (0.4)	0 (0)	2.3 (0.9)	0 (0)	1.3 (0.8)	0 (0)	7.2 (2.2)	0 (0)	6.2 (1.5)	0 (0)	5.8 (1.7)	0.5 (0.5)	8.2 (3.2)	0 (0)	5.5 (1.1)	1.7 (0.6)	2.7 (1.0)	0 (0)
Nacellidae																		
Cellana tramoserica	0.5 (0.3)	0 (0)	1.2 (0.5)	1.2 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	4.2 (0.6)	1.2 (0.5)	1.2 (0.3)	2.5 (0.3)	0.5 (0.3)	0 (0)	1.8 (0.5)	0 (0)
Neritidae																		
Nerita atramentosa	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0 (0)	0 (0)	0 (0)	0.8 (0.8)	0.4 (0.2)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0.8 (0.5)	0 (0)	0 (0)	0 (0)
Nystiellidae																		
Murdochella macrina	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)
Onchidiidae																		
Onchidella nigricans	6.7 (4.0)	0.3 (0.2)	2.2 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4.2 (0.4)	0 (0)	14.7 (4.5)	1.5 (0.7)	18.3 (4.1)	0 (0)	0.3 (0.3)	42.8 (39.0)	4.2 (1.4)	1.5 (0.6)

Patellidae

Scutellastra chapmani	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.4 (0.4)	0 (0)	0.2 (0.2)	0 (0)	0.3 (0.2)	0 (0)	0.2 (0.2)	0 (0)	1.3 (0.9)	0 (0)
Scutellastra peronii	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)
Phyramidellidae																		
Cingulina magna	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)
Seguenzioidea																		
Microcarina surgerea	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Siphonarioidea																		
Siphonaria denticulata	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.4 (0.9)	38.8 (31.5)	1.3 (1.3)	30.8 (13.8)	1.7 (1.1)	8.0 (3.6)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Siphonaria funiculata	0.5 (0.3)	20.7 (5.7)	5.3 (4.7)	9.5 (3.9)	2.2 (1.2)	11.2 (5.6)	0.2 (0.2)	8.2 (5.2)	4.8 (3.3)	0 (0)	1.8 (1.2)	0 (0)	11.7 (6.4)	0 (0)	8.3 (7.9)	0.5 (0.3)	1.5 (1.1)	0 (0)
Siphonaria laciniosa	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.4 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Triphoridae																		
Coriophora fusca	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)
Seila spp.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.2)	0 (0)	0 (0)	0 (0)
Trochidae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.7 (1.5)	0 (0)	0 (0)	0 (0)
Austrocochlea constricta	0.5 (0.5)	0 (0)	0.2 (0.2)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0.2 (0.2)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.3 (1.3)	10.2 (1.6)	0 (0)	0 (0)
Austrocochlea porcata	1.2 (0.8)	1.2 (1.0)	3.3 (1.0)	1.5 (0.8)	4.8 (2.6)	0.3 (0.3)	0.4 (0.2)	0.3 (0.3)	4.8 (1.9)	0 (0)	3.3 (1.0)	0.3 (0.2)	0 (0)	0.7 (0.7)	8.8 (3.2)	22.7 (4.9)	0.7 (0.4)	3.0 (1.3)
Cantharidella picturata	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chlorodiloma odontis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)
Eurytrochus strangei	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fossarina patula	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.8 (0.4)	0 (0)	0 (0)	0 (0)	0.8 (0.5)	0 (0)	0.8 (0.3)	0 (0)
Unidentified Mollusc 1	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0.2 (0.2)	0 (0)	0.4 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unidentified Mollusc 2	0.8 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.0 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	0 (0)

CLASS POLYPLACOPHORA

Acanthochitonidae

Acanthochitona pilsbryi	0.5 (0.3)	0 (0)	8.8 (1.7)	0 (0)	0.7 (0.5)	0 (0)	1.0 (0.8)	0 (0)	1.6 (0.5)	0 (0)	13.7 (2.1)	0 (0)	3.2 (1.1)	0 (0)	8.0 (6.4)	0 (0)	23.5 (5.4)	0.3 (0.3)

Chitonidae

Acanthopleura gaimardi	0 (0)	0 (0)	0.5 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.2)	0 (0)	0.2 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sypharochiton pelliserpentis	0.8 (0.5)	0 (0)	21.8 (2.2)	0.5 (0.2)	3.8 (0.8)	0 (0)	5.8 (2.8)	0.2 (0.2)	8.0 (2.8)	0.5 (0.3)	6.7 (2.2)	0 (0)	13.7 (2.1)	0.3 (0.3)	16.3 (6.6)	0 (0)	22.5 (3.6)	0.3 (0.2)

SIPUNCULA

CLASS PHASCOLOSOMATIDEA

Pascolosomatidae

Phascolosoma noduliferum	4.8 (1.6)	0 (0)	0.5 (0.2)	0 (0)	1.5 (0.8)	0 (0)	3.0 (2.0)	0 (0)	2.6 (1.3)	0 (0)	0 (0)	0 (0)	1.2 (0.7)	0 (0)	0.8 (0.5)	0 (0)	0.8 (0.5)	0 (0)