Ecological impacts of QX oyster disease and its management strategies

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Summary

As a consequence of rising global consumption of seafood and over-extraction of wild fin- and shell-fisheries, aquaculture is the fastest growing food production method in the world. Although in some instances aquaculture may benefit wild populations by deflecting harvest pressure away from these, in others it may have negative impacts as a consequence of facilitation of disease or biological invasion or where it results in genetic pollution of wild populations with selectively-bred genotypes. The oyster industry of New South Wales (NSW), Australia, is presently entirely dependent on aquaculture. Although the NSW industry historically cultured wild-caught native Sydney rock oysters (*Saccostrea glomerata*), in recent years high mortality of cultured oysters from QX disease has led to a shift towards culture of Sydney rock oysters selectively bred for disease resistance and non-native Pacific oysters (*Crassostrea gigas*) that are unaffected by the disease. It is unknown how QX disease or the resulting strategies of management that have been applied to the aquaculture industry (culture of disease resistant Sydney rock or Pacific oysters) may impact wild populations of Sydney rock oysters, and their important ecosystem services , which include provision of habitat and food for associated communities of fish and invertebrates. This thesis determined: (1) how QX disease has impacted wild populations of Sydney rock oysters, both directly and indirectly as a result of any facilitation of Pacific oysters, and (2) whether wild-stock Sydney rock oysters, disease resistant Sydney rock oysters, and Pacific oysters are functionally equivalent in their provision of habitat and food to associated communities of invertebrates.

Sampling in the Hawkesbury River, NSW, where QX disease has produced aquaculture mortality of Sydney rock oysters of up to 90%, revealed only a low (<14%) prevalence of the disease-causing parasite among wild Sydney rock oysters on rocky shores. Consequently, mortality rates of wild Sydney rock oysters remained low and there was no evidence of

replacement of populations of Sydney rock oysters with disease-resistant Pacific oysters. Field and laboratory experiments showed that even under the scenario that aquaculture facilitates invasion of Pacific oysters or causes genetic modification of wild Sydney rock oyster populations, little change in the ecosystem services provided by oysters would be expected. Despite the more rapid growth and mortality rates of Pacific than Sydney rock oysters, the two produced habitat of similar complexity and supported similar communities of invertebrates, irrespective of genotype. Settlement of Sydney rock oyster larvae did not differ between Pacific and Sydney rock oysters in mixed species beds and when in mono-specific patches, the non-native oyster enhanced larval settlement of Sydney rock oysters in high-flow environments, but decreased its settlement under low-flow, high-density conditions. The rapid growth of Pacific oysters had little impact on investment in anti-predator defences, such as shell strength and thickness, relative to Sydney rock oysters. Consequently, native oyster-boring whelks were capable of consuming Pacific oysters, although they consumed more of the Sydney rock oyster when both species were available. These results suggest that changes to the oyster aquaculture industry of NSW Australia are at present having little impact on wild oyster populations and their ecosystem services. The findings are consistent with other studies done elsewhere , which suggest there is often a high degree of redundancy in the services provided by native and non-native oysters.

Statement of Sources Declaration

I declare that this thesis 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 only original material. Any additional help received during the preparation of this work has been indicated in the 'Contributors' section.

Signature

Contributors

This thesis contains material that has been submitted or prepared for publication, as follows:

Chapter 1:General Introduction

I have done the literature review and writing of this chapter with constructive feedback from my supervisor Melanie Bishop, and important suggestions from my co-supervisor Wayne O'Connor.

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1 Introduction

1.1 Preface

In New South Wales, Australia, the Sydney rock oyster *Saccostrea glomerata* is a commercially valuable species, contributing to an aquaculture industry worth $AUS 35 million per year (O'Connor and Dove 2009). In addition, *S. glomerata* is an essential component of coastal environments. Wild *S. glomerata* populations provide habitat for diverse invertebrate and fish communities, form parts of complex food webs, and facilitate water clarity and nutrient cycling during filter feeding (Anderson and Connell 1999; Bayne and Svensson 2006; Jackson et al., 2008).

The *S. glomerata* industry has been under increasing threat from disease, commonly called QX (Queensland unknown) disease responsible for mass mortalities of oysters and industry closures (Nell 2007). In an attempt to restore the industry farmers are cultivating *S. glomerata* that have been selectively bred for QX disease resistance, and non-native Pacific oysters *Crassostrea gigas*, which are naturally unaffected by QX disease (Nell and Hand 2003; Nell and Perkins 2005a). Both types of oysters are produced in land based hatcheries, and then juvenile oysters are distributed to mature on farms. The aim of this research was to investigate whether QX disease and resultant changes to the aquaculture industry are influencing the structure and function of wild oyster assemblages.

1.2 The rise of aquaculture

The consumption of seafood has doubled since the early 1970s accelerating the depletion of the ocean's fish and shellfish resources (Naylor and Burke 2005). Over 50 % of capture stocks

have reached their maximum production potential, 16 % are overfished, 7 % are completely depleted, and most remaining fisheries are predicted to collapse within a few decades (Frid et al., 2005; Garcia and Grainger 2005; Costello et al., 2008). To meet the growing demand for seafood, aquaculture is the fastest growing food producing method in the world, increasing in average production by 6.2% per year (FAO 2010) and contributing to a US $1 trillion per year industry (Smith et al., 2010). The main differences between aquaculture to capture fisheries are: 1) the individual or corporate ownership of stocks, and 2) technologies used to enhance production such as husbandry, and 3) use of non-native species (Naylor et al., 2000). This means that the hunter-gatherer approach, which depends on natural ecosystem oscillations is shifting to more manageable seafood farming practices, analogous to the development of agriculture centuries ago.

Although aquaculture can relieve fishing pressures on wild stocks (Naylor et al., 2000; Shumway et al., 2003; Diana 2009), whether it contributes to the recovery of depleted species and degraded ecosystems is unclear. Positive effects of aquaculture resulting from enhancement of spawning stock biomass and removal of destructive fishing practices, such as dredging, can be offset by negative impacts (Naylor et al., 2005). At the local scale, aquaculture can result in organic enrichment (Delgado et al., 1999; Mazzola et al., 2000; Machias et al., 2004). At larger scales it can contribute to facilitation of diseases among wild populations, genetic homogenization of wild populations and invasion of cultured species that were not previously present in the environment (Hutchings and Fraser 2008; Rosenberg 2008; Diana 2009).

1.3 Impacts of aquaculture: facilitation of emerging diseases

Aquaculture farms can serve as reservoirs for unnaturally high densities of pathogens (Harvell et al., 1999). The high densities at which cultured animals are maintained can increase host-pathogen contact rates, the frequency of disease transmission and pathogen persistence (Lafferty et al., 2004; Krkošek et al., 2005; Peeler et al., 2011). Additionally, anthropogenic stressors innate to farming practices negatively influence host resistance to parasites and other disease causing agents, thus increase susceptibility to infection among farmed animals (Murray and Peeler 2005). For example, levels of sea lice infection have been four orders of magnitude greater among farms of juvenile Pacific salmon, *Oncorhynchus gorbuscha*, compared to ambient levels of infection (Krkošek et al., 2005). Introduction of non-native species for aquaculture can also increase the risk of introducing exotic pathogens (Dunn 2009).

Where disease-causing agents spill-over from farms into wild populations, the level of infection among wild populations can increase. Spill-over of disease from fish farms has in several instances induced mass mortalities and possible extinctions of wild fish species (Johnson et al, 2004; Krkošek et al., 2005; Rosenberg 2008). There are, however, no documented cases of disease spill-over from industrial shellfish culture operations to wild populations. As emerging diseases increasingly induce mass mortalities among commercially produced bivalves around the world (Sindermann and Rosenfield 1967; Friedman et al., 2005; Nell and Perkins 2006; Maloy et al., 2007; Dégremont et al., 2010), assessments of wild bivalves that occupy habitats in the vicinity of cultured stock are essential to determine disease spill-over.

1.4 Impacts of aquaculture: genetic pollution

Over thirty years ago, Newkirk (1980) stressed that "The domestication of species used in aquaculture will not be complete until we have control over all aspects of their biology including their genetics" (Newkirk 1980). Since then, the contribution of genetically improved species to aquaculture has grown substantially (Sheridan 1997). Genetic improvement of fish and shellfish is ultimately geared toward the enhancement of stock production. Characteristics such as faster growth, disease resistance and aesthetically pleasing qualities may be selected for through breeding processes (Myhr and Dalmo 2005; Gaffney 2006).

Introducing selectively bred fish and shellfish into natural aquatic environments, however, increases the risk of 'genetically polluting' wild populations (Hutchings and Fraser 2008; Diana 2009). Several studies have provided evidence that successive selection of breeders from a small number of parents over multiple generations, and inbreeding among hatchery reared animals reduces the genetic variation of cultured fish (Aho et al., 2006; Frost et al., 2006) and shellfish (Gaffney 2006; Yu and Li 2007). Hybridization between aquaculture and wild counterparts may reduce the genetic diversity among wild populations, reducing the adaptive capacity and reproductive fitness of a species (Browman 2000; Utter and Epifanio 2002; Reed and Frankhan 2003; Gaffney 2006; Frost et al., 2006).

Reductions in a population's genetic diversity may also extend to ecosystem impacts. This occurs where genetic changes to populations of plants or animals translate to phenotypic changes in traits (e.g.; average canopy height, grassland chemical composition) that are important in determining the structure and function of associated biological communities (Schweitzer et al., 2008; Whitham et al., 2008; Bailey et al., 2009). Early examples of the so-called 'genes to

ecosystems' phenomenon came from terrestrial ecosystems (e.g. Marrelli et al., 2006; Bailey et al., 2009), but an increasing number of examples of genetic changes that translate to ecosystem impacts are also emerging from marine environments (Hughes and Stachowicz 2004; Naylor et al., 2005; Walsh et al., 2006). For example, wild hybrid salmon that have inherited aggressive behavioural genes from hatchery reared escapee parents produce ecosystem level changes through the cascading effects of their competitive exclusion of native salmon (Naylor et al., 2005). Patches of seagrass *Zostera marina* are more resistant to disturbances if they contain a higher genotypic diversity (Hughes and Stachowicz 2004). Whether shellfish aquaculture can produce genetic changes in wild populations that cascade to ecosystem level impacts remains unknown, but is plausible given the important ecosystem services provided by many bivalves such as oysters and mussels (Bartley 2007; Camara and Vadopalas 2009).

1.5 Impacts of aquaculture: Biological invasions

Coastal habitats are among the most invaded environments in the world (Vitousek et al., 1996; Rilov and Crooks 2009). Biological invasions represent one of the greatest threats to ecosystems, causing biodiversity loss, habitat destruction and altered food web dynamics (Grosholz 2002; Ruiz et al., 1997; Byrnes et al., 2007; Galil et al., 2007; Molnar et al., 2008). The total environmental, social and economic costs of the damage and disruptions caused by invasions in the USA alone are an estimated $120 million per year (Pimentel et al., 2005).

Aquaculture is the second greatest cause of human-assisted bio-invasion of coasts after unintentional shipping transportation (Levine and D'Antonio 2003; Molnar et al., 2008). Of the species introduced for aquaculture, 64% have produced environmental impacts as a consequence of escape of cultured animals into the wild (Naylor et al., 2001; Molnar et al., 2008; Diana

2009). Aquaculture species are pre-disposed to become invasive pests because many of the exact same traits that are typically sought after for the optimization of aquaculture production are also characteristics that are likely to facilitate a successful invasive species. These traits include rapid growth, early sexual maturation, short generation time and broad environmental tolerance (Ricciardi and Rasmussen 1998; Diana 2009). For example, the short generation time and broad environmental tolerances of tilapia have resulted in its invasion of all systems on earth where it has been introduced for aquaculture, causing native fish extinctions and biodiversity loss (Peterson et al., 2004; Canonico et al., 2005). Similarly, the fast growing Pacific oyster *Crassostrea gigas* rapidly spread throughout mudflats of North Western Europe following introduction for aquaculture, outcompeting and replacing some native mussel *Mytilius edulis* beds (Diederich et al., 2005; Brandt et al., 2008; Troost 2010).

In addition to intentionally introducing commercially important exotic species, aquaculture can also lead to unintentional introductions of exotic hitchhiker species that are transported with aquaculture stock (Minchin 1996; Ruesink et al., 2005; Weigle et al., 2005). The majority of emerging diseases are caused by introduction of non-native disease causing agents that are unintentionally transported with live aquaculture fish and shellfish (Bartley 2007; Crowl et al., 2008). For example, the hitchhiking virus *Bonamia ostreae* accompanied shipments of the European oyster *Ostrea edulis* from Washington State to France in 1979 resulting in the decimation of native French oyster stocks (Weigle et al., 2005).

1.6 Ecosystem impacts of oyster aquaculture

Oysters have a long history of commercial exploitation for meat and limestone, contributing an estimated US $3 billion to global fisheries per year (Jackson et al., 2001;Ogburn

2007; FAO 2010). Due to a history of overexploitation dating back to pre-industrial times, the majority of the world's wild oyster reefs have been removed (Kirby 2004), with a mere 5 % of ecological function remaining globally (Beck et al., 2009). Consequently, the oyster industry is today almost entirely (97%; FAO 2010) reliant on aquaculture.

Historically, oyster aquaculture industries utilised wild-caught native oyster spat, but increasingly they utilise hatchery-produced stock that may be of native or exotic origin (Breese and Malouf 1977; Nell 2002a; O'Conner and Dove 2009). In hatcheries, environmental conditions (light, salinity and water temperature) can be carefully controlled to produce juvenile oysters of higher biomass than wild-caught recruits, and at times outside of the usual breeding season (Rene and Andre 1999; O'Conner and Dove 2009). Furthermore, oysters can be selectively bred for disease resistance and rapid growth (Ragone Calvo et al., 2003;Dove and O'Connor 2009).

Although hatchery supplementation of wild recruitment might reduce dependence on depleted wild oyster populations (Shumway et al., 2003; Gaffney 2006), it nevertheless involves the translocation of oysters from a foreign (hatchery) to a novel (natural) environment. The interaction of hatchery produced oyster stock with wild oysters is particularly hard to control, as oysters are broadcast spawners that release millions of eggs and sperm into the water column that may hybridize with wild oyster gametes (Luttikhuizen et al., 2003). Non-native parasites hosted by cultivated oysters may be transmitted to wild oyster hosts (Peeler et al., 2011), non-native oysters may develop self-sustaining naturalized invasive oyster populations (Diederich et al., 2005), while genetic bottlenecks may form from hybridization between selectively bred and wild

stocks of native oyster species and compromise natural oyster restoration (Camara and Vadopalas 2009).

Understandably, oyster aquaculture management prioritises human health, and commercial interest, but it can also have considerable ecological ramifications (Gaffney 2006; Kingsley-Smith et al., 2009). This is because oysters are important bioengineers of marine ecosystems that contribute to coastal and estuarine goods and services worth US $4 trillion per annum (Costanza et al., 1997). Oysters can form dense reefs that can cover kilometres of rocky subtidal, intertidal and mangrove dominated shoreline (Gutiérrez et al., 2003; Kirby 2004; Ruesink et al., 2005; Ogburn 2007). The complex habitat provided by oysters offers invertebrates and juvenile fish refuge from predators and environmental stressors such as desiccation and hydrodynamic forces, enhancing their biodiversity and biomass (Lenihan et al., 2001; Glancy et al., 2003; Grabowski 2004; Jackson et al., 2008). As suspension feeders, oysters remove suspended organic solids and phytoplankton, which improves water clarity (Jones et al., 2001), facilitates benthic pelagic nutrient cycling (Newell 2004) and influences pelagic larval recruitment and distribution through feeding currents and predation (Troost 2010). Oysters also serve as an important source of nutrition for crabs, fish, birds and gastropods (Fairweather et al., 1984; Grabowski 2004; Bishop and Peterson 2006). Where aquaculture promotes growth of wild oyster populations in areas of previously low habitat heterogeneity, positive effects on biodiversity may be seen (Shumway et al., 2003). Where, however, oysters replace other heterogeneous habitat, impacts to biodiversity may be negative, particularly if filtration by dense beds reduces the abundance of pelagic larvae of native species (Troost et al., 2009).

1.7 Aquaculture of Pacific oyster *Crassostrea gigas*

Native to Japan, the Pacific oyster *Crassostrea gigas* (Thunberg, 1793; Ostreidae) is the most translocated marine taxon in the world for aquaculture (Ruesink et al., 2005; Padilla, 2010). It ranks first in terms of global weight and second in terms of value of landings among all other global aquaculture fish and shellfish species (FAO 2010). *C. gigas* has been introduced to 66 non-native regions, including Australia (Nell 2001), West Coast USA (Langdon et al., 2003), German Wadden Sea (Diederich et al., 2005), Dutch Wadden Sea (Troost 2010) and France (Grizel and Héral 1991), and has become a nuisance invasive species among 24 regions (Ruesink et al., 2005).

C. gigas displays rapid growth and maturation and broad environmental tolerances. The oyster can reach marketable size (40-60 g) within 20 months faster than most other cultured species (Nell and Perkins 2005a) and reaches maturity faster in the warmer waters of lower latitudes (30 °C; Nell 2002b). Being hermaphoditic, *C. gigas* normally converts from male to female, with a high reproductive effort as spawning females produce between 20-100 million eggs during summer, when water temperatures are optimally 15 - 20 °C (Mason and Nell 1995). Survival and growth rates are highest when salinity levels range between 19-27 ppt (Nell and Holliday 1988), although *C. gigas* can temporarily resist wider salinity ranges (5-55 ppt; Nell and Gibbs 1986). Although optimal grow out conditions occur within intertidal and subtidal habitats (Ruesink 2007; Krassoi et al., 2008), *C. gigas* can tolerate up to 20 days emersion (Thomson 1952).

The invasion of *C. gigas* has contributed to negative and positive modifications of several natural ecosystem processes at a variety of locations. In Willapa Bay, Washington State USA,

C. gigas impedes native oyster, *Ostrea lurida*, restoration through predation on the native oyster's larvae (Trimble et al., 2009). In New Zealand, *C. gigas* has replaced the native oyster *Saccostrea glomerata* in several estuaries (Dinamani 1991). In the Wadden Sea, Europe, suspension feeding by *C. gigas* reduces the carrying capacity of the Oosterschelde estuary, increasing competition for food, while simultaneously predating on native mussel, *Mytilus edulis*, larvae (Troost et al., 2009). Hard substrate provided by invasive *C. gigas* populations occupying mudflats of the Wadden Sea has, however, increased the local biodiversity of benthic invertebrates through enhancement of habitat heterogeneity (Markert et al., 2010).

In Australia, *C. gigas* was initially introduced in Tasmania, South Australia and south Western Australia in 1947 and 1948 to develop an oyster industry (Thomson 1959; Medcof and Wolf 1975). This facilitated the proliferation of feral *C. gigas* populations in several Tasmanian estuaries (Mitchell et al., 2000). In 1984-85, however, heavy *C. gigas* settlement was also observed along natural shores of the Port Stephens estuary, on the New South Wales (NSW) mid coast, most probably a result of a suspected illegal introduction (Holliday and Nell 1985). By 1988, the naturalized population of *C. gigas* in Port Stephens had reached an estimated 26 million individuals (Nell 1993), and by 1991 wild *C. gigas* was so prolific in this estuary that laws to ban its commercial cultivation in Port Stephens were lifted (Nell 2001). Today, wild *C. gigas* are found at low abundance in many New South Wales estuaries (Summerhayes et al., 2009a; Bishop et al., 2010), although cultivation of reproductively capable diploids remains confined to Port Stephens. Studies indicate that at high density, fast growing *C. gigas* is capable of outcompeting the native *S. glomerata* for space (Krassoi et al., 2008). Community and ecosystem-level impacts of *C. gigas* in Australian estuaries remain largely unknown.

1.8 Australian story: Sydney rock oyster *Saccostrea glomerata* and QX disease

In Australia, oyster aquaculture is a highly valued commercial fishery. Unlike commercial oyster aquaculture industries in many other countries, including France, USA, Canada, and Germany, Australia has continued to commercially culture its native oyster, the Sydney rock oyster *Saccostrea glomerata* (Ostreidae; Gould, 1850). As one of the nation's oldest commercial fisheries, it contributes nearly half of Australia's edible oyster industry, valued at AUS $35 million each year (O'Connor and Dove 2009), while the Pacific oyster *C. gigas* constitutes the rest (Nell 2001). Predominantly, farmed oysters have been derived from natural seed, although within the past decade major progress in hatchery supply has increased the proportion of selectively bred stock cultured from hatchery supplemented seed (O'Conner and Dove 2009).

In recent years the *S. glomerata* industry has been under increasing threat from QX disease. QX (short for Queensland unknown) disease is caused by infection and sporulation of *Marteilia sydneyi*, a paramyxean protozoan parasite (Peters and Raftos 2003). During suspension feeding, the parasite enters the gills and palps of *S. glomerata*, where it travels to connective tissues, haemolymph spaces and digestive tubules, ultimately occupying 100% of the digestive cavity, effectively starving the oyster (Kleeman et al., 2002).

The first outbreaks of QX disease occurred in the early 1970s in warmer waters of southern Queensland, then in 1994 QX disease caused a 94 % loss of stock in the southern most outbreak at Sydney's Georges River (lat. 34 °S; Nell 2007). The most recent (2004) outbreak in the Hawkesbury River, 40 km north of Sydney, NSW (Nell 2007) reduced the estuary's $3.66 million industry, previously the fourth largest in NSW, to virtually nil by 2006 (Nell 2007).

Overall, QX disease has contributed to a 40% decline in industry production since the 1970's (Heasman et al., 2000).

Disease outbreaks have been particularly severe in the upper reaches of urbanized estuaries following heavy rain, at which time the immune system of oysters, specifically phenoloxidase production, is compromised (Butt and Raftos 2007). The disease is not directly transmissible and an intermediate host, the polychaete *Nephtys australiensis* has recently been identified as an intermediate host of the parasite (NSW Primary Industries Fishing and Aquaculture). Infection is seasonal, normally occurring from January to April, with diseased oysters dying over the winter months (Nell 2007).

1.9 QX disease management

Growing pressures of QX disease on Australia's oyster industry demand rigorous management of commercial oyster production. There are currently two primary disease management techniques being used. The first involves a breeding program for QX disease resistance. The second management technique is the cultivation of triploid *C. gigas*.

In 1990 the NSW Department of Primary Industries established an oyster breeding program. Although the program was initially intended to select oysters for fast growth, after two generations, in 1997, the breeding program was modified to include selection for QX ('Queensland Unknown') disease resistance. Presently, fifth generation QX disease resistant *S. glomerata* spawned from 3 breeding lines are circulating among disease impacted aquaculture leases (Dove and O'Connor 2009) while overall, hatchery supplemented seed now accounts for

nearly 30 % of industry spat (O'Conner and Dove 2009). This program has been very effective, with *S. glomerata* experiencing a 63 % survival rate of cultured stock within four generations (Nell and Perkins 2006) and farming has returned to many QX-affected estuaries (Green et al., 2011).

In contrast to *S. glomerata*, *C. gigas* is not affected by QX disease. Culture of reproductively capable, diploid, *C. gigas* is in New South Wales only permitted in Port Stephens, but permits allowing culture of sterile triploid *C. gigas* have recently been issued for several NSW estuaries, including those where the *S. glomerata* industry has been hit hard by QX disease (O'Connor and Dove 2009). Triploid individuals have three sets of chromosomes, and are therefore reproductively incapable. Triploids are produced by either breeding tetraploid males (4 chromosomes) with diploid females, or through chemical induction (Nell 2002b; Guo et al., 1996). Triploid *C. gigas* grow at twice the rate of QX disease resistant *S. glomerata* and three times that of wild-stock *S. glomerata*, reaching marketable size by 15 months or less (Nell and Perkins 2005b).The differing rates of growth among oyster types reflects both a greater feeding efficiency of *C. gigas* than *S. glomerata* (Bayne 2002) and also a greater allocation by triploids, that invest only minimally in production of gametes, to growth (Nell 2002b).

1.10 Impacts of QX disease management

Each of the strategies of management put in place to combat impacts of QX-disease on the NSW oyster industry has the potential to influence the ecosystem goods and services provided by wild *S. glomerata* populations, particularly where the abundance of wild *S. glomerata* populations is simultaneously being reduced by QX disease. *S. glomerata* is an important ecosystem engineer and a dominant part of east Australia's temperate coastal bays and estuaries,

often forming patches metres long of 90-100% cover (Krassoi 2001). On rocky shores and mud flats densely clustered *S. glomerata* create complex three dimensional habitats for epibenthic invertebrates (Underwood and Barrett 1990; Jackson et al., 2008; Summerhayes et al., 2009b), and provide a food source for predators such as gastropod whelks, fish, rays and birds (Fairweather et al., 1984; Anderson and Connell 1999). Their suspension feeding removal of plankton and suspended particles facilitate benthic-pelagic coupling and maintain water clarity (Bayne and Svensson 2006).

First, culture of QX-resistant genotypes of *S. glomerata* may influence the ecosystem goods and services provided by changing the genotypes and phenotypes of wild *S. glomerata* populations through hybridization between cultured and aquaculture stocks. In disease-afflicted estuaries, QX-resistant oyster genotypes typically grow faster than wild stocks (Nell and Perkins 2006). A shift from slower- to faster-growing phenotypes of wild oysters would likely influence the size-structure of wild populations and the complexity of habitat that *S. glomerata* provides. Many predators select prey items based on size (Silva et al., 2010), with larger oysters commonly experiencing a size-refuge from predation (Moran et al., 1984). Faster-growing oysters that spend a shorter period of time in smaller size classes may become less accessible to invertebrate and vertebrate predators as a food resource. The shift in population size structure might consequently modify trophic interactions. Small changes in habitat complexity resulting from shifts in population-size structure might also have large impacts on associated invertebrate communities that live between the interstices.

Second, aquaculture of triploid *C. gigas* may accelerate invasion of wild populations. Although triploids are sterile (Nell 2002b), contamination issues in production of triploids and

the gradual reversion of some triploids to diploidy can lead to small numbers of diploid, reproductively capable animals, on aquaculture leases (Allen 1987; Gong et al., 2004; Luo and Opaluch 2011). Although the proportion of diploids amongst a triploid batch would be small, at the scales of aquaculture, where hundreds of thousands of oysters are often in close proximity, the probability of having two reproductively capable *C. gigas* in sufficient proximity that they may reproduce with one another is non-zero (The National Academies 2003). Particularly where QX disease is negatively impacting wild *S. glomerata* populations and freeing space on rocky shores and other hard substrata usually dominated by the native oyster, invasion of non-native *C. gigas* may result.

If disease-mediated invasion of *C. gigas* is indeed occurring, this raises the question: how might ecosystems services change and to what extent will such changes ensue ecological impacts? Previous studies suggest that *C. gigas* filter twice as fast as *S. glomerata* oysters (Bayne 2002), and consequently grow almost 2 times faster than *S. glomerata*, and attain an average 150% larger size (Nell 2002a). Consequently, *S. glomerata* and *C. gigas* might conceivably differ in the habitat they provide to other organisms, their predation of larvae through filtration, and their role as prey items for vertebrate and invertebrate predators.

1.11 Research aims and approach

Although cultured *S. glomerata* are clearly susceptible to QX disease, the impact of QX disease on wild *S. glomerata* populations remains unknown. Furthermore, it is unclear whether genetic modification of wild *S. glomerata* populations by QX-resistant oyster genotypes, or enhanced invasion of *S. glomerata* populations by *C. gigas* as a consequence of QX disease and its management strategies might impact ecosystem processes supported by oysters.

This research addresses the following broad hypotheses:

1) QX disease will be infectious and cause high levels of mortality among wild *S. glomerata* populations of rocky shores;
2) By compromising the health of wild *S. glomerata* populations, QX disease will facilitate further proliferation of *C. gigas* and invasion of wild oyster populations in eastern Australia;
3) Ecosystem services provided by *C. gigas* will be functionally dissimilar to *S. glomerata*; therefore where *C. gigas* invasion is facilitated by QX disease and its management strategies it will potentially induce large ecological changes, and:
4) Differences in growth between QX disease resistant *S. glomerata* and natural *S. glomerata* will be evident, but differences in ecosystem processes between genotypes will not be as large as differences between *C. gigas* and *S. glomerata*.

1.12 References

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2 Status of the Sydney rock oyster in a disease-afflicted estuary: persistence of wild populations despite severe impacts on cultured counterparts.

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2.1 Abstract

Marine diseases represent a significant threat to wild organisms and the ecosystems they support. Nevertheless, most research has focussed on impacts of disease on cultivated organisms. In eastern Australia, the Sydney rock oyster (*Saccostrea glomerata*) aquaculture industry is increasingly impacted by unprecedented outbreaks of QX oyster disease caused by parasitic *Marteilia sydneyi*. This study for the first time considered impacts of the disease on wild oyster populations. We predicted that in a QX-afflicted estuary: (1) prevalence of the disease-causing parasite would be similar among wild *S. glomerata* on rocky shores as among aquaculture oysters; (2) QX-infection would lead to detectable seasonal mortality of wild *S. glomerata*, coincident with the timing of QX infection, and of greatest effect size at the most severely disease-afflicted sites; and (3) recruitment of the non-native Pacific oyster, *Crassostrea gigas,* would be positively correlated to the impact of QX disease on its competitor, *S. glomerata*. In the Hawkesbury River estuary, where QX disease has caused up to 90 % mortality among cultured *S. glomerata* we found a comparatively low prevalence of the disease among wild oysters, peaking at 14%. Annual infection of *S. glomerata* oysters did not cause seasonal patterns of mortality, and the native oyster's density remained comparable to other estuaries unaffected by the disease. Although *C. gigas* had a wide and similar distribution to the native oyster, its abundance was generally low. However, one site, repeatedly exposed to QX disease, supported significantly more *C. gigas* than all the other sites. Overall, our results indicate that unlike cultured counterparts, wild *S. glomerata* in the Hawkesbury river estuary are not at present significantly impacted by QX disease, or proliferation of disease-resistant, non-native*C. gigas*. Nevertheless, our study shows that diseases of aquaculture stocks have the capacity to infect wild populations, and that longer-term assessment of wild populations at risk is essential.

Keywords: intertidal, invasive species, Pacific oyster, *Saccostrea glomerata*, rocky shore

2.2 Introduction

The prevalence of disease amongst terrestrial and aquatic organisms is increasing around the globe (Lafferty and Holt 2003; Mydlarz et al., 2006). Pollution, habitat degradation and climatic change are weakening the immune systems of host organisms, rendering them more susceptible to disease (Lafferty et al., 2004; Torchin and Mitchell 2004). Simultaneously, climate change and species translocations are extending the range of parasites (Harvell et al., 2002; Lafferty et al., 2004). Infectious diseases that have increased in prevalence over the past 20 years and threaten to increase in the near future have been termed emerging infectious diseases (Harvell et al., 1999).

In marine habitats, awareness of emerging infectious diseases has grown, and with it, interest in the role of diseases in structuring populations and communities (Dunn 2009; Torchin et al., 2002). Yet although the impacts of emerging infectious diseases on populations of cultivated organisms have been extensively researched (see Harvell et al., 1999; Krkošek 2010; Peeler et al., 2011), their impacts on wild counterparts have received comparatively little attention (but see Friedman et al., 2005; Krkošek et al., 2005). In both natural and cultivated ecosystems, the susceptibility of organisms to disease is determined by the distribution of the parasite, the distribution of the host(s), and environmental factors that influence the ability of organisms to resist disease (Mydlarz et al., 2006). Differences between natural and cultivated systems in biodiversity (Thieltges et al., 2008; Faust et al., 2009), abiotic conditions (Lenihan et al., 1999; Mydlarz et al., 2006) and the distribution and abundance of host organisms (Krkošek et al., 2005) may lead to differences in the susceptibility of individuals to infection between these environments and hence, impacts of the disease.

In estuaries of New South Wales, Australia, cultivated populations of the native Sydney Rock oyster, *Saccostrea glomerata*, are increasingly being impacted by QX disease, caused by the protozoan parasite *Marteilia sydneyi*. The disease, first detected in 1974, was originally confined to Queensland, but in 1994 killed up to 80% of *S. glomerata* in aquaculture leasesin the upper reaches of the Georges River, near Sydney (Nell and Perkins 2006).The parasite has subsequently been detected in twelve New South Wales estuaries, causing the cessation of *S. glomerata* oyster culture within six of these (Nell 2007). The infective period for *M. sydneyi* in NSW is generally January to April, and QX disease-induced mortality of *S. glomerata* is particularly severe within aquaculture leases situated in the upper reaches of estuaries following periods of heavy rain that, with concurrent decreases in salinity, compromises oyster immunity (Butt et al., 2006).

The extent to which QX disease is infecting and impacting wild *S. glomerata* populations is unknown. Wild *S. glomerata* populations are abundant along intertidal and shallow subtidal rocky reefs of south-eastern Australia, commonly accounting for >90% cover (Bishop et al., 2010; Summerhayes et al., 2009). The three-dimensional structure of wild *S. glomerata* provides habitat for diverse invertebrate and fish communities, its suspension feeding facilitates water clarity and nutrient cycling and it provides an important food resource for crabs, whelks and fish (Anderson and Connell 1999; Bayne and Svensson 2006; Jackson et al., 2008). Additionally, the native oyster pre-empts space that may otherwise be occupied by the non-native Pacific oyster, *Crassostrea gigas*. The two species co-occur at mid intertidal to shallow subtidal elevations of the New South Wales coastline, where they overlap substantially in salinity tolerances (*C. gigas*:waters of 5 to 55 ppt; *S. glomerata*: 15 to 50 ppt; Nell and Holliday 1988). Where *C. gigas* is able to successfully settle at high abundance at mid to low intertidal elevations, it can outcompete native *S. glomerata* through overgrowth (Krassoi et al., 2008). In New South Wales, such levels of *C. gigas* proliferation

have not yet occurred since it was first recorded in the region in the mid 1970s (Wolf and Medcof 1974) however, invasion remains a concern as wild rogue populations exist in several New South Wales estuaries. *C. gigas* is unaffected by QX disease (Nell and Perkins 2005). Hence, if infection induces mortality of wild *S. glomerata*, impacts to estuarine ecosystems may be wide-reaching.

Here we assess the impact of QX disease on wild oyster populations in an east Australian estuary in which the disease has severely impacted the *S. glomerata* aquaculture industry. We predict that sampling of wild-oyster assemblages along a gradient of salinity will reveal: 1) widespread QX disease among *S. glomerata,* increasing in prevalence with distance away from the estuarine mouth; 2) a greater incidence of *S. glomerata* mortality and a reduced abundance of live oysters at sites with greatest QX infection; and; 3) a greater abundance of *C. gigas* at sites where QX diseases causes *S. glomerata* mortality, than at sites with no evidence of QX disease.

2.3 Methods

2.3.1 Study sites

To assess spatial patterns of QX infection among wild *S. glomerata* and to assess any impacts of the disease on wild oyster community structure, we studied oyster populations at five intertidal rocky shore sites along the Hawkesbury River Estuary, New South Wales, Australia (33°34'S, 151°18'E; Fig. 1) and two along the adjacent Pittwater estuary (33°38'S, 151°16'E; Fig. 2.1). The Hawkesbury River estuary has been subject to annual QX outbreaks among aquaculture oysters since 2004 (Nell 2007). In the marine embayment of Pittwater, by contrast, QX disease has never been recorded. Each estuary was a drowned river valley, with a tidal range of ~1.5 m. Within each estuary, sites were selected in areas of largely horizontal

sandstone substrate, supporting wild oyster populations. All sampling was done at 0.5m – 1.0m above low water springs, a tidal elevation at which *S. glomerata* and *C. gigas* co-occur (Summerhayes et al., 2009).

Our sites within the Pittwater estuary were 10 km from the ocean and of 34 ppt salinity (Fig. 1). Sites in the Hawkesbury estuary were situated 11-28 km from the ocean (Fig. 2.1), along a salinity gradient of 34 to 10 ppt because, among cultured oysters, QX disease increases in prevalence with distance from the estuary mouth and increasing freshwater influence (Butt and Raftos 2008). Sites in the upper estuary (30 km upstream) received greatest rainfall from January to March, with salinity sometimes dropping below 5 ppt for short periods during our study (A. Rubio pers. comm.). The upstream sites were interspersed among former locations of *S. glomerata* culture that had experienced up to 90% mortality due to QX disease in 2004. One upstream site, 7, was also adjacent to a site, at which certified disease-free oysters were sequentially deployed every 3 weeks, between November to June of the years 2005-2009, to assess temporal windows of QX infection (A. Rubio, unpublished data). At the peak of QX infection, between January and April of each year, 47-98% of oysters deployed at that study site (n = 150) were QX-positive within 6 weeks (A. Rubio, unpublished data).

2.3.1 QX disease among wild oysters

To assess the prevalence of QX infection among wild *S. glomerata*, we examined randomly-selected live oysters (n = 14-30, shell height ~18 – 67 mm) from each site in mid April of 2009 and 2010. Sample sizes were considered large enough to detect QX infection as previous studies in the Hawkesbury River detected QX infection among groups of 12 experimentally deployed *S. glomerata* (Butt and Raftos 2007). We defined QX infection as

the presence of sporulating *M. sydneyi* in the digestive gland of *S. glomerata* (Kleeman et al., 2002). Disease intensity could not be measured in this study because natural variation in the size of oysters sampled, and very low prevalence (see results) restricted assessment to presence/absence of the parasite, *M. sydneyi*. Testing was done in April because monthly sampling of experimentally deployed oysters in the Hawkesbury estuary revealed that sporulation of *M. sydneyi* was maximal at this time, in 2009, the first year of this study, when 47% of experimental oysters were QX-positive, as well as in previous years (A. Rubio, unpublished data). Digestive gland tissue was isolated from each oyster and a digestive gland imprint was made on a microscope slide. Slides were stained with modified Wright's stain (Wright's stain 6 g $L^{-1;}$ Giemsa stain 0.6 g L^{-1} in 1 L methanol), air-dried, mounted with a coverslip (Ultramount No. 7; Fronine PTY LTD, Riverstone, NSW Australia) and examined with an Olympus BH-2 microscope (40 × magnification). Infection was diagnosed by the presence of at least one mature *M. sydneyi* sporont per individual (Kleeman et al., 2002) and the percentage of infected oysters sampled at each site was determined.

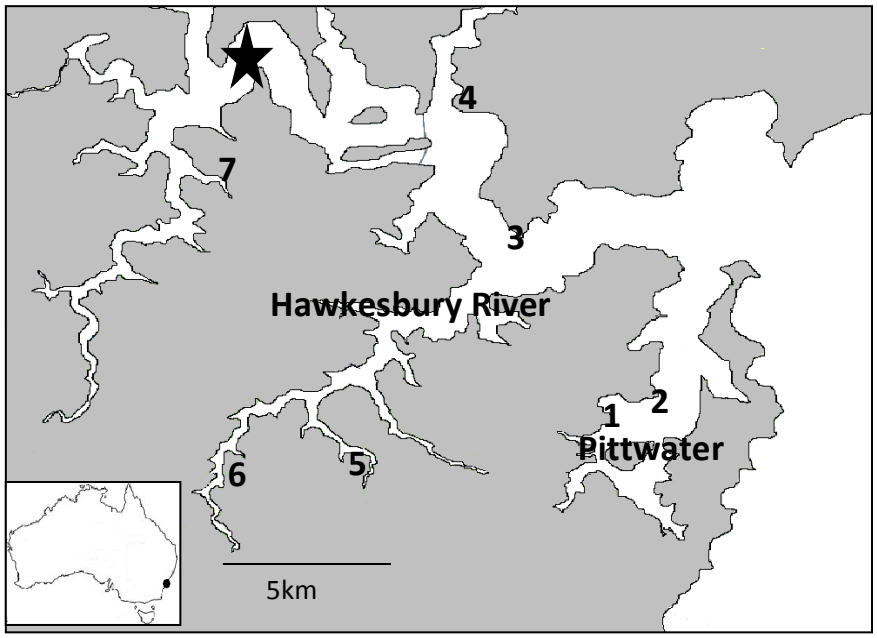

Figure2.1. Map of Hawkesbury Estuary, New South Wales, Australia, showing the location of sites sampled in the Hawkesbury River estuary, and the Pittwater estuary. Site numbers correspond to labelling on column graphs. The star shows the location of a 2009 deployment of cultured *S. glomerata* among which 47% became infected by QX disease (A. Rubio et al. unpublished data).

2.3.2 Sampling of oyster populations

To test whether at sites experiencing QX outbreaks, (1) there is annual mortality of *S. glomerata* caused by the disease and (2) this causes reductions in the abundance of *S. glomerata* that are not seen at unaffected sites we sampled at each of the sites biannually in 2009 and 2010. We sampled in January of each year to assess *S. glomerata* population structure immediately prior to each QX outbreak and then in July, to assess any oyster mortality and change in *S. glomerata* population structure from before to after each QX event. During each sampling time, we also assessed the abundance of *C. gigas* at each site. *C. gigas* overcatch on farmed oysters was reported by local oyster farmers during both sampling years (J. Stubbs, pers. comm.).

To assess changes to the population structure of *S. glomerata* from before to after QX events, and to determine the abundance of *C. gigas* at each site, we destructively sampled oyster communities on each sampling date. Destructive sampling was necessary for accurate taxonomic discrimination between *S. glomerata* and *C. gigas,* which requires examination for denticles, hinge teeth that are present in *Saccostrea* spp. but not *Crassostrea* spp. (Thomson 1954). It was also necessary for assessment of population size-structure, given that oysters can form complex three-dimensional habitat. We removed all oysters from six randomly selected 0.25 × 0.25 m quadrats within ~ 100 m rocky shore. This level of replication was

sufficient for accurate and precise measurements of species-specific densities (Dethier and Schoch 2005; Summerhayes et al., 2009). At the laboratory, the dorso-ventral shell height (to the nearest mm) of each oyster was measured with vernier callipers and each was opened to facilitate identification to species. Oysters too small for identification to species using morphological features were recorded as 'spat' (Anderson and Connell 1999). We documented all oysters as either live (with the hinge secure and flesh intact), boxed (dead oysters with two articulated valves) or dead (open shells without the top valve). We considered boxed oysters as evidence of recent mortality, while dead, disarticulated oysters were assumed to have been long dead (Ford et al., 2006). Previous studies have shown that QX infection causes mortality within a fortnight (Butt and Raftos 2007) hence any QX disease-inflicted mortality among wild oysters would have presumably manifested during the time since the infectious periods and sampling times in our study.

2.3.3 Analyses

We used mixed model ANOVAs with three orthogonal factors; year (random: 2009, 2010), season (fixed: January and July) and site (fixed: 1-7; see Fig. 2.1) to determine whether there were seasonal changes in oyster populations that were most pronounced at the sites experiencing greatest QX infection of oysters. Separate tests were run on the abundance of live, box and dead *S. glomerata* and the abundance of live *C. gigas*. Cochran's test was run before each analysis to test homogeneity of variance and heterogeneous data was ln (x + 1) transformed. Where variances remained heterogeneous even after transformation data were still analyzed because analysis of variance is relatively robust to heterogeneous variances (Box 1953; Underwood 1997). To account for the enhanced probability of Type I error, these analyses were only considered significant at $\alpha = 0.01$. Sources of significant differences

among means were investigated using Student-Newman-Keuls (SNK) tests. WinGMAV5 (Underwood & Chapman 1998) was used for analyses.

2.4 Results

2.4.1 QX disease among wild oysters

The infection of wild *S. glomerata* by *M. sydneyi* varied between years and among sites. In 2009, infection was only detected at Site 3, in two out of 30 oysters sampled, and at Site 6, in four out of 29 oysters sampled (see Fig. 2.1 for site locations). In 2010, among the 20 oysters per site that were sampled, QX infection was detected in one individual from each of Sites 6 and 7.

2.4.2 *S. glomerata* populations

Study sites supported abundant populations of live *S. glomerata* ranging from 0-532 individuals per 0.0625 m^2quadrat, with an average (±SE) of 78.7 ± 5.9 per 0.0625 m^2. At each of the seven sites there were significantly more live *S. glomerata* sampled in 2010 than in 2009 (Table 2.1, Fig. 2.2a). There was no detectable change in the abundance of live *S. glomerata* from before (January) to after (July) the season of QX infection in either year (Table 2.1, Fig. 2.2a). Samples collected at the most upstream site (7) contained significantly less *S. glomerata* than all other sites irrespective of year and season (Table 2.1; Fig. 2.2a). Site 5 contained fewer live oysters than all sites but site 7 (Table 2.1; Fig. 2.2a).

Significantly more box *S. glomerata* were sampled in 2010 than 2009 but there was no detectable change in the abundance of box *S. glomerata* from January (before QX season) to

July (after QX season) of either year (Table 2.1, Fig. 2.2b). Among sites, fewer box oysters were sampled at Site 7 than the remaining six sites, among which abundances of box *S. glomerata* were similar. Dead *S. glomerata*, by contrast, varied in abundance according to Site and Year, but were similarly unaffected by season (Table 2.1, Fig. 2.2c). Within four sites, the number of dead oysters increased between sampling in 2009 and 2010 and at two, the number did not change. Only at Site 6 were fewer dead oysters detected in 2010 than 2009 (Table 2.1, Fig. 2.2c). In both years, samples collected at site 7 contained significantly less dead *S. glomerata* than all other sites. Additionally, in 2010 sites 3 and 4 contained the greatest number of dead *S. glomerata* (Table 2.1, Fig. 2.2c).

At six of the seven sites, live *S. glomerata* displayed a unimodal size frequency distribution on each of the four sampling dates (Fig. 2.3). The largest oysters sampled at these sites were 70-80 mm shell height and the most frequently encountered size class was 10 – 20 mm. At each of these sites, juvenile oysters, <10 mm in shell height, were present on all four sampling occasions. Oysters <20 mm shell height were, by contrast, absent from samples collected from site 7, the furthest upstream. Instead, the live *S. glomerata* population at that site was dominated by oysters of 50 – 70 mm shell height. The smallest and largest *S. glomerata* sampled at Site 7 were 27 mm and 103 mm shell height respectively (Fig. 2.3). Box and dead *S. glomerata* displayed unimodal size frequency distributions that at sites 1-6 closely matched those of live oysters, although abundances of live oysters were much greater. Among these six sites, only at site 6 and only in January 2009 were more dead and box than live *S. glomerata* seen, within the 20 – 30 mm shell height range (Fig. 2.3). Site 7, by contrast, contained very few box but more dead than live *S. glomerata*. As with live *S. glomerata*, dead *S. glomerata* at site 7 were in the larger size ranges (i.e. from 71 – 80 and from 81 – 90 mm shell height) at each sampling time.

Table 2.1. Analyses of variance testing for differences in the abundance of live, box and dead *Saccostrea glomerata,* and live *Crassostrea gigas* among sites in each of two seasons of 2009 and 2010. Yr = year (2 levels, random: 2009, 2010); Se = season (2 levels, fixed orthogonal to Yr and Si: January and July); Si = sites (7 levels, 1-7, fixed). SNK = Student-Newman Keul's post hoc tests. * indicates ln(x + 1) transformation and ** indicates heteroscedastic data (Cochran's C = 0.15, $p < 0.05$) despite ln (x + 1) transformation. Significant differences (at $\alpha = 0.05$ for tests with homogeneous variances and $\alpha = 0.01$ for heterogeneous variances) are highlighted in bold.

		**Live			**Box			Dead			*C. gigas		
Source	df	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*P*	MS	*F*	*p*
Yr	1	9.69	29.40	**<0.01**	4.59	7.28	**<0.01**	11.10	18.02	**<0.01**	3.02	10.1	**<0.01**
Se	1	0.83	2.73	0.35	1.13	1.85	0.40	0.04	0.05	0.87	0.00	0.01	0.94
Si	6	51.60	131.54	**<0.01**	23.08	26.34	**<0.01**	27.29	7.24	**<0.05**	7.96	3.88	0.06
Yr × Si	6	0.39	1.19	0.38	0.88	1.39	0.22	3.77	6.11	**<0.01**	1.98	6.61	**<0.01**
Yr × Se	1	0.30	0.92	0.33	0.61	0.97	0.33	0.97	1.58	0.21	0.48	1.60	0.21
Se × Si	6	0.72	1.69	0.27	0.44	1.15	0.44	0.18	1.34	1.37	1.07	0.41	0.85
Yr × Si × Se	6	0.43	1.30	0.26	0.38	0.61	0.73	0.14	0.22	0.97	2.58	8.60	**<0.01**
Res	140	0.33			0.63			0.62			0.30		
SNK		(Yr) 2009<2010 (Si) (1=2=3=4=6)>5>7			(Yr) 2009<2010 (Si) (1=2=3=4=5=6)>7			(Yr × Si) 1,3,4,5: 2009<2010 6: 2009>2010 2,7: 2009=2010 2009: (1=2=3=4=5=6)>7 2010: (3=4)>(1=2=5=6)>7			(Yr × Se × Si) 5, Jan; 5, Jul: 2009>2010 6, Jan; 4,Jul: 2009<2010 Other Si x Se combinations: 2009=2010 5, 2009; 6, 2010: Jan>Jul 6, 2009; 4, 2010: Jan<Jul Other Si x Se combinations: Jan = Jul Jan 2009: (1=2=3=4=6=7)<5 Jul 2009: (1=2=3=4=5=7)<6 Jan 2010: (1=2=3=5=7)<(4=6) Jul 2010:(1=2=3=5=7)<(4=6)		

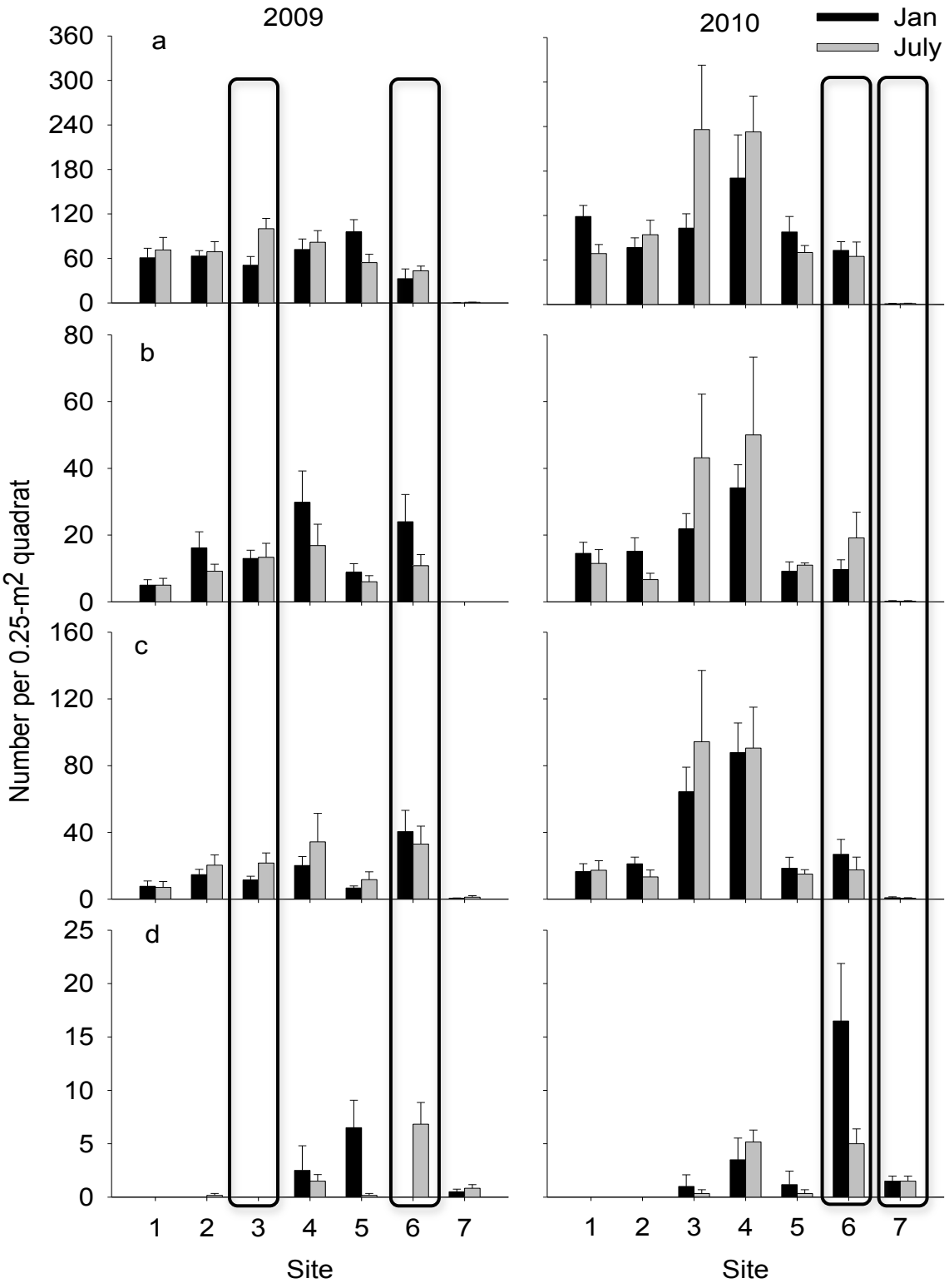

Figure 2.2. Mean (± 1 S.E) abundance of (a) live *S. glomerata*, (b) box *S. glomerata*, (c) dead *S. glomerata* and (d) live *C. gigas* collected in January (black bars) and July (grey bars) of 2009 and 2010. Sites are ordered from left to right according to decreasing salinity (ppt), as per Figure 2.1. n = 6. Outlined columns indicate sites where QX-infection was detected.

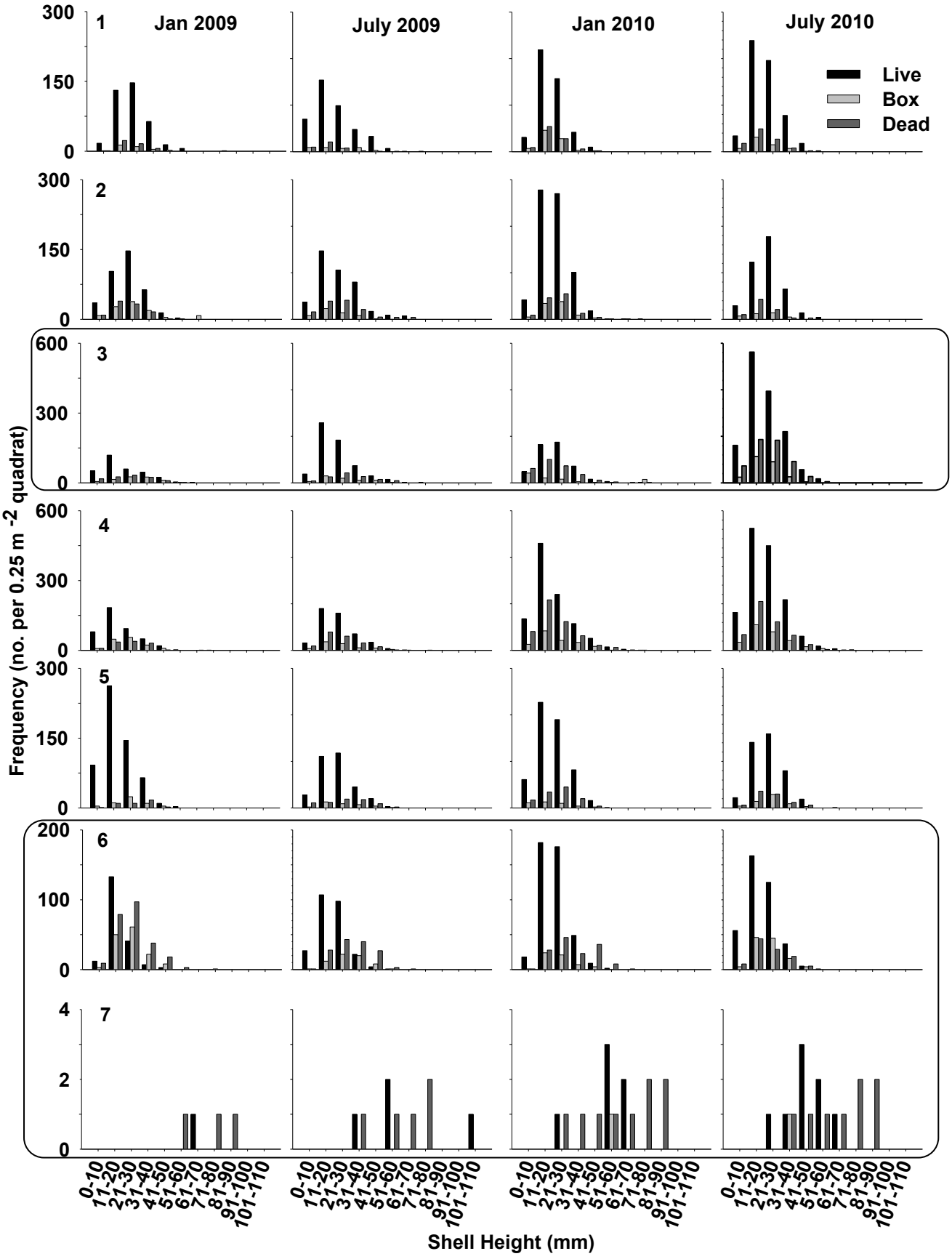

Figure 2.3. Frequency histograms showing the size distribution of live, box and dead *Saccostrea glomerata* at sites 1 – 7, sampled in January and July of 2009 and 2010. Data were pooled from

six 0.0625-m^2 quadrats. Note the difference in scale of the y axis among sites. Outlined panels indicate sites where QX-infection was detected during this study.

2.4.3 *C. gigas* populations

Sites within the Hawkesbury River (i.e. 3-7) supported populations of live *Crassostrea gigas* that ranged in density from 0 to 33 individuals per 0.25m^2 quadrat (Fig. 2.4), with an average of 1.97 ± 0.35 per 0.0625 m^2. By contrast, in Pittwater (i.e. sites 1 and 2) only two *C. gigas* were sampled, both live, and at Site 2 (Fig. 2.2d). The density of live *C. gigas* in the Hawkesbury River varied among sites, according to year and season (Table 2.1, Fig. 2.2d). Site 5 supported significantly more *C. gigas* than all other sites in January 2009, while Site 6 contained the highest density of *C. gigas* in July 2009 and January 2010 (Fig. 2.2d). Sites 4 and 6 had the highest densities of *C. gigas* in July 2010 (Fig. 2d). Differences between seasons in *C. gigas* abundance were not consistent between years and sites. More *C. gigas* were sampled in January than in July at Site 5 in 2009 and at Site 6 in 2010, whereas July supported a higher density of oysters than January at Site 6 in 2009 and Site 4 in 2010.

At not one of our study sites did we detect live *C. gigas* less than 10 mm in shell height (Fig. 2.4). Populations of live *C. gigas* were instead dominated by oysters within the size class of 20 – 60 mm shell height, with individuals as large as 110 mm sampled at Site 7 (Fig. 2.4). Box and dead *C. gigas*, although generally less abundant than live oysters, followed a similar size range to the latter. Only at Site 4, and only in Jan 2009 was evidence of recent unsuccessful cohort establishment found, indicated by a greater number of dead juveniles (Fig. 2.4). Only at

Site 7, the site with the highest proportion of *C. gigas*, did dead and box *C. gigas* consistently outnumber live *C. gigas* (Fig. 2.4).

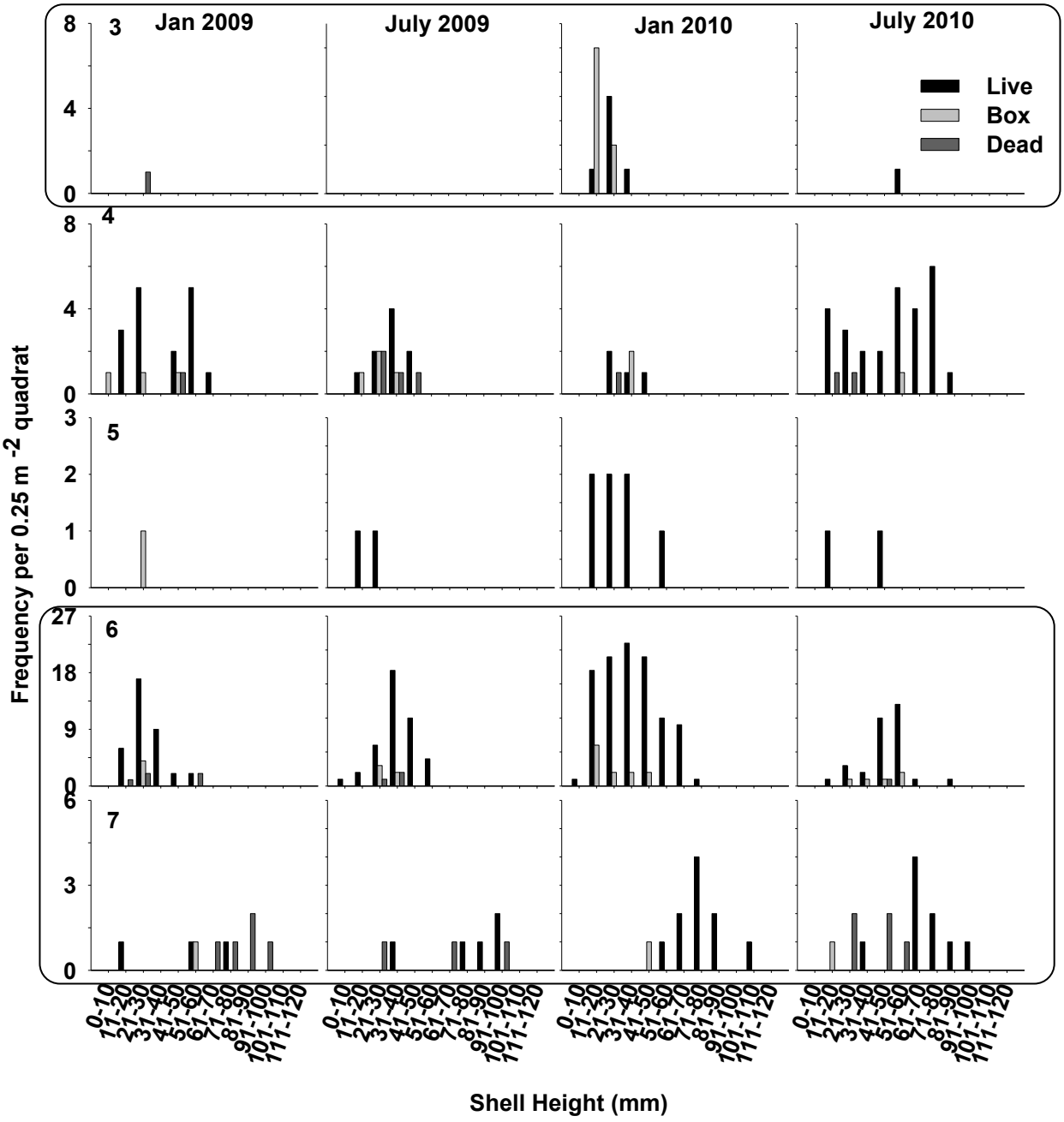

Figure 2.4. Frequency histograms showing the size distribution of live, box and dead *Crassostrea gigas* at sites 3 – 7, sampled in January and July of 2009 and 2010. Data were pooled from six 0.0625 m^2 quadrats. Note the difference in scale of the y axis among sites. Outlined panels indicate sites where QX-infection was detected during this study.

2.5 Discussion

During out two-year study, we found no evidence that QX disease was negatively affecting populations of wild *S. glomerata* in the Hawkesbury River estuary, New South Wales (NSW), Australia. Although we detected *M. sydneyi* among wild oysters, the prevalence of QX disease was much lower than in cultured oysters prior to (Nell 2007) or during this study (A. Rubio, unpublished data). Consequently, there was no detectable mortality of *S. glomerata* from the start to the end of QX infection periods of each of the two years of sampling. The abundance and size structure of *S. glomerata* were similar between QX-affected sites, and those in Pittwater, from which QX disease is not known. Populations of wild *C. gigas* remained small or absent from most study sites, and generally patterns of distribution and abundance did not appear correlated to QX disease.

The prevalence of QX disease among wild *S. glomerata* was low, and infection did not follow the predicted pattern of increasing prevalence along the upstream-gradient in salinity. Among the seven rocky shores sampled, prevalence of QX disease peaked at 14% at one of the upstream sites. A contemporaneous monitoring study in the upper Hawkesbury River estuary revealed a peak prevalence of QX disease of 47% at a nearby location (A. Rubio, unpublished data) and historical records place QX mortality of aquaculture oysters in this estuary at up to 98 % (Nell and Perkins 2006). As predicted, we found no infected oysters at higher-salinity sites in the Pittwater Estuary, however, infection was not isolated to upstream sites. Within the Hawkesbury, the site closest to the mouth contained a similar number of infected oysters as did the furthermost upstream site. This was unexpected given that lower salinity at upstream sites has been implicated in the facilitation of QX disease by weakening the immune system of *S. glomerata*, rendering it more susceptible to *M. sydneyi* (Butt et al., 2006).

A lower-than-expected incidence of QX disease among our field sites may reflect differences in the frequency of disease-resistance genes between wild oyster populations and/or environmental differences between rocky shores and oyster leases. Although historically based on wild-caught recruits (Nell 1993; Nell 2001a), the Hawkesbury River oyster industry today uses hatchery-bred spat as its main supply of juvenile oysters (Nell 2007). Naïve oysters (grown in a QX uninfected estuary) such as those deployed in monitoring surveys (A. Rubio, unpublished data), would, presumably, be more susceptible than wild oyster populations to QX-mortality in estuaries suffering from recurrent outbreaks. The recent success of breeding programs targeting QX resistance (Simonian et al., 2009) indicates that there is natural variation in susceptibility among individuals in wild populations to QX disease, on which selection could act.

Additionally, it is possible that environmental conditions on rocky shores lower the encounter rate of *S. glomerata* with the QX-causing parasite and/or the oyster's susceptibility to infection. *M. sydneyi* is not horizontally transferred among oysters in the laboratory, which suggests that an intermediate host may be involved in the life cycle (Roubal et al., 1989). The sediment-dwelling polychaete, *Nephtys australiensis*, has been found to carry the parasite and may serve as an intermediate host (Adlard and Nolan 2009). In the Hawkesbury River, and in other NSW estuaries, oysters are typically cultured on racks above muddy bottoms. Because oysters on racks are in closer proximity to the estuary floor they filter material resuspended from the benthos, and might experience an enhanced encounter rate with a sediment-dwelling intermediate host (Rubio 2007). Furthermore, at the mid tidal elevation of rocky shores at which our sampling was done, oysters might spend a reduced period of time inundated, compared to cultured oysters, potentially reducing rates of QX infection. The prevalence of *Bonamia roughleyi*, the protozoan parasite responsible for *S. glomerata* winter mortality, decreases with

tidal elevation (Nell 2007). On lower shores such as cultured oyster rack heights, increased QX infection among wild *S. glomerata* might facilitate invasion of the lower shores by C. *gigas*. However, further research is needed to determine the effects of shore height and inundation time on QX disease. Furthermore, at the high densities typical of aquaculture leases it is possible that oysters experience stressors that increase their susceptibility to the parasite (see Lafferty and Holt 2003).

Perhaps not surprisingly, given the low incidence of QX infection among wild *S. glomerata*, our biannual sampling of oyster assemblages did not provide evidence for QX mortality of *S. glomerata* at the Hawkesbury river study sites during the two years of our study. Numbers of newly-dead (box oysters) did not increase from before to after the season of QX infection in either of the two years of sampling. Although QX induced mortality can persist until at least August, and has been recorded throughout most of the year (Nell 2001b), all rocky shores, except the one furthest upstream, contained average *S. glomerata* densities (314 ± 5.9 per m^2) that were comparable to those in other New South Wales estuaries that do not have QX disease (Bishop et al., 2010). This suggests that even prior to this study, between the first reported QX outbreak in 2004 and 2009, significant mortality of wild *S. glomerata* did not occur. Furthermore, oyster populations had similar size frequency distributions among sampling times, including both newly recruited oysters and individuals of sufficient size to be > 1 year-old (Hand and Nell 1999), and indicative of stable *S. glomerata* populations.

Although *C. gigas* was found at six of the 7 sites in very low abundance, the non-native was generally more abundant at upstream study sites. This observation of confinement of *C. gigas* to lower-salinity waters is consistent with its distribution in other New South Wales

estuaries (Bishop et al., 2010) and is concordant with studies indicating that the Miyagi strain of Pacific oysters that was introduced to Australia, does not tolerate salinities of <4 or >33 (Imai 1980). Nevertheless, it is noteworthy that among upstream sites, *C. gigas* was most abundant at the site at which we detected greatest QX infection. The link between QX infection and *C. gigas* abundance, however, remains weak because populations of wild *S. glomerata* were no less abundant at this study site than at other locations, and numbers of newly or long-dead *S. glomerata* were no greater. Furthermore, at the study site at which the *S. glomerata* population was smallest, *C. gigas* were no more abundant than elsewhere and the low *S. glomerata* abundance is likely to have been caused by the inability of *S. glomerata* spat to survive prolonged exposure to salinities less than 19 ppt (Dove and O'Connor 2007) because QX infection and mortality appeared low. Possibly, differences in timing of recruitment between species (*S. glomerata* spawn in summer, while *C. gigas* spawn earlier in spring, Nell 1993; Mason and Nell 1995) explains why *C. gigas* were found in low abundance. For example, even if *S. glomerata* experienced QX induced mortality, settling of new recruits observed from autumn to winter (i.e., during and after QX infection season) might have pre-empted *C. gigas* recruitment in spring (before the following QX infection season).

The site with greatest *C. gigas* abundance was more sheltered from direct sunlight by trees, and received less wave action than other sites (E. Wilkie, pers. obs.), possibly minimising desiccation and providing conditions typically more suitable for *C. gigas* (e.g,. Melo et al., 2010; Troost 2010). When compared to the native oyster, *S. glomerata*, the non-native *C. gigas* is much less resistant to desiccation stress (Krassoi et al., 2008). The site of greatest *C. gigas* abundance was also the most heavily modified by human interference containing a boat marina and many moored vessels possibly creating a stressed environment that facilitates colonization of the invasive (e.g,.Occhipinti-Ambrogi and Savini 2003).

Although our study found no evidence of significant impacts of QX-disease on wild *S. glomerata*, changes in environmental conditions may facilitate this process in the future. Expression of QX disease reflects an interaction between environmental conditions that weaken the immune system of oysters, and the presence of *M. sydneyi* (Butt and Raftos 2008). Hence, environmental changes that weaken the immune system of *S. glomerata* may increase the susceptibility of ecosystems based on *S. glomerata* to collapse.

Elsewhere in the world, declining water quality and climatic change are also facilitating range expansions of other parasites of aquatic organisms (e.g., MSX and Dermo oyster diseases in the USA; Harvell et al., 1999). Although in our study, QX disease was not found to significantly impact wild *S. glomerata* populations, diseases of cultured organisms nevertheless have the potential to compromise wild aquatic populations, thereby impacting the functional ecological roles of such species (Dunn 2009; Krkošek et al., 2005; Torchinet al., 2002). As the incidence of aquatic diseases continues to increase, it will be important to incorporate the role of parasites into models of community structure and invasion.

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3 Are native *Saccostrea glomerata* and invasive *Crassostrea gigas* oysters habitat equivalents for epibenthic communities in south-eastern Australia?

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3.1 Abstract

Introduced species that alter the physical structure of marine habitats can have large impacts on biodiversity. We assessed whether in south-eastern Australia the invasive Pacific oyster, *Crassostrea gigas,* differs from the native Sydney rock oyster, *Saccostrea glomerata,* in the biogenic habitat that it provides to epibiotic communities. We also assessed how within a species (*S. glomerata*), genotype influences habitat provision. First, we conducted a field experiment, in which we compared recruitment of epibiota to concrete plates with either *C. gigas*, wild-stock *S. glomerata*, selectively bred *S. glomerata* or glue (control). Second, we assessed whether within wild *S. glomerata* populations invaded by *C. gigas*, communities of epibenthos are correlated to the ratio of non-native to native oysters. On experimental plates *C. gigas* grew larger, and experienced higher mortality than both selectively-bred and wild-stock *S. glomerata* at each of two heights on the shore. The two genotypes of *S. glomerata*, by contrast, displayed similar rates of growth and mortality. The differing growth patterns among oyster types had not, however, translated to consistent differences in the composition of associated benthic communities by 12 months following establishment of experimental treatments. Within established wild oyster assemblages*, C. gigas* were typically much larger than on our experimental plates, and the non-native influenced the abundance of several epibiotic taxa, although not the identity of species present. Where impacts of *C. gigas* on the abundance of associated benthic species occurred, they were generally negative. Overall, our results indicate a high degree of functional equivalency between *S. glomerata* and *C. gigas*. Hence, where native species are displaced by invasive species of similar morphology, the impacts of the non-native species on local diversity may be minimal.

Keywords: Aquaculture; Ecosystem engineers; Habitat complexity; Pacific oyster; Sydney rock oyster

3.2 Introduction

Ecosystem engineers create, modify and maintain habitats by controlling the availability of resources to other organisms (Jones et al., 1994). In doing so they regulate important ecosystem processes, such as species' interactions, and are key determinants of biodiversity (Jones et al., 1994). Although ecosystem engineers may have positive or negative effects on other organisms, those that increase the physical complexity of habitats tend to increase biodiversity (Bruno et al., 2003). In marine ecosystems, species such as gregarious bivalves, seagrasses, macroalgae, corals, and tube worms form complex biogenic habitats that facilitate dense and diverse communities by providing shelter from waves and desiccation stress, protection from predators, substrate for attachment and foraging, and sites of increased organic matter deposition due to modification of current flow (Lenihan et al., 2001; Bruno et al., 2003; Grabowski and Powers 2004).

Biological invasions that alter the distribution and abundance of ecosystem engineers have the potential to dramatically impact community structures and ecosystem processes (Crooks 2002; Ruesink et al., 2006; Kochmann et al., 2008; Markert et al., 2010). Where a non-native species adds habitat complexity or heterogeneity, the abundances and species richness of associated organisms, and their associated ecosystem functions, generally increase (Crooks 2002). This might occur where the invader is more complex than native organisms or replaces biogenic habitat that has been lost due to disturbances like overfishing (Harwell et al., 2010). For example, in the Wadden Sea replacement of native (*Mytilus edulis*) mussel beds with more complex Pacific oyster (*Crassostrea gigas*) matrices increased biodiversity (Markert et al., 2010). By contrast, where an invader displaces a native species that is of greater complexity, a reduction in the abundance, richness and identity of associated species may result. The third possibility is that the non-native closely matches the structure and function of the native being

replaced, in which case little change in associated biodiversity may be predicted (Ruesink et al., 2006).

In Australia, the Sydney rock oyster *Saccostrea glomerata* is an important ecosystem engineer. It is widely distributed along temperate and tropical coastlines (Nell 2001). On rocky shores and mud flats its densely clustered aggregations create complex three dimensional habitats for epibenthic invertebrates (Underwood and Barrett 1990; Jackson et al., 2008; Summerhayes et al., 2009a), provide a food source for predators (Anderson and Connell 1999) and facilitate benthic-pelagic coupling (Bayne and Svensson 2006). It also supports an aquaculture industry in the state of New South Wales that is worth AUS $35 million year (NSW DPI 2009/2010). Increasingly, the provision of habitat and services by Sydney rock oysters is being threatened by QX disease (Nell and Perkins 2006). QX disease, caused by the paramyxean protistan parasite, *Marteilia sydneyi*, invades the digestive gland of oysters that have been immuno-suppressed by sub-optimal environmental conditions (Butt and Raftos 2007). Mortality from starvation typically occurs within 60 days of infection, and has lead to up to 98% mortality of oysters on affected aquaculture leases (Peters and Raftos 2003) and lesser amount of mortality on natural substrates (Wilkie et al., unpublished data).

To replace oyster production lost to QX disease, hatchery reared QX disease resistant *S. glomerata* and non-native *C. gigas* are increasingly being cultivated in affected estuaries (O'Connor and Dove 2009). In most instances cultured *C. gigas* are typically triploids that are not reproductively viable. As a consequence of previous translocations of diploid *C. gigas* wild self sustaining populations of the non-native are, however, present on natural substrates of several NSW estuaries (Summerhayes et al., 2009a; Bishop et al., 2010), and the possibility of

some triploid oysters reverting to diploidy, and reproducing, remains (Nell 2002). Once established, the rapid growth of wild *C. gigas* on the low and mid intertidal shore can result in overgrowth and displacement of native *S. glomerata* (Krassoi et al., 2008).

Both *C. gigas* and QX-resistant Sydney rock oysters display markedly different growth characteristics to wild stock Sydney rock oysters when grown as single, free-individuals in aquaculture facilities (Bayne 2002, Nell and Perkins 2006). The faster rates of feeding, and greater metabolic feeding and growth efficiencies of *C. gigas* than *S. glomerata* typically result in the non-native growing at a much greater rate than the native species (Bayne 2002). However, *C. gigas* appears less resistant to desiccation stress than *S. glomerata* such that this growth differential decreases with increasing intertidal elevation (Krassoi et al., 2008). On leases in QX affected estuaries, QX disease resistant *S. glomerata* has a higher growth and survival rate than wild-stock counterparts (Nell and Perkins 2006). How these differences in physiology influence the quality of the habitat they provide on hard substrates is unknown.

Growth and survival patterns of bivalves are important factors that characterize and regulate the structure and complexity of biogenic habitats (Gutiérrez et al., 2003; Commito et al., 2008). Therefore, we might expect that replacement of slow growing *S. glomerata* with faster growing QX resistant *S. glomerata* and non-native *C. gigas* might modify the habitat architecture, and consequently associated communities. Here we assess: 1) differences in morphological development of oyster habitat provided by wild-stock *S. glomerata*, QX disease resistant *S. glomerata* and *C. gigas* at two elevations of an intertidal rocky shore, 2) whether differences in morphological development of oyster matrices influence associated communities, and 3) whether among *S. glomerata* populations invaded by *C. gigas*, communities of epibenthos

are correlated to the ratio of non-native to native oysters. We predict that differences in growth and mortality of the three oyster types will be evident, and will be greater at lower than higher intertidal elevations. Consequently, we predict that oyster type will have a larger effect on associated community structure on the low than the mid intertidal shore.

3.3 Methods

3.3.1 Colonization experiment

To assess: (1) whether wild-stock *S. glomerata* (SR), selectively bred QX-resistant *S. glomerata* (QX) and *C. gigas* (PO) provide structurally different habitats as a result of differing rates of growth and mortality and (2) whether the three oyster types support distinct benthic assemblages to one another, monotypic plates of juvenile oysters were deployed at Wanda Wanda Head, Port Stephens, New South Wales Australia (32°43'S, 151°59'E, Fig. 3.1). This east-facing site was dominated by a rocky revetment wall of boulders approximately 0.3 m in diameter that supported abundant natural populations of *S. glomerata*. It was approximately 10 km upstream of the estuarine mouth, had a tidal range of 1.3 m and an average salinity of 28 ppt. To test hypotheses about effects of tidal elevation on habitat provision, oysters of each type were deployed at two heights on the shore, low (L: 0.0 – 0.2 m above low spring water tide [LSWT]) and high (H: 0.3-0.6 m above LSWT).

Four month old oysters were deployed on concrete plates, 230 × 230 × 40 mm in area. To each plate, we glued 30 oysters of a single type using a drop of non toxic 2-part epoxy resin Megapoxy HT (Vivacity Engineering, Thornleigh NSW). Oysters were arranged evenly on one surface each plate, leaving a 15 mm margin around each edge. This arrangement gave a density of 750 oyster spat m^{-2}, which is representative of the naturally occurring density of oysters at

Port Stephens (Krassoi et al., 2008). To assess the extent to which patterns of algal and invertebrate colonization could be attributed to oysters, we also established control plates (C), receiving glue but no oysters. Oysters were all diploids: SR and QX were provided by the Department of Primary Industries Fisheries, Port Stephens NSW; PO were provided by Diemar's Oysters, Salamander Bay, Port Stephens, NSW. As a result of holding age of oysters (4 mo) constant across all treatments, the initial size of oysters varied slightly among oyster types, with faster growing PO (mean starting shell height [± SE]: 9.5 ± 2.1 mm) and QX (7.8 ± 2.0 mm) larger than SR oysters (7.0 ± 2.5 mm). In the absence of the protective structure normally supplied by adult oysters, each plate was initially covered with 5 mm plastic mesh to protect the juvenile oysters. After 7 months the oysters had grown sufficiently to experience a size refuge from many predators and the mesh was removed.

Thirty plates of each type (PO, QX, SR, C) were deployed at each of the tidal elevations on 3 May 2008. Plates were randomly deployed along ~ 300 m shoreline, by securely positioning them between rocks of the artificial rock wall, with their oyster-containing surface extending outwards.

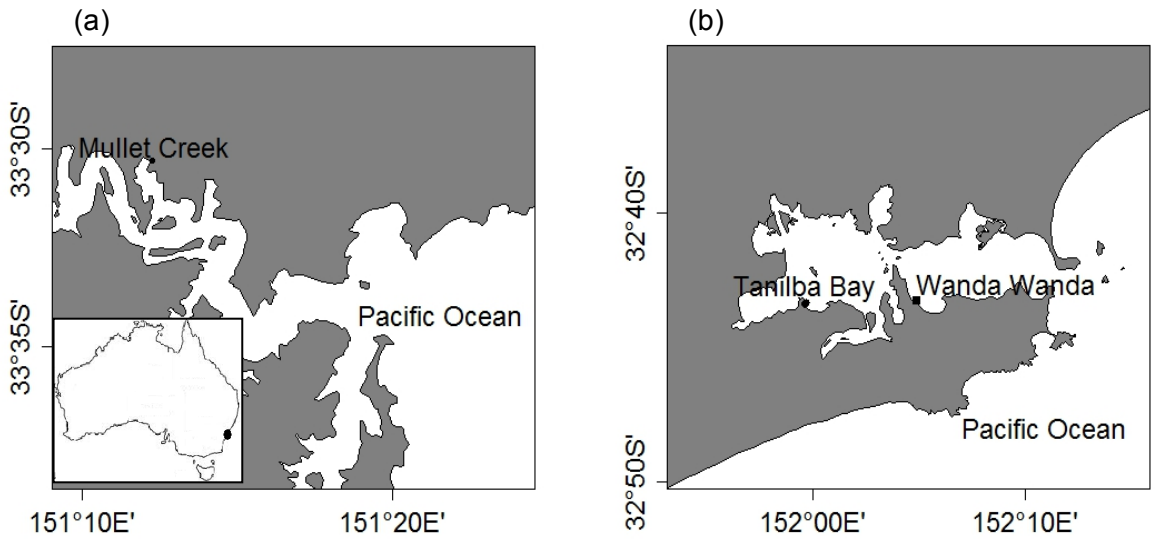

Figure3.1. Map of (a) the Hawkesbury River and (b) Port Stephens estuary showing the locations of wild oyster sampling (Mullet Creek, Hawkesbury River; Tanilba Bay, Port Stephens) and of the manipulative experiment (Wanda Wanda, Port Stephens).

3.3.2 Sampling

To test hypotheses about differences in growth, mortality and habitat provision among the three oyster treatments at each elevation, we sampled plates at five times after deployment: 2 months, 3 months, 7 months, 10 months, and 12 months. At each of the five times, 6 randomly selected plates of each oyster type from each shore height were sampled. Randomly sampling a different 6 plates at each time increased the power of detecting effects of oyster type above the noise of individual plate factors, such as cumulative biofouling over time, which can influence assemblage patterns (Chapman et al., 2005). At each sampling time, the percent mortality of oysters since the start of the experiment was determined by counting the number of live oysters

remaining on each plate, and determining the percentage of the original 30 that were missing. The shell height (to the nearest mm) of all live individuals from each plate was recorded using vernier calipers. After 10 and 12 months, the habitat complexity and spatial coverage of oysters was also quantified, and we assessed the benthic assemblage composition of plates. We assessed habitat complexity by calculating the ratio of the contoured outline of the oyster reef to the linear length between diagonal corners of each plate. Measurements were made using a piece of string, which could be easily moulded into the crevices between oysters. To quantify cover of oysters, and colonization by sessile organisms, the percentage covers of bare concrete, algae, naturally recruited and glued oysters, and colonies of spirorbid worms were assessed under a grid of 100 intersection points within a quadrat 200 × 200 mm, which was placed within the centre of each 230 x 230 mm plate so as to avoid edge effects. The total number of other epifauna (> 1 mm in diameter) was counted from within the entire quadrat. Organisms were classified as primary (attached to the concrete plate) or secondary (attached to an oyster) and identified to the lowest practical taxon (in most cases species).

3.3.3 Statistical analyses

Analyses of variance (ANOVAs) tested hypotheses about the effect of tidal elevation and species identity on the shell height and % mortality of oysters, the complexity provided by their structure, and the % oyster cover of live oysters. These had three orthogonal factors, time (5 levels for analyses of shell height and % mortality: 2, 3, 7, 10, 12 months; 2 levels for analyses of complexity and oyster cover: 10, 12 months), tidal elevation (2 levels: low, high) and oyster type (3 levels: SR, QX, PO). Prior to each analysis, assumptions of homogeneity of variances were tested using Cochran's *C*-test. Where variances were heterogeneous, data were ln (x + 1) transformed. In some instances, variances remained heterogeneous even after transformation. Data were still analysed because analysis of variance is relatively robust to heterogeneous

variances if there are many independent estimates of variance (Box 1953; Underwood 1997). In such instances, only terms of the ANOVA significant at $p<0.01$ were interpreted as differences, to account for the inflated rate of Type I error. In other instances, p-values of less than 0.05 were considered significant. Where ANOVAs indicated significant differences among treatment means, they were followed by Student Newman Keuls (SNK) tests to identify sources of difference.

To test hypotheses about multivariate differences in communities among oyster types, we ran multifactorial non-parametric permutational analyses of variance (PERMANOVA; Anderson 2001) on Bray-Curtis similarity measures calculated using fourth-root transformed data. Separate analyses were conducted on data collected at 7, 10 and 12 months. These had two orthogonal factors: tidal elevation (2 levels: low, high), and oyster type (4 levels: SR, QX, PO, C). Fourth-root transformation of data allowed all organisms (including those that were counted and those that were quantified as percentage cover) to have similar weighting in the analysis (Underwood and Chapman 1998). We did not analyze assemblages at 2 and 3 months because recruitment of epibiota was minimal at this stage. Patterns among assemblages were visualized using two-dimensional non-metric multidimensional scaling (nMDS) of centroids (points corresponding to the averages for each taxon within each treatment) calculated from fourth-root transformed data. SIMPER analyses (Clarke 1993) were used to identify discriminating taxa contributing to multivariate differences. Taxa with a dissimilarity to standard deviation ratio >1.3 were considered good discriminators (Clarke and Warwick 1994). Two-way orthogonal ANOVAs (tidal elevation: high, low; oyster type: SR, QX, PO, C) examined differences in the abundance of these discriminating taxa among treatments, as well as the total abundance and species richness of taxa. The RELATE procedure of PRIMER was used to determine the Spearman's rank correlation between the Bray-Curtis rank similarity matrix of benthic

community structure among plates with oysters, and the habitat complexity of each plate at 10 and 12 months.

3.3.4 Wild oyster assemblages

To assess how in established oyster habitat the ratio of *S. glomerata* to *C. gigas* oysters influences the structure of associated communities of fauna, sampling was conducted on rocky shores of Mullet Creek, Hawkesbury River (HR: 33°34'S, 151°18'E) and Tanilba Bay, Port Stephens (PS: 32°43'S, 151°59'E), New South Wales, Australia (Fig 3.1). Each of these locations are situated within drowned river valleys of tidal range of ~1.5 m and contain populations of *S. glomerata* on largely horizontal rocky substrate that is being invaded by *C. gigas* (Summerhayes et al., 2009a; Bishop et al., 2010). At each location, the contribution of *C. gigas* to oyster matrices varies at the scale of meters, resulting in heterogeneous habitat of variable *S. glomerata* to *C. gigas* ratio (Summerhayes et al., 2009a; Bishop et al., 2010). All sampling was done at 0.5 m – 1.0 m above low water springs, the tidal elevation at which *S. glomerata* and *C. gigas* co-occur (Summerhayes et al., 2009a).

At each location, oysters and associated organisms were enumerated within 20, 0.25 x 0.25 m quadrats, randomly positioned within oyster beds (comprised of live and dead oysters). First, the habitat complexity provided by oysters was quantified within each quadrat using the method outlined above (i.e. ratio of the contoured to straight line distance between diagonally opposite corners of the quadrat). Second, all oysters and associated organisms falling within the quadrat were chiselled from the rock surface and transported back to the laboratory for analysis. In the laboratory, oysters were separated and washed over a 1 mm sieve, and retained macrofauna were enumerated and identified to mixed taxonomic resolution (species for gastropods, bivalves

[except juvenile oysters classified to family], barnacles, chitons and crabs, family for polychaetes and sipunculids, and order for all other groups). Oysters were inspected under a dissecting microscope to identify epibenthic organisms that remained attached to the oyster shells after washing. Oysters were opened, and identified as either *S. glomerata* or *C. gigas* according to the presence of denticles, hinge teeth that are present in *Saccostrea* spp. but not *Crassostrea* spp. (Thomson 1954). The percent contribution of *C. gigas* to total oysters in each sample was calculated. The shell height (to the nearest mm) of each oyster was measured with vernier calipers. Oysters were classified as live, dead (open shells without the top valve) or boxed (dead oysters with two articulated valves) because empty oyster shells provide different habitat than live oysters and may influence epifaunal assemblages (Summerhayes et al., 2009b).

To test for a relationship between the percent contribution of *C. gigas* to oyster assemblages and associated communities of macroinvertebrates we conducted a Spearman's rank correlation between Bray-Curtis rank similarity matrices of these two data sets using the RELATE procedure of PRIMER. A separate analysis was conducted on data from each of the two locations, with the faunal data matrix fourth-root transformed prior to analysis. To visualize the relationship between the proportionate contribution of *C. gigas* to total oysters, and associated epifaunal communities, we used two-dimensional non-metric multidimensional scaling (nMDS) ordinations of epifaunal communities superimposed with symbols scaled in size according to the percent of *C. gigas* within each quadrat. Pearson's correlations assessed relationships between the percent contribution of *C. gigas* to oyster assemblages and: (1) habitat complexity, (2) oyster density, (3) percent cover, (4) total macroinvertebrate abundance, (5) macroinvertebrate species richness and (6) the most abundant taxa.

3.4 Results

3.4.1 Colonization experiment

3.4.1.1 Oysters

Tidal elevation had no influence on the shell height of any of the oyster types, at any of the sampling times (Table 3.1, Fig. 3.2). Pacific oysters (PO) were consistently larger than Sydney rock (SR) or QX resistant Sydney rock oysters (QX), and the shell height of SR and QX were, in turn, statistically indistinguishable (Table 3.1, Fig. 3.2). By the end of the 12 month experiment, PO had attained double the mean shell height of SR or QX (Fig. 3.2).

Whether oyster mortality differed among PO, SR and QX was dependent on time since the start of the experiment, but not on height on the shore (Table 3.1, Fig. 3.2). At 2 and 3 months, PO had a higher proportionate mortality than SR or QX. By 7 months, differences in proportionate mortality among oysters had disappeared (Table 3.1, Fig 3.2). The proportionate mortality of SR and QX increased significantly in the later part of the experiment (7, 10, 12 months) but the proportionate mortality of PO, which was high even early in the experiment, did not change through time (Table 3.1, Fig 3.2).

Habitat complexity did not significantly differ among oyster types on either the low or the high shore, after 10 or 12 months (Table 3.1). Among plates with oysters, the percentage cover of oysters was significantly greater at 12 months than 10 months, but did not differ by oyster type or shore height (Table 3.1).

Table 3.1 Analyses of variance testing for differences in shell height, percent mortality, habitat complexity and oyster cover among oyster types (Oy; 3 levels, fixed: Pacific oysters [PO], Sydney rock oysters [SR], QX-resistant Sydney rock oysters [QX]), at each sampling time (Ti; 5 levels for shell height and mortality: 2, 3, 7 10, 12 mo; and 2 levels for complexity and oyster cover: 10, 12 mo) and at two heights on the shore (He; 2 levels, fixed: L = low and H = High shore). Terms that are considered statistically significant ($p < 0.05$ unless otherwise stated) are highlighted in bold. * indicates heterogeneity of variances remained after $\ln(x + 1)$ transformation and more stringent alpha value of 0.01 was applied. SNK = Student Newman Keuls post hoc tests. $n = 6$.

	*Shell height (mm)				*Mortality (%)			*Complexity				Oyster cover		
Source of variation	*d.f*	MS	*F*	*p*	MS	*F*	*p*	*d.f*	MS	*F*	*p*	MS	*F*	*p*
Ti	4	9.4	262.7	**<0.01**	22.8	78.0	**<0.01**	1	0.002	3.5	0.06	232.6	5.7	0.05
He	1	0.2	5.1	0.09	0.0	0.1	0.76	1	0.001	0.2	0.77	0.7	<0.1	0.90
Oy	2	8.8	38.1	**<0.01**	9.8	4.4	0.05	2	0.001	2.1	0.33	85.1	2.1	0.17
Ti × He	4	0.1	1.4	0.26	0.3	1.2	0.32	1	0.001	2.5	0.12	3.8	0.1	0.76
Ti × Oy	8	0.2	6.5	**<0.01**	2.2	7.8	**<0.01**	2	<0.001	0.8	0.44	18.0	0.4	0.65
He × Oy	2	0.1	1.0	0.40	0.3	0.6	0.59	2	<0.001	0.4	0.73	141.8	3.5	0.29
Ti × He × Oy	8	0.1	1.4	0.21	0.59	2.0	0.05	2	0.001	0.9	0.40	59.9	1.5	0.24
Res	150	0.2		0.3				60	0.001			41.1		
SNK		Ti × Oy:			Ti:									
		Ti (Oy):			2 = 3 < 7 = 10 = 12									
		PO: 2 < 3 < 7 < 10 = 12			Ti × Oy:									
		SR: 2 = 3 < 7 < 10 < 12			Ti (Oy):									
		QX: 2 = 3 < 7 < 10 = 12			PO: 2 = 3 = 7= 10 = 12									
		Oy (Ti):			SR, QX: 2 = 3 < 7 = 10 = 12									
		2, 3, 7, 10, 12: S = QX < PO			Oy (Ti):									
					2, 3: QX = SR < PO									
					7, 10, 12: SR = QX = PO									

3.4.1.2 Epibiotic assemblages

Over the 12 month experiment, 12 algal and 24 invertebrate taxa were identified. Species contributing most to cover were the algae *Enteromorpha* sp. (35% of cover), *Cladophora* sp. (27%), *Corallina officinalis* (9%), and *Ralfsia* sp. (6%). Of the individual organisms counted, 10 different species of gastropods, 2 families of polychaete, 2 species of chiton, 2 species of barnacle, and 1 oyster family were identified. The most abundant epifauna were the tube worm *Galeolaria caespitosa* (15% of total individual epifaunal abundance), the barnacle *Elminius* sp. (12%), the limpet *Patelloida* sp. (9%), and the gastropods *Cantharidella picturata* (8%) and *Cacozeliana granaria* (8%).

Assemblages of epiobiota significantly differed among oyster treatments at each of the times of sampling (Table 3.2, Fig. 3.3). At 7 and 12 months, control plates without oysters supported distinctly different assemblages to those with oysters, but among plates with oysters, effects of oyster identity were not seen, with the exception of a significant difference between QX and SR at 7 months. At 10 months, differences among oyster treatments were dependent on height on the shore (Table 3.2; sig. Oy × He interaction). On the low shore, assemblages supported by plates with QX or SR differed from those on control plates but not to those on plates with PO, and QX and SR plates in turn supported different assemblages to one another. On the high shore, however, only the difference between PO and control plates was significant. At 12 months, a main effect of tidal elevation on assemblages was also seen (Table 3.2). There was no significant correlation between the benthic community structure and habitat complexity of plates regardless of oyster type after 10 (RELATE: þs = 0.01, $p = 0.52$) or 12 months (RELATE: þs = 0.29 , $p = 0.99$).

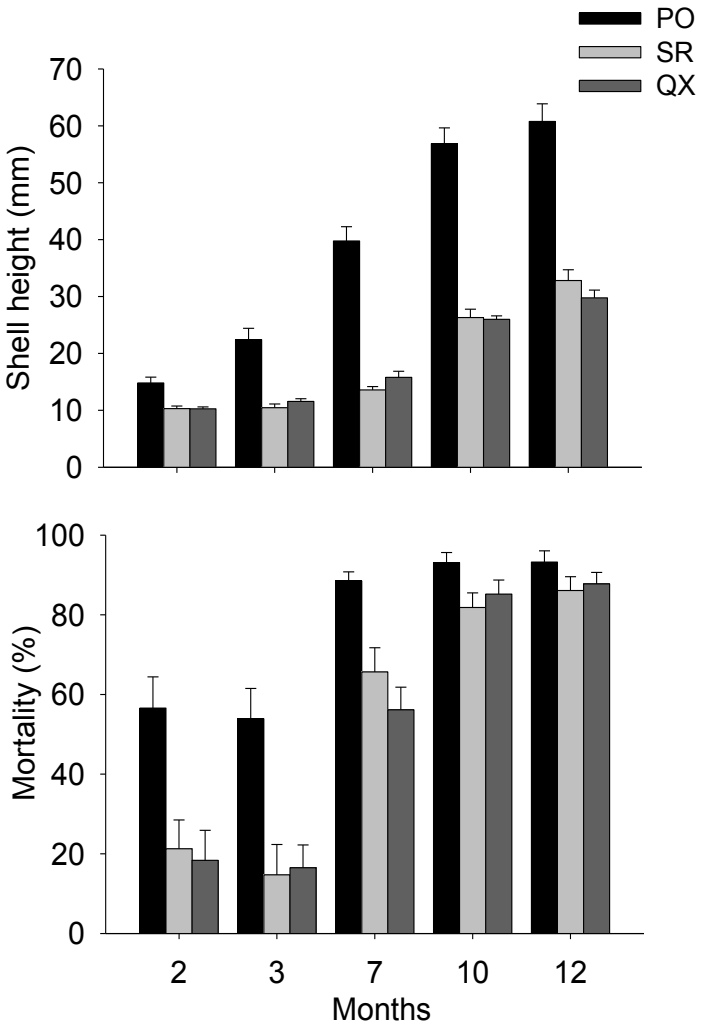

Figure 3.2. Mean (± 1 SE) shell height (mm) and cumulative mortality (%) of *Crassostrea gigas* (PO), *Saccostrea glomerata* (SR) and QX disease resistant *S. glomerata* (QX) 2, 3, 7, 10 and 12 months after deployment on the low and high intertidal shore. *n*= 12.

The total species richness of epibiota did not differ among oyster treatments or between heights on the shore 7 months after the start of the experiment, but at 10 and 12 months significant effects were seen (Table 3.2). At 10 months, the total species richness of epibiota was determined by interacting effects of oyster type and shore height (Table 3.2). On plates containing SR, total species richness was greater on the low than the high shore, but such effects

of elevation were not seen on any of the other plate types, and species richness did not differ among plate types at either tidal elevation. At 12 months, assemblages were more species rich on treatments with than without oysters, but did not differ among PO, SR and QX. The total abundance of epiobiota did not differ among oyster treatments or between heights on the shore after 7 and 12 months (Table 3. 3). At 10 months, differences in the abundance of epifauna among treatments were dependent on the interacting effects of oyster treatment and shore height (Table 3.3; sig. Oy × He). Among plates with no oysters, total abundance was greater on the low shore than on the high shore. Total species abundance did not differ among plates containing oysters irrespective of shore height (Table 3.3).

Table 3.2. Summaries of non-parametric permutational multivariate analyses of variance testing for sources of spatial variation in epibiotic assemblages on experimental plates 7, 10 and 12 months after deployment. Oy, oyster (4 levels: Pacific oysters [PO], Sydney rock oysters [SR], QX-resistant Sydney rock oysters [QX], and controls [C]).He, height on the shore(2 levels: high [H], and low [L]).*n* = 6. Comp = pairwise comparison. Terms significant at $p<0.05$ are highlighted in bold.

	7 months			10 months			12months		
	df	*Pseudo-F*	*p*	*df*	*Pseudo-F*	*p*	*df*	*Pseudo-F*	*p*
Oy	3	3.38	**<0.01**	3	2.53	**<0.01**	3	4.44	**<0.01**
He	1	1.66	0.17	1	12.53	**<0.01**	1	13.57	**<0.01**
Oy x He	3	0.59	0.85	3	1.97	**<0.05**	3	1.21	0.29
Res	40			39			38		

A posteriori tests

	Comp	*t*	*p*	Comp	*t*	*p*	Comp	*t*	*p*
	PO v SR	0.9	0.55	PO v SR	1.4	0.32	PO v SR	1.1	0.30
	PO v QX	1.5	0.24	PO v QX	1.3	0.10	PO v QX	0.8	0.62
	PO v C	2.1	**<0.01**	PO v C	1.7	**<0.05**	PO v C	2.8	**<0.01**
	SR v QX	1.8	**<0.05**	SR v QX	1.5	**<0.05**	SR v QX	0.8	0.63
	SR v C	2.1	**<0.05**	SR v C	1.8	**<0.05**	SR v C	3.0	**<0.01**
	QX v C	2.6	**<0.01**	QX v C	2.0	**<0.01**	QX v C	3.0	**<0.01**

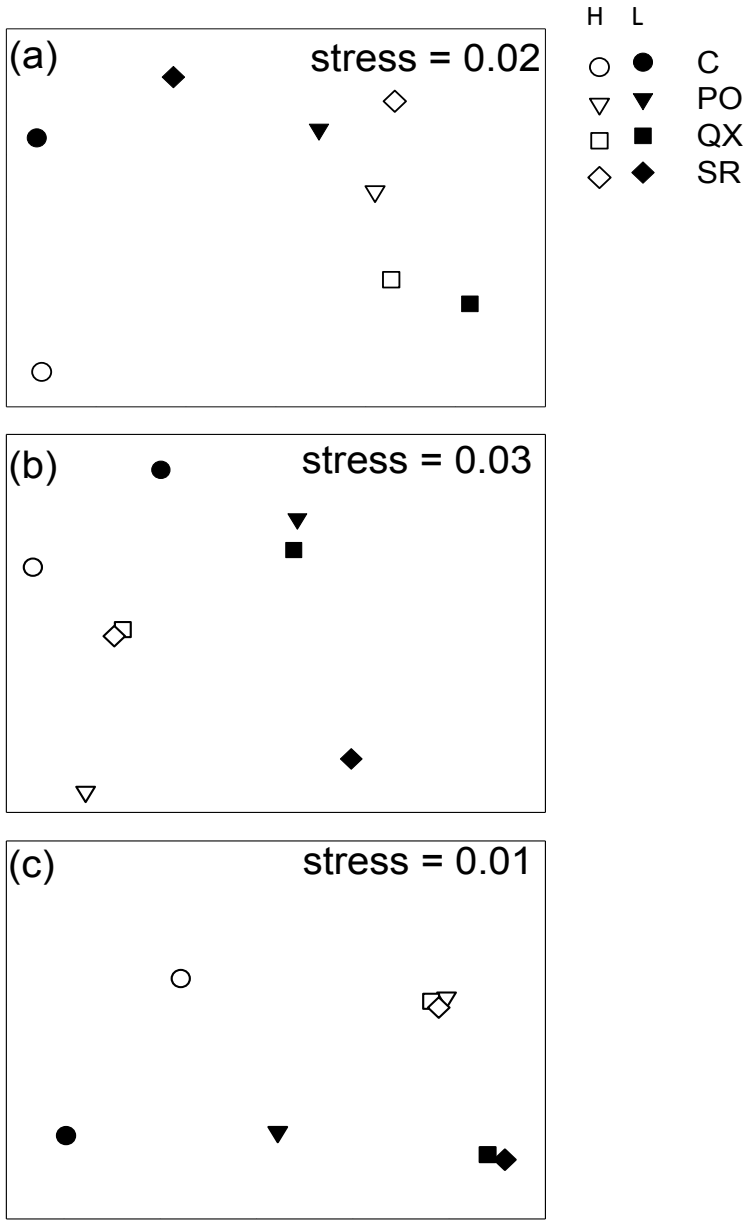

Figure 3.3. MDS ordinations of assemblages of epibiota on control plates (C; circles), and on plates with *Crassostrea gigas* (PO; triangles), *Saccostrea glomerata* (SR; squares) or QX disease resistant *S. glomerata* (QX; diamonds) at (a) 7 months, (b) 10 months and (c) 12 months after deployment at Wanda Wanda Head. Plates were deployed on the low (filled symbols) or the high (unfilled symbols) shore. Points represent centroids of fourth-root transformed data (calculated using $n = 6$). Bray-Curtis measures of dissimilarity were used.

Table 3.3. Analyses of variance testing for sources of spatial difference in the species richness of algae and invertebrates and the total abundance of invertebrates on experimental plates 7 10, and 12 months after deployment. Oy, oyster (4 levels: Pacific oysters [PO], QX-resistant Sydney rock oysters [QX], Sydney rock oysters [SR], and controls [C]).He, height on the shore(2 levels: high [H], and low [L]).n = 6. Terms significant at p <0.05 are highlighted in bold. SNK = Student Newman Keuls post hoc tests. *n* = 6

Time	*df*	Richness			Abundance		
7 months		MS	*F*	*p*	MS	*F*	*p*
Oy	3	0.96	0.32	0.81	0.49	0.90	0.45
He	1	0.52	0.17	0.68	0.21	0.38	0.54
Oy × He	3	2.08	0.69	0.56	0.55	0.81	0.50
Res	40	3.02			0.55		
10 months		MS	*F*	*p*	MS	*F*	*p*
Oy	3	6.14	2.31	0.09	20.57	2.72	0.06
He	1	0.16	0.06	0.81	1.69	0.20	0.65
Oy × He	3	9.45	3.56	**<0.05**	30.24	4.11	**<0.05**
Res	40	2.66					
SNK		Oy x He:			Oy x He:		
		He(Oy):			He(Oy):		
		SR: L > H			C: L < H		
		C,PO,QX: L = H			SR,PO,QX: L = H		
		Oy(He):			Oy(He):		
		L,H: PO = SR = QX = C			L, H: PO = SR = QX = C		
12 months		MS	*F*	*p*	MS	*F*	*p*
Oy	3	5.73	5.09	**<0.01**	0.25	0.47	0.70
He	1	2.71	2.40	0.13	1.21	2.26	0.14
Oy × He	3	2.03	1.80	0.16	0.91	1.17	0.18
Res	40	1.13			0.53		
SNK		Oy:C< (PO = SR = QX)					

SIMPER analyses indicated that the key discriminating taxa driving multivariate differences among oyster types were *Ulva.* sp (mean dissimilarity [Diss] to standard deviation ratio Diss/SD = 2.95), *Galeolaria caespitosa* (Diss/SD = 1.40), *Enteromorpha* sp. (Diss/SD = 1.39), *Cladophora* sp. (Diss/SD = 1.34), *C. officinalis* (Diss/SD = 1.33), and *Elminius sp* (Diss/SD = 1.31). Of these, the cover of *Enteromorpha* sp. at 7 months, the number of *C. officinalis*, *Galeolaria* sp, and *Elminius* sp, at 10 months, and *C. officinalis* at 12 months significantly differed among treatments (Table 3.4). At 7 months, *Enteromorpha* sp. was more

abundant on control plates than on any of the plates with oysters (Table 3.4). At 10 months, *C. officinalis* was more abundant on plates containing oysters on the low than the high shore (irrespective of type), but did not differ between tidal elevations on control plates. On the low shore, the alga was equally abundant among all treatments, but on the high shore, the alga was more abundant on QX and PO than on SR or control plates. By 12 months, there was more *C. officinalis* on the low than the high shore irrespective of treatment (Table 3.4). *Galeolaria* sp (at 10 months) was more abundant on SR and control plates on the low than the high shore. On the low shore, the tube worm was more abundant on SR plates than other oyster and control plates. In contrast, on the high shore, *Galeolaria* sp was more abundant on control than oyster plates (Table 3.4).

3.4.2 Wild oyster assemblages

3.4.2.1 Oysters

Oyster cover ranged from 90 - 100% in the Hawkesbury River (HR), and from 40 - 96% in Port Stephens (PS). The total density of oysters was roughly twice as great in PS than in the HR (mean ± 1 SE per 0.25 m^2; PS: 60 ± 7; HR: 29 ± 2) but the proportionate contribution of live *C. gigas* to oyster assemblages displayed the opposite pattern (HR: 58 ± 5%; PS: 13 ± 4%). The proportion of *C. gigas* was negatively correlated to total oyster density in HR (r = -0.83, p <0.01) and PS (r = -0.63, p < 0.01) assemblages, but there was no relationhip between the total percentage cover of oysters and % *C. gigas* in either estuary (Pearson's correlation: HR: r = -0.37, p = 0.10; PS: r = -0.16, p = 0.50). Oyster habitat was of similar complexity within each of the estuaries (HR: 1.52 ± 0.04; PS: 1.54 ± 0.05) and, within locations, did not vary among quadrats according to the proportionate contribution of *C. gigas* to oyster assembalges (Pearson's correlation; HR: r = -0.05, p = 0.83; PS: r = -0.2, p = 0.40).

In each of the estuaries, both oyster species displayed a unimodal size frequency distribution. *C. gigas* were on average 100% (HR) and 50 % (PS) larger than *S. glomerata* (Fig. 4). In HR, *S. glomerata* ranged in shell height from 10-81 mm, with the most frequently encountered size class of *S. glomerata,* 21-30 mm. The most abundant size class of *C. gigas* was 51-60 mm shell height and individuals ranged from 11 – 152 mm (Fig. 3.4). In PS, *S. glomerata* shell heights of 31-40 mm were most common, but ranged from 3 to 89 mm (Fig. 3.4). *C. gigas* were most commonly 41-50 mm (Fig. 3.4), but individuals as small as 11 mm and as large as 98 mm were sampled.

Table 3.4. Analyses of variance testing for sources of spatial difference in the abundance of discriminating taxa on experimental plates 7, 10, and 12 months after deployment. Oy, oyster (4 levels: Pacific oysters [PO], QX-resistant Sydney rock oysters [QX], Sydney rock oysters [SR], and controls [C]). He, height on the shore(2 levels: high [H], and low [L])). SNK = Student Newman Keuls post hoc tests. * indicates heterogeneity of variances remained after ln(x + 1) transformation and more stringent alpha value of 0.01 was applied. SNK = Student Newman Keuls post hoc tests. n = 6.

		Enteromorhpa sp.			*Ulva* sp.			*Cladophora* sp.				*Corallina officinalis*			
7 mo	*df*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*	**12 mo**	*df*	MS	*F*	*p*
Oy	3	1369	3.85	**<0.05**	341	0.98	0.41	461	2.00	0.13	Oy	3	1010	2.57	0.07
He	1	1	<0.01	9.86	201	0.75	0.39	44	0.19	0.66	He	1	2996	7.62	**<0.01**
Oy × He	3	171	0.48	0.70	35	0.10	0.96	307	1.34	0.28	Oy × He	3	249	0.63	0.60
Res	40	356			348			230			Res	40	393		
SNK		Oy: C>SR=QX=PO									SNK	He: L > H			
		He: L = H													

		Corallina officinalis			**Galeolaria* sp.			*Elminius* sp.		
10 mo	*df*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*
Oy	3	5	7.81	**<0.01**	1	3.30	0.03	0	0.47	0.70
He	1	42	63.4	**<0.01**	0	<0.01	0.97	3	19.74	**<0.01**
Oy × He	3	2	3.02	**<0.05**	1	8.84	**<0.01**	1	3.88	**<0.05**
Res	40	0.67			0.16			0.13		
SNK		Oy x He:			Oy x He:			Oy x He:		
		He(Oy):			He(Oy):			He(Oy):		
		PO,SR,QX: L > H			S,C: L > H			PO,SR,QX: L > H		
		C: L = H			P,Q: L = H			C: L = H		
		Oy (He):			Oy(He):			Oy(He):		
		L: C = SR = PO = QX			L: S > (PO = C = QX)			L, H: PO = SR = QX = C		
		H: (C = SR) < (PO = QX)			H: C > (PO = SR = QX)					

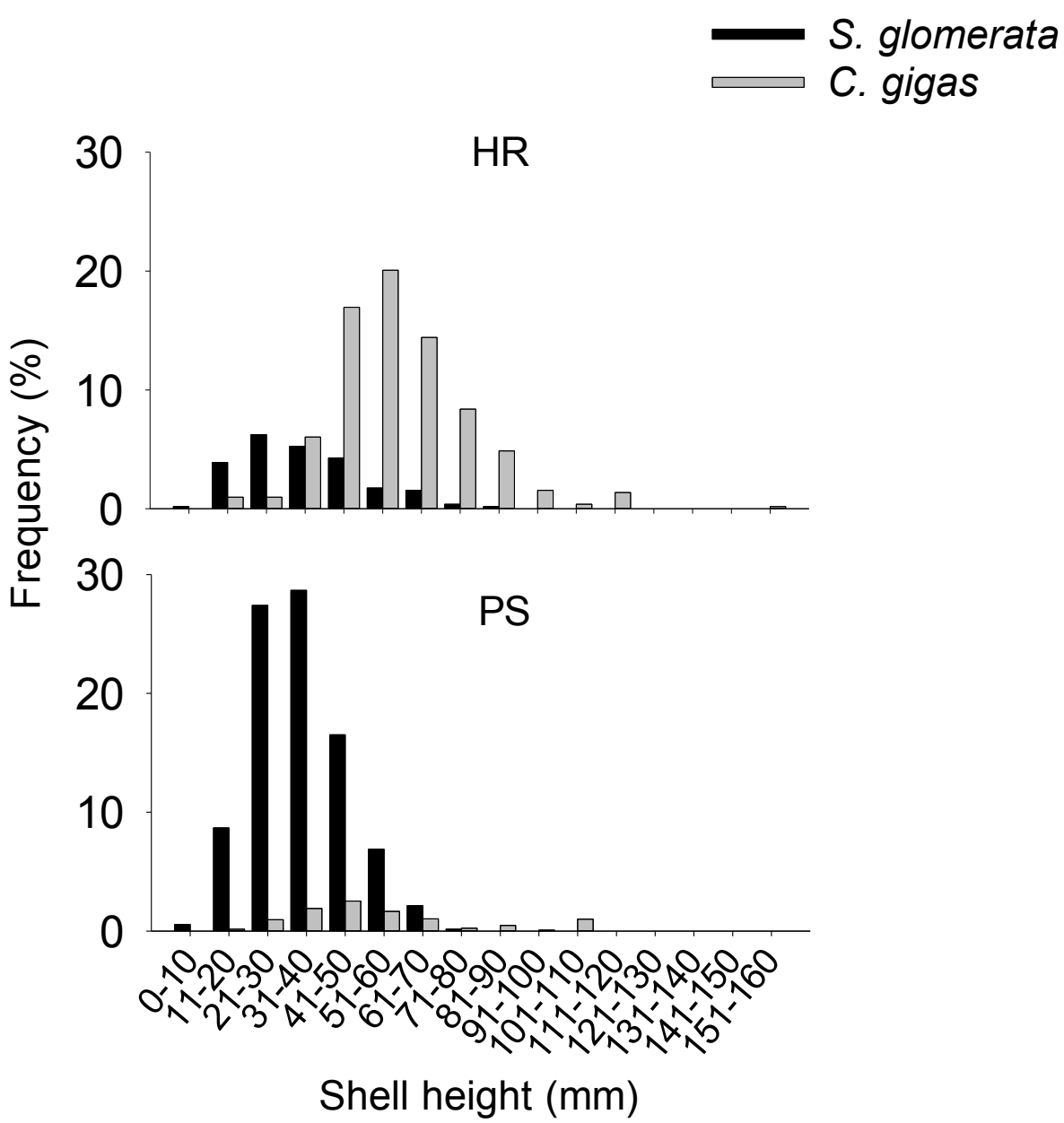

Figure 3.4. Frequency histograms showing the size frequency distributions of *Saccostrea glomerata* and *Crassostrea gigas* in Hawkesbury River (HR) and Port Stephens (PS) oyster assemblages.

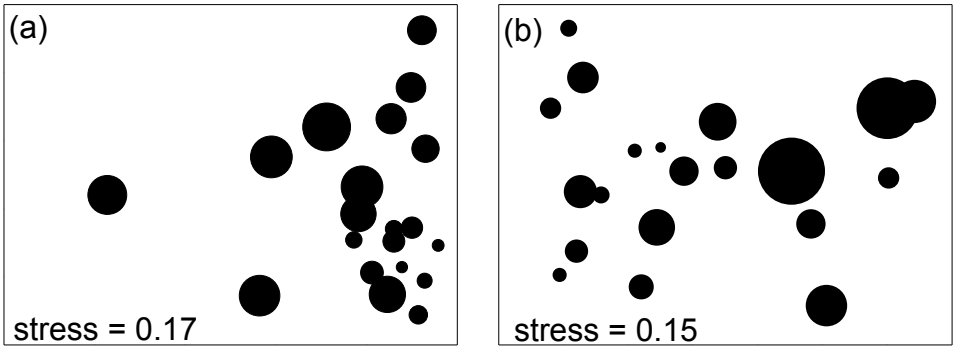

Figure 3.5. nMDS ordinations showing the relationship between the assemblage composition of epibiota (points) and the % contribution of live *Crassostrea gigas* to total oyster abundance (bubble size, where larger bubbles indicate a greater % of *C. gigas*), at the scale of quadrats (*n* = 20). Sampling was replicated in (a) in the Hawkesbury River and (b) at Port Stephens.

3.4.2.2 Epifaunal communities

Among HR assemblages, a total of 33 different invertebrates across 11 major taxa were identified. The most abundant species was *Xenostrobus pulex* (mean ± 1 SE: 155 ± 15 per 0.25 m^2) representing on average 54% of all oyster-dwelling epifauna. Other abundant species were *Bembicium aurutumn* (34 ± 4; 11%), *Patelloida mimula* (31 ± 4; 10 %) and *Elminius modestus* (24± 5; 8 %), *E. covertus* (7 ± 2; 2%), juvenile oysters (6 ± 1; 1 %) and *Irus crenatus* (4 ± 1; 1%). In PS, a total of 29 different invertebrates from nine major taxonomic groups were identified. Juvenile oysters were the most abundant invertebrate (mean ± 1 SE: 188 ± 32 per 0.25 m^2) contibuting 54 % to communities. Other abundant invertebrates were *B. auratum* (70 ± 8; 20 %), *P. mimula* (52 ± 4; 15%), polychates from the families Nereidae (11 ± 1; 3%) and Spirorbidae (5 ± 2; 1%), *E. covertus* (7 ± 1; 2%), and the mussel*Trichomya hirsuta* (6 ± 2; 1%).

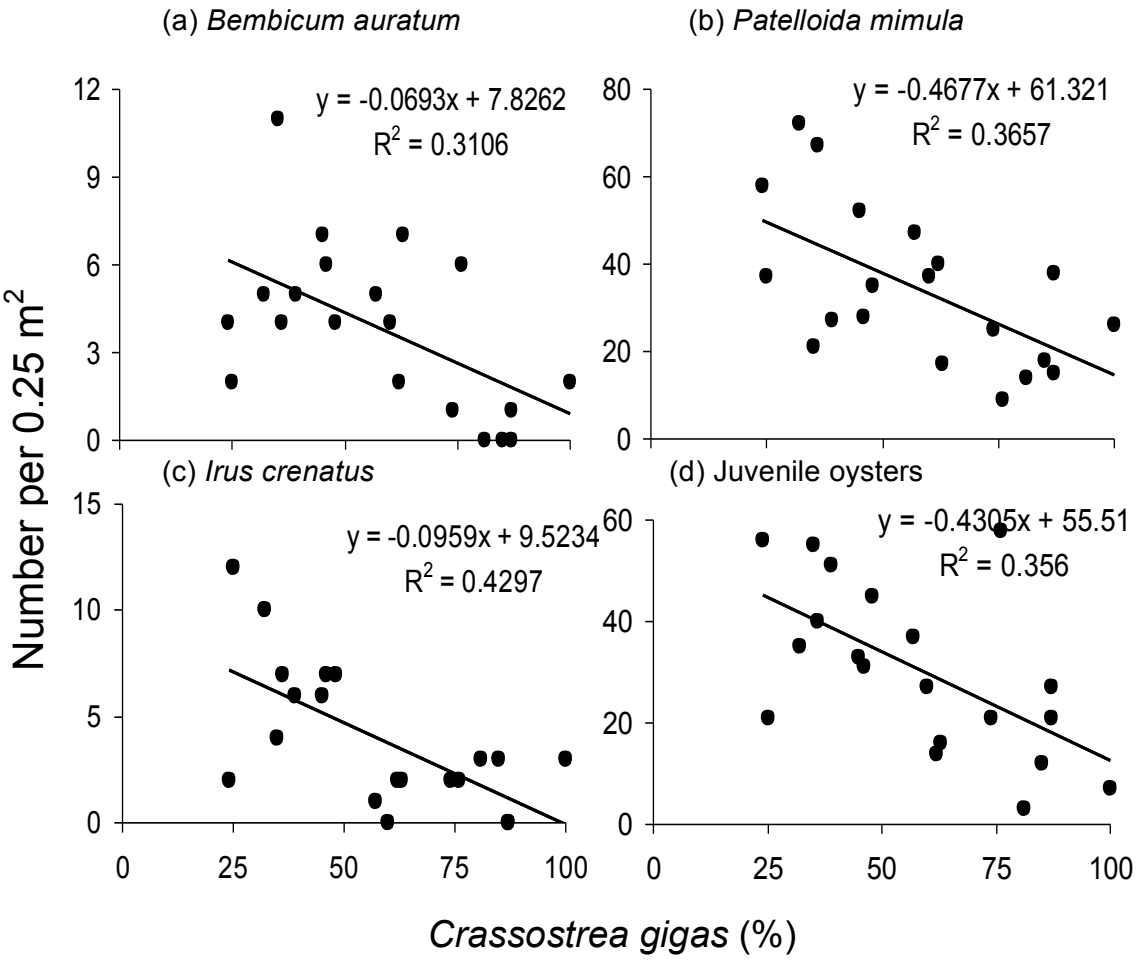

Figure 3.6. Relationship between the percent contribution of *Crassostrea gigas* to the total number of oysters per 0.0625 m^2 quadrat, and the density of (a) *Bembicium auratum*, (b) *Patelloida* mimula, (c) *Irus crenatus* and (d) juvenile oysters per quadrat in the Hawkesbury River. $n = 20$.

Differences among quadrats in the proportionate contribution of *C. gigas* to total oyster abundance did not correspond to differences in epifaunal community structure in either estuary (Fig. 3.5; RELATE - HR: þs = 0.108, $p = 0.15$; PS: þs = 0.122, $p = 0.14$). In HR, there was no correlation between the proportion of *C. gigas* and the total abundance of epifauna (Pearson's correlation: $r = 0.4$, $df = 18$, $p = 0.08$), and the proportion of *C. gigas* did not correlate to

species richness in either estuary (HR: $r = 0.4$, $df = 18$, $p = 0.08$; PS: $r = 0.4$, $df = 18$, $p = 0.07$). By contrast, in PS there was a significant positive correlation between the total abundance of epifauna and the proportion of *C. gigas* ($r = 0.5$, $df = 18$, $p < 0.05$) and in each of the estuaries correlations between the proportion of *C. gigas* and individual epifaunal taxa were detected. In HR, the abundance of each of *B. auratum, P. mimula*, and*I. crenatus* ($r = -0.6$, $df = 18$, $p < 0.01$) and juvenile oysters ($r = -0.7$, $df = 18$, $p < 0.01$) decreased with the increasing proportionate contribution of *C. gigas* to total oysters (Fig. 3.6). Within PS, there was a significantly positive correlation between the abundance of juvenile oysters and proportion of *C. gigas* ($r = 0.6$,$df = 18$, $p < 0.01$), but other common species did not show a relationship to the proportionate contribution of *C. gigas*.

3.5 Discussion

We hypothesised that by changing the physical architecture of habitat provided by oyster assemblages, invasion of native *Saccostrea glomerata* oyster populations by *Crassostrea gigas* would influence associated epibiotic communities along the east coast of Australia. Elsewhere, the modification of native biogenic habitats by *C. gigas* invasion has resulted in changes to associated benthic community structures (Kochmann et al., 2008; Markert et al., 2010; Troost 2010). Contrary to our hypothesis, and despite very different patterns of growth and mortality between native *S. glomerata* and non-native *C. gigas*, we found a large degree of redundancy between the two species in the benthic communities they support. On experimental plates of young oysters, temporally persistent differences in benthic communities among plates with wild stock *S. glomerata* (SR), QX-resistant *S. glomerata* (QX) and *C. gigas* (PO) did not develop, and where short-lived differences were apparent, they were generally confined to the low intertidal. Only among wild populations of oysters, where communities are more established, did we detect

impacts of *C. gigas*, and even then these were generally on species abundance rather than species identity.

3.5.1 Oyster morphology and habitat complexity

Our experimental deployments of oysters on plates confirmed that previous observations from cultured, clutchless oysters, of more rapid growth and greater mortality of *C. gigas* than *S. glomerata* (Bayne et al., 2002; Honkoop and Bayne 2002) also apply to oysters growing on hard substrate (see also Krassoi et al., 2008). Among wild populations this was apparent in a larger mean size of *C. gigas* than *S. glomerata* within each of two estuaries surveyed, and a lower density of oysters in patches dominated by *C. gigas* (see also Summerhayes et al., 2009a). The growth of QX-resistant and wild-stock *S. glomerata* was, by contrast, similar despite the 1.5 times greater growth of QX-resistant *S. glomerata* in aquaculture trials (Nell and Perkins 2006). Hence, in a natural setting, environmental factors overwhelmed genetic differences in determining growth of *S. glomerata*. Although survival rates are 4 times higher among QX-resistant than wild-stock *S. glomerata* in QX disease infected estuaries (Nell and Perkins 2006), in our study site where QX disease has not been detected, mortality rates between QX-resistant and wild-stock *S. glomerata* were similar, suggesting winter mortality caused by protozoan parasite *Bonamia roughleyi* that kills wild-stock but not QX- resistant *S. glomerata* (Nell and Perkins 2006; Nell 2007) has not impacted our results. Contrary to previous observations of negative effects of tidal elevation on *C. gigas* growth and survival (Krassoi et al., 2008), we observed no effect of height on the shore on oyster growth or mortality of *C. gigas* on our experimental plates. This may be because the boulders that dominated the shoreline of our Wanda Wanda study site reflected waves differently to a smooth shore and increased the tidal

elevation of wetting and spray. Additionally, the boulders may have caused shading, thereby reducing the desiccation stress usually experienced on the high rocky intertidal shore.

We predicted that the lower growth and mortality rates of *S. glomerata* than *C. gigas* would translate to differences in the complexity of habitat provided by the two species. Specifically, we predicted that within oyster assemblages dominated by *S. glomerata*, oysters would be densely packed, and interstitial space would be reduced relative to assemblages dominated by *C. gigas*, resulting in a smaller surface rugosity than oyster assemblages dominated by *C. gigas* that are larger and more angular. We also hypothesised that despite the lower density of oysters in assemblages dominated by *C. gigas*, the extent to which the rock surface is covered by oysters would be high because of the typically great horizontal extension of *C. gigas* (Crawford et al., 2006). Using surface rugosity methods, however, we were unable to detect any effect of *C. gigas* on habitat complexity, either on mono-type plates in the field or in naturally-recruited oyster assemblages in the wild. Similarly, oyster cover was not affected by oyster species composition among plates or wild assemblages. Harwell et al., (2010) similarly did not find any difference in the surface rugosity of habitat provided by two different species of oyster despite obvious differences in morphology. They suggested this might be because the rugosity index cannot discriminate between simple large scale topography and complex small scale topography (see also Roberts and Ormond 1987, Shumway et al., 2007).

3.5.2 Associated communities

Despite large differences in the morphology of *S. glomerata* and *C. gigas*, apparent from even an early age, effects of *C. gigas* on associated communities of epibiota were largely confined to established, wild oyster communities. Although, on experimental plates,

communities of epibiota differed markedly between plates with oysters and those (controls) without, among oyster types, few differences in community structure were seen, and where they occurred they were ephemeral. Consistent with the results of previous studies (e.g. Anderson and Underwood 1994) plates with oysters contained more species overall, but less opportunistic macroalgae (in this case *Enteromorpha* sp.) than those without. Differences in the abundance of mobile invertebrate taxa between plates with and without oysters may reflect the enhancement of surface area for attachment and grazing or the provision of protection from predators and desiccation by oysters (Wells 1961; Grabowski 2004; Summerhayes et al., 2009b). By contrast, the negative effect of oysters on *Enteromorpha* sp. is more likely to reflect consumption of algal propagules during oyster filtration (Tamburri and Zimmer-Faust 1996; Summerhayes et al., 2009b).

Within established wild oyster assemblages, *C. gigas* were typically much larger than on our experimental plates, and the non-native influenced the abundance of several epibiotic taxa. Where impacts of *C. gigas* on associated benthic species occurred, they were generally negative. Within the Hawkesbury River, where *C. gigas* accounted for up to 100 % of oyster assemblages, abundances of the snail, *Bembicium auratum*, the limpet *Patelloida mimula*, the bivalve *Irus crenatus* and oyster spat each declined as the contribution of *C. gigas* to oyster assemblages increased. In Port Stephens, *C. gigas* were less abundant and generally made a small contribution to oyster assemblages, and did not negatively influenced species richness.

Particularly for organisms that rely on hard substrate for attachment or interstitial space to forage, avoid predation and environmental stressors, even subtle modifications of habitat structure can have large influences on abundance (Kelaher et al., 2003; Willis et al., 2005). In

the Hawkesbury River, the decreasing abundances of the littorinid snail *Bembicium auratum* and the limpet *Patelloida mimula* with increasing *C. gigas* contribution to assemblages may have been associated with a reduction in surface area and/or interstitial space by the accompanying overall reduction in oyster density. Both *B. auratum* and *P. mimula* are epibenthic gastropods, abundant within *S. glomerata* patches. *P. mimula* are almost exclusively found on *S. glomerata* (Minchinton and Ross 1999). Hard substratum and complex convoluted *S. glomerata* shells also protect *B. auratum* from predation (Underwood and Barrett 1990).

An alternative explanation for the reduced abundance of several molluscs in patches of oyster dominated by *C. gigas* is that the non-native caused a reduced rate of larval survival. Particularly among taxa with a sessile benthic stage (e.g. oysters, mussels, barnacles, tube worms), patterns of distribution can be set at settlement (supply side ecology, Underwood and Fairweather 1989). In trapping larvae and algal propagules during suspension feeding, oysters influence benthic community structure (Tamburri and Zimmer-Faust 1996). Among bivalves, *C. gigas* has a rapid filtration rate, twice that of *S. glomerata* (Bayne 2002), and in Europe, high densities of exotic *C. gigas* reduced recruitment of the native mussel *Mytilus edulis* through ingestion of mussel larvae (Troost et al., 2009). Among the species most negatively impacted by *C. gigas* in the Hawkesbury River, all have a pelagic larval stage. *Patelloida mimula* displays minimal movement among oysters following settlement (Minchinton and Ross 1999), so may have a distribution that is particularly influenced by patterns of settlement.

Density-dependent differences in the settlement of *S. glomerata* larvae on *C. gigas* and *S. glomerata* have been observed as a consequence of differences in the rates at which the two oysters filter (E. Wilkie, unpublished data). At low densities, larval settlement was greater on *C.*

gigas than *S. glomerata*, perhaps because inhalant feeding currents draw larvae toward the oyster shell, whereas at high densities the pattern reversed, possibly from ingestion, or because increasingly turbulent currents restricted the larvae from reaching the shell surface (E. Wilkie, unpublished data). A similar pattern was seen here with increases in the proportionate contribution of *C. gigas* to oyster assemblages increasing oyster recruits at low *C. gigas* abundance (i.e. Port Stephens), and decreasing oyster recruits at high *C. gigas* abundance (Hawkesbury River). In Port Stephens, the positive relationship between *C. gigas* and juvenile oysters drove the relationship between the non-native and total epifaunal abundance.

3.5.3 Implications of results

In combination with other recent studies (e.g. Kelaher et al., 2007; Harwell et al., 2010) our results indicate a high degree of functional equivalency between morphologically similar ecosystem engineers. Hence, where native species are displaced by invasive species of similar engineering function, the impacts of the non-native species on the local diversity may be primarily quantitative effects of changes in density, rather than qualitative effects of changes in associated species identity (Padilla 2010). Here, *C. gigas* had minimal effects on the identity of species comprising benthic communities, although it affected their density by causing subtle changes in the availability of habitat. This contrasts to the invasion of soft-sediment habitats by *C. gigas* where state changes have occurred as a result of the introduction of new ecological functions (Ruesink et al., 2005; Molnar et al., 2008; Troost 2010). Therefore, although predicting the ecological impacts of invasive species that introduce new functions to an ecosystem will remain a challenge, in instances where there is a native analogue, impacts may be largely predictable from changes in the density of functional units.

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4 Substrate or sink? Differences in settlement of *Saccostreaglomerata* larvae on non-native *Crassostrea gigas* and conspecifics is determined by spatial arrangement

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4.1 Abstract

Biological invaders represent one of the greatest threats to coastal marine ecosystems, with their impact generally increasing with their abundance. Yet efforts to control impacts of invaders through reduction of their abundance may not be effective if locally high densities of the invader are sufficient to produce significant impacts. In New South Wales, Australia, the invasive, habitat-forming Pacific oyster, *Crassostrea gigas*, of low overall abundance, is most commonly found interspersed among native Sydney rock oysters, *Saccostrea glomerata*, but at some localities accounts for 100 % of oysters at the patch scale. We tested whether *C. gigas* provides equal settlement opportunities for *S. glomerata* larvae as conspecifics, and whether any impact of the invasive oyster on settlement of the native oyster is influenced by spatial arrangement, with impacts increasing at locally high density of the non-native species. In the laboratory, at high effective densities of mono-specific oysters, fewer *S. glomerata* larvae settled on *C. gigas* than *S. glomerata*. When, however, *C. gigas* were interspersed among *S. glomerata*, or presented at a lower effective density in the field, more *S. glomerata* settled on *C. gigas* than on conspecifics. Laboratory experiments revealed that at high density, the greater filtration rate of *C. gigas* – double the rate of *S. glomerata* – resulted in a reduction in larval settlement on the non-native oyster, possibly as a result of larval ingestion. Chemical cues and shell characteristics had no influence on larval settlement, and settlement on dead oyster shells was independent of substrate type, determined only by substrate area for attachment. These observations indicate that at low density or in mixed species beds *C. gigas* enhances *S. glomerata* larval settlement. Where, however, *C. gigas* is present at high local density, *S. glomerata* larval settlement may be negatively impacted. Hence, management strategies that prevent attainment of high local densities of *C. gigas* might be effective in minimizing impacts on native *S. glomerata*.

Keywords: density-dependent, invasion, oyster, settlement, spatial arrangement, filtration, chemical cue

4.2 Introduction

Biological invasions represent one of the greatest threats to coastal marine ecosystems and their important economic values (Grosholz 2002). They can reduce marine biodiversity, cause fisheries collapses, foul infrastructure and produce changes to ecosystem processes, such as nutrient cycling and productivity (Vitousek et al., 1996: Bax et al., 2003; Rilov and Crooks 2009). Despite increasingly rigorous quarantine procedures, transport via international shipping and deliberate introduction for fisheries and aquaculture continue to contribute to new invasions every year (Bax et al., 2003).

The establishment of non-native species poses a considerable problem to managers because, once there, they are often impossible to eradicate (Vitousek et al., 1996). Consequently management of invasive species often necessarily revolves around keeping invader populations at sufficiently low abundance that the economic benefits of the population control exceed the financial costs (Finnoff et al., 2005; Whittle et al., 2007). Although impacts of invaders generally increase with abundance (Escapa et al., 2004; Griffen and Byers 2009; Padilla 2010), this relationship can be linear or non-linear (see Yokomizo et al., 2009). Impact may be evident even at low abundance where the spatial arrangement of an invasive species within a landscape produces locally high density (Bell et al., 1995; Flather and Bevers 2002; Matias et al., 2010; Matias et al., 2011). Disentangling the relative importance in determining impact of spatial arrangement of an invader versus abundance is, therefore, critical if limited management resources are to be most effectively put towards invader control.

Invasive habitat-forming bivalves are among the taxa that can have disproportionately large ecological impacts relative to their abundance. In environments where hard surfaces are

limited, bivalve shells increase attachment substrate, and interstitial spaces that protect organisms from predators, waves, sedimentation and desiccation (Gutierrez et al., 2003; Commito et al., 2008). Consequently, where habitat-forming invaders increase the habitat complexity of an environment they are predicted to increase the abundance and diversity of associated organisms (Crooks 2002) – a function that would, presumably, increase with their abundance.

As suspension-feeders, bivalves, however, also have the potential to impact communities by influencing patterns of larval settlement (Tamburri et al., 2007; Troost et al., 2009). The majority (~70%) of coastal sessile species have a planktonic stage in their life history (Jones et al., 1999), and invasive bivalves may transform the distributions and abundance of such organisms by altering dispersal trajectories and survival through to settlement (Gribben et al., 2009; Woodford and McIntosh 2010). Whereas solitary bivalves may enhance settlement by producing feeding currents that entrain larvae (Tamburri et al., 2007) the stronger currents of clusters of bivalves might lead to ingestion and, in some cases, consumption of larvae (Smaal et al., 2005; Troost 2010). Where negative effects of larval filtration overwhelm positive effects of bivalve habitat complexity on associated organisms, reductions in the abundance and diversity of native flora and fauna may result.

The Pacific oyster *Crassostrea gigas*, (Ostreidae) is among the most translocated marine organisms in the world, introduced to over 66 non-native regions for aquaculture (Ruesink et al., 2005). It has established dense self-sustaining populations in some, but not all, of its introduced range that have lead to major shifts in associated community structure (Troost et al., 2009; Markert et al., 2010). In Australia, *C. gigas* was initially introduced to the southern states for

aquaculture in the 1940s and 1950s (Medcof and Wolf 1975). In 1984-85 it appeared in Port Stephens, New South Wales, after a suspected rogue introduction, growing to a population of 26 million by 1988 (Nell 1993). Subsequently, the Pacific oyster has established breeding populations in most NSW estuaries south of the Macleay River. Reproductively capable, diploid *C. gigas*, are cultured in Port Stephens, with culture of largely sterile, triploid *C. gigas*, being trialled in several other NSW estuaries (O'Connor and Dove 2009).

In many east Australian estuaries *C. gigas* now lives alongside the native Sydney rock oyster, *Saccostrea glomerata* (Summerhayes et al., 2009; Bishop et al., 2010). Although in most estuaries the total abundance of the non-native is low, it can form monospecific clumps, accounting for up to 100 % of oysters at the patch scale (Bishop et al., 2010; Wilkie unpublished data). *S. glomerata* and *C. gigas* each form habitat of similar complexity (E. Wilkie unpublished data), but *C. gigas* is a more rapid feeder (Bayne 2002). The two species may also produce different chemical cues, or have different shell micro-structure that might influence settlement processes. Hence, the two species may not be equivalent in their net effect on settlement processes of marine invertebrates. In addition, selective breeding for QX disease resistance is producing lines of *S. glomerata* that grow ~ 30 % faster than natural counterparts (Nell and Perkins 2005) partly attributable to faster feeding rates of selected oysters (Bayne 2000), which may influence larval settlement.

Here, we investigate: (1) whether the two oyster species, *S. glomerata* and *C. gigas*, provide equal settlement opportunities for *S. glomerata* larvae, when present in mixtures and in monocultures and when at higher and lower density; (2) whether any differences in larval settlement on the two species is influenced by the genotype of the *S. glomerata* substrate, which

is increasingly being modified by selective breeding practices; and (3) whether any differences in *S. glomerata* settlement between the two species is caused by differences in chemical cues, shell characteristics and/or filtration process between the two oysters. We predict that at low densities, or in mixed oyster beds, the strong feeding currents produced by live *C. gigas* may enhance larval settlement over that on low density *S. glomerata*. Where live *C. gigas* are present at high density, or as a monoculture, we expect, however, that the net effect of *C. gigas* filtration will be larval ingestion, resulting in decreased larval settlement on the non-native as compared to *S. glomerata*. We expect that settlement on dead oyster shells will be proportional to substrate area for attachment and that this relationship will be similar between *C. gigas* and *S. glomerata*. Understanding the functional equivalency of the two species is critical in developing management strategies to minimise impacts of non-native *C. gigas* on native biodiversity.

4.3 Methods

4.3.1 Larval cultures and adult oysters

The *S. glomerata* oyster larvae used in experiments were produced in the New South Wales Primary Industries Port Stephens Fisheries Institute (Taylors Beach, NSW, Australia). Larvae were large enough to be retained on a 212 μm screen, could swim, had developed eye spots, 3 -5 gill buds and a protruding foot, which indicates that they are competent to settle. They typically reach this stage at 20 – 22 days (O'Connor and Dove 2009).

Our experiments utilised three types of adult oyster as substrate for settlement: wild stock *S. glomerata*, *S. glomerata* selectively bred over six generations for fast growth and then QX disease resistance (Nell and Perkins 2006) and *C. gigas*. Oysters were sourced from aquaculture

leases in Port Stephens, Brisbane Waters and the Hawkesbury River. Experiments conducted at the Port Stephens Fisheries Institute used diploid oysters. The experiment at the Kangaroo Point mesocosms used sterile triploid *C. gigas* because *C. gigas* is considered a noxious pest in the adjacent Hawkesbury River.

Unless otherwise indicated, all oysters were cleaned of fouling organisms by scrubbing with a wire brush prior to experiments. The average shell height (distance between hinge and ventral margin) of each oyster type, within each experiment, was calculated from 30 individuals. The average shell surface area was calculated from 10 oysters per type, per experiment. We calculated shell surface area by wrapping replicate oysters in aluminium foil, so that it covered all exposed surface, and measuring the surface area (mm^2) of the resulting piece of foil using ImageJ software. Although, within each experiment, the shell-height of the various types of oyster shell-substrate were matched, we standardized larval settlement by mean shell surface area of each treatment to remove any confounding effect of slight differences.

4.3.2 Equivalence of native and non-native oysters as substrates for *Saccostrea glomerata* settlement, in the field and in mesocosms

We conducted experimental field deployments of live and dead oysters to address (1) whether natural settlement of oyster larvae differs between *C. gigas* and *S. glomerata* and (2) how differences in settlement between substrates is dependent on spatial arrangement of oysters. Deployments were within Salamander Bay, Port Stephens, NSW (32 ° 43'S, 152 ° 07 E) at a site at which oyster farmers collect *S. glomerata* recruits for farming due to the good larval supply. We hypothesised that differences in larval settlement would be more pronounced between *C. gigas* and *S. glomerata* when the oysters were live and actively filtering than dead, and when

they were presented in monocultures, rather than mixed species arrangements that may dilute chemical cues and other substrate-specific effects.

Differences in settlement between monospecific clumps of *S. glomerata* and *C. gigas* were addressed by deploying nine oysters of a single species (shell-height [mean ± S.E.]: *S. glomerata* 79 ± 1.0 mm; *C. gigas* 81 ± 1.5 mm), which were all either live or dead, and randomly dispersed in polyethylene mesh bags (n = 4 for each species and status combination) suspended at 0.5 m above low water springs. To assess whether any differences in settlement of oysters between substrates were still apparent when the oyster substrates were interspersed, such as in a mixed species bed, we deployed 20 oysters of each species and of each status (live or dead) in a single (850 × 400 × 220mm) SEAPA long-line oyster basket, at a tidal elevation of 0.5 m above low water springs. For each of the deployments, dead oysters were produced by carefully opening live oysters, so as to avoid shell damage, removing all tissue and gluing shut the two valves with non-toxic two-part epoxy resin adhesive (Araldite). A similar quantity of glue was applied to each live oyster, without sealing the valves shut, to control for any effect of the resin on oyster settlement. Consequently, dead oysters were structural mimics of live oysters, but without the capacity to filter. Oyster substrates were deployed in February 2011, and retrieved after four months. The number of recruits on the external surface of each oyster was enumerated. At the time of retrieval, recruits were still too small to morphologically distinguish between *S. glomerata* and *C. gigas*. Hence, although from historical patterns of larval settlement at this site it would appear that *S. glomerata* likely dominated new recruits (W. Diemar pers. com), *C. gigas* may also have contributed.

Consequently, a follow-up mesocosm experiment was conducted to specifically consider the response of *S. glomerata* larvae to *C. gigas* and *S. glomerata* substrates offered as monocultures and in mixtures, where they are interspersed. We predicted that if most of the larvae settling in the field were *S. glomerata*, we would observe similar differences in settlement among oyster substrates in flow-through mesocosms as in the field experiment. Among *S. glomerata* substrates, we not only considered wild-type oysters, but also oysters selectively bred for resistance to QX disease and fast growth (hereafter, QX). We hypothesised that differences in settlement would be greater between than within species.

Experiments were conducted within 250 L flow-through mesocosms situated at Kangaroo Point, adjacent to the Hawkesbury River, NSW, Australia. First, to establish differences in larval settlement among monocultures, we placed nine live SR (shell-height [mean ± SE]: 72 ± 1.3mm), QX (75 ± 0.8mm) or PO (81 ± 0.7 mm) oysters at the bottom of replicate tanks (n = 4 per treatment). Second, to determine if differences in larval settlement persisted among oyster types when they were presented in a mixture, we also conducted mixed-species trials, with three live individuals of each of the three oyster types so as to maintain a density of nine per tank (n = 5 per treatment). The tanks received seawater, filtered through 25 μm filter to remove larvae and other macroinvertebrates, at a rate of 90 L h^{-1}. Oysters were arranged in the centre of each tank so that their left valves rested on the bottom, and they were close but not touching. Adults were acclimatized in the tanks for 24 h, before we introduced 13,000 (0.052 larvae mL^{-1}) larvae in filtered seawater to the centre of each tank. Nine days after addition, the number of larvae that had settled on each oyster was counted under a stereomicroscope.

Data from field and mesocosm experiments were analysed using analyses of variance (ANOVAs). We compared larval settlement among field-deployed monoculture bags using a 3-way ANOVA with the factors: Species (2 levels: *S. glomerata*, *C. gigas*); Status (2 levels: live, dead) and Bags (nested within species and status; 4 levels, random), with n = 9 oysters per bag. Settlement was compared among treatments contributing to the field-deployed mixed species basket using a 2-way ANOVA with orthogonal factors: Species (2 levels: *S. glomerata*, *C. gigas*) and Status (2 levels: live, dead), with n = 20 oysters. For the mesocosm experiment, settlement was compared among monoculture treatments using 2-way ANOVA with factors, Oyster type (3 levels *S. glomerata*, *C. gigas*, QX, fixed) and Tank (4 levels, nested within oyster type, random), with n = 9 oysters. To test for differences in larval settlement among oyster substrates in the mixed species arrangements we ran a 2-way orthogonal ANOVA with factors; Oyster type (3 levels: *S. glomerata*, *C. gigas*, QX, fixed) and Tank (5 levels, random), with n = 3 oysters. Cochran's C-tests were run prior to each test to assess homogeneity of variance. Where data were not homogeneous, we performed Sq (x + 1) transformations. Post-hoc Student Newman Kuels tests examined the sources of significant treatment effects.

4.3.3 The role of chemical cues and shell type in determining larval settlement rates

To determine if differences in *S. glomerata* larval settlement among oyster types is influenced by chemical cues emitted by oysters, the structure of the substrate they provide, or an interaction between the two, we designed a fully factorial experiment. The factor, chemical cue, had four levels: *S. glomerata*, *C. gigas*, QX or none. Substrate type had 3 levels: *S. glomerata*, *C. gigas*, or QX. To extract chemical cues, we bathed 26 similar-sized adults of a single oyster type (shell height; *S. glomerata*: 78 ± 1 mm, *C. gigas*: 77 ± 1 mm, QX: 76 ± 1 mm) overnight and at a constant temperature of 23°C in 400 L of aerated filtered sea water (as per Tamburri et

al., 2008). The control treatment contained only filtered seawater. Each solution was then distributed among five 8 L buckets, so that each replicate held 750 mL of aerated water. There were 15 buckets per water type.

To create settlement substrates, oysters were killed by heating in a 1000 W microwave oven for ~30 seconds. This method was chosen instead of shucking because it also removed living organisms associated with the shell surface (i.e. in biofilms and micro invertebrates that may influence the chemical composition of the test solution). Dead oysters were rinsed with a fresh water pressure hose, scrubbed with a wire brush and rinsed again with sterile filtered seawater. Their two valves were then glued shut with a non-toxic, insoluble, two part epoxy resin (Megapoxy, Megabond Thornleigh NSW, Australia) so that larvae could not settle on the inside of the valves. Glue was left to dry for 12 h before commencement of the experiment.

Each bucket of water was randomly assigned one of the three substrate treatments to give a total of five replicates for each of the 12 treatments. Three shells of the assigned type were positioned in the centre of the bucket. In addition to the 15 buckets of each water-type that received dead oysters, we also established an additional fifteen buckets with no chemical cue, into which we placed either three live *S. glomerata*, *C. gigas* or, QX to give n = 5. The live oyster treatments allowed us to test whether settlement of *S. glomerata* on dead substrate with matching chemical cue could replicate settlement on live oysters of the same type. Live oysters received a small amount of epoxy on their lip to control for any chemical influences of the resin and received the same scrubbing and hosing as dead shells.

We pipetted 1000 *S. glomerata* larvae (0.125 mL^{-1}) into the centre of each bucket, at the water line. After 2 days, the water from each bucket was changed with a fresh supply of the same type of water. The larvae in each bucket were retained on a 212 µm sieve then washed back into the same bucket to continue the experiment. The experiment lasted 4 days, which is sufficient time for larvae to settle (O'Connor et al., 2008). At the conclusion of the experiment, each shell was examined under a stereomicroscope and the settled larvae were counted.

To test the hypothesis that species-specific waterborne chemical cues and substrate type interact to determine larval settlement, we ran two fully orthogonal, two factor analysis of variance (ANOVA) on the number of larvae that had settled per bucket. The first ANOVA with factors bathwater (4 levels: *S. glomerata*, *C. gigas*, QX, none) and substrate (3 levels: *S. glomerata*, *C. gigas*, QX) and the second ANOVA with factors oyster status (2 levels: live vs dead) and substrate (3 levels: *S. glomerata*, *C. gigas*, QX) assessed whether dead oyster shells with corresponding chemical cue could replicate the effects of live oysters.

4.3.4 Effects of oyster filtration on larval settlement

To test whether differences in filtration rate between live *C. gigas* and *S. glomerata* contributes to differences in numbers of *S. glomerata* recruiting to their surfaces, we (1) ascertained differences in filtration rate between the two species, and (2) assessed whether the difference in the number of larvae settling on live *S. glomerata* and *C. gigas* adults was less when feeding was inhibited than when it was allowed.

Live oysters were restricted from filtering by tightly binding individuals shut with a rubber band (herein 'banded' oysters). We compared the rate at which *S. glomerata* and *C. gigas* filter and determined the efficacy of the rubber banding in preventing filtration using an algal depletion experiment. Twenty similar-sized oysters of each species (shell height; SR: 87 ± 2 mm; PO: 96 ± 2 mm) were placed in filtered sea water and the first fifteen of each to gape and commence filter feeding were selected for use in the experiment. Individuals were randomly assigned to one of two treatments: untouched and banded (n = 5 oysters of each species per treatment). Each individual was placed in a separate 8 L bucket with aerated filtered sea water. Oysters were fed a concentration of 100,000 Tahitian *Isochryisis* algae cells mL^{-1}. Every 30 min, a 1 mL aliquot of water from each individual bucket was collected to determine the algal concentration, and hence the number of algal cells consumed. Following each sample collection, consumed algae were replenished to maintain a constant concentration of 100,000 T. *Isochryisis* algae cells mL^{-1} throughout the experiment. After 3 h the experiment was terminated and the total number of algal cells consumed was calculated. This was used to determine oyster filtration rate ($L\ h^{-1}$) (Coughlan 1969).

To assess species-specific effects of filtration on *S. glomerata* settlement, we conducted a fully orthogonal experiment, with the factors; species (2 levels: *S. glomerata*, *C. gigas*) and treatment (3 levels: non-banded, banded, and banding control). We hypothesised that a greater number of larvae would settle on the banded than the non-banded oysters, and the difference between banded and non-banded treatments would be greater for *C. gigas* than *S. glomerata*. The banding control treatment, where oysters were loosely bound by rubber bands that did not restrict filtering, allowed us to ascertain whether the structure or composition of the rubber influenced larval settlement. Three oysters of a single species, and all receiving the same banding treatment, were used per replicate. There were five replicates of each treatment.

Oysters were positioned in the centre of an 8 L bucket with 750 mL aerated filtered sea water. Methods for the experiment were otherwise as described for the chemical cue experiment (described above), but we used fresh filtered sea water in water changes, after two days. At the conclusion of the experiment, we counted the number of larvae that had settled on each oyster under a stereomicroscope.

A two-way fully orthogonal ANOVA with the factors banding (2 levels: banded vs untouched) and species (2 levels: *S. glomerata* vs *C. gigas*) tested whether there were differences in filtration rate (L h^{-1}) between the two species that were reduced by banding the oysters. Analogous ANOVAs, but also including a third level of the factor banding (banding control), tested for effects of oyster type and treatment on total larval settlement.

4.3.5 Effect of substrate density on larval settlement and survival

To test whether (1) similar relationships are seen between substrate density and settlement of *S. glomerata* larvae, irrespective of whether the substrate is *S. glomerata* or *C. gigas* and (2) the status of oysters as live or dead influences density-dependencies, we conducted two laboratory experiments. We predicted that among live oysters, the relationship between density and larval settlement would be non-linear because at low oyster densities, filtration would facilitate larval settlement by drawing larvae in, but at high oyster densities, filtration would inhibit larval settlement through larval ingestion and, in some instances, larviphagy. We expected the switch from positive to negative effects to be at a lower density threshold for *C. gigas*, which has a greater filtration rate. Among dead oyster shells, we predicted that patterns of larval settlement would primarily be a function of substrate area and hence settlement would display similar increases with substrate area, irrespective of substrate type. The first had two

factors, species (2 levels: *S. glomerata*, *C. gigas*) and density (3 levels: high [H], 3 oysters; medium [M], 2 oysters; and low [L] 1 oyster per 8 L bucket). To determine to what extent patterns were apparent even in the absence of oyster filtration (and were hence related to the available substrate area) we repeated the same experiment using dead oyster shells by shucking live oysters and removing all tissue. This method was used so that the biofilm, that may influence larval settlement (Tamburri et al., 1992), was maintained and the main source of difference between live and dead oysters was the ability to filter. Methods for the experiments were otherwise as described for the banding experiment.

To test for interacting effects of the identity of the substrate and the density of the substrate on larval settlement, we performed fully-orthogonal ANOVAs with the two factors species (2 levels: *S. glomerata*, *C. gigas*) and density (3 levels: H, M and L). Results from the experiments utilizing live and dead oysters were analysed separately because of slight differences in the area of substrate available for settlement between the two experiments. Counts of larvae settling on the live oysters were ranked across all replicates of all treatments prior to analysis to eliminate issues of heterogeneous variances resulting from highly variable settlement. Raw counts were analysed to test hypotheses about settlement on dead oyster shells.

4.4 Results

4.4.1 Equivalence of native and non-native oysters as substrates for *Saccostrea glomerata* settlement, in the field and in mesocosms

In field and mesocosm experiments, the spatial arrangement of oyster substrates influenced patterns of settlement on *C. gigas* and *S. glomerata* (Fig. 4.1 a,b; Fig. 4.2). In the field, despite significant variation in larval settlement among bags, effects of oyster species and status were

evident (Table 4.1). More larvae settled in monospecific bags of *C. gigas* than in monospecific bags of *S. glomerata* irrespective of status as live or dead (Table 4.1a, Fig. 4.1a), and for each species settlement was higher on live than dead oysters (Table 4.1a, Fig.4.1a). When, however, oyster treatments were interspersed in a basket, differences in settlement were not apparent between *C. gigas* or *S. glomerata* between live and dead substrates, or an interaction of the two (Table 4.1b, Fig. 4.1 b).

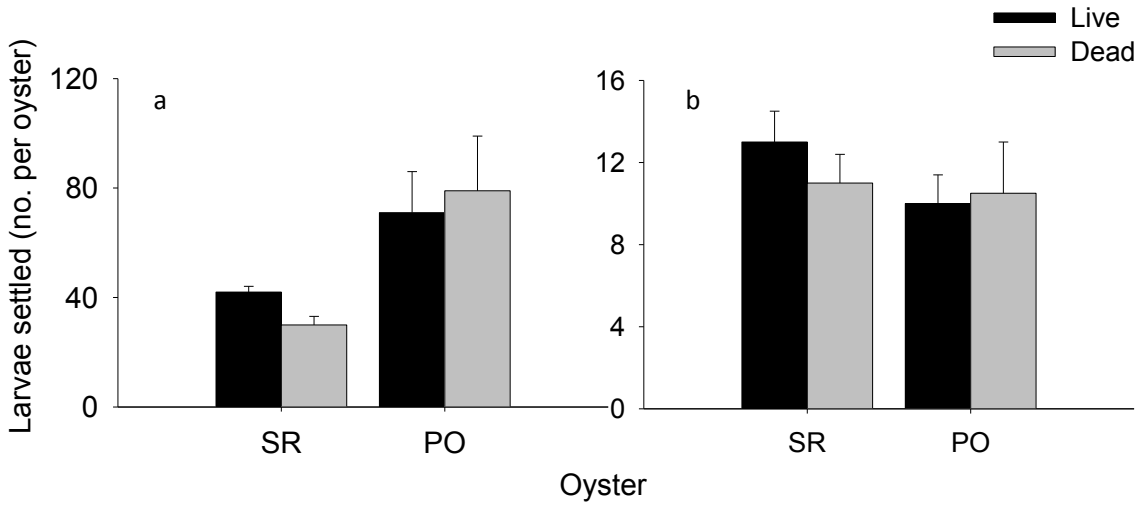

Figure 4.1. Mean (± SE) *S. glomerata* recruits per oyster in a) monospecific bags each containing nine *S. glomerata* (SR) or *C. gigas* (PO) oysters, that are either live or dead (n = 4 bags), and b) a mixed species basket containing live and dead oysters of each species, with n = 20 individual oysters per treatment, thus a total of 80 oysters.

Among flow-through mesocosms containing a particular type of oyster, significant variation in settlement of *S. glomerata* larvae was seen at the scale of tank (Table 4.2). Nevertheless, an effect of oyster type was evident over and above this small-scale variation, with significantly greater larval settlement in tanks containing only *C. gigas* than those with wild or QX-resistant *S. glomerata* (Table 4.2a; SNK: Oy, Fig. 4.2). Larval settlement was more variable among tanks containing *C. gigas* than *S. glomerata* or QX (Table 4.2a; SNK: T(Oy)). Within tanks containing

a mixture of substrates, more larvae settled on *S. glomerata* and *C. gigas* than QX, despite some variation in settlement among tanks (Table 4.2b Fig. 4.2). Settlement on *C. gigas* was much less in mixed than single-species tanks (Fig. 4.2).

Table 4.1. Analyses of variance testing for differences in the number of *S. glomerata* larvae settled on wild stock *S. glomerata* (SR), and *C. gigas* (PO) oyster substrates in field deployed A) nested mono-cultures (n = 9 oysters) and B) orthogonal mixed-species (n= 20 oysters). Oy = oyster (2 levels: *S. glomerata*, *C. gigas*); S = status (2 levels, live or dead), and B = bag (4 levels, random). SNK = Student-Newman Keul's post hoc tests. * indicates Sq (x + 1) transformation. Significant differences at are highlighted in bold.

A) *Mono- specific				
Source	*df*	MS	*F*	*p*
Oy	1	126.09	16.00	**<0.01**
S	1	43.41	5.51	**<0.05**
B (Oy,S)	12	7.88	3.20	**<0.01**
Oy × S	1	22.60	2.87	0.11
Res	128	2.46		
SNK				
Oy	*C. gigas >S. Glomerata*			
S	Live > Dead			

B) Mixed				
Source	*df*	MS	*F*	*p*
Oy	1	0.18	4.81	0.27
S	1	0.60	17.06	0.15
Oy × S	1	0.04	0.04	0.85
Res	76	1		

Table 4.2. Analyses of variance testing for differences in the number of *S. glomerata* larvae settled on wild stock *S. glomerata* (SR), selectively bred *S. glomerata* (QX) and *C. gigas* (PO) oyster substrates in A) nested mono-specific (n = 9 oysters) and B) orthogonal mixed-species (n= 3 oysters) mesocosms. Oy = oyster (3 levels: SR, QX, PO); T = tank (random: 4 levels in mono-specific, 5 levels for mixed-species mesocosm) SNK = Student-Newman Keul's post hoc tests. * indicates Sq (x + 1) transformation. Significant differences at are highlighted in bold.

A) *Mono- specific				
Source	*df*	MS	*F*	*p*
Oy	2	147.07	4.48	**<0.05**
T	9	32.10	33.70	**<0.01**
Res	120	0.93		
SNK	PO > SR = QX			

B) Mixed				
Source	*df*	MS	*F*	*p*
Oy	2	850.20	7.91	**<0.05**
T	4	615.09	5.72	<0.05
Oy × T	8	107.51	1.60	0.17
SNK	SR = PO > QX			

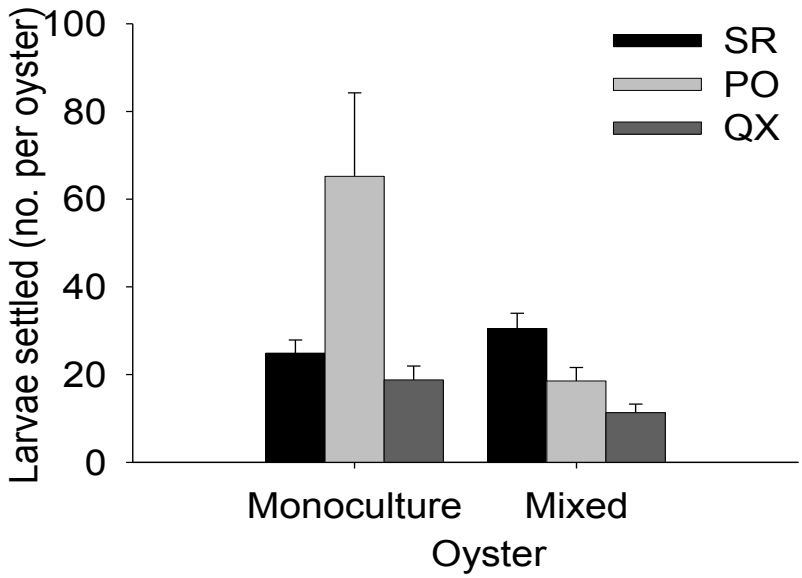

Figure 4.2. Mean (± SE) settlement per oyster of *S. glomerata* larvae on wild-type *S. glomerata* (SR), *C. gigas* (PO) and QX resistant *S. glomerata* (QX) in monoculture (n = 4) and mixed culture (n = 5) substrates of replicate tanks.

4.4.2 The role of chemical cues and shell type in determining larval settlement rates

In still-water tanks, settlement of *S. glomerata* larvae on dead oyster shells was not influenced by chemical cues (ANOVA; $F_{3,48} = 0.93$, $p > 0.05$), the substrate type (ANOVA; $F_{2,48} = 2.69$, $p > 0.05$), or an interaction between the two factors ($F_{6,48} = 0.57$, $p > 0.05$). Whether oysters were alive or dead did, however, have an influence on *S. glomerata* settlement on *C. gigas*, but not on wild or QX-resistant *S. glomerata* (ANOVA; sig. status x species interaction: $F_{2,24} = 4.60$, $p < 0.05$). More larvae settled on the dead than the live *C. gigas* (SNK, $p < 0.05$, Fig. 4.3). Among live oysters, *C. gigas* supported the fewest larvae.

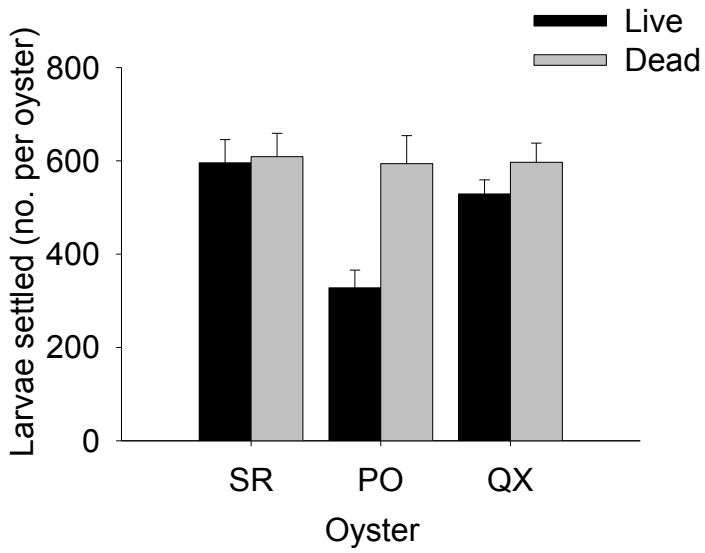

Figure 4.3. Mean (± SE) number of *S. glomerata* larvae settled on three Live (L) and Dead (D) wild-type *S. glomerata* (SR), *C. gigas* (PO), and QX resistant *S. glomerata* (QX). n = 5.

4.4.3 Effects of oyster filtration on larval settlement

Rates of water filtration differed between oyster species (ANOVA: F = 53.89, $p < 0.001$). *C. gigas* filtered water approximately twice as fast *S. glomerata* (mean ± SE; *C. gigas*: 1.09 ± 0.08 l h^{-1}; *S. glomerata*: 0.46 ± 0.05 l h^{-1}). The banding shut of oysters produced species-specific reductions in their filtration rate (ANOVA, sig. banding x species interaction: $F_{2,24} = 5.01$, $p < 0.05$). Banded *C. gigas* filtered water significantly slower than non-banded *C. gigas* (banded: 0.00 ± 0.28 l h^{-1}; non-banded: 1.09 ± 0.08 l h^{-1}, SNK, $p < 0.05$), but the filtration rate of *S. glomerata*, which was much lower than that of *C. gigas*, did not significantly differ between banded and non-banded oysters, despite banding halving the mean filtration rate (banded: 0.23 ± 0.06 l h^{-1}; *S. glomerata*: 0.46 ± 0.05 l h^{-1}; SNK: $p > 0.05$).

Settlement of *S. glomerata* on live oysters was determined by the interacting effect of oyster species and the banding treatment of the live oysters (ANOVA: $F_{2,24} = 5.01$, $p < 0.05$, Fig. 4.4). For neither of the species was there a difference in settlement between the non-banded and the loosely banded control oysters (SNK tests, $p > 0.05$). Consequently, the effects of banding could be interpreted as an effect of reducing filtration, and not of other confounding factors. A significantly greater number of larvae settled on banded than on control or non-banded *C. gigas* (SNK tests, $p<0.05$). There was no significant difference in the number of larvae that settled on banded, control or non-banded SR (SNK tests, $p > 0.05$). Between species, significantly more larvae settled on non-banded *S. glomerata* than *C. gigas* (SNK tests, $p < 0.01$) while among banded oysters there were no differences in larval settlement between species (SNK tests, $p > 0.05$).

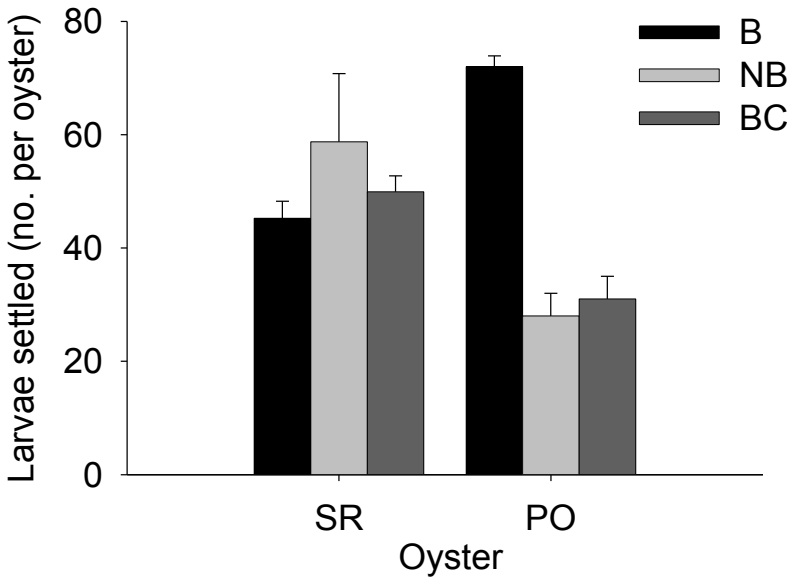

Figure 4.4. Mean (± SE) *S. glomerata* larvae settled on three *S. glomerata* (SR) and *C. gigas* (PO) that are banded and unable to filter (B), banded control that are able to filter (BC) and non-banded able to filter (NB). n = 5.

4.4.4 Effect of substrate density on larval settlement and survival

The influence of density and type of substrate on larval settlement was dependent on whether oyster substrates were live or dead. In 8 L buckets, among treatments with live oysters, larval settlement was greater on *S. glomerata* than *C. gigas* (ANOVA: $F_{1,24} = 7.61$, $p < 0.05$; Fig. 4.5a). This pattern was driven by the difference in settlement on SR and PO at the low density of live oysters (SNK, $p < 0.05$). There was no significant difference in larval settlement between the two species at the medium or the high density of live oysters ($p > 0.05$). There was no effect of species on settlement when the oysters were dead (ANOVA: $F_{1,24} = 0.86$, $p > 0.05$, Fig. 4.5b). Among dead oyster treatments, significantly more larvae settled in the high density treatments than in the medium or low density treatments, irrespective of species (ANOVA: $F_{2,24} = 5.23$, $p < 0.01$, Fig. 4.5b).

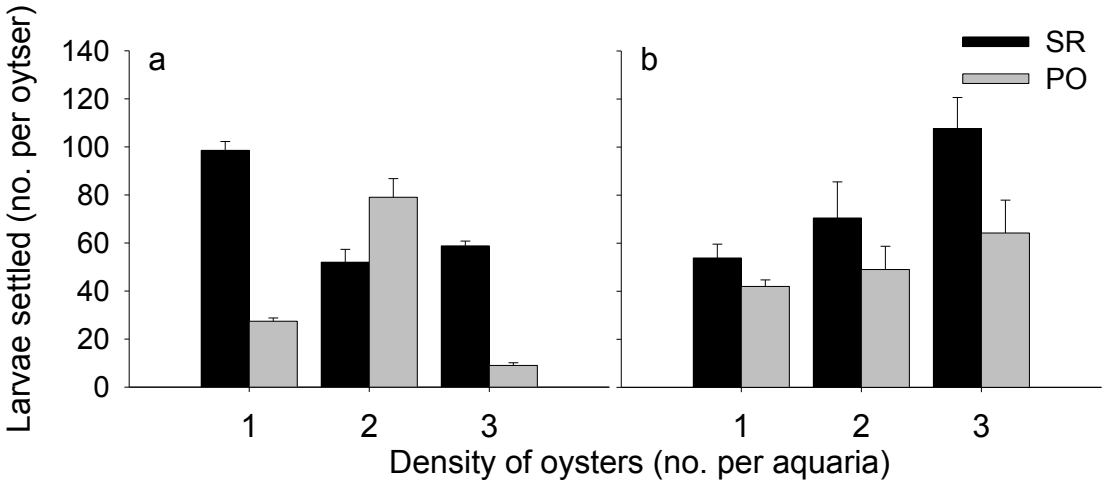

Figure 4.5. Mean (± SE) *S. glomerata* larvae settling in 8l buckets with 1, 2 or 3 a) Live or b) Dead *S. glomerata* (SR) and *C. gigas* (PO). n = 5.

4.5 Discussion

Through a combination of laboratory and field experiments we have demonstrated that the impact of non-native *Crassostrea gigas* on settlement of native *Saccostrea glomerata* larvae is dependent on the non-native oyster's spatial arrangement and density. In the field and in flow-through mesocosms, ~ 25- 200% more larvae settled on *C. gigas* than *S. glomerata*, when the two oyster species were presented as clusters of a single-species. When, however, *C. gigas* was interspersed with *S. glomerata*, settlement of *S. glomerata* did not differ between the two oyster substrates and was similar to settlement on single species clusters of *S. glomerata*, of equivalent density. At the higher effective density and low-flow environment within aquaria the impact of *C. gigas* reversed, with 600% less settlement of *S. glomerata* larvae on *C. gigas* relative to *S. glomerata*.

The reason why, among still-water aquaria containing only a single oyster substrate, fewer *S. glomerata* larvae settled in tanks with *C. gigas* than *S. glomerata* appeared to be the greater filtration rate of the non-native than the native oyster. In the absence of external currents, localized currents produced by suspension feeders can be the primary determinants of settlement patterns (Abelson and Denny 1997). In concordance with a previous study (Bayne et al., 2002), we found that the filtration rate of *C. gigas* was roughly twice that of *S. glomerata*. When monocultures of oysters were free to filter, greater numbers of larvae settled on clusters of *S. glomerata* than *C. gigas*. When, however, filtration was inhibited, either by killing the oysters, or banding shut their valves, this pattern disappeared and, in some cases, was reversed with greater settlement on dead *C. gigas* than *S. glomerata*. The reduced settlement of larvae on high densities of filtering *C. gigas* than *S. glomerata* may be because the strong filtration currents produced by *C. gigas* result in some *S. glomerata* larvae being ingested and killed by the adult oysters. In the Oosterschelde estuary, Germany, an increase in the filter feeding biomass

following invasion of *C. gigas* reduced the abundance of the mussel *Mytilus edulis* due to consumption of the mussel's larvae (Troost et al., 2009). Our study did not, however, examine the gut contents of *C. gigas* and *S. glomerata* for *S. glomerata* larvae, which would be required to test this hypothesis (see Troost 2008).

The explanation for the reverse pattern in the flow-through mesocosms and the field deployments of greater numbers of *S. glomerata* larvae settling on mono-specific patches of *C. gigas* than *S. glomerata* was less clear. Although many species of oyster use waterborne chemical cues to select substrata for settlement (Turner et al., 1994; Zimmer-Faust and Tamburri 1994; Anderson 1996; Tamburri et al., 2008), and transport of cues may be enhanced by currents (Burke 1986), experiments suggested that cues from neither *S. glomerata* nor *C. gigas* influence settlement of *S. glomerata* larvae. Under conditions of flow, oyster patch size, shape and orientation can each be important factors that influence settlement rates, due to their influence on processes of larval transport (Bell et al., 1995; Tanner 2003; Grabowski et al., 2005). It is possible that the deeper cup-shape of *C. gigas* may intercept currents differently to *S. glomerata*, in such a way that enhances passive deposition of larvae. Alternatively at low densities, and in conditions of low flow, the stronger ciliary currents of *C. gigas* than *S. glomerata* may be more effective at drawing in passing larvae in and promoting larval settlement. Ciliary currents are known to play an important role in determining encounter rates between larvae and adults (Tamburri et al., 2007) and, in the field, more larvae settled on live than dead oysters of each species. Subtle differences in shell microstructure between the two species do not appear to play a role. In experiments utilising dead oysters, settlement of *S. glomerata* larvae displayed the same density-dependent pattern of increase in settlement with increase in shell area irrespective of the oyster species that was present.

Our observation that more *S. glomerata* larvae settled on *C. gigas* than *S. glomerata* in field and mesocosm experiments was unexpected given that *S. glomerata* settle gregariously. Indeed, many other marine invertebrates and fishes display greater settlement on conspecifics than on bare or dissimilar habitats (Dobretsov and Wahl, 2001; Scardiña et al., 2009), different families of the same order (Booth 1992) and even different genotypes of their species (Bierne et al., 2003). In field and mesocosm experiments, however, the pattern of greater larval settlement on *C. gigas* than *S. glomerata* disappeared when the non-native oyster was interspersed with *S. glomerata*. This indicates that a threshold density of *S. glomerata* was required for effects of the non-native to be seen.

What will be the net outcome of *C. gigas* invasion on *S. glomerata* populations in east Australian estuaries? Recent surveys of several estuaries indicate that densities of *C. gigas* at the estuary- and site-scale remain low. A 2006 survey found that in the Hawkesbury River estuary, the mean density of *C. gigas* on rocky shores was 17 ± 13 m^{-2} (Summerhayes et al., 2009). In Port Stephens, the mean density of *C. gigas* in 2008 was 4 ± 1 m^{-2} and had not increased since the early 1990s (Bishop et al., 2010). In each estuary, *C. gigas* typically constituted < 5% of oysters at a site, and even in the most invaded areas *C. gigas* constituted <20% of total oyster abundance (Summerhayes et al., 2009, Bishop et al., 2010). At these densities, negative impacts of *C. gigas* on larval settlement of the magnitude that have been seen in the Wadden Sea and Oosterchelde estuary (Smaal et al., 2005; Troost et al., 2009) are unlikely. Instead, where the two species coexist in well mixed waters, positive effects of the non-native oyster on *S. glomerata* settlement may be seen. At the patch scale, however, negative impacts of *C. gigas* on *S. glomerata* larval settlement may occur where *C. gigas* are locally very dense and found in low-flow environments. Sampling of mixed oyster beds of *C. gigas* and *S. glomerata* at the patch scale, revealed that along a tributary of the Hawkesbury River, NSW the abundance of

oyster spat, *Bembicium auratum*, *Patelloida mimula* and *Irus crenatus* each decreased with the increasing proportionate contribution of *C. gigas* to total oysters (Wilkie et al., submitted). Mono-sepcific patches of *C. gigas* are likely to be more common lower on the shore, where *C. gigas* experiences a growth advantage over *S. glomerata* (Krassoi et al., 2008).

Management of invasions requires knowledge of the densities and spatial scales at which the non-native species impacts native communities and ecosystems. Our research adds to an increasing number of studies showing non-linear density-dependent effects of non-native species on native biota (e.g. Finnoff et al., 2005; Yokomizo et al., 2009). In order to set realistic and cost-effective goals for management of invaders and their impacts, such density- and scale-dependencies need to be understood (Yokomizo et al., 2009). Particularly in instances where relationships between invader density and impact are non-linear, ignorance of the density–impact curve will lead to goals and management strategies that are mis-matched.

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5 Differences in the shell strength of native and non-native oysters do not influence their susceptibility to a generalist predator

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5.1 Abstract

Proliferation of non-native species in a novel environment can depend on the degree to which native predators exert top-down control on the novel prey item. Among non-native epifaunal molluscs, rates of predatory mortality may be determined by the ease with which generalist predators can penetrate the shell, a main line of anti-predator defence. We assessed whether in eastern Australia the faster growth of non-native Pacific oyster *Crassostrea gigas*, compared to the native *Saccostrea glomerata*, comes at the cost of reduced investment in shell thickening, rendering the non-native oyster weaker and more susceptible to a generalist predator, the muricid gastropod *Morula marginalba*. Laboratory tests confirmed that among larger (>50 mm shell height) oysters, the shells of *C. gigas* were weaker than *S. glomerata* of equal size. There were, however, no differences in shell strength or thickness between smaller *S. glomerata* and *C. gigas* of the size range consumed by *M. marginalba*. In the absence of shell strength differences among smaller oysters, *M. marginalba* preferentially consumed familiar native over non-native oysters. When *M. marginalba* had no choice of prey item, *C. gigas* were consumed at the same rate as the native oyster. The ability of *M. marginalba* to consume *C. gigas* when the native oyster is not available suggests that under the scenario that *C. gigas* proliferates and out-competes *S. glomerata* the whelk may be able to exert some top-down control on the invasive species.

Keywords: Biological invasions, *Crassostrea gigas*, *r* – selection, *Saccostrea glomerata*

5.2 Introduction

Coastal habitats are among the most invaded environments in the world (Vitousek et al., 1996; Rilov and Crooks 2009). International shipping activities and species translocations for aquaculture have resulted in unintentional and deliberate species introductions (Bax et al., 2003; Naylor 2005). Only a small fraction of introduced species survive and proliferate in new environments (Molnar et al., 2008). Those that do (termed invasive species) can, however, go on to cause costly economic and ecological impacts (Pimentel et al., 2005; Molnar et al., 2008) such as biodiversity loss, modification of fundamental ecosystem processes, fisheries collapses, damage to property and even human health (Vitousek et al., 1996; Grosholz 2002; Bax et al., 2003; Rilov and Crooks 2009). Consequently, understanding when and where introduced species become invasive, and under what circumstances they cause large ecosystem impacts is a key concern to coastal managers.

Invasion is determined by traits of the non-native species, and by characteristics of the recipient environment. Introduced species that successfully establish and proliferate frequently have *r* – selected life history traits, including rapid growth, early maturity, short generation time, high fecundity, (McMahon 2002; Erlandsson et al., 2006), as well as eurytopy and a broad native range (Lodge 1993; Sakai et al., 2001). These traits allow non-native species to rapidly colonize disturbed environments, pre-empting space so it is no-longer available for colonization of native species, and to out-grow and out-compete native species (McMahon 2002). Characteristics of the recipient environment, which predispose it to invasion include abiotic similarity to the invader's native range, frequent and intense disturbance, a low diversity of native species, structurally simple food webs and a lack of abundant native predators capable of consuming the non-native species (Levine and D'Antonia 1999; Occhipinti-Ambrogi and Savini 2003; de Rivera et al., 2005). Invasion is

predicted to occur where traits of the non-native species match well to the recipient environment (Facon et al., 2006, Krassoi et al., 2008).

Previous studies have demonstrated an important role for native predators in limiting invasion success (Byers 2002; de Rivera 2005). Invasive *r* - selected species that allocate a large fraction of resources to rapid growth and high reproductive output might, potentially, have less energy to invest in anti-predator defences (Bazzaz et al., 1987). In coastal environments, epibenthic bivalve molluscs constitute a significant proportion of introduced marine species, as a consequence of translocation for aquaculture (e.g. Ruesink et al., 2005) and the *r*-selected life history characteristics (including early maturation, broad-cast spawning and rapid growth) of many of this group (Karatayev et al., 2007; Trimble et al., 2009). Epibenthic bivalve molluscs that are sessile and unable to actively escape predation rely on shell armour (Vermeij 1976) and habitat complexity (Graboswki 2004) for anti-predator defences. In environments with abundant generalist predators that frequently select prey items on the basis of shell strength (Moran 1985; Micheli 1995; Buhle and Ruesink 2009), any trade off among bivalves of investment in anti-predator defence to accommodate rapid growth and reproduction might come at the cost of enhanced predatory mortality (Bishop & Peterson 2006; Newell et al., 2007). Enhanced predation on invasive species with limited investment in anti-predator defences, however, requires that native species in the novel environment are able to recognise the invasive species as prey items. Furthermore, predators might select prey items that they are more accustomed to consuming (Hughes and Dunkin 1984).

The Pacific oyster *Crassostrea gigas* is one of the most notorious marine invasive species in the world. Native to Japan, *C. gigas* has been introduced to 66 non-native regions,

of which it has become a nuisance species among 24 regions (Ruesink et al., 2005). Within Australia, *C. gigas* has been present since the mid 1900s when it was introduced to southern Australia for aquaculture and fisheries (Thomson 1959). In New South Wales it has been present since the mid 1980s, at which time the oyster appeared in Port Stephens after a suspected rogue introduction from the southern states (Holliday and Nell 1985). Despite occupying similar intertidal and shallow subtidal habitats to the native Sydney rock oyster, *Saccostrea glomerata*, and a history of out-competition of *S. glomerata* in New Zealand (Dinamani 1991), *C. gigas* has to date failed to proliferate in New South Wales to abundances that negatively influence *S. glomerata* (Summerhayes et al., 2009; Bishop et al., 2010). It has been suggested that the failure of *C. gigas* to proliferate might be because of its much more rapid rate of growth than *S. glomerata*, double that of the native species (Bayne 2002), and its larger maximal size (105 mm compared with 86 mm for *S. glomerata*) comes at the cost of a thinner shell, which renders it more susceptible to predation (Bishop et al., 2010).

The muricid gastropod *Morula marginalba* is an abundant predatory gastropod on rocky shores of southeastern Australia, which consumes *S. glomerata* among its prey items (Moran et al., 1984). To eat its selected prey item, *M. marginalba* drills a hole in the shell of its prey using its radula (Fairweather and Underwood, 1983). Although *M. marginalba* is a generalist predator, capable of consuming many species of sessile shelled mollusc in its environment, it often selects prey on the basis of shell thickness (Morton 2003; Morton 2008) and can quickly adapt to novel prey with a weaker shell, irrespective of the presence of familiar prey (Rilov et al., 2001; Morton 2005; Morton 2008).

Here we test the hypotheses that (1) *C. gigas* will have thinner shells than similar-sized *S. glomerata,* (2) the generalist predator *M. marginalba* will be able to recognise *C. gigas* and consume them as prey items, and (3) where *C. gigas* is thinner shelled than *S. glomerata*, a greater number of the non-native oyster will be consumed by *M. marginalba* offered a choice of the two due to the shorter handling of the non-native species; and (4) in the absence of a difference in shell strength between *S. glomerata* and *C. gigas*, *M. marginalba* will consume the two species at similar rates because predation is determined by handling times, not differences in substrate recognition.

5.3 Methods

5.3.1 Oysters and whelks

Experiments (1) assessing morphological differences between the two species and (2) comparing the susceptibility of *Saccostrea glomerata* and *Crassostrea gigas* to predation were replicated with aquaculture and wild oysters. We hypothesised that the greater environmental stresses that act on wild oysters might lead to greater differences in morphology and, consequently, differences in susceptibility to predation than between aquaculture oysters. Cultured oysters of each species were obtained from Diemar's Oysters, Salamander Bay, Port Stephens, New South Wales (NSW), Australia. To prevent differences in grow-out environment from confounding differences in the biology of the two species, the two species were collected from the same aquaculture lease. Wild oysters were collected from a single site in Mullet Creek, a tributary of the Hawkesbury River (HR: 33°34'S, 151°18'E) where the two species form mixed beds, from a tidal elevation of 0.0 – 0.2 m above LWST. Immediately prior to each experiment, oysters were held in closed tanks of aerated filtered seawater. Seawater was changed every 2 d, and oysters were fed a mixed diet of *Isochrysis*, *Pavlova*, *Thalassiosira weissflogii*, and *Tetraselmis*.

For experiments examining how the oyster shell substrate to which *Saccostrea glomerata* are attached influences their susceptibility to predation, we used 6-month old *S. glomerata* that had been settled onto the shells of 3 year old oysters. The adult *S. glomerata* (shell-height [mean ± SE]: 72 ± 1 mm) and *C. gigas* (81 ± 1 mm) were obtained from aquaculture leases in the Hawkesbury River and Brisbane Waters, NSW, Australia.

The *Morula marginalba* used in predation experiments were collected from the intertidal rock platform (0 – 0.5 m above low water spring tide [LWST]) at Long Reef Aquatic Reserve, NSW (33°44'S, 151°18'E). The *M. marginalba* had a shell height of 19 – 25 mm (mean ± SE: 21 ± 0.27 mm) and were with the size-range known to consume *S. glomerata* (Moran 1985). Whelks were starved for 48 hr prior to experiments.

5.3.2 Differences in shell strength between *S. glomerata* and *C. gigas*

To test the hypotheses that (1) for a given shell height of oyster, *C. gigas* will have a weaker shell than *S. glomerata* and (2) differences in shell will be greater among wild than cultured oysters, we measured the force (Newtons) required to crack shells of *S. glomerata* and *C. gigas* across a range of sizes of each (10 – 120 mm). Oysters (n = 70 *C. gigas* and n = 80 *S. glomerata* for wild oysters; n =130 *C. gigas* and n = 110 *S. glomerata* for aquaculture oysters) were shucked and dried to constant weight at 60^{o}C. Drying valves standardised moisture levels and should not have altered their relative resistance to crushing (Currey 1979). Left valves were used for measurements because our observations of *M. marginalba* feeding suggest that left valves are most often drilled by whelks to extract meat. The thickness of the left valve of every oyster shell was measured (to the nearest 0.00 mm), as close to its centre as possible, using outside dial calipers. An Instron 5542 electromechanical materials testing

machine (Instron, Canton, MA, USA) was then used to conduct flexure tests using a flat-ended cylindrical steel punch (1.0 mm diameter) load. Single left valves of oysters were placed exterior surface-up on a horizontal platform positioned at the centre of the machine's punch receiving ledge - 100 mm diameter, and 300 mm below the punch end. Valves were orientated such that the punch would contact as close to the centre of each as possible. A suspended mechanical arm lowered the punch at 10 mm sec^{-1} until the shell was cracked. The maximum force (in Newtons) required to crack the shell was recorded.

Linear regressions assessed relationships between 1) oyster shell height and shell strength (force in Newtons) and 2) oyster shell height and shell thickness. For each relationship and source of oysters (i.e. aquaculture or wild), a student *t*-test assessed differences in regression slopes between species.

5.3.3 Consumption of alternate oyster species by *Morula marginalba*

To test whether non-native *C. gigas* is equally as susceptible to *M. marginalba* predation as native *S. glomerata*, a more familiar prey item, we performed choice experiments whereby we simultaneously offered equal numbers (2 each) of similar-sized *C. gigas* and *S. glomerata* (27 ± 0.2 mm) to *M. marginalba*. This size-range of oysters was chosen for our experiments because it is towards the upper size limit of *Saccostrea* spp. that *Morula* spp. can handle (Moran et al., 1984) and *M. marginalba* may consequently be particularly responsive to small differences in the energy profit of oysters within this range. The oysters used in experiments were from an aquaculture lease in Port Stephens. It was not possible to replicate the experiment with wild oysters because: (1) the two species are from their exterior morphology indistinguishable in the field, and shucking of wild oysters would be required prior to the

experiment to confirm oyster identity from denticles; and (2) sufficient wild oysters of each type, within the size range consumed by *M. marginalba*, were not available. In any case, measurements of shell strength indicated that within the size-range of oysters that can be consumed by *M. marginalba*, there was no difference in shell strength between *C. gigas* and *S. glomerata,* when either aquaculture or wild sources of oysters were considered (see Results).

Oysters were placed in replicate 1 L glass beakers containing ~ 300 ml aerated filtered sea water and a single whelk that had been starved for 48 hours prior to the study. Beakers were randomly positioned under a fluorescent light set on a 12 h light/dark cycle and household petroleum jelly was smeared ~ 2 cm above the waters surface to contain whelks. Beakers were checked for predation after 2 h and every 12 h thereafter over a period of four weeks (trial 1, n = 10) or three weeks (trial 2, n =10; shortened because rates of consumption were greater than in trial 1). Lengths of feeding trials were chosen to reduce the influence of variability of handling times among individual *M. marginalba* within each replicate (Faiweather and Underwood 1983). Any dead oysters were recorded (by species and status as drilled or undamaged) and replaced with a new individual of the same species. We also recorded whether each *M. marginalba* was sitting on an oyster, and if so, of what species. Water was changed once per 48 h, but should have represented minimal disturbance to whelks since on rocky shores they experience strong wave disturbances during foraging (Fairweather and Underwood 1983). Only the trials (n = 15) in which individual *M. marginalba* had consumed at least one oyster by the end of the experiment were included in analyses. We used a two-tailed paired t-test to assess any difference in the rate of consumption of the two oyster species, across the two trials.

Sometimes the consumption of prey is independent of predator choice, for example where mechanical limitations restrict consumption of better defended prey (Bishop and Peterson 2006; Brown and Haight 1992), or predators modify prey consumption according to availability (Fairweather 1988). To ascertain whether any differences in the rate at which *M. marginalba* consumed *C. gigas* and *S. glomerata* persisted even where no alternative prey items were available, we concurrently conducted a 'no-choice' experiment. We offered four oysters of the same species to a single *M. marginalba*, so that total oyster density was identical to the choice experiments. Methods were as for choice experiments, with n =10 replicates of each no-choice treatment run concurrently with each choice trial. Two-sample t-tests compared the rate at which *M. marginalba* consumed the two oyster species between the two no-choice treatments, within each trial. Replicates in which whelks demonstrated no interest in feeding, either during or after the trial, were excluded from analyses.

Additionally, we directly compared the proportions of each type of oyster consumed between choice and no-choice experiments using χ^2 goodness-of-fit tests. These used an equal number of replicates, randomly selected, of each treatment from within each trial, to give a total of n = 8. Under the null hypothesis that there is no effect of choice on the preference for one species over the other, the expected numbers of *S. glomerata* and *C. gigas* consumed in choice experiments were:

$$E_S = N_T[M_S/(M_S + M_C)]$$

$$E_C = N_T[M_C/(M_S + M_C)]$$

where M_S and M_C are the numbers of *S. glomerata* and *C. gigas* consumed when each is presented on its own (in equal numbers) and N_T is the total number of oysters consumed when offered together in choice experiments. We estimated M_S, and M_C by summing the number of

each oyster consumed in no-choice trials across independent replicates. We used χ^2 goodness-of-fit tests to compare these expected numbers with the numbers of oysters consumed when presented together (N_S, N_C for *S. glomerata* and *C. gigas* respectively; $N_S + N_C = N_T$). Because these χ^2 tests are subject to excessive Type I error (see Liszka and Underwood 1990), results were considered statistically significant only when $p < 0.01$.

To enable assessments of whether differences in energy profit might potentially contribute to any difference in the consumption of the two prey species, we compared the wet tissue mass between ten individuals of each of the two species, *C. gigas* and *S. glomerata,* randomly selected from the group used in experiments. Tissue was removed and oysters were dried to constant mass at 90°C in the oven, and empty shells reweighed. We calculated wet tissue mass using the difference between whole wet weight and shell dry weight. This method was used in preference to weighing wet tissue directly because it is often difficult to completely remove all soluble tissue from the oyster shell cavity, leading to underestimations of tissue mass using direct assessment. Under the null hypothesis of no preference by *M. marginalba* for one species over the other, we expected that the tissue reward (in terms of biomass) would be comparable between *C. gigas* and *S. glomerata* of similar size. Wet tissue weights between species were analysed using two-sample *t*-tests.

Additionally, we ascertained the shell density of seven oysters of each species to assess whether there were structural differences, other than strength, between *C. gigas* and *S. glomerata* that might influence predation. Shell density was calculated as valve surface area divided by valve total dry weight, and was expressed as $mg.mm^{-2}$. The surface area was measured by covering the top of the left valve in aluminium foil, so that all exposed surface

was covered, and measuring the surface area (mm^2) of the resulting piece of foil using ImageJ software. Shell weight was measured to the nearest mg after drying to constant weight at 60˚C using an AND GX400 milligram balance. A one-way analyses of variance (ANOVA) tested for differences in shell density between species.

5.3.4 Effects of substrate of attachment on susceptibility of *S. glomerata* to *Morula marginalba* predation

To test the hypothesis that the susceptibility of *S. glomerata* to predation would not be affected by the shell substrate to which they were attached, we compared rates of predation of *M. marginalba* on *S. glomerata* spat (shell-height [mean ± SE]: 12.2 ± 3.7mm) attached to dead adult *S. glomerata* and *C. gigas* shells. The number of spat was held constant at 10 per shell. Excess spat that were surviving since original settlement (mean ± SE spat per *S. glomerata*: 146 ± 16, *C. gigas:* 473 ± 53) were removed from oysters with a chisel. In the laboratory, two oyster shells of each species, *S. glomerata* and *C. gigas*, with attached spat were placed in replicate (n = 18) aquaria that were 300 × 100 × 100 mm and contained ~ 1 L aerated, filtered sea water. Adult oyster shells were arranged alternately by species at the centre of each tank. To each aquaria, one *M. marginalba* was added adjacent to the oyster group. To contain whelks inside the aquaria, but allow migration between submerged and emmersed habitats, a smear of household petroleum jelly was applied ~ 2 cm above the water's surface. Aquaria were randomly positioned under a fluorescent light set on a 12 h light/dark cycle. After 24 h, and then every 48 h thereafter, the position of the *M. marginalba* (i.e., attached to *S. glomerata* or *C. gigas* adult) and signs of predation were recorded. An oyster that had been consumed had a small drill hole (~0.5 mm diameter) on the right valve (as the left valve was attached to the adult), its valves were gapping and the tissue absent. Consumed oysters was quantified per adult and shell height of each consumed oyster

measured (nearest mm) with vernier callipers. The experiment lasted 15 d, with water changed once per 48 h. A paired t-test assessed significant (at $\alpha = 0.05$) differences in the number of times *M. marginalba* were observed attached to *S. glomerata* and *C. gigas* adults, and differences in the number of *S. glomerata* spat consumed on each of the substrate types per tank.

5.4 Results

5.4.1 Differences in shell strength between *S. glomerata* and *C. gigas*

Among aquaculture oysters, regressions slopes between shell strength and shell height did not differ between *C. gigas* and *S. glomerata* ($t = 0.08$, d.f = 63, $p = 0.46$; Fig 5.1a). Similarly, the relationship between shell thickness and height was statistically indistinguishable between the two species ($t = 0.08$, d.f = 63, $p = 0.49$; Fig. 5.1b). By contrast, among wild oysters, the relationship between shell height and shell strength was steeper for *S. glomerata* than *C. gigas* ($t = 75$, d.f = 48, $p = 0.00$), with no detectable difference in shell strength among small size classes, less than 25-30 mm shell height (Fig. 5.1c). There was no species-specific differences in shell thickness ($t = 0.12$, d.f = 48, $p = 0.45$; Fig. 5.1d).

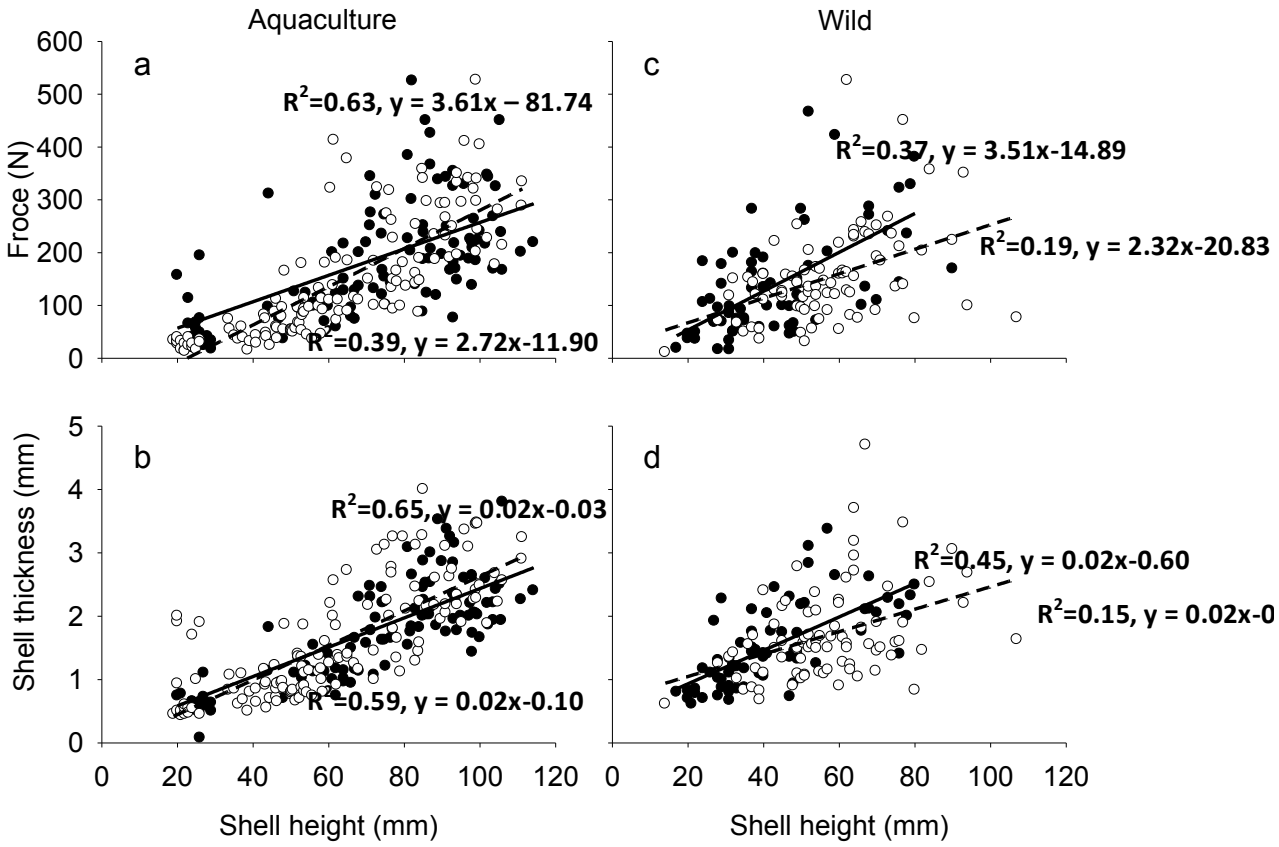

Figure 5.1. Relationships between shell height (mm) and force (in newtons) required to crack shells, and between shell height and thickness (mm), for *Saccostrea glomerata* (filled symbols) and *Crassostrea gigas* (non-filled symbols). Aquaculture and wild-collected oysters were considered separately.

5.4.2 Consumption of alternate oyster species by *Morula marginalba*

During the choice experiments, *M. marginalba* on average consumed 1.9 ± (1 SE) 0.2 oysters. In 12 of the 15 choice experiments, *M. marginalba* consumed more *S. glomerata* than *C. gigas.* In 2 of the 15 trials, *M. marginalba* consumed equal numbers of the two types of oyster and in only 1 trial were more *C. gigas* consumed. Consequently, across all trials, significantly more *S. glomerata* (1.5 ± 0.2) than *C. gigas* (0.3 ± 0.2) were consumed (paired t-test: t = 3.7, d.f = 15, $p = 0.002$; Fig. 5.2). In no-choice experiments, by contrast, there was no difference in the mean number of *S. glomerata* (1.8 ± 0.3) and *C. gigas* (1.6 ± 0.3)

consumed (two-sample t-test: t = 0.69, d.f = 14, $p = 0.5$). Consequently, *M. marginalba* consumed a significantly higher number of *S. glomerata* in choice experiments than predicted from the no-choice trials ($\chi^2 = 5.9$, d.f = 13, $p < 0.02$; Fig. 5.2). Wet tissue mass was slightly greater among *S. glomerata* (mean ± SE: 0.80 ± 0.05g) than *C. gigas* (0.71 ± 0.05g), however this difference was not statistically significant (t = 0.12, d.f = 8, $p = 0.45$). Similarly, the mean shell density (mg mm^{-2}) of *S. glomerata* (1406 ± 198) was slightly greater than that of *C. gigas* (1030 ± 122), although not significantly so (ANOVA: $F_{1,15} = 3.3$, $p = 0.09$).

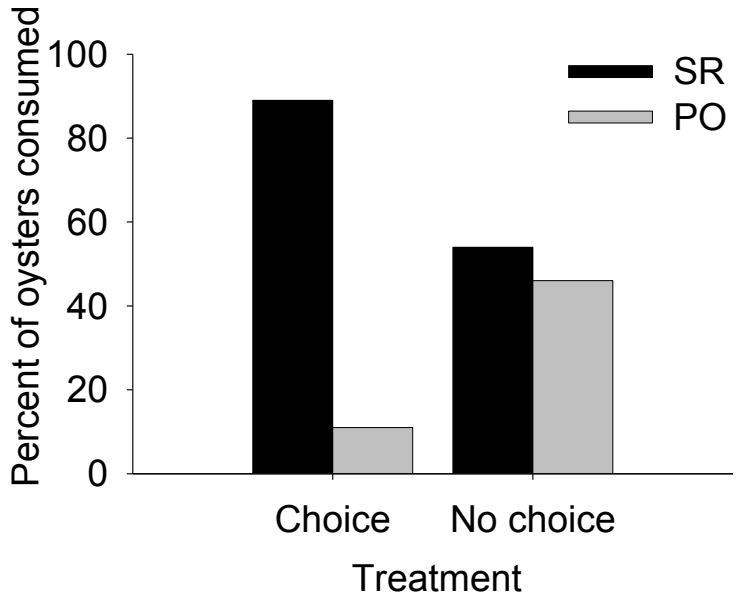

Figure 5.2. Proportionate contribution of *Saccostrea glomerata* (SR) and *Crassostrea gigas* (PO) to total oysters consumed by *Morula marginalba* in feeding trials where the two species were offered together (choice) and in mono-specific trials (no-choice). Oysters were pooled across the 8 replicates of each treatment.

5.4.3 Effects of substrate of attachment on susceptibility of *S. glomerata* to *Morula marginalba* predation

The number of *S. glomerata* spat consumed by *M. marginalba* did not differ between *S. glomerata* (mean ± S.E: 2.2 ± 0.30) or *C. gigas* (2.1 ± 0.58) shell substrates (paired t-test: t = 0.74, d.f = 17, $p = 0.47$). Among sampling times, *M. marginalba* was observed on each of the shell substrates on a similar number of occasions times (*S. glomerata:* 4.1 ± 0.42; *C. gigas:* 4.8 ± 0.54: paired t-test: t = 1.4, d.f = 17, $p = 0.18$).

5.5 Discussion

Our study confirmed predictions that among wild oysters, the relationship between shell height and shell strength would be steeper for slower-growing Sydney rock oysters, *S. glomerata*, than for faster growing Pacific oysters, *C. gigas*. Yet, among small size classes (< 20 mm shell height), within the size range consumed by the generalist predator *M. marginalba* (Moran et al., 1984), we found little difference in shell strength between the two oyster species. Consequently, rates of predation by *M. marginalba* were not greater on *C. gigas* than *S. glomerata.* Instead, contrary to our prediction, whelks displayed preference for the more familiar prey item, *S. glomerata.*

Our finding that, among wild oysters, there was a steeper relationship between shell height and strength for a slower- than a faster-growing species is consistent with ecological trade-off theory that posits that the benefits of performing one ecological task often comes at the cost of performing another (Stearns 1992). Yet whereas other similar studies have found that this may result in *r*-selected species suffering high predatory mortality that could compromise proliferation (e.g., Bishop and Peterson 2006, Newell et al., 2007) this was not the case here. It was the slower-growing species that suffered greater mortality by the

generalist predator, *M. marginalba*. We did not observe any differences in shell strength and thickness between oyster species at juvenile stages (~20 mm shell height), up to one year old (as predicted from age-length relationships, Nell and Perkins 2005). Only when oysters were > 50 mm were differences in shell strength and thickness apparent between species. The apparent lack of an early stage growth-defence trade off among *C. gigas* is potentially a critical factor in determining the success of the non-native oyster as an invader around the world (Micheli 1995; Boulding et al., 1999; Bishop and Peterson 2006).

Although large, wild *C. gigas* were generally weaker-shelled than similar sized *S. glomerata*, this pattern did not extend to aquaculture oysters. Many marine bivalves have an inducible element to the morphological defence (Reimer and Tedengren 1996; Bourdeau 2010) and it is possible that in the enclosed trays of aquaculture leases that exclude predators, *S. glomerata* invested less in morphological defence than genetically identical oysters would in the wild. Alternatively the differing results derived from analysis of aquaculture and wild oysters may be a consequence of genetic differences. Aquaculture oysters are frequently bred for rapid growth (Hand et al., 2004; Nell and Perkins 2005) so, irrespective of species may allocate less energy to anti-predator defence than wild oysters.

In the absence of any difference in shell strength between *S. glomerata* and *C. gigas*, *M. marginalba* preferentially consumed similar-sized native over non-native oysters. In choice experiments 800% more *S. glomerata* than *C. gigas* were consumed, despite equal rates of consumption of the two species when presented independently of one another. Preferential selection of *S. glomerata* did not appear to reflect a greater energy profit of *S. glomerata* than *C. gigas*, with similar-sized oysters of each species containing a similar tissue biomass. Similarly, differences in shell density likely did not play a role, with the preferred *S.*

glomerata containing a slightly denser, but not significantly so, shell than *C. gigas*. Had shell density played a role, it might be expected that the less dense, *C. gigas*, shell might be easier to penetrate given similar shell thicknesses. It remains possible, however, that there may have been differences in formation of the periostracum, the thin, flexible fibrous and sclerotinised protein layer that covers the calcified shell, between species, that influenced predation. The periostracum protects oysters from microborers such as sponges, and muricid whelks that prefer abraded regions of the shell (Mao Che et al., 1996; Guenther and Nys 2006). Periostracum develops at the shell lip as oysters grow; therefore, formation of periostracum might be slower among slower growing *S. glomerata* than fast growing *C. gigas*. Whether this influenced predation rates by *M. marginalba* requires analyses of shell periostracum of each species.

Instead, the preference of *M. marginalba* for *S. glomerata* may reflect a greater familiarity of the whelk with the native prey item, with which it has co-evolved. Among common molluscs on east Australian rocky shores, *S. glomerata* is a preferred prey item (Fairweather et al., 1984; Fairweather 1987). The whelk may be imprinted to follow a particular chemical or behavioural cue produced by the native but not the non-native oyster. Our experiments did not account for the fact that populations of *C. gigas* of similar age would be dominated by larger individuals than similar-aged, slower growing *S. glomerata*. It is, however, likely that had age, not size, of oyster been held constant, the selection by *M. marginalba* of *S. glomerata* over *C. gigas* would be exacerbated, with the non-native oyster more rapidly entering a size refuge from predation than the native oyster. Previous studies have demonstrated that predator preferences govern predation behaviour, within a single species of prey (Morton 2004). The predatory muricid whelk *Lepsiella venosa* prefers larger

over smaller *Xenostrobus pulex* and anterior over posterior sides of the prey's shell because of an increased energy rewards (Morton 2004).

This study considered consumption of *C. gigas* and *S. glomerata* by only a single predator, *M. marginalba*, under a single scenario that the two prey items are equally abundant. A similar study, utilising another whelk native to temperate eastern Australia, *Bedeva hanleyii* as the predator, found that it played an important role in limiting *C. gigas* recruitment to oyster leases due to its selection of non-native over native oysters (Rodley and Davis 2009). Furthermore, there are many other predators of oysters in east Australian estuaries, such as toadfish *Tetractenos glaber* and mud crabs *Scylla serrata* (Underwood and Barrett 1990; Anderson and Connell 1999). Like whelks, many fish and crabs consume bivalve prey items of a given size in proportion to their shell strength (Anderson and Connell 1999; Silva et al., 2010). Therefore, these predators, capable of consuming larger oysters within the size range where differences in the shell strength of the two species, *C. gigas* and *S. glomerata* are evident, may consume more of the non-native oysters, all else being equal. The opportunistic consumption of *C. gigas* by *M. marginalba* when *S. glomerata* were not available suggests that under the scenario that *C. gigas* overgrows and smothers *S. glomerata* (see Krassoi et al., 2008), the whelk may be able to exert some top-down control on the invasive species. Opportunistic foraging is a common phenomenon among whelks that can switch between several different prey items depending on availability (Fairweather 1987; Morton 2008). Future studies would be required to examine critical densities at which *M. marginalba* switches from *S. glomerata* to *C. gigas*.

Our study compared predation of *M. marginalba* between single *S. glomerata* and *C. gigas* oysters, unattached to hard substratum. In the wild, however, oysters of each species

may be found attached to a variety of substrates including conspecifics and the alternate oyster species. Experiments found that the substrate, adult *S. glomerata* or *C. gigas*, to which *S. glomerata* spat were attached, did not influence rates of their predation by whelks. This is not surprising considering that predation of oysters by whelks involves only drilling and does not require the prey item to first be removed from the substratum, as with predation by some crabs.

A lack of native predators has possibly influenced the establishment and proliferation of naturalized invasive *C. gigas* populations elsewhere around the world (Escapa et al., 2004; Trimble et al., 2009; Troost 2010). Our observations suggest that the rate of invasion of *C. gigas* in Australian estuaries will not be reduced by predation by *M. marginalba* where native prey are still available in abundance. Conversely, our results indicate that were *C. gigas* to replace *S. glomerata*, the non- *M. marginalba* could switch prey items to the non-native oyster. Therefore our results do not provide evidence for disruption of trophic relationships by the invasion of *C. gigas* in east Australian estuaries, although further studies, utilising different predator species, are recommended.

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6 General conclusions

6.1 Impacts of QX disease not resonated among wild oyster populations

The emergence of diseases among cultured fish and shellfish may facilitate an increase in the persistence and intensity of diseases affecting wild populations (Murray and Peeler 2005). However, this study has shown that in the Hawkesbury River estuary, despite QX disease-induced mass mortalities among cultured Sydney rock oysters, *S. glomerata*, wild counterparts remain relatively unaffected. This study provides the first evidence that wild *S. glomerata* occupying an estuary with a QX-disease impacted aquaculture industry are hosts to the disease causing parasite *Marteilia sydneyi*. Infection levels were, however, 7 orders of magnitude lower than among aquaculture counterparts (chapter 2). Together with previous observations (Hudson and Hill 1991), these results suggest that diseases associated with aquaculture oysters will not necessarily increase the prevalence or intensity of diseases among wild oyster populations, or produce harmful effects to wild oysters and the ecosystem services they provide.

Presumably *M. sydneyi* has been a natural component of the Hawkesbury River ecosystem but gone undetected until sudden disease outbreaks impacted *S. glomerata* cultured stock. Elsewhere, QX disease has been expressed and mortality of oysters induced in only six of the 17 New South Wales (NSW) estuaries known to contain the disease-causing parasite, *M. sydneyi* (NSW Primary Industries 2011a). This suggests that there is natural variation in susceptibility to QX disease among individuals in wild populations, on which selection could act (Culloty et al., 2004). It has also been suggested that environmental stressors that weaken the oyster's immune system and, in particular that reduce phenoloxidase production, may be required for disease expression (Butt and Raftos 2007; Green et al., 2011). Yet whereas among hatchery-produced oysters, QX disease is more prevalent at low salinity (Butt et al.,

2006), an increasing prevalence of the disease with distance upstream was not detected (chapter 2). However, in the future, sources of stress such as floods (Butt et al., 2006), chemical pollution (Chu and Hale 1994), hypoxia (Boyd and Burnett 1999) and climate change (Harvell et al., 2002) may potentially facilitate greater QX mortality of wild populations.

Certain increases in *C. gigas* introductions for aquaculture may potentially induce a sudden emergence of QX disease among wild *S. glomerata* if parasite host-switching were to occur (Poulin et al., 2010; Peeler et al., 2011). In Europe, *C. gigas* hosts the cogener of *M. sydneyi*, *Marteilia refringens* (Cahour 1979). Hence, although *C. gigas* is unaffected by QX disease (Nell 2007), it is possible that *C. gigas* could be a competent host for *M. sydneyi*, facilitating the parasite and causing 'spill back' to *S. glomerata* populations (Poulin et al., 2010; Peeler et al., 2011). Furthermore, increasing oyster biomass towards or above the carrying capacity by increasing either *S. glomerata* or *C. gigas* cultivation may cause immunological stress to oysters that amplifies the virulence of the parasite (Lenihan et al., 1999; Murray and Peeler 2005; Krkošek 2010). Thus monitoring whether *M. sydneyi* may potentially become virulent with increasing aquaculture intensity is a priority.

Observations suggest that the *S. glomerata – M. sydneyi* host-parasite relationship is presently stable among wild oyster populations of the Hawkesbury estuary. However, regular long-term monitoring of parasitic infection among wild and cultured *S. glomerata* is required for successful management of aquaculture and natural oyster resources because it is possible that evolution of *M. sydneyi*, which for other parasites has been observed within decades (Altizer et al., 2003), or environmental change may trigger sudden epidemics

6.2 Is *C. gigas* invasion a ticking time bomb?

It was predicted that QX disease and farming of triploid *C. gigas* might facilitate proliferation of *C. gigas* populations along rocky intertidal shores of the Hawkesbury River estuary. Nevertheless, populations of *C. gigas* in this estuary remain small despite being present in this estuary for several decades (Marine Pollution Research 2005) and do not appear to be negatively impacting *S. glomerata* (chapter 2). This contrasts with parts of Europe, North America and Patagonia where proliferation of *C. gigas* has been seen within less than two decades of introduction of the non-native species (Escapa et al., 2004; Ruesink et al., 2006; Brandt et al., 2008; Trimble et al., 2009; Troost 2010) and adds to growing evidence that there may be natural controls on *C. gigas* abundance in New South Wales (NSW) estuaries (Bishop et al., 2010). These may include pre-emption of space by native *S. glomerata*, sub-optimal abiotic conditions, abundant native predators that exert top-down control or susceptibility to diseases specific to the *Crassostrea* genus.

Along the Hawkesbury River (chapter 3) and in other NSW estuaries (e.g., Bishop et al., 2010), *S. glomerata* reach densities as high as 1400 oysters m^{-2} and commonly 100% cover on intertidal and shallow subtidal rocky shores, (chapter 3). Although *C. gigas* can outcompete *S. glomerata* at high density (Krassoi et al., 2008), pre-emption of space by native oysters may restrict the initial establishment of *C. gigas*. Successful invasion of *C. gigas* elsewhere has occurred along comparably bare mudflats, or mudflats that contain sparse populations of native bivalves such as mussels *Mytilus edulis*, rocks and boulders, and man-made structures that provided a hard substrate for attachment of *C. gigas* recruits (Mitchell et al., 2000;Nehls et al., 2006; Ruesink et al., 2006; Trimble et al., 2009; Markert et al., 2010; Troost 2010).

The abiotic conditions of the Hawkesbury River may also limit *C. gigas* distribution. Among the greatest *C. gigas* invasions in the world have occurred on sheltered tidal estuaries protected from wind and wave action (Mitchell et al., 2000;Diederich et al., 2005; Trimble et al., 2009; Troost 2010) but the Hawkesbury River estuary is a drowned river valley carved through Hawkesbury sandstone bedrock, that experiences prologued periods of drought during El Niño periods of Southern Oscillation (Jones 1987, 1990). Its shorelines are dominated by wave-exposed rocky substrate, on which temperatures may reach 50^{o}C during summer low-tides that occur in the heat of the day (Krassoi 2001), and suspension-feeding by oysters may be inhibited during periods of significant wind. While the spat of native *S. glomerata*, which have evolved in this system, can tolerate warm temperatures of up to 44^{o}C for 6 hr periods, the duration of aerial exposure on the mid intertidal shore, *C. gigas* recruits experience in excess of 50% mortality at temperatures above 36^{o}C (Krassoi 2001). Optimal spawning temperatures for the oyster (15-20^{o} C; Diederich et al., 2005; Nehls et al., 2006) are reached during Australian winters, when *C. gigas* do not spawn (Mason and Nell 1995) and are commonly exceeded during summer, the key reproductive period. Furthermore, whereas *S. glomerata* can persist in sub-optimal environments by keeping its valves closed for weeks at a time, the faster-filtering *C. gigas* cannot do this (Nell and Dunkley 1984; Krassoi et al., 2008). In dry periods, the lower reaches of the estuary become very marine (salinities close to 35 ppt), and the high salinities appear to limit *C. gigas* distribution (see Bishop et al., 2010). The Kumamoto strain of *C. gigas*, introduced to Tasmania and presumed to have been translocated to NSW, is not found in waters with salinities <5 or >33 in its native Japan (Thomson 1952; Imai 1980). Sampling by the present study along a salinity gradient confirmed the absence of *C. gigas* at high salinity sites, closest to the estuarine mouth.

Although results from laboratory studies indicate that *C. gigas* is not a preferred prey item of the native predatory oyster drill *M. marginalba* (chapter 5), it remains possible that other generalist predators may exert significant top-down control on *C. gigas* populations of east Australian estuaries. Adult *C. gigas* are weaker-shelled than equivalently sized *S. glomerata* (chapter 5) and may be more susceptible to larger vertebrate and invertebrate predators than the native oyster. Along rocky shores in Vancouver, Canada predation by crabs, seastars and whelks has been identified as a key control on the abundance of *C. gigas* (Ruesink 2007). Some native Australian predators are functionally equivalent to predators found within the native range of *C. gigas* (Fujiya 1970; Korringa 1976; Galleni et al., 1980), such as the oyster drill *Thais orbita* (Fairweather 1987), toad fish *Tetractenos spp* (Anderson and Connell 1999), and stylochid flatworm or 'oyster leach' *Imogine mcgrathi* (O'Connor and Newman 2001), and may contribute to the limited *C. gigas* distribution in the present study system.

Of particular recent importance in what might constitute a form of biotic resistance to *C. gigas* invasion is the recent outbreak of Pacific Oyster Mortality Syndrome (POMS), which causes mass mortality (up to 100%) among cultured and wild *C. gigas* in the Georges River, Sydney NSW (lat. 34 °S; 151° E). The causative agent of POMS is an Ostreid herpes virus (OsHV1; Zippel et al., 2011). A similar strain of virus has been associated with high mortalities of cultured *C. gigas* in France (Segarra et al., 2010) and Ireland (Zippel et al., 2011). Whether POMS has persisted but gone undetected, while playing a key role in limiting *C. gigas* invasion of rocky shores is unknown.

Although invasive species can require a time-lag of decades to centuries following introduction to proliferate (Rilov et al., 2004; Crooks 2005; Simberloff 2009), current

observations suggest that the innate abiotic and biotic characteristics that presently restrict proliferation of *C. gigas* in east Australian estuaries will continue to impede proliferation into the future. In estuaries of New Zealand, *C. gigas* outcompeted populations of *S. glomerata* within 10 years of introduction (Dinamani 1991). Hence, if detrimental effects of *C. gigas* were to occur in eastern Australia, we might expect they would already be evident. Instead, this system may be like Victoria Harbour, Hong Kong, that has arguably, analogous biological and geophysical characteristics to Australia, and relatively resistant to *C. gigas* invasion (Baker et al., 2003; Lam and Morton 2005). Despite an 800 year long history of aquaculture of *Crassostrea* spp. (*C. ariakensis* and *C. hongkongiensis*), in Hong Kong, to this day abundant populations of native rock oyster *Saccostrea cucullata* continue to dominate adjacent rocky shores (Lam and Morton 2004). The major difference between Hong Kong and east Australia is the presence of the parasitic disease (QX) in Australia, which could over time facilitate the non-native oyster (Bell et al., 2009). Yet even under this scenario of loss of *S. glomerata*, replacement by *C. gigas* would be expected to be restricted to sheltered shores where conditions would support the non-native.

6.3 Context dependent impacts of *C. gigas* invasion

As *C. gigas* has not become a prolific pest in eastern Australia (see also Summerhayes et al., 2009; Bishop et al., 2010), it is not surprising that in the estuaries examined, populations of *C. gigas* have not produced large ecosystem impacts. In the Hawkesbury and Port Stephens estuaries, populations of *C. gigas* formed relatively analogous habitats to those provided by *S. glomerata* (chapter 3). By contrast, in Europe, North America, and Patagonia *C. gigas* constitutes an entirely novel biogenic habitat on tidal flats that facilitates large changes in associated biodiversity, increasing the diversity and abundance of many associated organisms (Escapa et al., 2004; Ruesink et al., 2006; Markert et al., 2010; Troost 2010).

Laboratory experiments indicated that dense patches of *C. gigas* are capable of acting as sinks for *S. glomerata* larvae (chapter 4), reducing larval settlement, possibly through predation. Presently, in the NSW estuaries examined, wild *C. gigas* have not yet attained this density required to create negative impacts to larval settlement. Negative effects of *C. gigas* on *S. glomerata* larval settlement could, however, arise if the dominant species composition were to shift from *S. glomerata* to *C. gigas* such as is occurring in the Wadden Sea (Troost et al., 2008; Troost et al., 2009).

Exotic species may facilitate novel parasite transmission to native hosts (Peeler et al., 2011). Although there have been no reports of *S. glomerata* infection of the *C. gigas* POMS virus (NSW Primary Industries 2011b), the ramifications for *S. glomerata*, and associated ecosystems may be potentially very serious if host switching occurred. Indeed non-lethal POMS transmission to other oyster and clam species have been recorded (Arzul et al., 2001). Moreover, analysing *S. glomerata* for POMS infection now would indicate whether the virus stays dormant and unnoticed to predict any potential ecosystem impacts were virulence to manifest years after initial infection (Peeler et al., 2011). While strict government regulations prevent movement of oysters and thus associated parasites from estuary to estuary, monitoring parasitic loads among cultured and wild oysters are necessarily critical as boating activities, illegal translocations and hitchhiking on fishing gear, for example are not completely controllable.

In combination with the results of previous studies (see Padilla 2010), the results of the present study suggest that impacts of *C. gigas* invasion are highly context dependent, and sometimes unreasonably stigmatized. Whether or not populations of wild *C. gigas* were to expand in Australia, the ecosystem response is not predicted to be drastic as the non-native

species appears to exhibit a high degree of functional redundancy with *S. glomerata*. Indeed, some impacts elsewhere are considered positive where biodiversity and productivity increase due to *C. gigas* invasion (Diederich 2006; Markert et al., 2010). Large ecosystem impacts are less likely to be caused by the establishment of *C. gigas*, but rather its decline. Only after *C. gigas* has already caused persistent ecological change through replacement of *S. glomerata* and associated ecosystem goods and services, will a spontaneous decline in the abundance of *C. gigas* be conducive to potentially large ecosystem impacts (see McMahon 2002; Simberloff and Gibbons 2004).

6.4 Deeper unknowns: impacts of *C. gigas* invasion in the subtidal zone.

This and most previous studies on *C. gigas* in NSW estuaries (e.g. Krassoi 2001; Summerhayes et al., 2009; Bishop et al., 2010) only considered abundances and impacts of the non-native on the intertidal shore. There is, however, strong evidence to suggest that tidal elevation influences the distribution and abundance of *C. gigas* (chapter 3; Mitchell et al., 2000; Krassoi et al., 2008), with survival, growth (chapter 3) and competitive ability against *S. glomerata* (Krassoi et al., 2008) all increasing with decreasing elevation on the intertidal shore. Hence, it is possible that in the subtidal zone, where food is not as limiting and desiccation stress is not present, *C. gigas* might experience enhanced growth and survival (see Bayne 2002; Ruesink 2007) that allows persistence of much larger populations than seen in the intertidal. This would, however, be contingent on a favourable salinity and the absence of significant top-down control of oysters by fin-fish and swimming crustacean predators, that exert stronger predation pressure in the subtidal than the intertidal, which they can only access at high tide.

This study provides two lines of evidence to suggest that in the subtidal zone *C. gigas* might experience sufficient predation to limit proliferation. First, laboratory experiments found that a generalist predator, *Morula marginalba*, readily consumed *C. gigas* when *S. glomerata* was not available (chapter 5). Hence, if *C. gigas* proliferated in places where *S. glomerata* is less abundant, such as the lower intertidal zone, it is likely that *C. gigas* would experience significant predation by other generalist native predators. Second, shell strength testing revealed that among oysters greater than 50 mm in shell height, *C. gigas* was weaker-shelled than *S. glomerata* (chapter 5). Consequently, generalist predators, such as crabs, fish and rays, that target larger bivalves and commonly consume prey items in accordance with their energetic cost of opening versus energy benefit (Brown and Haight 1992; Brown 1998; Silva et al., 2010), may consume *C. gigas* at a greater rate than similar sized S. *glomerata*. Smooth toadfish *Tetractenos glaborus*, yellow fin bream *Acanothopagrus australis* and mud crabs *Scylla serrata* are among the abundant subtidal predators of larger oysters in NSW estuaries (Hill et al., 1982; Edgar and Shaw 1995; Holloway and Connell 2002) that may target weaker-shelled *C. gigas*.

If, however, *C. gigas* is able to proliferate in the subtidal, its impacts may be greater than seen in the intertidal for several reasons. First, it is likely that *C. gigas* would attain larger size in the subtidal than the intertidal, where it closely matches the habitat formed by *S. glomerata*. Larger oysters would, presumably, provide greater surface area of attachment for epibenthic organisms (Kochmann et al., 2008). Furthermore, in the subtidal, where oysters can filter for the entire tidal cycle, the negative effects of *C .gigas* on larval settlement, seen at high oyster density (chapter 4), might be more pronounced. Impacts on larval settlement may extend to other organisms with pelagic larvae such as spirorbid and serpulid polychaetes, barnacles, bryozoans, sponges, and mussels (Glasby 2000). Reduced velocity and exposure to

tidal fluctuations at the subtidal zones may also augment the larval sink effect of *C. gigas* substrates where the vertical movements of larvae are not as influenced by turbulence (Turner et al., 1994; Zimmer-Faust and Tamburri 1994; North et al., 2008).

Further studies comparing the ecosystem goods and services provided by *C. gigas* and *S. glomerata* at the subtidal zone, particularly on muddy bottoms underneath and adjacent to oyster leases where *C. gigas* may thrive (Markert et al., 2010) are needed. The benthic layers of the subtidal zone also receive a greater load of oyster biodeposition from leases (Crawford et al., 2003) therefore investigating processes in the benthos is also a crucial next step in predicting the impacts of *C. gigas* invasion in Australian estuaries.

6.5 Impacts of farming selectively bred oysters

This study did not find evidence that genetic pollution of wild *S. glomerata*, which could result from farming selectively bred *S. glomerata*, impacts associated biological communities. Contrary to what was predicted, *S. glomerata* selectively bred for QX disease resistance and rapid growth did not attain greater sizes than natural oysters when grown on rocky shores free of disease, and consequently supported similar epibiotic communities (chapter 3). Similarly, results suggested that oyster genotype does not influence its role as a substrate for *S. glomerata* larval settlement. Although water borne cues, normally in the form of proteins released from adult conspecifics may increase larval settlement rates on conspecifics parents (Zimmer-Faust and Tamburri 1994), cues specifically associated with QX disease resistant *S. glomerata*, that are not present in natural counterparts (Simonian et al., 2009) did not have any detectable effect on *S. glomerata* larval settlement patters in this study. Despite its physiological role in improved growth rates of QX disease resistant *S.*

glomerata (Bayne 2000), the faster feeding rates of QX disease resistant *S. glomerata* compared to natural counterparts had no effect on larval settlement (chapter 4). While suction currents created by bivalve species that filter-feed more quickly than other species may positively influence pelagic larval settlement (Troost et al., 2008; Troost et al., 2009), the physiological intra-species differences in feeding rates on larval settlement in our experiments appear to be less pronounced. Presumably, among wild populations, differences in feeding currents produced by the various genotypes would be further depressed be external influences. It is clear that generalizations about the impacts of genotype on wild oyster population phenotype are therefore very hard to make as *S. glomerata* are highly phenotypically plastic and capable of modifying shell growth, shell shape, somatic growth and feeding rates in response to natural environmental conditions (Paterson et al., 2003; Bayne and Svensson 2006; Parker et al., 2011).

In an estuary where the Sydney rock oyster industry has now for 8 years been entirely based on culture of *S. glomerata* selectively bred for disease resistance, wild oyster populations remained relatively stable (chapter 2). A succession of *S. glomerata* cohorts continued to contribute to adult populations that were comparable in population structure to *S. glomerata* occupying shores of Port Stephens where QX disease resistant oysters are not farmed (Bishop et al., 2010). Contrary to observations among some other selected lines of oysters (Carlsson et al., 2006) and fish (Aho et al., 2006; Frost et al., 2006), genetic diversity of third generation mass selected for faster growth (English et al., 2000) and sixth generation QX disease resistant *S. glomerata* (Wayne O'Connor, unpublished data) is not reduced through successional hatchery breeding. Several studies have indicated that genetic variation may not be necessarily reduced by mass selection of oysters and that hatchery bred lines show comparably similar genetic heterogeneity as observed among wild counterparts (Appleyard

and Ward 2006; Hong and Li 2007). Therefore the genotypic diversity of wild *S. glomerata* and the adaptive capacity of future generations to environmental stressors are unlikely to be affected by selective breeding for aquaculture. Indeed, mass selection might actually benefit the wild *S. glomerata* populations in the likely onset of climate change related stressors, as selected lines of *S. glomerata* are more resilient than natural counterparts to ocean acidification (Parker et al., 2011).

Present results add to previous observations that suggest it is not the genetic pre-disposition alone that determines wild oyster phenotype, but the interaction of environmental conditions and genetics (Evans and Langdon 2006). Although the impacts of genetic modification in this study are presently unrecognizable from these experiments, impacts may augment after a few generations.

6.6 Implications for Australian estuaries and oyster aquaculture

Present observations suggest that efforts to restore the oyster industry of a QX-impacted estuary whilst minimising *C. gigas* invasion through culture of sterile triploid oysters have been to this point, successful. The industry has benefited, while there is no evidence of proliferation of the non-native oyster in the wild, despite the possibility of reversion to diploidy. The cultivation of triploid *C. gigas* in Australia alongside *S. glomerata* has allowed farmers to diversify stock as a risk management initiative to counter potential impacts from QX disease and winter mortality. Since research for this thesis began, the contribution of commercial production of triploid *C. gigas* has grown from virtually nothing to $2.9 million, as its aquaculture is now permitted in the Shoalhaven/Crookhaven estuary (2008), Wallis

Lake (2009) and Wapengo Lake (2011) (NSW Primary Industries 2011c). It is probable that further expansion of the *C. gigas* industry will occur.

This study suggests that from an ecological point of view, triploid *C. gigas* aquaculture does not present significant risk. In the Hawkesbury River, there is no evidence that the farming of triploid *C. gigas* has negatively impacted wild *S. glomerata* populations, and experiments suggest that even if reversion of triploid *C. gigas* to diploidy were to occur, ecological impacts would be minimal. To the contrary, invasion of *C. gigas* may benefit coastal landscapes where ecosystem goods and services are lost following declines in native oysters (Zavaleta et al., 2001; Bishop and Peterson 2006). The important ecosystem goods and services provided by native *S. glomerata* oysters including habitat provision, larval settlement substrate and predator-prey relationships are generally matched by *C. gigas*. Any differences observed were quantitative not qualitative, thus estuarine ecosystems are unlikely to be drastically modified by the proliferation of wild *C. gigas*. This study therefore adds to growing evidence that the cultivation of *C. gigas* in Australia has little environmental impact (Crawford et al., 2003), and provides additional support to the growing body of literature explaining how *C. gigas* invasions are not necessarily harmful to recipient environments (Padilla 2010).

Food security is a major global priority. Aquaculture is predicted to contribute 50 % of total fisheries product by 2012 (FAO 2010). It is essential that fisheries can rely on stable, manageable and sustainable aquaculture technologies, particularly now as diseases among native species emerge through increasing pressures of climate change, pollution and coastal development. In the interest of oyster aquaculture, this thesis suggests that selective breeding programs that are designed to maintain genetic diversity, and introduction of triploid exotic

species might be part of the solution, rather than the problem, for improving sustainable aquaculture industries.

6.7 References

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