

**Abiotic and biotic factors influencing the invasion of *Carcinus maenas* in southern New
South Wales, Australia**

By

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This thesis is presented as partial fulfilment to the requirements for the degree of Doctor of
Philosophy in the School of Biological Sciences, Macquarie University

Submitted February 2014

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General summary

Non-indigenous species are among the greatest threats to global biodiversity and collectively cause significant economic damage. The European shore crab *Carcinus maenas* is a global marine pest that has been present in Australia for over 100 years, but has been little studied in this part of the world. This thesis aimed to enhance our knowledge of the distribution and abundance of *C. maenas* in New South Wales (NSW) and the role that the unique abiotic and biotic features of the Intermittently Open and Closed Lakes and Lagoons (ICOLLs) that dominate the NSW coastline play in the invasion. ICOLLs can spend periods of days to years disconnected from the ocean by a sand bar, and display high spatio-temporal variability in their abiotic and biotic conditions. Trapping surveys across 14 estuaries (including ICOLLs) and spanning 8 dates found that in NSW the abundance of *C. maenas* is low and displays considerable spatial variation at the scales of estuaries and habitat. The detection of crabs in estuaries was positively correlated with the proportion of time estuarine entrances were open. Within estuaries, abundances were greater in mangroves than in saltmarsh, seagrass, oyster or bare sediments and were negatively correlated with the abundance of several native predators. Hence the opening and closing of estuarine entrances influenced *C. maenas* invasion by determining opportunities for recruitment, and possibly by determining the predator and habitat assemblages within these. Recruitment and cohort analyses indicated that *C. maenas* larvae primarily settle in the late Austral winter to spring, retaining the reproductive phenology of the northern hemisphere. Overall, this study suggests that although *C. maenas* has been present in Australia for over a century its abundance, and hence impact, remains low along the NSW coast due to a combination of abiotic and biotic factors.

Certification

I hereby declare that this work has not been submitted for a higher degree at this or any other University or institution.

Clifford J. Garside

February 2014

Chapter contributions

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

Chapter 1

General Introduction

I have completed this literature review and writing for this chapter with constructive help from my supervisor Melanie Bishop and my co-supervisors Melinda Coleman, Brendan Kelaher and Tim Glasby.

Chapter 2

The frequency of connection of coastal water bodies to the ocean predicts *Carcinus maenas* invasion

Garside, C.J., Coleman, M.A., Glasby, T.M., Kelaher, B.P. and M.J. Bishop

My contribution to the research and paper: Concept - 30%; Data collection - 100%; Analysis - 95%; Writing - 90%; Total – 78.75%

The original concept was developed prior to my involvement by the co-authors; experimental design, data analysis and editorial assistance was in conjunction with my supervisor Melanie Bishop. Further editorial help was given by Melinda Coleman, Brendan Kelaher, and Tim Glasby. Tim Glasby also helped with an alternative concept in data analysis.

This paper has been accepted for publication in the international peer reviewed journal, *Limnology and Oceanography*.

I have presented this chapter at the Eighth International Conference on Marine Bioinvasions in Vancouver, British Columbia, Canada in 2013.

It has also been presented at the AMSA 50th Anniversary Golden Jubilee Conference on the Gold Coast, Queensland in 2013.

Chapter 3

The timing of *Carcinus maenas* recruitment to a south-east Australian estuary differs to that of native crabs

Garside, C.J., Stone, L.J., Glasby, T.M., and M.J. Bishop

My contribution to the research and paper: Concept - 70%; Data collection - 100%; Analysis - 100%; Writing - 85%; Total – 88.75%

The original concept was developed with information from Tim Glasby. Luke Stone helped in the field and in the lab, and in completing a third year project on *Carcinus maenas*, raised some interesting discussion points that benefited the manuscript. Experimental design, data analysis and editorial assistance was in conjunction with Melanie Bishop my primary supervisor. Some data used was donated by the Sapphire Coast Marine Discovery Centre. Crab species identification was confirmed by Dr. Shane Ahyong from the Australian History Museum. Further editorial help was given by Melinda Coleman, Brendan Kelaher, and Tim Glasby.

This chapter was presented at the Sapphire Coast Marine Conservation Society; Eat the Invader session, at the Wharf and Aquarium in Merimbula New South Wales, in 2013.

Chapter 4

The distribution of the European shore crab, *Carcinus maenas*, with respect to mangrove forests in south-eastern Australia

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My contribution to the research and paper: Concept - 90%; Data collection - 100%; Analysis - 100%; Writing - 85%; Total – 93.75%

Experimental design, data analysis and editorial assistance was in conjunction with Melanie Bishop. Further editorial help was given by Melinda Coleman, Brendan Kelaher and Tim Glasby.

This Chapter has been accepted for publication by the international journal, *Journal of Experimental Marine Biology and Ecology*.

Chapter 5

Putative predators of *Carcinus maenas* in eastern Australia

Garside, C.J., Coleman, M.A., Kelaher, B.P. and M.J. Bishop

My contribution to the research and paper: Concept - 95%; Data collection - 100%; Analysis - 100%; Writing - 85%; Total – 95%

Experimental design, data analysis and editorial assistance was in conjunction with Melanie Bishop. Fish identification help was received from Dr Culum Brown (Macquarie University), Mark McGrouther and Sally Reader (Australian History Museum). Further editorial help was given by Melinda Coleman, Brendan Kelaher and Tim Glasby.

Chapter 6

General Discussion

I have completed this synthesis on the chapter findings with constructive feedback from my supervisor Melanie Bishop and co-supervisors Melinda Coleman, Brendan Kelaher and Tim Glasby.

Ethics approval

Ethics committee approval was obtained (2011/056–3). Ethics certificate in Appendix 2.

Acknowledgements

I would like to acknowledge the contributions of Mary, Boden and Brielle Garside who tirelessly helped with fieldwork. I would like to thank my primary supervisor Melanie Bishop for her support and guidance throughout this thesis, my co-supervisors Melinda Coleman, Tim Glasby and Brendan Kelaher for improving each chapter and the three reviewers who's comments have improved the quality of this Thesis. I also acknowledge the researchers and staff in the Benthic Ecology Group at Macquarie University during my candidature: Emma Wilkie, Brad Coates, Andreas Nicastro, Mirella Verhoven, Lara Ainley, Dan Bateman, Joe Kenworthy, Jen Rowland, Ramilla Furtado, Valter Amaral, Belinda Cooke, Dominic McAfee and Peter Mahoney. I also want to thank my parents and siblings. Further acknowledgements are included in each of the data chapters.

CHAPTER 1

General introduction

Biological invasions

Invasive species are, along with habitat destruction, pollution, overfishing and climate change, significant modifiers of global marine biodiversity (Lodge 1993, Williamson 1996, Stachowicz et al. 2002, Li and Daler 2004, Worm et al. 2006, Brockerhoff and McLay 2011). Species invasions occur when non-indigenous species (NIS) are translocated to novel environments, establish, proliferate and spread (Sakai et al. 2001). Although not all NIS produce large negative impacts (Davis et al. 2011), collectively invasive species are considered an increasing threat to the structure and function of ecosystems (Mack et al. 2000). Additionally, marine invasive species can cause billions of dollars of economic damage (Pimentel et al. 2005). For example, they can foul the hulls of ships, decreasing their efficiency, clog pipes and foul infrastructure, negatively impact economically important wild fisheries and aquaculture and contribute to losses of important ecosystem functions (Vitousek et al. 1996, Pimentel et al. 2005, Pejchar and Mooney 2009).

Invasion rates have increased exponentially with the growth of world trade (Ruiz et al. 1997, Mack et al. 2000). At any one time, it is estimated that over 7,000 species are being transported by commercial shipping to new environments (Rilov and Crooks 2009). Of translocated species, approximately 10% establish and of those establishing, approximately 10% become invasive (Williamson and Fitter 1996). New invasions can spread quickly over large areas from a point of introduction (Grosholz and Ruiz 1996, de Rivera et al. 2007), through human-mediated and natural mechanisms (Wasson et al. 2001, de Rivera et al. 2007, Blakeslee et al. 2010). Minimizing the establishment, proliferation and spread of NIS is dependent on a comprehensive understanding of factors that influence each stage of the invasion process.

The establishment, proliferation and spread of NIS are determined by the life history of the invading species, the vector of introduction, the supply of propagules, the match between the biology of the NIS and the novel abiotic environment, and characteristics of the native community to which the invader is introduced (Brockerhoff and McLay 2011). Following

arrival at a novel location, abiotic factors act as ‘filters’, determining those NIS whose environmental tolerances will enable them to establish in the recipient environment (Carlton 1996, Sakai et al. 2001, Olyarnik et al. 2009). Biotic interactions then determine whether the NIS is able to establish and proliferate (Olyarnik et al. 2009). NIS may be more likely to successfully establish and proliferate in environments where they escape parasites, fill empty niches and/or gain refuge from competition and predation (Lodge 1993, Torchin et al. 2003). The invasion resistance theory proposed by Elton (1958) suggests that the more diverse a receiving community, the more resistance it has to invasion. Empirical studies, however, suggest that the relationship between native community diversity and invasibility depends on spatial scale (Fridley et al. 2007). Furthermore, causality of the relationship is often difficult to establish because low native biodiversity is often caused by disturbance and the disturbance itself may facilitate the invader (Crooks et al. 2011). Consequently, knowledge of why the distribution of certain invaders remains relatively localized around the point of introduction, while others spread widely, remains limited. Furthermore, it remains unclear why specific NIS successfully invade some locations more than others.

***Carcinus maenas* – a global invader**

The European shore crab (*Carcinus maenas*, Linnaeus 1758) is one of the most widespread marine invaders (Lowe et al. 2000). Native to northwest Europe and North Africa, the crab has established non-native populations on the Atlantic and Pacific coasts of North America, Japan, South America, South Africa and southern Australia (Carlton and Cohen 2003, Thresher et al. 2003). The shore crabs’ euryhalinity, broad thermal tolerance and high fecundity enable it to be particularly adept at colonising new areas and establishing permanent populations (Cohen et al. 1995, Rodriguez and Suarez 2001, Thresher et al. 2003). Vectors for *C. maenas* distribution around the globe have been linked to ship fouling, ballast water, dry ballast, bait and the seafood trade, as well as aquaculture (Ruiz et al. 1997, Carlton and Cohen 2003).

Carcinus maenas is a generalist predator of invertebrates known to consume prey from more than 150 genera (Cohen et al. 1995). It is commonly found on hard and soft substrata in the littoral and sub-littoral zone, down to 60m (Broekhuysen 1936, Almaca 1962, Naylor 1962, Crothers 1967). One of the largest impacts of *C. maenas* invasions has been on

commercial shellfish and aquaculture industries, for example the venerid bivalves *Transennella tantilla* and *T. confusa* in Bodega Harbour, California (Grosholz and Ruiz 1995), the soft-shell clam *Mya arenaria* in New Hampshire, Maine and Plum Island Sound, Massachusetts (Glude 1955, Ropes 1968), the bay scallop (*Argopecten irradians*) in the Niantic River, Connecticut (Marshall 1960), and the Manila clam (*Venerupis philppinarum*) in Tomales Bay, California (Grosholz et al. 2001). Other impacts of *C. maenas* include modification of habitat (Garbary et al. 2013), competition with *Cancer* spp. crabs (McDonald et al. 2001) and American lobster (*Homarus americanus*; Williams et al. 2009), and induced changes in the behaviour and morphology of several prey species (Trussell and Smith 2000, Klassen and Locke 2007).

Carcinus maenas naturally disperses during a pelagic larval stage and subsequent active migration of adults up to 15 km (Thorson 1961, Gomes 1991, Queiroga 1996, 1998, Yamada and Gillespie 2008). *Carcinus maenas* produces planktonic larvae that pass through six developmental stages; a prezoa, 4 zoeal stages, and megalopae, before metamorphosis into a benthic juvenile. The crab grows through 18–20 moult cycles before reaching its maximum size (~86 mm male; ~70 mm females) and terminal anecdysis (Crothers 1967). *Carcinus maenas* has been shown to reach larger sizes in its introduced ranges especially on the west coast on North America (McGaw 2001). Following migration of ovigerous females down estuaries towards the more stable environmental conditions of the mouth, larvae are hatched from eggs during night time ebbing tides (Queiroga et al. 1994). Larvae are typically transported to the sea shortly after spawning where they pass through the four zoeal and megalopal stage (Crothers 1967). The distance that *C. maenas* travel during their planktonic stage is determined by ocean currents, temperature, salinity, tides, nutrients, and predation (see; de Rivera et al. 2007, Yamada and Kosro 2010). Larvae may be found up to 40 km offshore (Queiroga 1996). The megalopae reinvade estuaries, settle and metamorphose into benthic juveniles (Queiroga 1996).

Carcinus maenas utilise different habitats during its life stages and among seasons. In the warmer months, juveniles and small adults are most common in complex habitats of the intertidal, while adults are most abundant in the subtidal habitats (Crothers 1968). In colder months, populations become less active and most of the population remain in the subtidal zone (Naylor 1962, Crothers 1968). Adult *C. maenas* make tidal migrations to feed with the flood tide and most migrate back to the subtidal zone with the ebb tide (Hunter and Naylor

1993). In North America and South Africa, *C. maenas* is more common in protected embayments and on moderately exposed shores, than at wave-exposed locations (Grosholz and Ruiz 1996). Where present on the outer coast, *C. maenas* is absent from soft sediments, instead occupying hard substrates at low abundance (Grosholz and Ruiz 1996). The availability of prey resources is thought to contribute to its habitat utilization (Grosholz and Ruiz 1996).

The wide ranging thermal and saline tolerances of *C. maenas* have contributed to its success as an invader. Adult *C. maenas* can survive in salinities ranging from 4–54‰ (Crothers 1967, Beukema 1991) while larvae do not survive below 20‰ (Nagaraj 1993, Anger et al. 1998, Bravo et al. 2007). The thermal tolerance of adults is also wide ranging from 0–30°C and narrower for larvae from 6–25°C (Dawirs and Dietrich 1986, Nagaraj 1993, Harms et al. 1994).

Range expansion of *Carcinus maenas* following introduction

Within its non-native range, *C. maenas* has been observed to spread from its point of introduction at rates of 1.7–200 km/yr (Grosholz 1996, Thresher et al. 2003; Table 1.). This variable pattern of spread may be dictated by several mechanisms including current direction and speed (Pringle et al. 2011), predation (de Rivera et al. 2005), weather patterns, such as El Niño that warm coastal waters and increase current speeds (Yamada and Kosro 2010), as well as the presence of secondary vectors for translocation, such as recreational boating (Darbyson et al. 2009), intra-regional transport (Wasson et al. 2001) and aquaculture (Cohen et al. 1995). In some instances, where propagule pressure has been high, spread of populations has also occurred in the opposite direction to dominant flows (Byers and Pringle 2008, Pringle et al. 2011).

Table 1. Post-introduction rates of range expansion for non-native populations of the European shore crab *Carcinus maenas*¹, *Carcinus aestuarii*² and hybrids³. *Carcinus aestuarii* has been included in the table as it is from the same genus and can produce hybrids with *C. maenas*. Distances and rates of range expansion are based on the assumption of a single introduction.

Locations of range expansion	Time period	Distance of range expansion (km)	Range expansion per year (km y ⁻¹)	Source of data
South Africa ^{1,3}				
Table Bay Harbour, Cape Town to Saldanha Bay South Africa	1983–1990	15	1.9	Le Roux et al. 1990
Table Bay Harbour, Cape Town to Saldanha Bay South Africa	1983–1990	117	16.7	Griffiths et al. 1992,
East coast of N. America ¹				
Cape Cod to southern Canada	1872–1951	690	8.7	Glude 1955
Passamaquoddy Bay to south-eastern Gulf of St. Lawrence, CA.	1953–2000	~1000	21.3	Audet et al. 2003
West coast of N. America ¹				
San Francisco Bay, California	1989–1992	80	26.6	Cohen et al. 1995
San Francisco Bay to Bodega Bay, California	1993–1994	120	120	Grosholz and Ruiz 1995
Humbolt Bay to Monterey Bay, California	1989–1996	*	55	Grosholz and Ruiz 1996
Western North America from San Francisco north	1993–1999	1200	200	Yamada et al. 2000 in Thresher et al. 2003
San Francisco Bay, California to Barkley Sound B.C. CA.	1989–1998	1200	133	See and Feist 2010
Patagonia, South America ¹				
Comodoro Rivadavia, Patagonia	2000–2004	200	50	Hidalgo et al. 2005
Australia ¹				
Adelaide, South Australia	1970s–1998	40	1.7	Thresher et al. 2003
Port Phillip Bay, Victoria to Two Fold Bay, Eden NSW	1890–1986	~620	6.5	Hutchings et al. 1989, Carlton and Cohen 2003
Port Phillip Bay Victoria to Tasmania	1993	200		Gardner et al. 1994
Tokyo Bay to Sagami, Osaka and Dokai Bay, Japan	1984–1996	~500	41.6	Carlton and Cohen 2003
Tokyo Bay to Suo-nada Sea	1984–2005	*	1.55	Koike and Iwasaki 2010
~ distances were personally calculated *total distance was not given				

Although *C. maenas* has established and rapidly spread from its point of introduction along several coastlines (Table 1), its spread has been less than predicted in some places (Carlton and Cohen 2003, Thresher et al. 2003, Hampton and Griffiths 2007). This could be due to

abiotic conditions, top-down control by the native community (Hunt and Yamada 2003), and competition with native species (de Rivera et al. 2005) or disease (Sakai et al. 2001).

C. maenas is susceptible to predation during its benthic and pelagic phases (Crothers 1968). Along the east coast of the USA, predation by the blue crab *Callinectes sapidus*, perhaps in combination with other factors, is thought to have restricted the southward spread of *C. maenas* (de Rivera et al. 2005). Rock crabs (*Cancer irroratus*), Jonah crabs (*Cancer borealis*) and American lobster (*Homarus americanus*) have been hypothesised to contribute to the non-native crab's mostly intertidal habitat distribution along this coastline by preying upon and competing with *C. maenas* in subtidal habitats (Williamson 1996, Ruesink et al. 2005), although *C. maenas* can out-compete and prey upon juvenile *H. americanus* (Rossong et al. 2006, Williams et al. 2006). On the West Coast of North America, aggressive rock crabs of the genus, *Cancer*, have been identified as predators of *C. maenas* that potentially reduce the non-native species abundance (Hunt and Yamada 2003, Jensen et al. 2007). Several other species have been shown to predate *C. maenas* in the crab's native (Crothers 1968) and invasive ranges (Cohen et al. 1995, Klassen and Locke 2007).

The Australian *Carcinus maenas* invasion

Within Australia, *C. maenas* has been present since at least the late 1890s (Fulton and Grant 1900, 1902). In Victoria, the crab was first observed in Port Phillip Bay, a major shipping port and hot-spot for biological invasions (Hewitt et al. 2004). It is suspected that the crab arrived in the dry ballast used by ships (Proctor and Thresher 1997). In New South Wales (NSW), the crab was first observed in Port Jackson (near Sydney) in the 1890s (Ahyong 2005), although recent records from this location are lacking. *Carcinus maenas* was observed on the south coast of New South Wales in 1971 (Hutchings et al. 1989), in South Australia in 1976 (Zeidler 1978) and Tasmania in 1993 (Gardner et al. 1994). *Carcinus maenas* was detected in Western Australia in 1965 (in Zeidler 1978) but has not since been observed (Wells et al. 2010).

Within its Australian range, *C. maenas* occupies a variety of habitats, ranging from intertidal to subtidal habitats (Day and Hutchings 1984, Hutchings et al. 1989, Proctor 1997, Thresher et al. 2003). In Tasmania, the crab is found from shallow subtidal habitats down to 4 m and is

found in the full range of habitats present in estuaries and embayments, from seagrass beds to anoxic mud (Proctor 1997, Thresher et al. 2003). In South Australia, existing records of *C. maenas* largely come from degraded subtidal habitats around Adelaide (Zeidler 1978). In Victoria, *C. maenas* is considered a shallow water species that has not been recorded in trawls or benthic sleds in deeper parts of Port Phillip Bay (Cohen et al. 2001). It is common on the Victorian coast in sheltered bays and absent from exposed western shores (Cohen et al. 2001). At Lakes Entrance and Mallacoota Inlet in Victoria, *C. maenas* have been found in water 2 m deep and juveniles up to 3 cm in carapace width have been found under boulders in lower intertidal habitats (D.C. Bateman, unpublished data). Port surveys in Two Fold Bay, NSW, found *C. maenas* were common amongst intertidal rocks and mud (Hutchings et al. 1989). Another investigation in 1996 found the crab to be restricted to the brackish estuarine tributaries of the Bay (Pollard and Rankin 2003). *Carcinus maenas* has also been found on intertidal sandflats at Merimbula Lake and subtidally in Back Lake, NSW (Day and Hutchings 1984).

Although *C. maenas* has been present in Australia for over a century (Fulton and Grant 1900, 1902, Ahyong 2005), its impacts (if any) remain largely unknown. A paucity of baseline data, required for before and after comparisons, may have contributed to the small number of impact assessments. Although impact assessments can be done without pre-invasion data incorporating proper replication using reference sites, to our knowledge, these have not been done for *C. maenas* in Australia. Furthermore, there have been few investigations of impacts of the crab outside Tasmania, because it has been assumed that the low density of *C. maenas* is insufficient to cause significant impacts on native populations. In Tasmania, Walton et al. (2002) predicted *C. maenas* to have a significant negative effect on the venerid clam *Katelysia scalarina* fishery and induced morphological changes in a predatory whelk *Haustorium vinosum* (Freeman et al. 2013). There is increasing concern about the threat that *C. maenas* poses to the Australian aquaculture industry, valued at more than \$84 million dollars (AUD) per year (Australian Government 2008).

Despite the lack of evidence for widespread impacts of *C. maenas* in Australia, it is regarded as a pest species and is subject to a National Control Plan (NCP), developed in 2008 (Australian Government 2008). The purpose of the NCP is to determine a nationally agreed response to *C. maenas* management and ongoing strategies that give the best options to control spread and deleterious effects (Australian Government 2008). Included in the plan

are frameworks for implementing management strategies, at local and regional scales that: (1) prevent further incursion of the pest; (2) respond to new incursions; and (3) manage impacts of the pest. Strategies for preventing further incursions include ballast water management, guidelines for biofouling and restrictions on translocation of aquaculture stock. Trapping, biological control and chemical control are discussed as potential options to eradicate new incursions or control abundance (Australian Government 2008). This comprehensive plan highlights the lack of knowledge about the Australian *C. maenas* invasion. In particular, the plan highlights that major research gaps exist in Australia in (1) understanding the invasion process of *C. maenas*, and in particular, the role of native predators in influencing its distribution and (2) the capacity for *C. maenas* to expand its range. Given *C. maenas*' history of spread and proliferation elsewhere in the world, it is important to understand what factors are limiting *C. maenas*' distribution along the coastline of mainland Australia to understand the potential for range expansion of this crab.

Factors influencing the distribution of *C. maenas* in Australia

Based on its environmental tolerances, the projected range expansion of *C. maenas* along the east coast of Australia is to southern Queensland (28°S Latitude; Carlton and Cohen 2003, Summerson et al. 2007, Compton et al. 2010). Yet, despite being present in Australia for >100 years, *C. maenas* has not realised this full predicted range (Carlton and Cohen 2003, Summerson et al. 2007, Compton et al. 2010). Instead, the established range of *C. maenas* remains limited to south of Batemans Bay, NSW (35.74°S, T.M. Glasby, unpublished data), although there have been ephemeral observations of adult *C. maenas* as far north as Botany Bay, NSW (Ahyong 2005). Thresher et al. (2003) determined the rate of spread of *C. maenas* up the east coast of Australia to be 1.7 km/yr, which is one of the lowest rates of spread for *C. maenas* anywhere in the world (Table 1).

In other parts of its range, boundary currents have been implicated in limiting the 'upstream' (i.e. counter current) range expansion of *C. maenas* (Pringle et al. 2011). Along the east coast of Australia, the southward flowing East Australian Current (EAC) dominates circulation patterns, moving warm water down the east coast of Australia from its source in the Coral Sea to the east coast of Tasmania at speeds of up to 3.6 ms⁻¹ (Mata et al. 2006). Yet, although migration of *C. maenas* up the east coast of Australia is against the prevailing direction of this boundary current, large cyclonic and anti-cyclonic eddies generated by the

current in summer and autumn (Mata et al. 2006), combined with its weakened flow in winter (Ridgway and Godfrey 1997), can facilitate counter-current flow. Oceanographic modeling by Roughan et al. (2011) indicated that larvae of *C. maenas* could be transported up to 200 km in a northerly direction along the NSW coast. Other marine invertebrates found along the east coast of Australia display genetic structures that are consistent with northward as well as southward larval dispersal (e.g. sea anemone: Hunt and Ayre 1989, sea urchin: Banks et al. 2007, damsel fish: Curley and Gillings 2009, kelp: Coleman et al. 2011) and it is unlikely that this is a key factor limiting northward range expansion of this non-native crab.

Instead, the east Australian expansion of *C. maenas* may be slowed by the periodic closure of the entrances of estuaries along this coastline to the sea. Along the NSW coast, Intermittently Closed and Open Lakes or Lagoons (ICOLLs) are the dominant feature and account for 92% of the total number of estuaries (Roy et al. 2001, Haines et al. 2006). ICOLLs have a variable connection to the sea and can spend prolonged periods as closed systems during dry periods such as those typified by El Niño years (Roy et al. 2001, Haines et al. 2006). The northerly longshore drift of sand forms and maintains a berm above high tide, closing off ICOLLs to the sea for days to years (Roy et al. 2001, Haines et al. 2006). Storm events and heavy rain can breach the bar built lagoons and restore tidal inundation. Additionally, some ICOLL entrances are artificially opened for flood mitigation and to maintain water quality (Everett et al. 2007).

The entrance status of an ICOLL influences its physical, chemical and biological environment (Haines 2006). During prolonged periods of connectivity with the coastal environment, environmental conditions within the ICOLL remain relatively constant, there is consistent tidal inundation and dispersal of biota can occur between the ICOLL and adjacent coastal systems. When ICOLL entrances are closed, water quality is highly influenced by patterns of rainfall, evaporation and input of nutrients and other pollutants from the catchment. The water temperature is no longer buffered by tidal exchange and is instead heavily influenced by air temperature, and the ICOLL is a closed system into and out of which dispersal cannot occur. Within ICOLLs that are mostly open to the ocean, habitat forming species such as mangroves, oyster reef and saltmarsh that require a tidal cycle or good flow can establish. These species are important detrital contributors and provide substrate for epiphytic organisms, shelter for small molluscs, crustaceans and fish (Roy et al. 2001). Within ICOLLs

that are mostly closed, the species requiring a tide or good flow are absent. An investigation into fish communities within ICOLLs revealed entrance condition played a more important role in determining their composition than biogeographic processes (Jones and West 2005, Hadwen et al. 2007). Extended periods of entrance closure have seen the collapse of fish communities in several ICOLLs (Bennett 1985, Pollard 1994; in Griffiths, 1999) and recruitment failure in species that need pelagic environments to spawn (Kok and Whitfield 1986; in Griffiths, 1999). On short term time scales, however, artificial opening of ICOLLs can also contribute to fish kills (Becker et al. 2009). Macrobenthic communities have been shown to be less diverse in closed ICOLLs than open ones (Dye and Barros 2005).

ICOLL entrance dynamics may influence spatio-temporal patterns in the abundance of *C. maenas* by affecting opportunities for recruitment, by influencing abiotic conditions or by changing the structure and function of native biological communities. When an ICOLL is closed to the ocean, natural recruitment of *C. maenas* into the ICOLL from other sources cannot occur. Anthropogenic vectors, such as translocation by the movement of aquaculture stock (Ruiz et al. 1997) or boats, fishing nets or landward migration of individuals may be the only means of introduction. Hence, during periods of ICOLL closure, the presence of *C. maenas* in the ICOLL may be dependent on: (1) previous introduction during open periods; (2) the ability of *C. maenas* to survive and reproduce within the closed ICOLL; or (3) human mediated introductions. During periods of entrance closure, for example, the salinity may rapidly rise or fall, depending on the ratio of rainfall to evaporation (Kjerfve 1994, Haines et al. 2006). In several NSW ICOLLs the salinity has been observed to exceed 70‰ during periods of closure that have coincided with hot dry summers and caused mass mortality of *C. maenas* (M.A. Coleman, unpublished data). Furthermore, during hot dry periods, the water temperature of shallow ICOLLs may be greatly influenced by air temperatures. Such environmental conditions may challenge the survivorship of even the most euryhaline and thermally tolerant species such as *C. maenas*. The shift in the ecology of ICOLLs between periods of connectivity with the ocean and periods of entrance closure may also influence prey availability and predation on *C. maenas*.

This thesis

Homogenisation of the world's oceans is an increasing threat to biodiversity. Invasive species cause ecological and economic damage on a global scale. Factors controlling dispersion and subsequent invasion have been well described. The extent to which natural or anthropogenic mechanisms facilitate and/or disrupt the spread of individual species requires substantially more empirical study. Knowing which factors contribute to the proliferation and spread of a species can inform environmental managers of where to focus efforts for control or eradication.

The general aim of this thesis was to determine the abiotic and biotic factors influencing the distribution and abundance of *C. maenas* along the south-east coast of New South Wales.

In Chapter 2 the spatio-temporal patterns in the abundance of *C. maenas* in south-east Australia were investigated in five habitat types common in south-east Australian estuaries and ICOLLS. Given the variability in the opening regimes of ICOLLS we investigated the effect this may have on *C. maenas* population dynamics. It was hypothesised that the presence and abundance of *C. maenas* in estuaries would decrease with the proportion of time their entrance spends closed. This chapter also assessed whether differences in *C. maenas* abundance among estuaries reflected differences in their habitat availability and whether biogenic habitats supported a greater abundance of *C. maenas* than unvegetated habitats.

In Chapter 3 the focus of the study was to understand some of the reproductive life history traits of *C. maenas*. This study aimed to develop a method for detecting *C. maenas* recruitment to benthic habitats in south-east Australian estuaries and identify the timing of recruitment. It was predicted *C. maenas* would recruit in greater numbers to structured than unstructured habitats during the Austral spring and autumn, and timing would be similar to native crabs. These observations of recruitment were coupled with a cohort analysis of *C. maenas* trapped in another south-eastern Australian estuary.

In Chapter 4, some of the causes for the differential distribution of *C. maenas* among habitats were investigated. It was hypothesised that the abundances of *C. maenas* would be greater inside than outside of mangrove forests and under the canopy than in the pneumatophore zone, both as a consequence of reduced rates of predation and also as a consequence of reduced summertime temperatures under the shaded mangrove canopy.

Experiments were done to determine whether the structure provided by the mangrove forest resulted in lower rates of predation on tethered *C. maenas* than in adjacent unstructured habitats. We also assessed whether, as a consequence of the reduced summertime temperature under the mangrove canopy, *C. maenas* displayed a preference for shaded versus non-shaded habitat.

In the final data chapter 5, the potential role of predation in controlling populations of *C. maenas* in east Australian estuaries was investigated. We predicted the suite of native putative predators on the east coast of Australia may have an effect on the distribution of *C. maenas*. This chapter combined a trapping survey with feeding trials to identify potential predators of *C. maenas* in south-eastern Australia, which are negatively correlated with the abundance of the non-native crab.

The final chapter summarises how this thesis has enhanced our understanding of processes influencing the distribution and abundance of *C. maenas* in NSW, Australia. It discusses the implications of the research findings for management of the invasive species and identifies future research needs.

Each chapter in this thesis, with the exception of the final one, has been developed as a manuscript for submission to an international peer reviewed journal. This created some overlap within the thesis.

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CHAPTER 2

The frequency of connection of coastal water bodies to the ocean predicts *Carcinus maenas* invasion

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Abstract

Semi-enclosed coastal water bodies range from permanently open estuaries to lagoons that are periodically isolated from the ocean. The proportion of time these are connected to the ocean may influence biological invasions by determining recruitment opportunities, environmental conditions, and available biogenic habitats. Along the south coast of New South Wales, Australia, we tested whether the abundance and presence of the non-native crab, *Carcinus maenas*, varies among coastal water bodies according to the proportion of time their entrance is open to the ocean. In quarterly sampling over two years in 14 estuaries and lagoons varying in the percentage of time they were closed to the ocean from 0–100% crab presence and abundance was correlated with the proportion of time the entrances were open. Crabs were absent from lagoons with entrances closed to the ocean > 60% of the time and consistently detected in open estuaries. Crab abundance increased with the connectivity of coastal water bodies to the ocean and was influenced by habitat availability. In water bodies where conditions supported mangroves, this habitat contained higher abundances of crabs than seagrass, saltmarsh, oyster habitat, or bare substrate.

Status of the entrance as open or closed initially predicts the likelihood of the crabs' arrival by influencing their population connectivity between estuaries or lagoons and the open coast, and the probability of establishment by influencing abiotic conditions. Entrance morphology may also affect crab abundance by influencing the available habitats. Hence, the geomorphology of semi-enclosed coastal water bodies should be considered when managing marine bioinvasions.

Introduction

Along with pollution, habitat destruction and climate change (Harley et al. 2006; Lotze et al. 2006), non-indigenous species (NIS) are an increasing threat to the structure and function of ecosystems (Molnar et al. 2008). Invasion rates have increased exponentially with the growth of world trade over the past 200 years (Ruiz et al. 1997; Molnar et al. 2008). Irrespective of natural barriers tens of thousands of potentially invasive species are being transported within and between oceans annually (Ruiz et al. 1997). Although only 1% of all species introductions are estimated to result in invasions (Williamson and Fitter 1996), where invasions do occur, they can cause significant ecological and economic effects (Pimentel et al. 2005; Molnar et al. 2008).

The establishment, proliferation and spread of NIS are determined by the match between the recipient abiotic environment and the biology of the species. Initially, abiotic factors act as a 'filter', determining those NIS whose environmental tolerances will enable them to establish in the recipient environment (Sakai et al. 2001). Following the abiotic filter, biotic interactions then determine the species' proliferation (Olyarnik et al. 2009). Both biotic and abiotic factors influence the spread of an NIS from the initial point of introduction (Ruiz et al. 1997; Sakai et al. 2001).

Semi-enclosed coastal water bodies are among the most invaded habitats on earth due to their proximity to ports and human activities (Lotze et al. 2006). Semi-enclosed coastal water bodies range from permanently open estuaries to lagoons that are only periodically connected to the ocean. Intermittently closed and open lakes and lagoons (ICOLLs) make up 13% of the world's coastline (Kjerfve 1994), but the role of their unique abiotic and biotic features in influencing biological invasion is poorly understood. ICOLLs occur along coastlines

with high inter-annual variability in rainfall and wave climate and, although found throughout the world, are particularly common on the African (18% of the coastline) and North American continents (18% of the coastline; Kjerfve 1994) and are locally common in south-eastern Australia, Western Australia, South Africa, New Zealand, Mexico, Texas, and the Atlantic coast of Brazil and Uruguay (Haines 2006). During periods of low rainfall, longshore drift of sand forms and maintains a berm above high tide closing off ICOLLs to the sea from days to years (Roy et al. 2001; Haines et al. 2006). Storm or flood events can breach the berm and restore tidal inundation. In addition many ICOLLs are opened artificially for flood or water quality mitigation. ICOLLs can remain open from days to years, although increasingly ICOLL entrances are being artificially trained to keep them permanently open to maintain water quality and minimise risk of coastal flooding as well as to facilitate maritime activities.

The entrance dynamics of ICOLLs may influence the ability of biological invaders to arrive, establish and persist. When the ICOLL entrance is closed, the arrival of new recruits into the estuarine system will only be possible where the organism is capable of crossing the semi-terrestrial berm or where anthropogenic vectors, such as translocation by the movement of aquaculture stock, still persist (Ruiz et al. 1997). During periods of entrance opening, by contrast, marine species can recruit to ICOLLs from the ocean environment, generally enhancing the abundance and diversity of species over periods of entrance closure (Pollard 1994a; Dye and Barros 2005). Entrance closure, however, not only influences connectivity between ICOLLs and the adjacent coastal waters, but also the physico-chemistry and biology of the ICOLL. When the entrance of the ICOLL is closed during drought, high rates of evaporation can lead to hypersaline waters (Kjerfve 1994). Conversely, when the entrance is closed during periods of high rainfall, salinity can drop markedly (Pollard 1994a,b). In this shallow water environment, water temperatures are strongly influenced by air temperature, particularly during periods of entrance closure (Gale et al. 2006). This considerable environmental variability may challenge the survival of even the most eurythermal or euryhaline species. Moreover, ICOLLs, due to their periodic closure, may not support some habitat forming species that require a tidal cycle, such as mangroves. The availability of structured estuarine habitat may, in turn, influence the ability of NIS to establish following arrival.

The European shore crab (*Carcinus maenas*, Linnaeus 1758) is an epibenthic omnivore native to northwest Europe and North Africa, where it is common on hard and soft substrata in the littoral and sub-littoral zone, down to 60 m (Naylor 1962; Crothers 1967). It has established populations on the Atlantic and Pacific coasts of North America, Japan, South Africa, and southern Australia (Carlton and Cohen 2003; Thresher et al. 2003). In Australia, *C. maenas* was first recorded in Port Philip Bay, Victoria, in the late 1800s (Thresher et al. 2003). Despite being present in Australia for >100 yr, and having a predicted range to a latitude of 28°S, in southern Queensland (Carlton and Cohen 2003; Summerson et al. 2007), persistent populations appear to be limited to south of Batemans Bay, New South Wales (NSW), at latitudes below 35.7°S (T. Glasby unpublished data). Ephemeral populations have, however, been reported as far north as Botany Bay, NSW (Ahyong 2005), at a latitude of 34°S.

The south-east Australian coastline is dominated by ICOLLs (Haines 2006). Hence, one potential hypothesis for the ephemeral populations of *C. maenas* along the south-east Australian coastline is that ICOLL entrance dynamics limit the recruitment of *C. maenas* and produce highly variable conditions that challenge the ability of the crab to establish persistent populations. In its native range, female *C. maenas* release larvae, often on an ebbing tide, and the larvae develop in coastal waters before returning to the coast or estuary to settle (Queiroga et al. 1997). Hence, closed ICOLL entrances would severely limit the recruitment opportunities and dispersal capability of the invader. Further, the crab's temperature (adults: 0-30°C; juveniles: 6-25°C) and salinity (adults: 4-54; juveniles: > 20) tolerances may be exceeded within ICOLLs when entrance closure overlaps with hot, dry periods or flood. Thus, crabs that have recruited during previous periods of entrance opening or that have been introduced via other vectors may not survive.

Along other coastlines where there are high numbers of ICOLLs, limited range expansion and proliferation of *C. maenas* has also been reported. In South Africa, *C. maenas* has not established beyond the greater Cape Town region following its first record in 1983, despite evidence that mating pairs are dispersing further afield (Carlton and Cohen 2003; Hampton and Griffiths 2007). Along the Atlantic coast of Brazil and Uruguay, which is also characterised by high numbers of ICOLLs, the crab has not established despite several historic records (Carlton and Cohen 2003). In California where there are significant numbers of ICOLLs in the south of the state, but predominantly drowned river valleys to the north, spread of the crab from San Francisco Bay has been much greater in a northerly (>2000 km)

than a southerly (125 km) direction, although other factors may also explain this pattern (Grosholz and Ruiz 1996).

South-east Australian ICOLLs vary in the proportion of time their entrances are open to the ocean (Fig. 2), according to their orientation relative to prevailing wave patterns (Roy et al. 2001; Haines et al. 2006) and management interventions, such as the construction of training walls and dredging operations that are designed to keep their entrances open. Here we conducted quarterly sampling of adult *C. maenas* over two years, in 14 estuaries and ICOLLs which varied in the proportion of time their entrance was closed to the ocean. We hypothesised that: 1) the presence and abundance of *C. maenas* in estuaries and ICOLLs would decrease as the proportion of time their entrance spends closed increases; 2) among water bodies with *C. maenas* there would be a greater abundance of the crab in permanently open estuaries than in ICOLLs; 3) differences in *C. maenas* abundance among estuaries and ICOLLs would, in part, be underpinned by differences in habitat availability among these; and 4) within estuaries and ICOLLs, biogenic habitats, such as mangroves, seagrass, and saltmarsh, would support greater abundances of *C. maenas* than unvegetated habitats.

Methods

Experimental design

The relationship between the entrance dynamics of semi-enclosed coastal water bodies (i.e., estuaries and ICOLLs) and the presence and the abundance of *C. maenas* was assessed through trapping surveys, performed quarterly from December 2010 to September 2012. Fourteen estuaries and ICOLLs were selected for study between Durras Lake (35.64°S, 150.31°E) and Merimbula Lake (36.90°S, 149.92°E), on the south coast of NSW, Australia (Table 1; Fig. 1). These coastal water bodies were chosen to represent the full spectrum of entrance dynamics seen in NSW (Fig. 2), varying in the proportion of time their entrances are closed from 0–99%, historically (according to multiple decadal analysis of historical photographs, council records, long-term water level records and literature; Haines 2006) and 0–100% during this study, which spanned an uncharacteristically wet period (Table 1). Each

estuary and ICOLL was within the known distribution of *C. maenas* in east Australia (T. Glasby unpublished data)

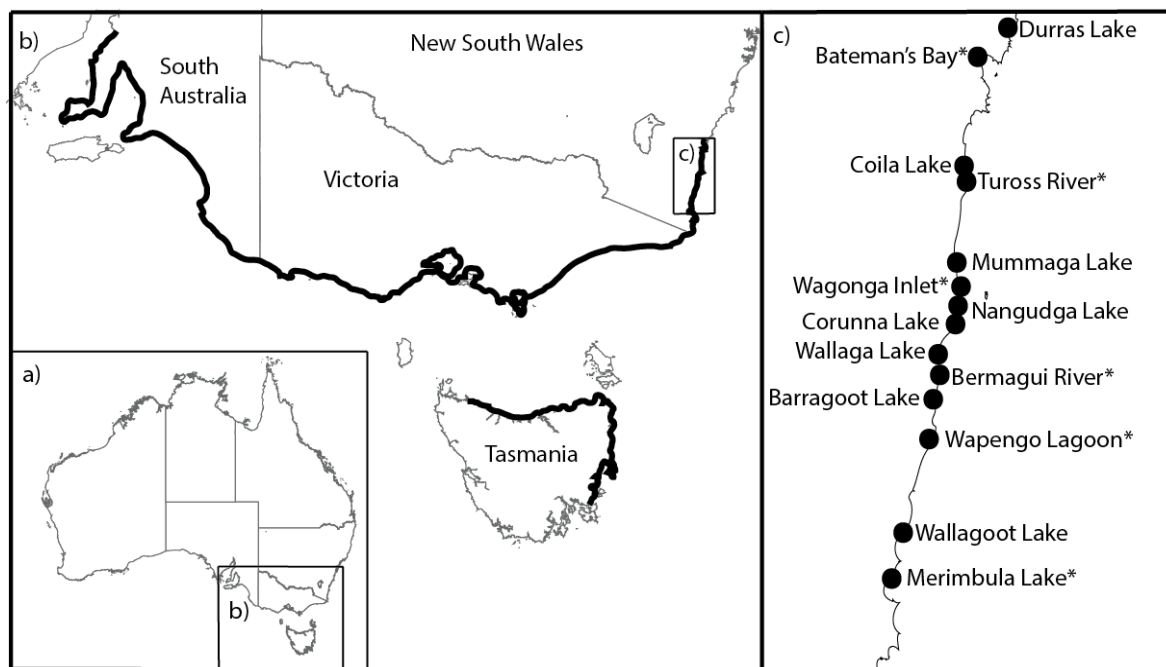


Figure 1. Map of Australia (a) showing the present distribution of *Carcinus maenas* (bold line) in Australia (b) and the estuaries surveyed (c). * denotes estuaries in which five habitats were sampled (mangrove, oyster, seagrass, saltmarsh and unvegetated sediments). In all others, three habitats (seagrass, saltmarsh and unvegetated sediment) were sampled.

Table 1. The locations (listed from north to south), size, and entrance characteristics of the 14 semi-enclosed coastal water bodies surveyed. Each was characterised as either a permanently open estuary (E) or as an intermittently open and closed lake or lagoon (ICOLL; I). In each it was noted whether mangroves were absent (A) or present (P) and whether at each sampling time the entrance was closed (C) or open (O).

Water body	Classification ^a	Longitude (°)	Latitude (°)	Size ^a (km ²)	Mangrove presence	% time closed ^b	2010	2011				2012		
							Dec	Mar	Jun	Sep	Dec	Mar	Jun	Sep
Durras Lake	I	150.30	-35.63	3.9	A	52	C	C	C	O	O	O	C	C
Clyde River	E	150.25	-35.74	48.6	P	0	O	O	O	O	O	O	O	O
Coila Lake	I	150.13	-36.04	6.3	A	95	C	C	C	C	C	C	C	C
Tuross River	I	150.13	-36.06	13	P	1	O	O	O	O	O	O	O	O
Mummaga Lake	I	150.15	-36.09	1.4	P	64	O	O	O	O	O	O	O	O
Wagonga Inlet	E	150.13	-36.21	5.6	P	0	O	O	O	O	O	O	O	O
Nangudga Lake	I	150.14	-36.26	0.5	A	73	O	O	O	O	O	O	O	O
Corunna Lake	I	150.13	-36.28	1.7	A	93	O	O	O	C	C	O	O	C
Wallaga Lake	I	150.07	-36.36	7.8	A	20	O	O	O	O	O	O	O	O
Bermagui River	E	150.06	-36.42	1.4	P	0	O	O	O	O	O	O	O	O
Baragoot Lake	I	150.06	-36.47	0.4	A	99	C	C	C	C	C	C	C	C
Wapengo Lagoon	I	150.01	-36.37	4	P	3	O	O	O	O	O	O	O	O
Wallagoot Lake	I	149.95	-36.79	4	A	96	C	C	C	C	C	O	O	C
Merimbula Lake	I	149.92	-36.89	4.5	P	1	O	O	O	O	O	O	O	O

Source: ^aGeoscience Australia 2013; ^bHaines 2006: percentages are based on analysis of historical photography, council records, long term water level records, and literature over multiple decades.

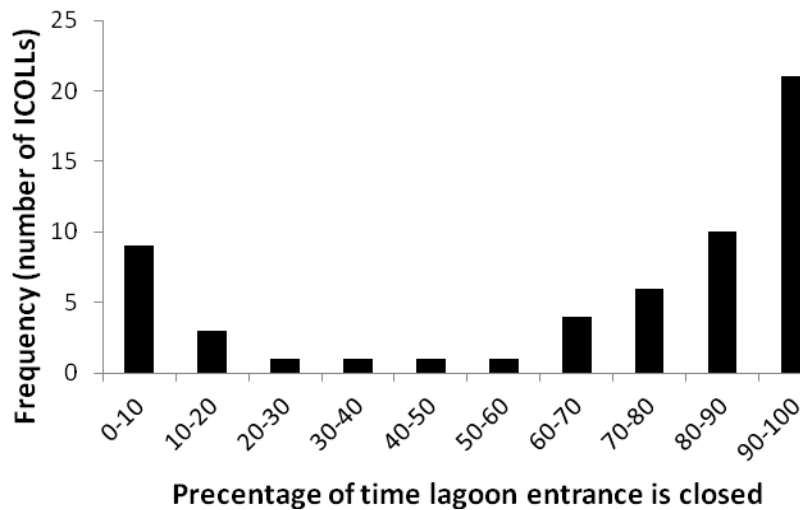


Figure 2. Frequency distribution categorising New South Wales ICOLLs greater than 0.01 km² in area according to the percentage of time their entrance spends closed (Haines et al. 2006). $n = 70$ ICOLLs.

Within each water body, sampling of *C. maenas* was done with replicate traps, stratified according to habitat and each placed at least 20 m apart. For every water body, traps were randomly placed in or directly adjacent to 1) saltmarsh (dominated by *Sarcocornia* spp., *Sporobolus* spp., and *Juncus* spp.) and 2) seagrass (a mixture of *Posidonia australis*, *Zostera* spp., and *Halophila* spp.), and on 3) unvegetated habitat near the mouth of the water body, comprised of a mixture of rocky and sandy substrate ($n = 10$ within each). Sampling of unvegetated habitat was not conducted because a pilot study failed to detect *C. maenas* at the back of estuaries, adjacent to freshwater inputs in any of the estuaries or ICOLLs. Where present, trapping was also conducted 4) in *Avicennia marina* mangroves, and 5) in oyster leases or oyster reef (mixed beds of *Saccostrea glomerata* and *Crassostrea gigas*) at the same intensity ($n = 10$). Mangrove and oyster reefs were not present in ICOLLs with entrances that were closed to the ocean for more than 60% of the time, presumably due to the dependence of these habitat types on tides and water flow. Traps in seagrass and oyster habitats were set in the subtidal zone (with the exception of Nangudga Lake where only intertidal seagrass habitat was present), in water < 3 m deep, and traps in mangroves, were necessarily, in intertidal habitat. The traps in saltmarsh and unvegetated habitat were intertidal when estuaries were open, but subtidal (< 1 m depth) when estuaries were closed,

our design holding sampling areas but not depth constant between periods of opening and closure. Traps were randomly distributed across multiple habitat patches within each estuary. The design gave between 30 and 50 traps per estuary per time of sampling.

Sampling methods

Traps were 62 x 42 x 20 cm, constructed of 1.3 cm mesh netting and had two entrances extending the width of the traps at opposite ends, at the apex of two inward facing 45° panels. Similar traps have been used to detect *C. maenas* in Australian waters (Thresher et al. 2003). Traps were weighted with half a fired clay brick and baited with a single frozen 8–15 cm Pilchard (*Sardinops neopilchardus*) housed in a perforated bait-bag of 1.3 cm plastic mesh. The time of day at which traps were deployed was variable, but did not vary systematically among water bodies or habitats. Each trap was set for 22–24 h, which was based on a pilot study in an area of high *C. maenas* abundance for NSW that showed no significant difference in catch between traps deployed for 12 or 24 h (ANOVA, $F_{1,6} = 0.47$, $p = 0.517$), or between traps set during the morning or evening (ANOVA, $F_{1,1} = 0.98$, $p = 0.504$). The GPS coordinates and depth of each trap was recorded at the time of deployment. At the end of each deployment period, the number of *C. maenas* in each trap was counted. At most times of sampling, surface measurements of water temperature (with a thermometer) and salinity (with a refractometer) were taken at a random location in each estuary.

Statistical analyses

To examine whether the temporal presence of *C. maenas* in semi-enclosed coastal water bodies decreases with an increase in the percentage of time the entrance is closed, we used Spearman's rank correlations to test for a negative relationship between the proportion of the eight survey dates on which the entrance of each water body was open and the proportion of the eight survey dates on which crabs were detected within each water body. To assess whether the abundance of *C. maenas* similarly decreases with the frequency of water body entrance closure, we performed an additional Spearman's rank correlation between the Catch Per Unit Effort (CPUE) in each estuary, across all surveys, and the proportion of the eight survey times each water body entrance was closed. To test the hypothesis that among water bodies with crabs, estuaries would support greater abundances of *C. maenas* than ICOLLs, a single factor (2 levels, fixed: permanently open, intermittently open) univariate PERMANOVA (Anderson et al. 2008) was run on Euclidean

distance matrices produced from the average CPUE of *C. maenas* in each water body, across the eight sampling times. Although PERMANOVAs were originally designed for analysis of multivariate data, they can also be used to analyse univariate data and, unlike ANOVAs, do not assume that data are normally distributed (Anderson et al. 2008).

To determine the subsets of abiotic and biotic variables to which abundances of *C. maenas* were most closely correlated at the scale of water bodies, we used the Biological-Environmental Stepwise (BEST) routine in PRIMER6. The routine uses an iterative procedure of adding and removing variables (999 permutations) to assess those combinations of variables which produce a resemblance matrix most closely correlated (according to Spearman's rank correlations) to that of the *C. maenas* CPUE data (Clarke and Gorley 2006). The environmental data used in the analysis came from the NSW Office of Environment and Heritage (Roper et al. 2011) and the National Land and Water Resources Audit (Geoscience Australia 2013), as well as this study (the temperature and salinity data; Table 2). The analysis included: 1) geomorphological attributes of each water body (catchment area, total area, open water area, perimeter, volume, average depth, and the areas of each of: the barrier and back-barrier, central basin, fluvial bay head delta, flood ebb delta); 2) hydrodynamic variables (total flushing time, annual surface flow, annual base flow, total annual flow, runoff coefficient, water extraction); 3) other abiotic variables (mean wave height, maximum wave height, tidal range, average temperature, average salinity, latitude); as well as 4) the area of key natural and artificial habitats (seagrass, mangrove, saltmarsh, intertidal flats, rocky shore, channel, the length of perimeter that was bedrock and artificial structure, % estuary under aquaculture). Full explanations of these metrics are shown in Appendix 1. The analysis correlated a Euclidean distance matrix produced from normalised environmental variables (Clarke and Gorley 2006) with a Euclidean distance similarity matrix calculated from *C. maenas* CPUEs (calculated from all traps deployed within an estuary across multiple sampling times) and was set to investigate subsets of no more than five variables. Following analysis by BEST, Spearman's rank correlations were then conducted between each individual variable that was among the explanatory subset and *C. maenas* abundance.

To test the hypothesis that within water bodies, the abundance of *C. maenas* would differ among habitat types, two univariate PERMANOVAs (Anderson et al. 2008) were run on Euclidean distance matrices produced from pairwise comparisons of catches within

individual traps. The first PERMANOVA contrasted all five habitat types, including only the six water bodies in which all habitat types were present. The analysis had three orthogonal factors: water body (6 levels, random), habitat type (5 levels, fixed: seagrass, saltmarsh, unvegetated, mangrove, oyster) and sampling date (8 levels, random). Within each water body, patches of habitat were randomly selected for sampling on each date. The second PERMANOVA tested for differences in the abundance of crabs among the three habitat types (seagrass, saltmarsh, unvegetated) within the ICOLLs without mangrove or oysters. The four ICOLLs in which no crabs were caught were excluded from this analysis, leaving four ICOLLs in this second analysis, which had the same three orthogonal factors as the first. Each analysis used traps as an individual replicate. Where significant treatment effects were seen (at $\alpha = 0.05$), the analysis was followed by pair-wise post-hoc tests to assess the sources of differences.

Results

Of the 4300 traps set, 38 were stolen or tampered with leaving 4262 traps that caught a total of 268 *C. maenas*. Hence, across all water bodies and sampling dates the CPUE was 0.06 crabs per trap. The average size of *C. maenas* was $56 \pm (1 \text{ standard error (SE)})$ mm with a range of 22–83 mm.

Of the fourteen southern NSW semi-enclosed water bodies sampled, we detected *C. maenas* populations within ten. Nine of the water bodies (3 estuaries and 6 ICOLLs) in which *C. maenas* were caught had entrances that remained open for the duration of this study, while the tenth, the ICOLL Corunna Lake, opened and closed several times during the study, but in total had an entrance that was closed < 40% of the time. The four water bodies from which crabs were absent were the two ICOLLs, Coila Lake and Baragoot Lake, that were closed at each of the eight sampling times, as well as the two ICOLLs, Wallagoot Lake and Durras Lake, that spent > 60% of their time closed. This study provided the first record of *C. maenas* in the Tuross River and Mummaga Lake.

Among water bodies, there was a significant negative correlation between the proportion of survey dates on which a water body had a closed entrance and the proportion of survey dates on which *C. maenas* were caught (Spearman's $\rho = -0.682$, degrees of freedom (df) =

12, $p = 0.003$; Fig. 3A). Similarly, the catch of *C. maenas* per unit effort, averaged across all sampling times, was significantly negatively correlated to the proportion of survey dates on which the entrance of a water body was closed (Spearman's $\rho = -0.636$, $df = 12$, $p = 0.007$; Fig. 3B). Contrary to our hypothesis, among water bodies with *C. maenas*, CPUE was not significantly higher in estuaries than ICOLLs (PERMANOVA: $Pseudo-F_{1,9} = 1.41$, $p = 0.325$).

Of the environmental variables considered (see Methods), the subset that best explained spatial variation in the CPUE of *C. maenas* among the 14 water bodies was: % of estuary under aquaculture, central basin, bedrock perimeter, mean wave height and tidal range ($\rho = 0.665$, $p = 0.256$). In the top ten combinations of 5 variables (each with $\rho > 0.65$), three more variables were included: barrier and back-barrier area, % structure along perimeter and average salinity. Mean temperature varied little among estuaries and was not a contributing variable (Table 2). Quarterly measurements of salinity over the two years of this study detected a minimum salinity of 5 in Tuross River in December 2011 and a maximum of 38 in Durras Lake in September 2012 (Table 2). There was a significant positive relationship between salinity and CPUE (Spearman's $\rho = 0.440$, $df = 105$, $p = 0.001$; Fig. 4). Of the discriminating variables, the next largest correlation was between CPUE and the perimeter of bedrock (Spearman's $\rho = 0.521$, $df = 13$, $p = 0.056$).

Table 2. The average (avg, $\pm$ SE), maximum (max), and minimum (min) temperature (temp), and salinity in each of the estuaries, between December 2010 – September 2012. $n = 5-8$ sampling times within each estuary. Estuaries are ordered from north to south.

Estuary	Avg temp $\pm$ SE (°C)	Max temp (°C)	Min temp (°C)	Avg ‰ $\pm$ SE	Max (‰)	Min (‰)
Durras Lake	16.3(1.6)	25.1	10.5	27.3(3.4)	38	15
Clyde River	18.8(1.2)	23.4	13.8	28.8(3.1)	37	15
Coila Lake	17.7(1.7)	24	12.8	25.5(1.3)	33	22
Tuross River	18.5(1.6)	25.4	12.8	22.5(3.6)	35	5
Mummaga Lake	18.0(1.7)	25.2	11.9	35.4(0.5)	37	34
Wagonga Inlet	18.6(1.4)	24	14.3	35.4(0.5)	37	33
Nangudga Lake	17.1(2.2)	24	9.3	34.5(1.2)	37	27
Corunna Lake	16.3(1.6)	21.7	11.3	33.0(1.7)	37	25
Wallaga Lake	17.6(1.7)	22.7	12.4	34.7(0.6)	37	31
Bermagui River	16.5(1.3)	21	13	35.8(0.4)	37	34
Baragoot Lake	14.8(1.7)	20	9.5	23.1(2.9)	34	15
Wapengo Lagoon	16.6(1.7)	24	12.9	35.9(0.3)	37	34
Wallagoot Lake	16.7(1.8)	23.5	11.2	31.8(0.9)	35	28
Merimbula Lake	18.1(1.4)	23.5	12.2	35.2(0.3)	36	33

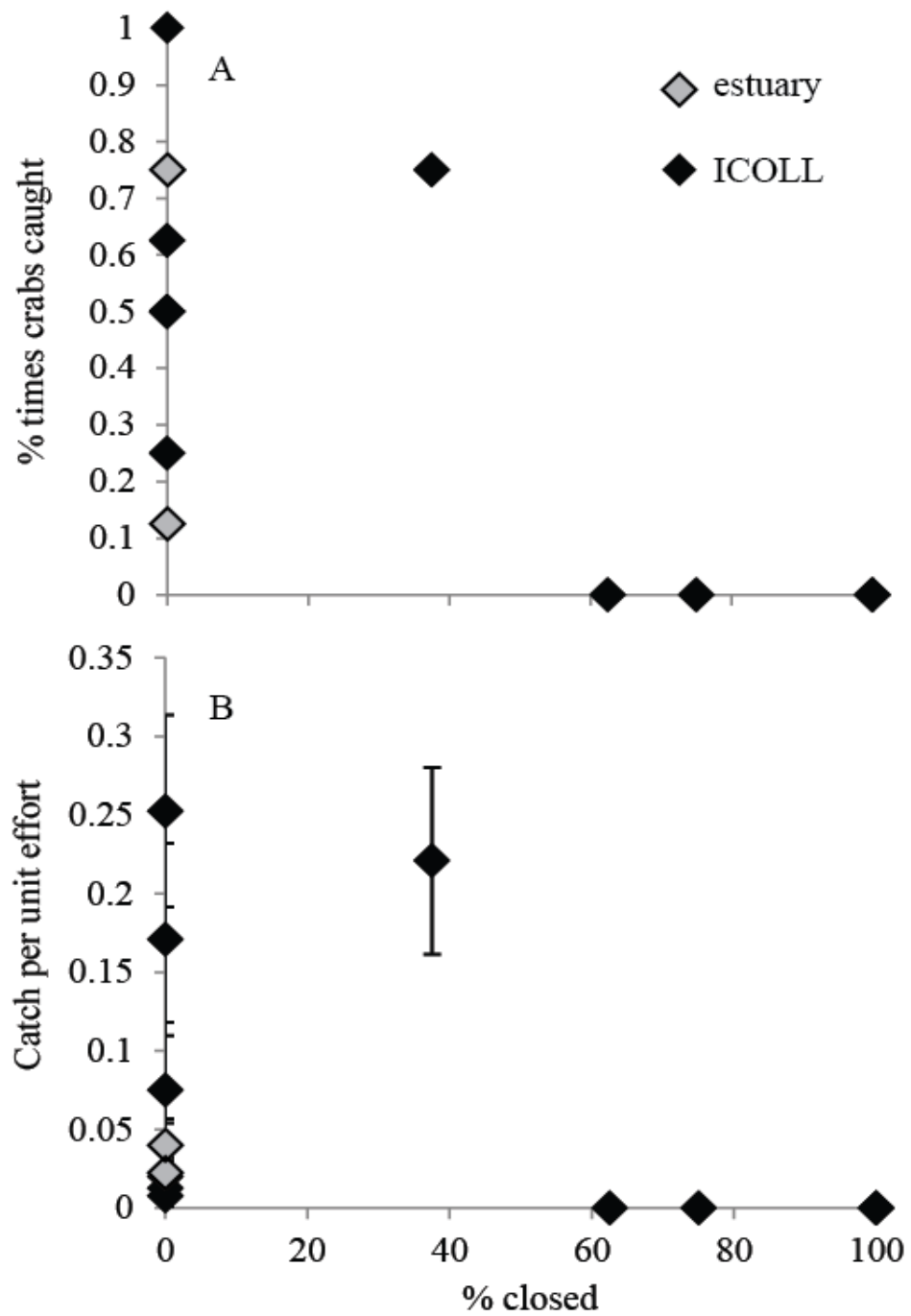


Figure 3. The relationship between the percentage of the eight sampling times at which an estuary or ICOLL had a closed entrance (% closed) and (A) the percentage of times at which *Carcinus maenas* were caught within it (% times crabs caught) and (B) the mean ($\pm$ SE) catch per unit effort of *C. maenas* across the eight sampling times. $n = 14$ water bodies.

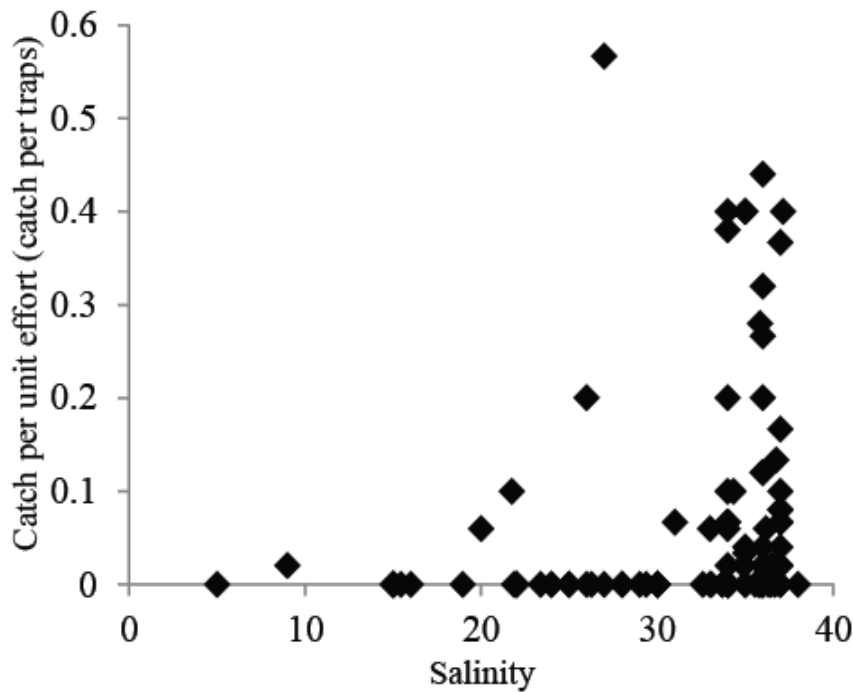


Figure 4. The relationship between the average salinity of an estuary or ICOLL at a given sampling time, and the catch per unit effort of *Carcinus maenas*. $n = 107$.

Across the six estuaries that contained all five habitat types, *C. maenas* abundance displayed differences among habitat types that varied according to survey date (PERMANOVA, significant Habitat x Survey interaction: $Pseudo-F_{28,2142} = 1.85$, $p = 0.008$) and water body (PERMANOVA, significant Habitat x Water Body interaction: $Pseudo-F_{20,2142} = 12.86$, $p = 0.001$). The three-way interaction between Habitat, Water Body and Survey was not significant ($Pseudo-F_{140,2142} = 1.20$, $p = 0.063$). On 6 of the 8 survey dates, significantly more crabs were found in the mangroves than in any of the other four habitat types (post-hoc tests, $p < 0.05$; Fig. 5A). Among the other habitat types, the unvegetated habitat had higher crab abundance than the seagrass or oyster beds on two sampling dates, and saltmarsh had the highest crab abundance of the four, followed by the unvegetated habitat, on one date (post-hoc tests, $p < 0.05$). When abundances were viewed across all survey dates, all but one of the estuaries (Tuross River) had significantly more crabs in mangroves than in the other habitats. Notably, across these six estuaries, not one crab was trapped within seagrass.

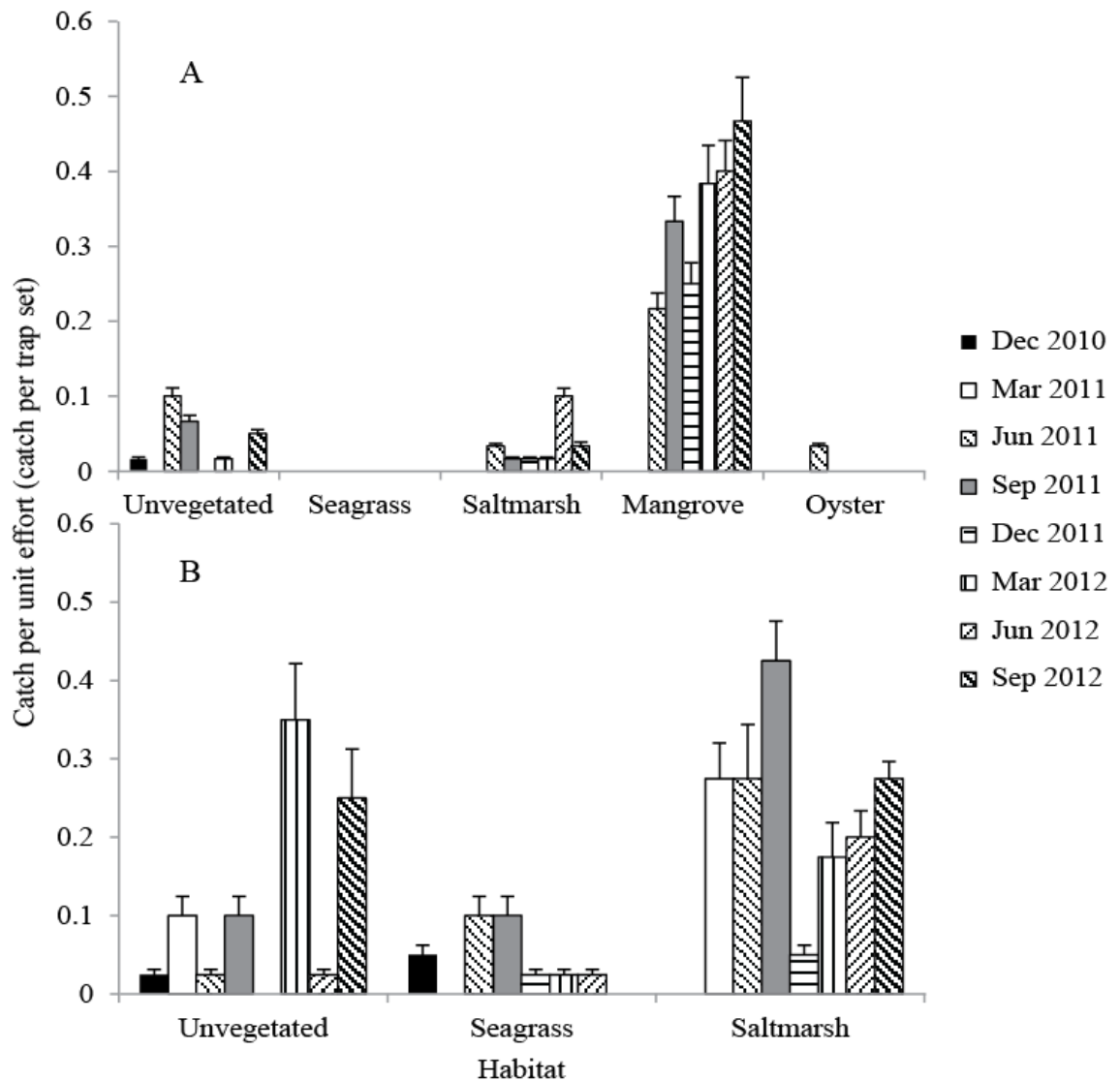


Figure 5. Mean (+ SE) catch per unit effort of *Carcinus maenas* in water bodies where (A) all five of the habitat types -- unvegetated, seagrass, saltmarsh, mangrove, and oyster -- were present and were sampled ($n = 6$ water bodies) and (B) where oyster and mangrove were absent, and only three of the habitats could be sampled ($n = 4$ water bodies).

Among the four estuaries lacking oyster or mangrove habitat, but in which *C. maenas* were trapped, crab abundances displayed differences among the three surveyed habitats that varied according to survey date, and estuary (PERMANOVA, significant Habitat x Survey x Water Body interaction; $Pseudo-F_{42,857} = 1.91$, $p = 0.002$; Fig. 5B). During one of the eight surveys, more crabs were caught in the unvegetated habitat than the seagrass or saltmarsh habitat of Wallaga Lake (post-hoc tests, $p < 0.05$). Otherwise, there were no significant differences.

Discussion

Our survey of 14 semi-enclosed water bodies representing a range of entrance morphologies supported the hypothesis that prolonged closure of ICOLL entrances impedes *C. maenas* invasion. As the proportion of time an ICOLL entrance was open decreased, so too did *C. maenas* abundance and the proportion of sampling times at which we detected the non-native crab in that ICOLL. Across the eight sampling times, we failed to detect any *C. maenas* in ICOLLs that had entrances that remained closed for > 60% of our study.

The frequency of entrance opening presumably influenced the presence of *C. maenas* by directly determining opportunities for recruitment from the external, coastal environment where, in other parts of the world, reproduction and early larval development typically occurs (Queiroga et al. 1997). Of the water bodies in which we failed to detect *C. maenas*, two were ICOLLs that remained closed for the duration of our study, and over the previous few decades spent > 95% of the time closed. This entrance opening regime provides few, if any, natural colonization opportunities. The other two water bodies in which we failed to detect *C. maenas* were ICOLLs open for < 1 yr during our study, and one had spent 96% of the previous several decades closed while the other had spent 52% of the time closed. During periods of entrance closure, the presence of crab populations in ICOLLs is dependent on: crabs having recruited to the ICOLL during past periods of entrance opening; and those individuals either surviving or successfully reproducing. Hence, in these two ICOLLs limited natural colonization opportunities, perhaps coupled with unfavourable environmental conditions for crab survival in the intervening periods of entrance closure likely prevented establishment.

Our study was conducted during a strong La Niña event, ending March 2012, which produced the wettest two years in Australian history for the region (Bureau of Meteorology 2012). Where this rainfall coincided with periods of estuary entrance closure, marked changes in salinity were observed (Table 2). Our quarterly sampling revealed that in the two estuaries that opened during the study, but remained free of crabs, salinity was as low as 15 during times of entrance closure, with the possibility of lower salinities being reached in the intervals between our sampling of salinity, which was only conducted every 3 mo, coincident with trapping (Table 2). Under such conditions, the reproduction of any surviving crabs is likely to have been unsuccessful, as larval *C. maenas* require salinities above 20 in order to

survive (Nagaraj 1993; Anger et al. 1998). Hence, long-term persistence of *C. maenas* populations in such ICOLLs would require opening of the ICOLL entrance to allow immigration of new individuals.

Of the ten semi-enclosed coastal water bodies in which we detected *C. maenas*, nine were open for the duration of our study and one opened and closed. Among these ten water bodies, the abundance of *C. maenas* did not differ between estuaries and ICOLLs. Instead, differences in habitat availability among water bodies may have influenced crab abundances. The availability of hard substrate was among the best environmental predictors of *C. maenas* abundance. In its native range, *C. maenas* is very abundant on rocky shores, with juveniles found on the high rocky intertidal shore throughout the tidal cycle and adults migrating with the tide as they forage (Naylor 1962; Crothers 1967). In its introduced range, *C. maenas* has been found on hard rocky substrates in several locations (Grosholz and Ruiz 1996). Furthermore, previous studies have found that greater numbers of megalopae settle in structurally complex habitats, compared to open sandy areas (Moksnes 2002). The present study did not specifically target rocky areas, but instead unvegetated habitat at the mouth of estuaries that was a mixture of sand and rock. Although our trapping consistently identified crabs in this mixed-substratum habitat, it was not where we observed abundances to peak.

Among the habitats examined in this study, more *C. maenas* were caught in mangroves than in other habitats. Mangrove forests support high abundances of invertebrates, such as oysters and mussels (Nagelkerken et al. 2008), which may serve as a prey resource for *C. maenas*, which is a generalist consumer of molluscs (Ropes 1968). The structural complexity provided by pneumatophores can decrease the foraging efficiency of predatory fish that forage at high tide (Sheridan and Hays 2003). Further, the mangrove canopy provides shading that may enable *C. maenas* to persist in environments where the temperature of unshaded substrates approaches or exceeds the maximum temperature the crab can tolerate (C. Garside unpublished data). Mangroves are rare or absent in ICOLLs which have entrances that are closed for > 20% of the time, so are more abundant in permanently open estuaries than ICOLLs (Geoscience Australia 2013). Hence, whether semi-enclosed coastal water bodies are permanently open estuaries or intermittently open lagoons may indirectly influence crab abundances by influencing the presence and extent of mangrove habitat. To our knowledge our study represents the first documentation of *C. maenas* in mangroves,

although there have been unpublished sightings of the crab in mangroves in South Australia (S. Dittman pers. comm.). Australia has the highest latitude mangroves in the world, so there are likely to be few other places where the climatic envelope of *C. maenas* overlaps with the distribution of mangroves. New Zealand, where the crab is yet to invade, is an obvious exception.

In this study, *C. maenas* were only caught in seagrass (*Zostera muelleri*, *Z. capricorni*) habitat within one of the 14 estuaries surveyed. This was an estuary with an average depth of 65 cm and where the seagrass is predominantly in the intertidal zone. In their native range, *C. maenas* have been found to settle in dense numbers in the structured habitat of seagrass, but tend to migrate away from these areas as they mature (Moksnes 2002). As our study did not use pit traps and targeted only adult *C. maenas*, we cannot comment on whether an ontogenetic shift in habitat may also occur in Australian estuaries. Alternatively, the non-native crab may experience predatory or antagonistic interactions with other inhabitants of seagrass beds such as Leatherjackets (*Meuschenia* spp.), Toadfish (*Tetractenos* spp.), Bream (*Acanthopagurus* spp.), or Blue Swimmer Crabs (*Portunus pelagicus*) (Miller and Skilleter 2006; Hadwen et al. 2007). In North America, *Callinectes sapidus*, a con-familial of *P. pelagicus*, has been found to limit the distribution of *C. maenas* through aggressive interactions (de Rivera et al. 2005).

We have shown that ICOLL entrance dynamics are related to patterns of *C. maenas* invasion along the coastline of NSW, Australia. The temporary closure of ICOLLs to the ocean is associated with reduced crab presence, probably as a net outcome of the periodic loss of connectivity to the sea and, hence, reduced opportunities for colonization. Regimes of ICOLL entrance opening may also influence environmental conditions such as salinity, which determine survivorship of larvae and of colonising crabs and the availability of biogenic habitats, such as mangroves, which are used by the crab. In reducing the number of water bodies that can serve as reservoirs for reproductive *C. maenas*, ICOLL entrance closure may also help to prevent *C. maenas* abundances reaching pest status at the regional level. Our finding that ICOLLs hinder *C. maenas* colonization raises the possibility that in South Africa, the Atlantic coast of Brazil and Uruguay and southern California where ICOLLs are a common feature of the coastline and *C. maenas* is limited in distribution or absent (Carlton and Cohen 2003), the periodic closure of ICOLL entrances may similarly play a role in limiting the crab's

establishment. Other abiotic and biotic factors, however, likely also contribute and further studies would be required to parse their effects.

The question, however, remains as to why *C. maenas* has not established on the north coast of NSW and in southern Queensland, where ICOLLs are less common. Although the southward-flowing East Australian Current does not appear to hinder connectivity of *C. maenas* among estuaries and ICOLLs of southern NSW and Victoria (Burden et al. 2014), it may hinder larval transport further to the north. Modelling of NSW ocean currents suggests that larvae could be transported up to 200 km north from the southern tip of NSW, but would be unlikely to be carried as far north as Sydney (Roughan et al. 2011). Additionally, warmer temperatures that are approaching the upper thermal limit of *C. maenas* may prevent survival and, hence, establishment of the crab in northern NSW and southern Queensland.

In summary, it appears that ICOLLs or estuaries that remain predominantly closed may experience a high degree of natural resistance to invasion by persistent populations of *C. maenas*. This study suggests that permanently opening an estuary to the ocean through means of a training wall may increase the probability of *C. maenas* invasion relative to closed estuaries. Hence, management strategies aimed at mitigating coastal flooding and maintaining water quality and navigation channels by artificially opening ICOLLs need to consider how perceived benefits of this intervention may be offset by the potential cost of enhanced biological invasion. Although abundances of *C. maenas* are presently low along the south-east Australian coastline, any significant changes in management strategies could lead to proliferation and spread of the invader as has been seen in many other parts of the world (Carlton and Cohen 2003).

Acknowledgements

We thank Bradley Coates, Jennifer Rowland, Jian Smith, and Michael McMaster for assistance with fieldwork and thank two anonymous reviewers whose helpful suggestions improved the manuscript. This study was funded by an Australian Research Council (ARC) Linkage Grant to MJB, MAC, TMG, and BPK with industry partners, the New South Wales Department of Primary Industries, Sapphire Coast Marine Discovery Centre, Southern Rivers

Catchment Authority, and Batemans Marine Park. This work was performed during a Ph.D. candidature at Macquarie University. CJG was in receipt of an Australian Postgraduate Award Industry (APAI) and an International Macquarie Research Excellence Scholarship (IMQRES).

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Appendix 1. Definitions for terms used in the BEST analysis in Chapter 2

Attribute	Measurement	Definition
Geomorphological		
Catchment area	Km ²	Extent of area that drains into each estuary.
Total area	Km ²	The area of each estuary.
Open water area	Km ²	Area of open water in each estuary.
Perimeter	Km ²	Length of perimeter of each estuary.
Volume	ML (millions of Litres)	Quantity of water in each estuary.
Average depth	metres	Average depth of each estuary.
The barrier and back-barrier		Known as bar, sand spit, barrier island, strand. Barrier environments are a distinctive component of wave-dominated estuaries and are common shore face features on any coastline subjected to high wave energy.
Central basin	Km ²	Central basins are uniform, lower energy environments in the deeper and quieter parts of estuaries, and are often formed landward of barrier bar deposits in wave-dominated estuaries.
Fluvial bay head delta	Km ²	Fluvial deltas are complex associations of geomorphological settings, sediment types and ecological habitats, at the point where a freshwater source enters an estuarine water body.
Flood ebb delta	Km ²	Flood and ebb tidal deltas are subtidal to supratidal dunes and channels, typically found in the entrances of wave-dominated estuaries and deltas (adjacent to the barrier), and are formed by redistribution of sediment by tidal movement in and out of the entrance.
Hydrodynamic variables		
Total flushing time	days	Result of dividing the lake volume by the flow in or out of the lake. It roughly expresses the amount of time taken for a substance introduced into a lake to flow out of it again.
Annual surface flow	ML (millions of Litres)	Surface water flows off the catchment into the estuary
Annual base flow	ML (millions of Litres)	The low flow into each estuary
Total annual flow	ML (millions of Litres)	The combined surface and base flow
Runoff coefficient	Ratio	Runoff coefficients representing the ratio of annual runoff to rainfall
Water extraction	ML (millions of Litres) per year	The process of taking water from each estuary for irrigation, industry, recreation, flood control or treatment to produce drinking water.
Other abiotic variables		
Mean wave height	metres	Average height of waves outside of the entrance of the estuary.
Maximum wave height	metres	Maximum height of waves outside of the entrance of the estuary.
Tidal range	metres	Amplitude of the tide outside of the estuary.
Average temperature	Centigrade	Average temperature of the estuary (see methods)
Average salinity	Practical Salinity Unit	Average salinity of the estuary (see methods)
Latitude	Degree	A geographic coordinate that specifies the north-

		south position of a point on the Earth's surface
	The area of key natural and artificial habitats	
Seagrass, mangrove, saltmarsh, intertidal flats, rocky shore	Km ²	Area of the estuary comprised of any specific biogenic habitat
Channel	Km ²	Channels are environments of frequently high energy, in terms of tidal movement (e.g. tidal channels) or fluvial flow (e.g. river channels).
the length of perimeter that was bedrock	km	The length of shoreline of an estuary composed of bedrock
Artificial structure	Km ²	The length of shoreline of an estuary composed of artificial structures
% estuary under aquaculture	Area percentage	Percent of the area of the estuary used for aquaculture

CHAPTER 3

The timing of *Carcinus maenas* recruitment to a south-east Australian estuary differs to that of native crabs

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Abstract

For meroplanktonic species, patterns of recruitment to benthic substrates can play an important role in influencing population dynamics. Although the invasive crab *Carcinus maenas* has been present in south-east Australian waters for over 100 years, little is known about when and where it recruits to benthic substrates in its introduced range where water temperatures, which can trigger reproduction, are typically elevated compared to its native range. This study assessed the timing of *C. maenas* recruitment from August 2011 through October 2013 relative to native crabs in Merimbula Lake (36.89°S, 149.92°E) south-eastern Australia, and compared the effectiveness of four different types of recruitment bags for detecting the non-native crab. *C. maenas* recruited in greater numbers to live or dead oyster substrate and to artificial turf than to less structured habitat. Recruitment of most native crabs peaked during the autumn, whereas *C. maenas* recruitment peaked in the late winter and spring, unlike in its native Europe where recruitment typically occurs in summer and autumn. Although likely triggered by the warmer water temperatures of south-eastern Australia, this differing reproductive phenology may also modify interactions between *C. maenas* and native crab recruits.

Introduction

Understanding processes that determine the distribution and abundance of species is a fundamental concern of ecology. For organisms that have a dispersive stage in their life-history, their distribution and abundance may be set by patterns of settlement as well as post-settlement processes, such as mortality and migration (Olafsson et al. 1994; Underwood 2000). Recruitment describes the survival of newly-settled organisms through to a particular point in time and is a function of settlement rate and early post-settlement mortality (Hatton 1938; Bertness et al. 1992). For many benthic marine species which transition from a planktonic larval to a benthic juvenile phase (i.e. meroplanktonic species), recruitment can be critical in determining the dynamics of juvenile and adult populations (Underwood and Denley 1984).

The timing of recruitment may influence survival through to reproduction by dictating the abiotic and biotic environment in which a species establishes. The juvenile life-history stages of many marine organisms have very stringent environmental requirements, outside of which considerable post-settlement mortality can occur (Connell 1985; Moksnes et al. 1998). Furthermore, the timing of an organism's recruitment relative to that of other organisms can influence patterns of competition, predation, and consequently succession (Anderson and Underwood 1994; Strasser and Gunther 2001). As a result, strong seasonal trends in the recruitment of many marine organisms are apparent (Sutherland and Karlson 1977; Keough 1983; Dawirs 1985; Turner and Todd 1993), in some instances reflecting temperature triggers to key stages of reproduction and the commencement of developmental stages (e.g. Thorson 1950; Olive 1995).

Given the role of recruitment in dictating survival through to reproduction, strong selection might be expected to optimize its timing. Indeed, some organisms display considerable spatial variation in the timing of recruitment among locations (Yamada and Kosro 2009; Sanford and Kelly 2011). Invasive species, in particular, that are often typified by high phenotypic plasticity and an *r*-selected life history strategy, might be expected to display changes in the timing of recruitment following translocation to novel environments (Lee 2002). This may occur in response to altered abiotic conditions or to an assemblage of predators or competitors that differ in phenology to those in the invader's native range

(Breen and Metaxas 2009). In an extreme case, for example, temperate species that invade habitats in different hemispheres need to overcome reversed seasonality.

The European shore crab (*Carcinus maenas*, Linnaeus 1758), native to northwest Europe and North Africa (Broekhuysen 1936; Naylor 1962; Crothers 1967), has established non-native populations on the Atlantic and Pacific coasts of North America, Japan, South America, South Africa and southern Australia (Carlton and Cohen 2003; Thresher et al. 2003). Although vectors such as ballast water have assisted in the translocation of *C. maenas* around the world, once established, *C. maenas* is able to extend its distribution through pelagic dispersal and adult migration (Thorson 1961; Gomes 1991; Queiroga 1996; 1998; See and Feist 2010). Generally, larvae are hatched from eggs in the lower parts of estuaries during night time ebbing tides, following migration of ovigerous females down the estuaries (Queiroga et al. 1994). Larvae are typically transported to the sea shortly after spawning where they pass through four zoeal and a megalopal stage (Crothers 1967). The megalopae reinvade estuaries, settle and metamorphose into benthic juveniles (Queiroga 1996). The settlement of *C. maenas* represents an active response by the megalopae to the substrates they encounter (Moksnes et al. 2003), with greater numbers typically recruiting to more structurally complex habitats (Eriksson and Edlund 1977; Sprung 2001; Moksnes 2002). Consequently, structured artificial collectors can provide a useful tool for the detection of the presence of megalopae, as they provide a substrate for settlement even in otherwise structureless habitat (Paula et al. 2006).

In both the native and invasive range of *C. maenas*, there is evidence that ovigerous females are present at multiple times of year (e.g. Crothers 1967, Queiroga 1996, Yamada and Gillespie 2008; Table 1). Successful settlement and, hence, recruitment is more temporally constrained, primarily occurring in summer and autumn, although this is not the case in Portugal where recruitment occurs year-round (e.g. Crothers 1967; Berrill 1982; Baeta et al. 2005; Table 1). Crab larvae start hatching when water temperatures exceed 10°C in salinities above 26‰ (Crothers 1967). Temperature also influences survival, growth and the food intake of *C. maenas* larvae, with larval development proceeding at temperatures of 12–25°C and time to settlement decreasing as temperature increases (Dawirs 1985; Nagaraj 1993; de Rivera et al. 2007). *Carcinus maenas* brood eggs for approximately 20 days (Berrill 1982; Vinuesa 2007), after eggs are released the planktonic duration, with a constant salinity of 35, is 53 days (at 10°C) to 27 days (at 25°C; Nagaraj 1993). Temperature has been shown to be a

good predictor of *C. maenas* presence around the globe and its potential to invade suitable habitats (Compton et al. 2010).

Table 1. The timing (Sp = Spring; Su = Summer; Au = Autumn; Wi = Winter) of key events in the reproductive cycle of *Carcinus maenas*, in its native and invasive ranges. The temperature (°C) at which each stage of development has been observed to occur at each location is also indicated (Lab = data from lab experiments only)

Place	Ovigerous female		Zoeae in Plankton		Megalopae found		Settlement		References
	Season	Temp °C	Season	Temp °C	Season	Temp °C	Season	Temp °C	
Menai Strait, Wales	Sp–Su	16–19 (lab)			Native range Su				Zeng and Naylor 1996
Swansea, Wales	Wi–Su		Sp–Su						Naylor 1962
Milford Haven, Wales	Wi–Sp		All year		Sp–Su				Crothers 1966, 1967
Ria de Aveiro, northern Portugal	Wi	12.5	Wi–Sp	12.5– 14.5	Sp	14.5	Sp		Queiroga 1996
Ria Formosa lagoon, Portugal			Au–Sp	12–24			Wi	12–16	Sprung 2001
Mondego estuary, Portugal	All year	12.7–23.3	All year	12.7– 23.3	All year	12.7–23.3	All year		Baeta et al. 2005
Helgoland, Germany			Sp–Au	11–17	Su–Au	9–15			Dawirs, 1985
Gullmar Fjord, Sweden							Su–Au	18	Eriksson and Edlund 1977
					Invasive range Su–Au				
Central Maine, United States	Sp–Su						Au	9.5	Berrill 1982
Prince Edward Island, Canada	Su	16–23	Su–Au	16–23			Au	5–19	Audet et al. 2008
Nova Scotia, Canada	Su–Au	8–23	Su	8–22	Su–Au	9–18			Cameron and Metaxas 2005
Northwest, North America	Wi–Sp		Wi		Wi				Yamada and Gillespie 2008
San Jorge Gulf, Argentina	Au–Sp	7–16.8	Sp	7–8.2					Vinuesa 2007
Eden, New South Wales, Australia	Au–Sp								Pollard and Rankin 2003

Little is known about the timing of *C. maenas* reproduction and recruitment in Australia, where the crab has been present since at least the late 1800s (Fulton and Grant 1900). As compared to many European estuaries where water temperature falls below 10°C during winter, water temperatures of New South Wales estuaries are much warmer with the average temperature for winter typically ranging from 11.5–20°C in winter and 15.5–25°C in summer (Poore 2004). Hence, the temperature required for reproduction may be met year round, potentially enabling successful recruitment in multiple seasons.

Carcinus maenas populations in NSW are persistent yet small; a recent trapping study found populations to be patchy and discrete yielding a catch per unit effort of 0.06 (Chapter 2). Due to the small population and lack of information on their planktonic phases a novel detection experiment was designed to: 1) develop a method for detecting *C. maenas* recruitment to benthic habitats in south-east Australian estuaries; 2) assess the timing of *C. maenas* recruitment in a south-east Australian estuary; and 3) use the method to compare the timing of *C. maenas* recruitment with that of native crabs. Specifically, following observations of recruitment of *C. maenas* to bags of commercially-cultured oysters in several estuaries (J. Mackay, pers comm), we assessed whether these may be more effective for detecting recruits than the artificial turf which has previously been suggested as a recruitment substrate (Amaral and Paula 2007). We hypothesised that all crabs would recruit in greater numbers to structured than unstructured habitats.

Observations of recruitment of *C. maenas* to different substrates were coupled with a cohort analysis of *C. maenas* sampled in a nearby estuary. We predicted that *C. maenas* would recruit during the Austral autumn and spring when temperatures are similar to the time of year at which *C. maenas* recruit in their native range.

Methods

Merimbula Lake recruitment study

Assessments of crab recruitment and growth were done on an oyster lease in Merimbula Lake, on the south coast of New South Wales (NSW) Australia (36.89°S, 149.92°E). The lease consisted of parallel wooden rails, separated by 1.5 m, between which trays of oyster bags could rest at a mid-intertidal elevation approximately 30cm above the substrate. The habitat

within the lease was dense seagrass (*Posidonia australis*). The lease was chosen for assessments of *Carcinus maenas* recruitment because: (1) recruits of this species have previously been observed at this site (J. Mackay, pers comm); and (2) Merimbula Lake has previously been demonstrated to have a persistent population of *C. maenas* (Day and Hutchings 1984). During the study, the water temperature at the study site ranged from 10–26°C, and salinity ranged from 20–38‰ (NSW Food Authority, unpublished data). Average monthly sea surface temperatures for Merimbula Lake, New South Wales are shown in Figure 1. The tidal amplitude of the lake is ~1.5 m.

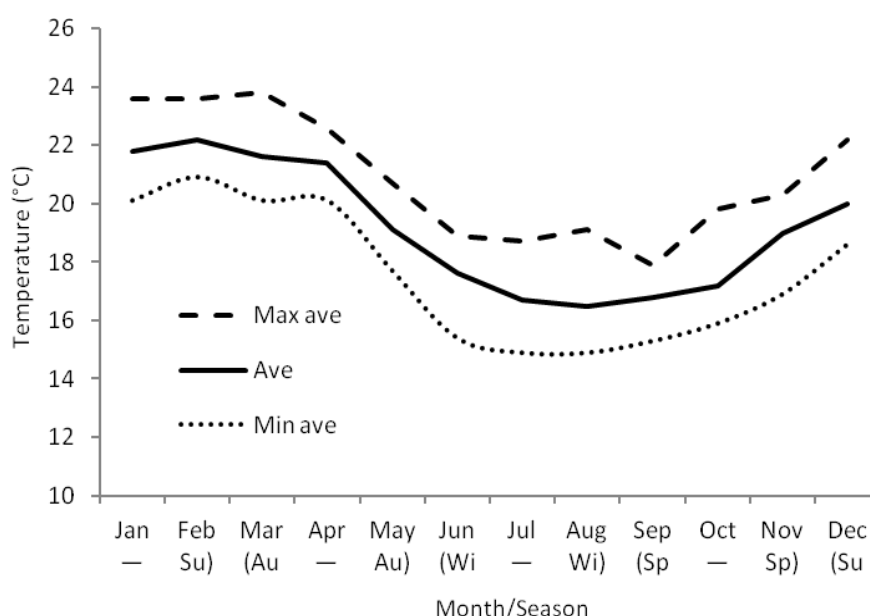


Figure 1. Mean monthly sea surface temperatures for Merimbula Lake, New South Wales (www.seatemperature.org). (Sp) Spring, (Su) Summer, (Au) Autumn, (Wi) Winter

Four substrate treatments were used in the assessments of *C. maenas* and native crab recruitment: empty, artificial turf, live oysters and dead oysters. It was hypothesised that crabs would recruit in greater numbers to the three structured treatments than to the empty treatment, and that among the structured treatments, crabs would recruit in greater numbers to live oysters than to dead oysters or turf, as oysters represent a potential food source to the crabs (Mascaró and Seed 2001). Each treatment was enclosed within 445 × 850 × 90 mm mesh bags, constructed of 3 mm mesh non-toxic UV stabilised plastic mesh (Zapco Aquaculture Ltd.). This mesh size was sufficient large that *C. maenas* megalopae could settle inside the mesh bags, but sufficiently small that following minimal growth the

crabs were unable to escape. The bags assigned to the empty treatment did not receive any substrate inside. The bags assigned to the artificial turf treatment received a 400 × 500 mm piece of green Kindy Turf, of 19 mm thickness (Synthetic grass & rubber surfaces, Australia Pty Ltd). This was based on a study by Amaral and Paula (2007) determining artificial habitat was just as efficient as natural fibres used to collect *C. maenas* in its native range. Bags assigned to the live oyster treatment received a mix of Sydney rock oysters (*Saccostrea glomerata*) and Pacific oysters (*Crassostrea gigas*), maintained at a volume of 5 L per bag. The dead oyster treatment received 5 L of oyster shell per bag, of a similar size distribution to the live oyster treatment.

So as to assess the timing of *C. maenas* and native crab recruitment, 10 bags of each treatment were deployed quarterly, from September 2011 to October 2013. Bags were deployed in random order on 2500 × 1000 × 40 mm oyster trays, with three bags per tray. Each set of bags was left in the field for 3 months. Hence, recruitment was assessed twice in each season; spring (September–November), summer (December–February), autumn (March–May), winter (June–August), over a 24 mo period.

At the end of each deployment period, bags were removed from the oyster lease and the contents of each washed separately through a 10 mm screen onto a 1 mm sieve. Material retained on the 1 mm sieve was transferred to a container and frozen. Material retained on the 10 mm screen was also visually checked for crabs and if found, they were added to the contents of the 1 mm sieve. Back at the laboratory, samples were thawed and benthic phase crabs were separated from small shell fragments and organic material under a lamp. Under a dissecting microscope the crabs were then identified (according to Poore 2004), enumerated by species, and sized by measuring the carapace width at its widest point (between anterolateral spines for species where they were present) to the nearest 0.1 mm, using digital callipers.

PERMANOVA (Anderson et al. 2008) was used to test the hypothesis that the abundance of (1) *C. maenas* recruits, and (2) the most abundant native crab recruits would vary among substrates and seasons. The analyses had three factors: treatment (4 levels: live oyster, dead oyster shell, artificial turf, empty); season (4 levels: summer, autumn, winter, spring); and year (2 levels: year 1, year 2), with $n = 10$ bags per treatment. Where significant effects were

seen (at $\alpha = 0.05$), the PERMANOVA analyses were followed by pair-wise post-hoc tests to assess differences between levels of treatments.

Fisheries Creek cohort analysis

To assess whether the timing of *C. maenas* recruitment was similar in a second NSW estuary, we: (1) determined the change in size over time for newly settled benthic phase *C. maenas* in eastern Australia and (2) used this to interpret the results of a trapping survey in Fisheries Creek, a small Intermittently Closed and Open Lagoon (ICOLL) 23 km south of Merimbula Lake, on the southern side of Twofold Bay, NSW (37.11°S, 149.92°E). This location was chosen for study because abundances of *C. maenas* are high in Fisheries Creek relative to elsewhere in southern NSW (Pollard and Rankin 2003). The change in size of newly settled *C. maenas* was estimated by calculating the difference in mean carapace width of crabs collected in settlement bags deployed at Merimbula for 3 months (from September – November 2011) and 6 mo (from September 2011 – February 2012). The difference in the mean size of crabs between these bags was interpreted as growth between November 2011 and February 2012, since no new recruits were detected in bags over this period and the abundance of *C. maenas* did not significantly differ between these two treatments, suggesting negligible mortality (PERMANOVA: $Pseudo-F_{(1,118)} = 0.065$, $p = 1$).

Trapping in Fisheries Creek was carried out on 20 sampling dates between February 2007 and November 2010. On each occasion, we set 10–12 ‘Opera House’ yabbie traps (640 × 460 × 210 mm), constructed of green 10 mm mesh with an 85 mm diameter hard circular opening at either end deployed at least 10 m apart.. These crabs were effective at capturing and retaining crabs >20 mm in carapace width, with crabs in the 16–20mm size class and smaller likely underrepresented (pers. obs.). Each trap was baited with an 8–15 cm Pilchard (*Sardinops neopilchardus*) and left to fish for 22–24 h. Traps were either set near the mouth of the estuary on sand or further up the creek on muddy sand in water < 1 m depth. At the end of each deployment period, we counted the number of crabs caught by each trap, sized them to the nearest mm using the method described above, and noted the number of gravid females. Using these data, we constructed size frequency histograms for each sampling time, standardised to trapping effort, with which we could track the recruitment of young of the year cohorts (Fig. 3).

Results

Merimbula recruitment study

Among the bags deployed for three months, the abundance of *Carcinus maenas* differed according to the three way interaction between Treatment $\times$ Season $\times$ Year (PERMANOVA: $Pseudo-F_{(9,288)} = 15.84$, $p = 0.001$; Fig. 2). In winter of Year 2, significantly more *C. maenas* were detected in bags of live oysters than in the other three treatments (post-hoc tests, $p < 0.05$; Fig. 2), but at all other times (when abundances of recruits were low) there was no significant difference among the four treatments (post-hoc tests, $p > 0.05$, Fig. 2). No crabs were observed in the empty bags at any of the sampling times. No significant difference among seasons was found for any of the other treatments, in year 1 or year 2, or for the live oysters in year 1 (post-hoc tests, $p > 0.05$; Fig. 2). Across all treatments, observations of *C. maenas* were confined to spring of Year 1, and spring and winter of Year 2.

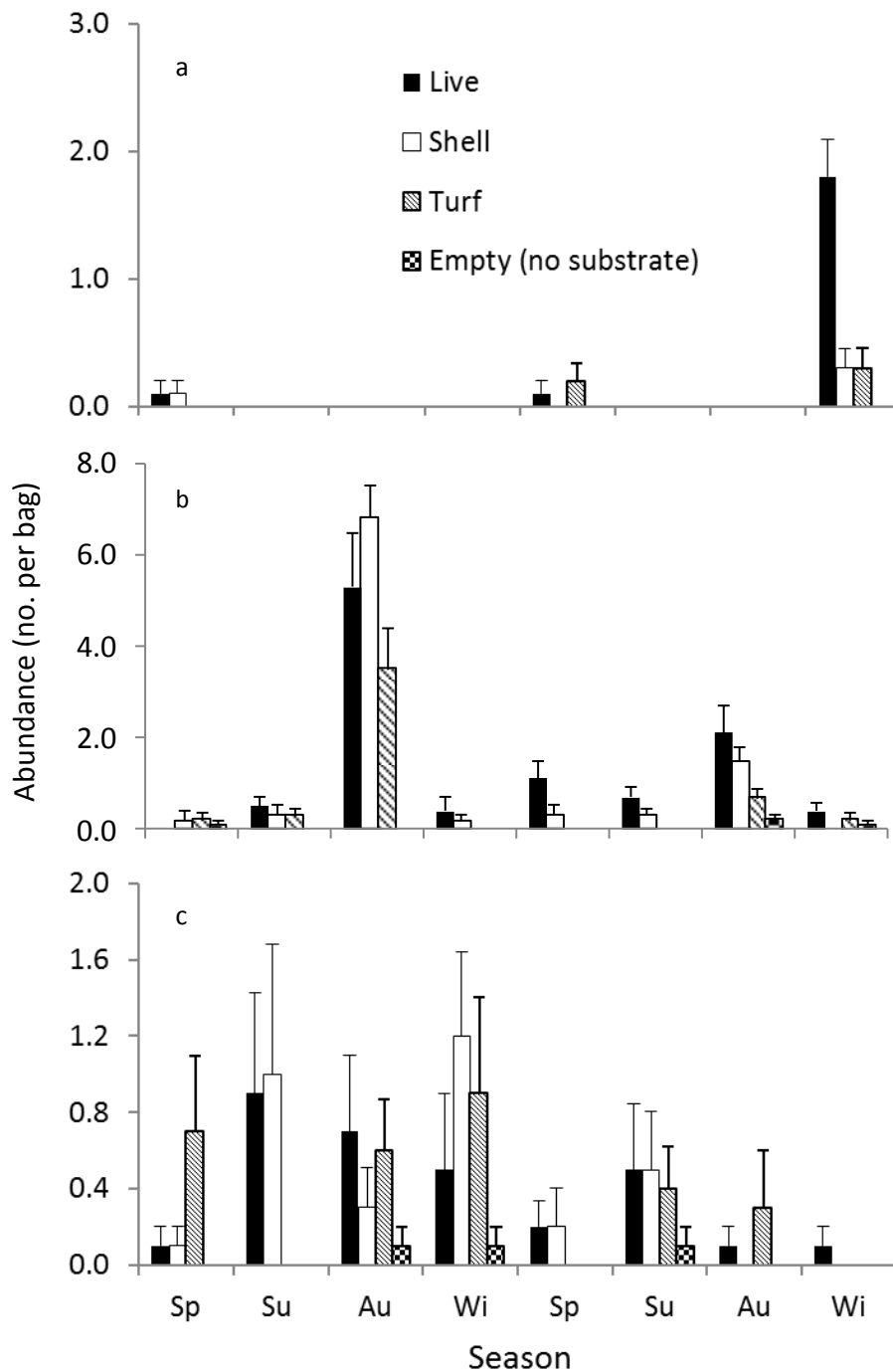


Figure 2. Mean (+SE) abundance per bag of (a) *Carcinus maenas*, (b) *Pilumnopus serratifrons*, and (c) *Halicarcinus ovatus* at the end of 3 mo deployment periods. Sp (Spring), Su (Summer), Au (Autumn), Wi (Winter). Yr 1 (September 2011 – August 2012), Yr 2 (September 2012 – October 2013). $n = 10$

In addition to *C. maenas*, 13 species of native crab were collected in bags during our study (Table 2). Among these, *Pilumnopus serratifrons* (Smooth Handed Crab) and *Halicarcinus ovatus* (False Spider Crab) were the most abundant, accounting for 55% and 21% of total crabs, respectively (Fig. 2). Of the 13 native species, six recruited only in autumn, one

recruited only in summer, two recruited in late summer and early autumn, one recruited only in winter one recruited in late winter and spring, while the other two recruited year round (Table 2).

Table 2. The total number, by species, of crabs detected in bags deployed for multiple 3 month periods (over 2 years), their mean ($\pm$ SE) size in the bags, their maximum (max) recorded size in Australia (from the literature) and their season of recruitment to the bags (Sp = Spring, Su = Summer, Au = Autumn, Wi = Winter)

Species	Total	Mean size (mm)	Max size (mm)	Season found
<i>Carcinus maenas</i>	30	6.7(1.3)	85 ^b	Sp ^{1,2} , Wi ²
<i>Pilumnopus serratifrons</i>	254	4.9(0.4)	20.1 ^b	All
<i>Halicarcinus ovatus</i>	96	5.0(0.3)	10 ^a	All
<i>Litocheira bispinosa</i>	41	3.0(0.1)	11 ^a	Sp ¹ , Wi ¹
<i>Brachynotus spinosus</i>	8	9.3(0.1)	17 ^a	Su ^{1,2} , Au ¹
<i>Amarinus lacustris</i>	8	3.5(0.1)	10 ^a	Su ^{1,2}
<i>Paragrapsus laevis</i>	6	7.5(0.5)	37 ^a	Su ¹ , Au ¹
<i>Thalamita sima</i>	2	5.4(0.2)	85 ^a	Au ¹
<i>Notomithrax minor</i>	1	5.8	40 ^a	Au ¹
<i>Ilygrapsus paludicola</i>	10	3.3(0.1)	9 ^a	Au ^{1,2}
<i>Ocypode convexa</i>	3	5.9(0.3)	25 ^a	Au ²
<i>Macrophthalmus crassipes</i>	5	4.4(0.3)	37 ^a	Au ¹
<i>Parasesarma erythrodractyla</i>	1	2.5	*	Wi ¹
<i>Charybdis orientalis</i>	1	8.7	46 ^a	Au ¹

^a(Poore 2004)
^b(C.J. Garside, unpublished data)

1= found in first year
2=found in second year
1,2= found in both years

The number of *P. serratifrons* that recruited to bags varied according to the three way interaction between Treatment $\times$ Season $\times$ Year (PERMANOVA: $Pseudo-F_{(9,288)} = 5.85$, $p = 0.001$; Fig. 2). In year 1, the abundance of *P. serratifrons* in bags with structured habitat (i.e. live oyster, dead oyster, or turf) was greater in autumn than at the other three times of year (post-hoc tests, $p < 0.05$; Fig. 2). In the empty bags, in which there were fewer of this crab, there was no significant difference in abundance among seasons (post-hoc tests, $p > 0.05$). In year 2, the abundance of *P. serratifrons* was greater in autumn than winter among the bags

with live oysters or shell and greater in autumn than in spring or summer among bags with shell or turf treatment (post-hoc tests, $p < 0.05$; Fig. 2).

The abundance of *H. ovatus* recruits in bags varied according to Treatment (PERMANOVA: $Pseudo-F_{(3,288)} = 3.51$, $p = 0.017$; Fig. 2) and Year (PERMANOVA: $Pseudo-F_{(1,288)} = 10.19$, $p = 0.004$; Fig. 2), but displayed no significant interaction among factors or a main effect of Season (PERMANOVA, $p > 0.05$). Pair-wise tests showed no difference in abundance for *H. ovatus* among the structured treatments (post-hoc tests, $p > 0.05$) but indicated significantly more *H. ovatus* in the bags of structured habitat than in the empty treatment (post-hoc tests, $p < 0.05$; Fig. 2). *H. ovatus* was significantly more abundant in Year 1 than Year 2 (post-hoc tests, $p < 0.05$; Fig. 2).

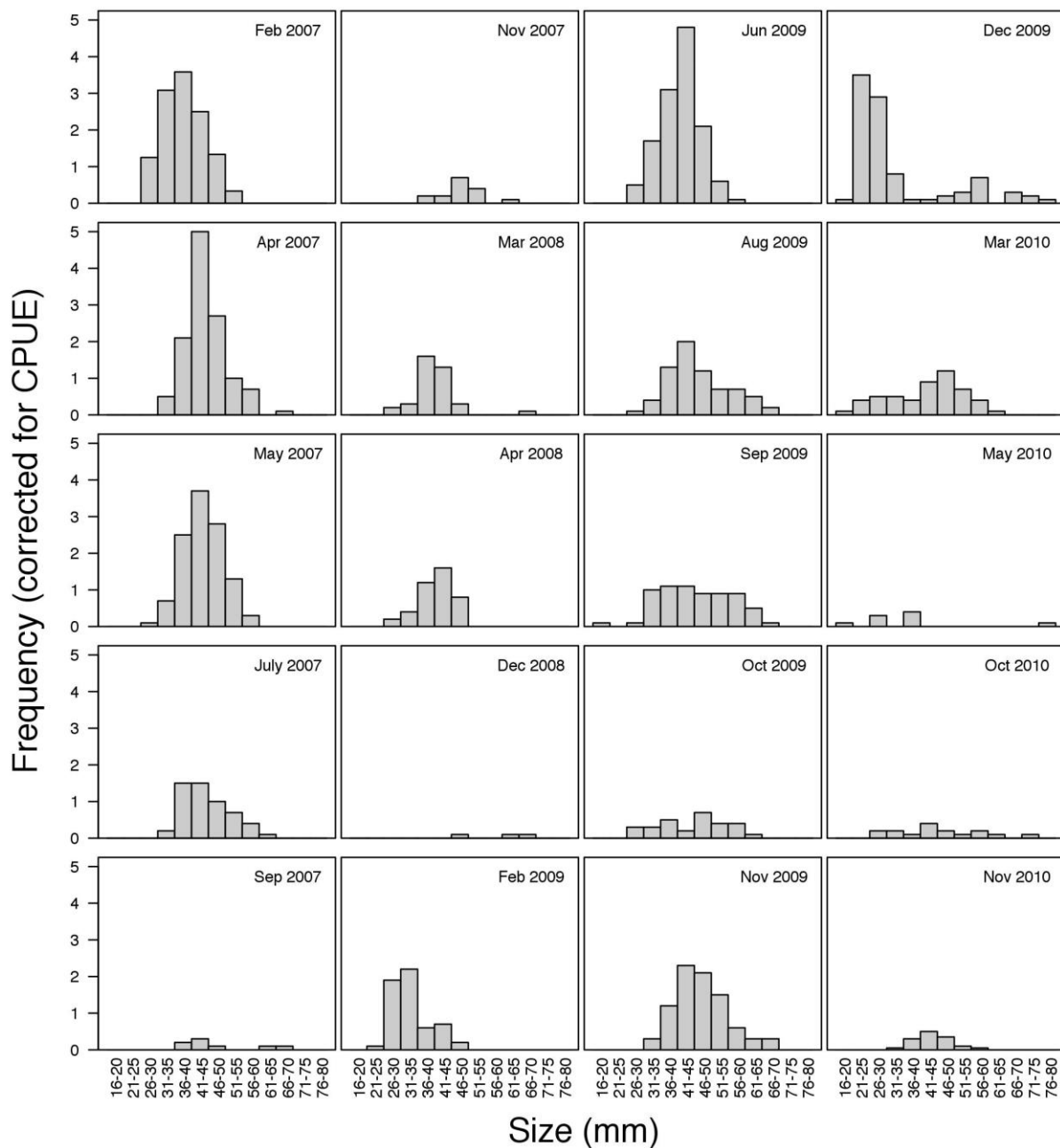


Figure 3. The size distribution of *Carcinus maenas* trapped within Fisheries Creek, NSW, on 20 sampling dates between February 2007 and November 2010. Size distributions are expressed as Catch Per Unit Effort (CPUE). $n = 1391$

Fisheries Creek cohort analysis

During 2011, the mean ($\pm$ SE) size of *C. maenas* was 6.7 ($\pm$ 1.3 mm, $n = 2$) in the bags deployed for 3 mo over spring and 27.8 ($\pm$ 2.2 mm, $n = 3$) for the bags deployed for 6 months over spring and summer, with no new recruits detected over summer. Hence, the change in

the size of the crabs over the 3 mo summertime period in which water temperature averaged 20.3°C (NSW Food Authority, unpublished data) was 7 mm/month.

Over our 45-mo study of Fisheries Creek *C. maenas* populations, spanning 20 sampling dates, we detected 5 main cohorts of crabs recruiting to a size class detectable by our traps (Fig. 3): (1) prior to February 2007; (2) between November 2007 and March 2008; (3) between December 2008 and February 2009; (4) September 2009; and (5) between December 2009 and March 2010. Based on the 7 mm/month change in size: the first and second cohorts, were 5–6 mo old at the time of their first detection in February 2007 and in March 2008, respectively, and hence settled in Sept–Oct (spring); the third cohort, was 4–5 mo old in February 2009, and hence settled in Oct–Nov (late spring); the fourth (small) cohort was 2–3 months in September 2009 and hence settled in Jun–Jul (winter); and the fifth cohort was 3–4 months old in December 2009, so settled in Jul–Sept (late winter–spring). In addition to these main recruitment events, small numbers of crabs of the smallest size class detectable by our traps were detected in March and May 2010, indicating, based on their size of 16–20 mm, settlement in January through to March (summer–early autumn).

Twenty seven gravid females were found on six occasions (May 07, Jun 09, Jul 07, Aug 08, Sep 09 and Nov 07) over the three year period.

Discussion

In combination, our recruitment study and cohort analysis indicate that in south-eastern Australia, *Carcinus maenas* megalopae are primarily settling in the late Austral winter to spring when average water temperatures are at their lowest (Fig. 1). By contrast, crabs native to south-eastern Australia primarily recruited in autumn when temperatures are higher (Fig. 1; Table 2). The timing of peak *C. maenas* recruitment in Australia, hence, differs from high latitude locations in its native and invasive range where recruitment primarily occurs in autumn (Table 1). This disparity likely reflects the warmer water temperatures within southern New South Wales, but this timing of recruitment may also modify interactions between *C. maenas* and native Australian crabs.

Quarterly deployments of natural and artificial substrate to Merimbula Lake over two years revealed that in each year, *C. maenas* recruitment peaked in late winter or early spring. The cohort analysis, conducted on data acquired from a nearby estuary, Fisheries Creek in

Twofold Bay, over nearly 4 years, revealed that the smallest size class of *C. maenas* trapped (16–20 mm) was sampled primarily in summer (four of the five occasions). Based on the change in size of *C. maenas* detected by this study, summer recruits of 16–20 mm would be derived from a spring settlement event. Hence, in south-eastern Australia the reproductive phenology of *C. maenas* may more closely match that displayed by the crab in Portugal, where settlement, although occurring year round (Baeta et al. 2005), tends to peak in winter and spring (Queiroga 1996; Sprung 2001), than at higher latitudes where settlement predominantly occurs in autumn (Eriksson and Edlund 1977; Berrill 1982; Audet et al. 2008).

Although in Merimbula Lake we only detected recruitment of *C. maenas* in late winter and early spring, ovigerous females were detected in nearby Fisheries Creek, from May to November and in previous studies in south-eastern Australia between April and November (NSW DPI unpublished data; Pollard and Rankin 2003). If *C. maenas* brood eggs for approximately 20 days (Berrill 1982; Vinuesa 2007), eggs may be released from June to December resulting in settlement from July to January if the planktonic duration is 25 days (at 20°C) to 40 days (at 15°C; Nagaraj 1993). A broader settlement period was supported by the trapping in Fisheries Creek, which although primarily detecting recruits of a size consistent with spring settlement, on several occasions also found small numbers of crabs that appeared to have come from settlement events at other times of the year. Hence, it seems that a broad window of settlement may be possible in south-eastern Australia; however, settlement peaks in late winter to early spring, perhaps due to pre-settlement mortality reducing the survival of larvae and megalopae through to settlement at other times of the year. The failure of our recruitment bags, deployed in Merimbula Lake, to detect recruitment outside of the peak event may have been a consequence of limited spatial replication.

Studies done elsewhere within the native and introduced ranges of *C. maenas* suggest that at high latitude locations, warming of waters following winter triggers reproduction of *C. maenas* (Crothers 1967; Audet et al. 2008; Table 1). In Portugal and Australia, however, the low temperatures of less than 10°C which prevent the hatching of eggs (Crothers 1967) and of less than 12°C that stall larval development (de Rivera et al. 2007) rarely occur. At Merimbula Lake, where our recruitment study was done, the average minimum sea temperature in the coolest months of July and August is 14.9°C (Fig. 1) and the lowest water temperature that we have recorded in the estuary is 9°C (C.J. Garside, unpublished data).

Instead, peak summer water temperatures may, by contrast, limit larval survival. Sprung (2001) found *C. maenas* larvae were present only at temperatures below 23°C. In the warmest month, March, the average maximum sea surface temperature at Merimbula is 23.8°C (Fig. 1). Ovigerous females were notably absent from our study site in the warmest, summer months. Late winter to early spring recruitment does, however, in south-eastern Australia overlap with the seasonal peak in rainfall and river flow that may reduce salinities below those suitable for larvae and newly settled juveniles.

Although cannibalism may be a more significant control on early survival of *C. maenas* than interspecific interactions (Moksnes 2004), in displaying a recruitment peak that differs in timing to many crabs native to south-eastern Australia, *C. maenas* may be able to minimize interactions with potential competitors. Of the thirteen species of native crab detected by this study, eleven displayed peak recruitment periods outside of the period of *C. maenas* recruitment in the substrates examined by this study. This included the most abundant taxon of native crab at our study site, *Pilumnopus serratifrons*, which accounted for over 50% of total crabs. Only three species of native crab were found to have temporal windows of recruitment that overlapped with the late winter–spring recruitment peak of *C. maenas*: *P. serratifrons*, which displayed some minimal recruitment year-round; *Halicarcinus ovatus*, which also recruited year-round; and *Litocheira bispinosa*, which although displaying a very similar pattern of recruitment to *C. maenas*, accounted for less than 10% of the native crabs recruiting to our bags. The size of *C. maenas* recruits exceeded all three of the species of native recruits, with which the invader overlapped.

The settlement of *C. maenas* represents an active response by the megalopae to the substrates they encounter (Moksnes et al. 2003). Previous studies have found greater recruitment of *C. maenas* to structured than unstructured habitats (Moksnes 2002). Greater recruitment to structured habitats may be driven by the strong selective pressure of reduced post-settlement mortality (Moksnes et al. 1998) or by a greater surface area for attachment of benthic invertebrate prey (Moksnes 2002). Here, we similarly found greater recruitment to our bags with structural habitat than those that were empty. Although live oyster bags collectively contained the greatest overall number of *C. maenas* when summed across the study, this pattern was driven by a difference among habitats at a single time point. Our study suggests that trays of commercially cultivated oysters, or specially deployed bags containing small oysters are likely to be appropriate for surveillance of *C. maenas* recruits

along the New South Wales coastline. It also reinforces the view that translocation of cultured oysters may potentially be a mechanism of *C. maenas* spread (Thresher et al. 2003). Our results do not necessarily suggest that natural oyster reef is a key site for settlement because artificial collectors, such as ours, provide substrate for settlement in areas where megalopae may normally pass over benthic substrates (Paula et al. 2006). A survey of natural substrates would be required to assess recruitment to natural habitats.

In summary, our study suggests that in south-eastern Australia, *C. maenas* displays a much longer reproductive season than in higher latitude locations that results in a late winter–spring recruitment peak. Although likely reflecting a release from the water temperature constraints of cooler climates, this strategy has the potential to benefit *C. maenas* by minimizing temporal overlap in recruitment with native crabs, and allowing temperature-sensitive stages in the reproductive cycle to occur outside of summer temperature extremes. In demonstrating that peak recruitment of *C. maenas* to benthic habitats of eastern Australia primarily occurs in late winter–spring, and occurs to oyster bags, our study provides guidance to surveillance programs aimed at early detection of the invader at new locations along the Australian coastline.

Acknowledgements

We thank the Sapphire Coast Marine Discovery Centre for providing the trapping data for Fisheries Creek. G. Meini, A. Gehin, J. Rowland, R. Furtado, J. Kenworthy, D. Bateman and S. Lamesa assisted with fieldwork. J. Mackay, C. and D. Boyton and S. Cullenward provided access to oyster leases and assisted with field logistics. S. Ahyong of the Australian Museum provided assistance with the identification of crabs. B. Kelaher M. Coleman, D. Barneche and D. Johnson provided helpful comments that improved the quality of the manuscript. This study was funded by an Australian Research Council (ARC) Linkage Grant to M. Bishop, M. Coleman, T. Glasby and B. Kelaher, with industry partners, the NSW Department of Primary Industries, Southern River Catchment Authority and Bateman's Marine Park. This work was performed during a Ph.D. candidature at Macquarie University during which C. Garside was in receipt of an Australian Postgraduate Award Industry (APAI) and an International Macquarie Research Excellence Scholarship (IMQRES).

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CHAPTER 4

The distribution of the European shore crab, *Carcinus maenas*, with respect to mangrove forests in south-eastern Australia

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Abstract

There is growing interest in associations between non-native species and native foundation species. Along the south-east coast of Australia, the European shore crab, *Carcinus maenas*, overlaps with the distribution of the grey mangrove, that provides refuge to many native invertebrates from predators and physiological stress. We tested the hypothesis that *C. maenas* would be more abundant under the canopy of mangrove forests than in adjacent unvegetated intertidal habitat. Trapping surveys within three estuaries found greater abundances of *C. maenas* in mangrove forests than in adjacent unvegetated habitat and, within mangrove forests, under the canopy than in the pneumatophores zone. Average temperatures under the mangrove canopy were up to 2°C lower than in unshaded habitats and maximum temperatures up to 5.7°C less. The results of tethering studies did not support the hypothesis that predatory mortality of *C. maenas* was reduced in mangroves. To the contrary, survivorship of tethered crabs was much lower under the mangrove canopy than in adjacent unvegetated habitat. Habitat choice experiments, however, indicated that crabs chose shaded and protected over unshaded habitat. Hence, along this coastline where summertime maximum air temperatures may approach the known LD₅₀ of *C. maenas* (40 minutes at 40°C), mangroves may help to facilitate persistence in intertidal habitats. Our study adds to a growing number indicating that foundation species may not only facilitate native species, but non-native species too. Additional studies are now needed to confirm the mechanism of the association between *C. maenas* and mangroves in eastern Australia.

Introduction

The introduction of non-native species to novel environments is recognized as a major driver of ecological change (Vitousek et al. 1996; Simberloff 2005). Non-native species are now found in every ecosystem, and have impacts ranging from negligible to transformative (Vitousek et al. 1996; Simberloff 2005). Given the potential for some non-native species to cause significant ecological and economic costs, understanding the ecological factors that regulate them has become a key concern of applied ecologists. For species that are known to have established in a novel environment, a first step in this process requires gathering information on their distribution and abundance, and then on the abiotic and biotic controls of these.

There is growing interest in how the distribution of non-native species follows distributions of foundation species (*sensu* Dayton 1972). Foundation species produce changes in environmental conditions that may be positive, negative or neutral to individual species, but on the whole tend to enhance biodiversity and species abundances (Dayton 1972; Bruno et al. 2003; Jones et al. 2004). Just as foundation species may make the environment more suitable for many native species, they might also have high associated abundances of non-native species (e.g. Bulleri and Benedetti-Cecchi 2008; Byers et al. 2012; Thompson and Schiel 2012).

The European shore crab, *Carcinus maenas* (Linnaeus 1758), is a global invader that has spread from northwest Europe and North Africa (Broekhuysen 1936; Naylor 1962; Crothers 1967) to the Atlantic and Pacific coasts of North America, Patagonian coast of South America, Japan, South Africa and southern Australia (Carlton and Cohen 2003; Thresher et al. 2003; Hidalgo et al. 2005). The crab is an omnivore and is found on hard and soft substrata in the upper littoral and sub-littoral zone, down to 60m, in protected marine and estuarine habitats (Crothers 1968; Grozholtz and Ruiz 1996; Thresher et al. 2003). In environments where the crab is vulnerable to high densities of predators, or to physiological stress, for example during periods of emersion at intertidal sites, it frequently is associated with foundation species such as marsh, algae or seagrass (e.g. Dijkstra et al. 2012; Ellis et al. 2012; Watt and Scrosati 2013; Bertness and Coverdale 2013). Along the east coast of Australia, mangroves are one of the most abundant foundation species in the intertidal environment and support dense and diverse native communities of epiphytic algae, terrestrial and aquatic

invertebrates, fish and mammals (Morrisey et al. 2010). In southern New South Wales and the east coast of Victoria, the distribution of the temperate grey mangrove, *Avicennia marina*, overlaps with that of *C. maenas* and it may provide an important habitat refuge for the crab in intertidal habitat.

Previous large scale-surveys in south-eastern Australia indicate that higher densities of *C. maenas* exist in mangroves than in saltmarsh, seagrass and oyster beds or in unvegetated sediment near the mouth of the estuary (Chapter 2). However, it is unclear whether at smaller scales mangroves also support greater abundances of the non-native crab than adjacent unvegetated intertidal habitat. The prop roots and peg roots (pneumatophores) of mangroves form a structurally complex habitat that greatly enhances the availability of hard substrata for molluscs to attach to (Nagelkerken et al. 2008) and can reduce the foraging efficiency of benthic predators such as toadfish (Tetraodontidae spp.) and bream (*Acanthopagrus australis*), which feed in mangrove forests at high tide (Warren, 1990; Primavera 1997; Smith and Hindell 2005). Furthermore, the mangrove canopy helps to maintain a shaded and humid environment which might be particularly important in enabling *C. maenas* which tolerates temperatures from < 0 to 35°C (Eriksson and Edlund 1977; Hidalgo et al. 2005) to withstand air temperatures that can exceed 44°C during the south-eastern Australian summer.

In this study, we investigate the distribution of *C. maenas* in intertidal waters of south-eastern Australia with respect to *A. marina* mangroves. Specifically, we assess whether: (1) within invaded south-east Australian estuaries, the European shore crab (*Carcinus maenas*) is more abundant within mangrove forests than in adjacent unvegetated habitat; (2) in mangrove forests *C. maenas* experiences reduced rates of mortality; (3) temperatures on the surface of sediments are cooler in mangrove forests than in unvegetated areas; and (4) when offered a choice between shaded and unshaded environments, *C. maenas* choose shaded habitat.

Methods

Spatial sampling

To test whether *Carcinus maenas* is more abundant (1) inside than outside of mangrove habitat patches and (2) under the mangrove canopy, where there is shading, than in the pneumatophore zone where there is structure but not shading, we conducted sampling within three estuaries on the south coast of New South Wales (NSW) Australia: Merimbula Lake (36.89°S, 149.92°E), Bermagui River (36.42°S, 150.06°E) and Wagonga Inlet (36.21°S, 150.13°E). Each of these estuaries is known to contain small populations of *C. maenas*, which is widely distributed, though not yet abundant, on the NSW coast (Chapter 2), and has patchy mangrove forest along its shoreline. Within each estuary, we randomly selected three study sites, each of which contained adjacent habitat patches (each at least 100 m in along-shore width) with mangrove and without (hereafter unvegetated). Within the mangrove habitat patch of each site, we set replicate traps ($n = 4-7$) at each of three elevations: under the mangrove canopy; in the pneumatophore zone; and 30 m seaward of the canopy. To control for effects of tidal elevation, traps were set at corresponding elevations in the adjacent unvegetated habitat. Traps were separated by at least 15 m.

Within each estuary, trapping was done on each of two consecutive days in spring of 2011, using 62 × 42 × 20 cm traps, constructed of 1.3 cm square plastic netting with two horizontal entrances at the apex of two inward facing 45° panels of netting at either end. These traps have been previously used for surveillance of *C. maenas* in Australian ports (Hewitt and Martin 2001; Thresher et al. 2003). Traps were baited with a single 8–15 cm pilchard (*Sardinops neopilchardus*), deployed on a day time low tide and left to fish for 22–24 hrs (as per Chapter 2). At the end of this period we enumerated the number of *C. maenas* per trap. Traps were then redeployed at different positions within each elevation of each habitat, and re-sampled after another 22–24 hrs.

PERMANOVA (Anderson et al. 2008) assessed differences in crab abundance among tidal elevations and habitats in each estuary. Separate analyses were conducted for each estuary because the abundance of *C. maenas* differed markedly among these. The analyses had the factors: site (3 levels, random); height on the shore (3 levels, fixed: high [a tidal elevation equivalent to that under the mangrove canopy], medium [a tidal elevation equivalent to that just seaward of the canopy, within the pneumatophore zone], low [30 m seaward of high]);

and habitat (2 levels, fixed: mangrove, unvegetated). There was no significant difference in crab catches between sampling dates ($p > 0.05$), so these were pooled to give 8–14 replicate traps per tidal elevation and habitat at each site. The analyses used unrestricted permutation of Euclidean distances calculated from raw data and were based on 999 permutations. Where significant treatment effects were seen (at $\alpha = 0.05$), the analyses were followed by PERMANOVA pair-wise post-hoc tests to assess sources of difference.

Survivorship experiment

To assess whether *C. maenas* experiences greater survivorship under the canopy of the mangrove forest than in adjacent unvegetated habitat, a tethering experiment was performed at the three sites in Merimbula Lake (36.89°S, 149.92°E) in December 2012. At each site, nine tethered crabs were deployed at each of the high and low elevations of the mangrove forest and the adjacent unvegetated habitat. The elevations were as described for the trapping study, such that in the mangrove patch, the high elevation was under the mangrove canopy, and the low elevation, below the pneumatophore zone, in unvegetated sediment. Tethered crabs were at least 15 m apart and in the intertidal zone.

The tethered crabs, collected by trap from intertidal habitats within Fisheries Creek, Twofold Bay, NSW, were 15–36 mm in carapace width, had a male to female ratio of 6:5 and were undamaged at the time of deployment. Individual crabs were randomly assigned to habitats and tidal elevations. They were tethered using 35 cm long pieces of nylon uncoated 14 kg test wire, one end of which was anchored to the sediment using a 15 cm long steel pin, and the other secured to the crab using a 3.2 mm diameter zinc-plated washer, glued (using Selleys® Tarzan's grip® shock-proof super glue) to the middle of the crab's carapace. Although the tethers restricted movement of crabs, they did not impede burial. Further, in a pilot study we found that in the absence of predation, the crabs remained attached to their tethers. We assessed the status of each tethered crab 4 and 11 days after deployment. Crabs were noted as alive and undamaged, alive and damaged (e.g. missing chelae), dead and undamaged, or dead and damaged, or missing. These data were used to calculate the proportion of crabs surviving at each elevation of each habitat and site.

We assessed the influence of habitat on the proportion of survival of *C. maenas* using a separate PERMANOVA for each of the two temporally non-independent sampling dates. The PERMANOVAs, which used sites as replicate blocks ($n = 3$), had two factors, height on the

shore (2 levels: high, low) and habitat (2 levels: mangrove, unvegetated) and used square-root transformed data. Statistical procedures were otherwise as described for the spatial survey.

Temperature data collection

To assess whether there are differences in the average or maximum temperature among the four habitat strata in which crabs were tethered, we deployed miniature Thermochron® iButton temperature loggers (Thermodata Pty. Ltd., Brisbane). At each of the three sites in Merimbula Lake where crabs were tethered, three waterproofed iButtons were randomly deployed on the sediment surface of each habitat strata, at least 15 m apart. The iButtons were programmed to take readings hourly throughout the tidal cycle for four consecutive days in December 2012 (i.e. summer). Three-way PERMANOVAs with the factors habitat (2 levels: mangrove, unvegetated), height on the shore (2 levels: high, low) and site (3 levels, random) tested for sources of spatial variation in the (a) average and (b) maximum temperature recorded by each logger.

Habitat preference experiment

We evaluated whether *C. maenas* displays a preference for shaded and more protected over unshaded and more exposed habitat using choice experiments on the shoreline of Merimbula Lake (36.89°S, 149.92°E). Experiments were conducted during a midday low tide of a sunny day in March 2013, during which the air temperature was 25°C. On an unshaded section of shoreline, at a tidal height of mean low water +1.3 m, we established a 50 cm wide and 100 cm long arena, enclosed with 20 cm high clear Perspex (Plexiglass). The long axis of the arena ran parallel to the waterline and was divided into two 50 cm-long halves. Each half of the enclosure could be shaded using a 50 × 50 cm cover, constructed of a Perspex sheet covered with 3 mm black plastic mesh.

We used an experimental design of the type proposed by Olabarria et al. (2002) to test for preference. We conducted four treatments: (1) shade + unshaded, with crabs initially placed on the shaded side; (2) shade + unshaded, with crabs initially placed on the unshaded side; (3) shade + shade; and (4) unshaded + unshaded. For each treatment, we recorded the proportion of time (over 5 min or until the crab buried, if sooner) that crabs spent on their starting side of the arena. The crabs, trapped in Merimbula Lake the previous night, had an

average carapace width $45 (\pm 0.7 \text{ standard error (SE)})$ mm and a male to female ratio of 1:1. Ten crabs were randomly assigned to each treatment, with each crab used only once. The starting position of each crab was the centre of the designated side of the arena.

In the event of a preference for the shaded and more protected microhabitat we expected to observe that (1) crabs spend a greater proportion of time in the preferred, shaded, microhabitat (i.e. [shaded'-unshaded = shaded'-shaded] > shaded-unshaded'; where ' denotes the starting microhabitat) and (2) crabs spend a smaller proportion of time in the non-preferred, unshaded, microhabitat when they have a choice (i.e. unshaded'-unshaded > unshaded'-shaded). These hypotheses were statistically tested using one-way PERMANOVAs.

Results

Spatial sampling

In Merimbula Lake, crabs were caught at each of the three sites, and in all of the habitat strata except the low shore of the mangrove habitat (i.e. below the pneumatophore zone; Table 1). In each of the other two estuaries, Wagonga Inlet and Bermagui River, crabs were only caught at one of the three sites and at each of these were present only on the high shore of the mangrove forest, under the canopy (Table 1). Due to the low number of crabs caught at Wagonga Inlet and Bermagui River, only the data from Merimbula Lake were statistically analysed.

PERMANOVAs revealed that within Merimbula Lake, there was a significant interaction between height on the shore and habitat type that differed among sites (Habitat $\times$ Height $\times$ Site; *Pseudo- $F_{4,234}$* = 4.55, p = 0.001). At two of the three sites, crabs were more abundant at the high tidal elevation of the mangrove habitat (i.e. under the canopy) than in any other habitat (Table 1). At the other site, crabs were most abundant at the mid elevation of the mangrove forest, in the pneumatophore zone (Table 1).

Table 1. Mean (SE) abundance of *Carcinus maenas* per trap, within patches of mangrove and unvegetated habitat at each of three sites (S1, S2, S3), in Merimbula Lake, Wagonga Inlet and Bermagui River. Within mangrove patches, traps were set under the mangrove canopy (high), seaward of the mangrove canopy in the pneumatophore zone (mid) and 30 m seaward of the mangroves, in front of the pneumatophore zone. Traps were deployed at equivalent tidal elevations of the unvegetated habitat. Merimbula and Bermagui $n = 7$, Wagonga $n = 4-7$.

Site	Mangrove			Unvegetated		
	High	Mid	Low	High	Mid	Low
Merimbula Lake (S1)	1(1)	0	0	0	0	0
Merimbula Lake (S2)	0	0.5(0.5)	0	0	0	0
Merimbula Lake (S3)	8.5(5.5)	1(1)	0	1.5(1.5)	0.5(0.5)	1.5(1.5)
Wagonga Inlet (S1)	0	0	0	0	0	0
Wagonga Inlet (S2)	0	0	0	0	0	0
Wagonga Inlet (S3)	1.5(0.5)	0	0	0	0	0
Bermagui River (S1)	1(1)	0	0	0	0	0
Bermagui River (S2)	0	0	0	0	0	0
Bermagui River (S3)	0	0	0	0	0	0

Survivorship experiment

After 4 days, the proportion of crabs that remained alive on tethers ranged from 0.78 at the high elevation of one of the unvegetated patches to 0.33 at the high elevation (i.e. under the canopy) of one of the mangrove patches (Fig. 1). After 11 days, the proportion of crabs remaining alive on tethers ranged from 0.75 at the high elevation of one of the unvegetated patches to 0 at the high elevation of the mangrove forest (Fig. 1). On neither of the sampling dates did we find a whole dead crab attached to a tether. Across all sampling times, crabs were either alive and undamaged (35% of all individuals), dead with only remnants of the carapace remaining attached to the tether (34%) or the crab was entirely missing (31%). Following pilot studies that revealed that in the absence of predators, crabs remained attached to tethers, missing crabs were interpreted as mortality as opposed to failure of the tethers.

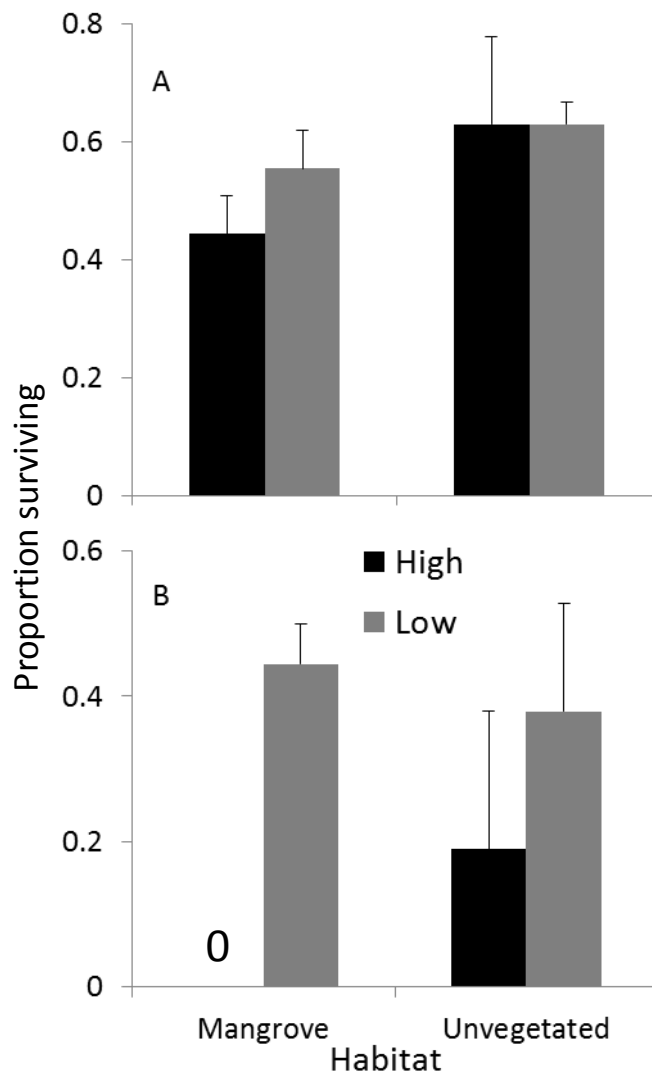


Figure 1. Mean (+SE) proportion of tethered *Carcinus maenas* (out of 9) remaining alive on tethers following 4 (A) and 11 (B) days of deployment at high and low elevations of mangrove and unvegetated habitat patches within Merimbula Lake. 0 = no survivors. $n = 3$ sites.

The added variance component due to site was not significant at any of the sampling times ($p > 0.05$) enabling analysis of data across these. Four days after the start of the experiment, there was no significant effect of height on the shore (PERMANOVA, $Pseudo-F_{1,8} = 0.56$, $p = 0.51$), habitat ($Pseudo-F_{1,8} = 1.85$, $p = 0.13$) or an interaction of these two factors ($Pseudo-F_{1,8} = 0.30$, $p = 0.61$) on crab survival. After 11 days, however, crab survival was determined by the interacting effects of height of the shore and habitat (PERMANOVA, $Pseudo-F_{1,8} = 10.78$, $p = 0.026$). At the high elevation on the shore, significantly fewer crabs were found in the mangrove than the unvegetated habitat, but at the low elevation, there was no difference in survival between the two habitats (post-hoc tests, $p > 0.05$).

Temperature data collection

Over four days in early summer, temperature loggers recorded temperatures, during flooded and non-flooded tidal states, ranging from 10.5-35.5°C at our study sites in Merimbula Lake. The highest temperatures were recorded at the high intertidal elevation of the unvegetated habitat patches, while the lowest temperatures were recorded at the high intertidal elevation of the mangrove and unvegetated habitat patches.

Independent of study site, average temperature displayed a difference between habitats that was dependent on tidal elevation (PERMANOVA, sig. Height $\times$ Habitat interaction; *Pseudo- $F_{1,24} = 118$, $p = 0.004$* ; Fig. 2a). High on the shore, average temperature was significantly lower under the canopy of the mangrove forest than in adjacent unvegetated habitat (post-hoc tests, $p < 0.05$), but lower on the shore, below the mangrove canopy, there was no significant difference in average temperature between habitat patches (post-hoc tests, $p > 0.05$). The average temperature under the mangrove canopy was at least 1°C cooler than outside of the mangrove forest at the high tide elevation (Fig. 2a). Maximum temperature displayed a weak but non-significant trend for being lower under the canopy of the mangrove forest than at similar tidal height of adjacent unvegetated habitat (Fig. 3b, PERMANOVA, *Pseudo- $F_{1,32} = 10.48$, $p = 0.085$*), but did not detectably differ from lower tidal elevations (Fig. 2b). The maximum temperature under the mangrove canopy was at least 2.3°C cooler than unvegetated habitat of a similar tidal elevation (Fig. 2b).

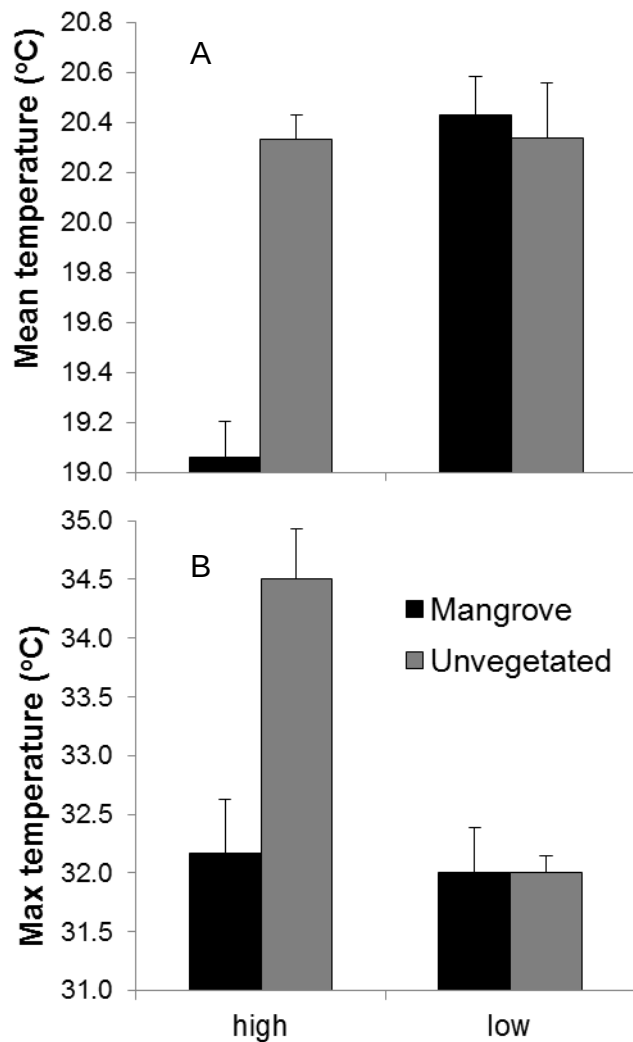


Figure 2. Mean (+SE) (A) and maximal (B) temperatures recorded on the sediment surface by loggers deployed on the high and on the low shore, in mangrove and unvegetated habitat, over a four day period in December 2012. $n = 3$ data loggers, within each of 3 sites.

Habitat preference experiment

When offered a choice of unshaded and shaded microhabitat, crabs spent over double the amount of time in the shade (83% shaded: 37% unshaded; Fig. 3). Among trials that included the hypothetically preferred microhabitat, shade, crabs spent a significantly greater amount of time in their starting side of the arena when it was shaded than when it was unshaded (i.e. [shaded'-unshaded = shaded'-shaded] > [unshaded'-shaded]; PERMANOVA: *Pseudo- $F_{2,27} = 5.69$, $p = 0.02$* ; Fig. 3). When started in the hypothetically non-preferred microhabitat, unshaded, there was a weak trend for crabs to spend significantly less time in this

microhabitat when offered a choice of shade than when not offered a choice, although this pattern was not statistically significant (PERMANOVA: $Pseudo-F_{1,17} = 2.18$, $p = 0.17$; Fig. 3).

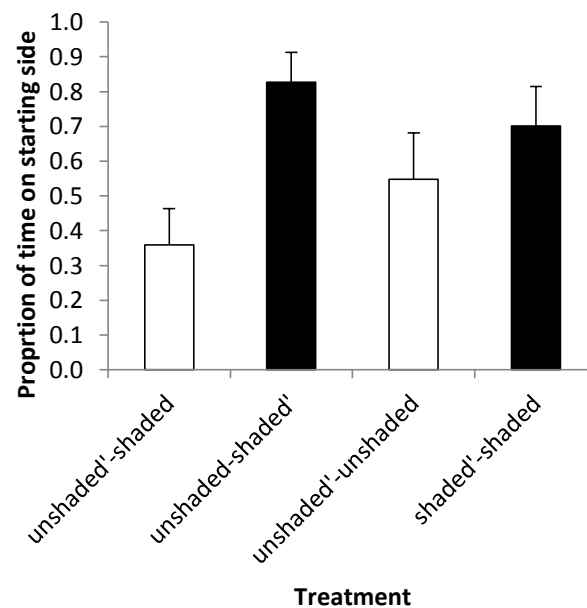


Figure 3. Mean (+SE) proportion of time spent by *Carcinus maenas* in the starting side (denoted by ') of experimental arenas in which pairs of habitats were offered to individual crabs. Crabs started in unshaded (white) or in shaded (black) habitat. $n = 10$ replicate trials.

Discussion

This study has revealed that in southern New South Wales (NSW), Australia, *C. maenas* is more abundant under the mangrove canopy than lower on the shore, or at a comparable tidal elevation in adjacent unvegetated habitat. This was despite greater rates of mortality under the mangrove canopy than in adjacent habitat strata and, instead, may reflect more favourable abiotic conditions for this cold-temperate species under the shaded mangrove canopy.

Consistent with previous studies along the south-east coast of Australia (Chapter 2), we found that the abundance of *C. maenas* was patchy and that the crab was not present at all sites sampled. As in previous studies conducted at different times of the year, overall abundances of *C. maenas* were lower in Bermagui River and Wagonga Inlet than in Merimbula Lake (Chapter 2). Larger scale factors such as the supply of recruits, salinity and

predator abundances likely contribute to differences among estuaries and sites in the abundance of crabs (Dethier and Schoch 2005). At sites where the crab was present, the non-native crab displayed a consistent pattern of greater abundance under the mangrove canopy than in adjacent unvegetated habitat. In a previous study, conducted across 14 New South Wales estuaries, we found higher abundances of *C. maenas* in mangrove forests than in saltmarsh, seagrass, oyster beds, or in unvegetated habitat near the mouth of the estuary (Chapter 2). Further, there have been unpublished sightings of the crab in mangroves in South Australia (S. Dittman, pers. comm.). Together, these studies suggest that in south-eastern Australia, mangroves are an important habitat of the non-native crab.

Australia has the highest latitude mangroves in the world and its south-east coastline is the only region of the world where the distribution of mangroves and *C. maenas* presently overlap. In parts of the world without mangroves, however, *C. maenas* has been found to utilize other types of biogenic intertidal habitat as shelter. For example, in New England, *C. maenas* are locally abundant in degraded salt-marshes where the burrows of the native crab *Sesarma reticulatum* provide refuge from physical stress and predation (Bertness and Coverdale, 2013; Coverdale et al., 2013). Within marshes and on intertidal rocky shores, the non-native crab is found in particularly high abundances under the canopies of macroalgae such as *Ascophyllum*, *Chondrus*, *Fucus* and *Mastocarpus* (e.g. Dijkstra et al., 2012; Ellis et al., 2012; Watt and Scrosati, 2013), the algae providing protection from predation and from temperature stress.

Mangrove forests support high abundances of invertebrates, such as gastropods and bivalves (Nagelkerken et al., 2008), which may serve as a prey resource for *C. maenas*, a generalist consumer of molluscs (Ropes, 1968). Additionally, mangroves may provide shading and the complex habitat provided by pneumatophores may lower the foraging efficiency of predators (Warren, 1990, Sheridan and Hays, 2003). We had hypothesised that the greater abundance of *C. maenas* crabs in mangroves than in adjacent habitats may be explained by reduced predatory mortality of crabs under the mangrove canopy. Previous studies have found that in intertidal habitats, biogenic habitat can serve as an important refuge for *C. maenas* from predators (e.g. Ellis et al., 2012) and the complexity of pneumatophores is known to decrease the foraging efficiency of some finfish at high tide (Sheridan and Hays, 2003). To the contrary, we found that survivorship of crabs was much lower in mangroves than in the unvegetated strata sampled. We did not quantify the

abundance of putative predators of *C. maenas* inside and outside of mangrove forests, and it is possible that other mesopredators, that may be intraguild predators of *C. maenas*, were also significantly higher in mangroves compared to bare sand (Nanjo et al., 2011). Where complex habitats provide mesopredators with refuge from higher order predators, and a foraging resource, mesopredators may select these over other habitats (Dill, 1987; Lima and Dill, 1990). Alternatively the greater mortality of *C. maenas* in the mangroves may be explained by a reduced capacity of large crabs to escape predators through burial due to the dense mat (Kelaher et al., 1998). Tethering of crabs may have heightened this effect by preventing crabs from migrating to areas where they can bury. In the salt-marshes of New England, *C. maenas* evict *Sesarma* from their burrows to gain refuge (Bertness and Coverdale, 2013).

Instead, it is possible that the greater abundances of *C. maenas* under the mangrove canopy were due to reduced temperature stress of *C. maenas* in this microhabitat as compared to the adjacent habitat strata. Although we did not measure the physiological stress of the crabs in the various habitat strata, the maximum temperature recorded by replicate loggers under the mangrove canopy was on average 2°C cooler and as much as 5.7°C less than on unshaded shoreline of similar intertidal height, and the average temperature they recorded was typically 1°C less (Fig. 3). In environments where the temperature of unshaded substrates approaches or exceeds the maximum temperature the crab can tolerate, shading by the mangrove canopy may be critical to persistence of the crab. Along the south coast of NSW, the crab's LD₅₀ of 40 minutes at 40°C (Ahsanullah and Newell, 1977) is occasionally exceeded intertidally. Below the lethal temperature, however, animals may also exhibit behavioural preferences for conditions that limit sub-lethal stress. Previous studies report that *C. maenas* acclimated to water temperatures of 8–22°C, the range experienced off the NSW coast, first start to show signs of heat stress at temperatures of 31–35°C (Cuculescu et al., 1998) and when exposed to temperatures exceeding 26.8°C display an emigration response to cooler areas or to air where they can thermoregulate via evaporation through the gills (Ahsanullah and Newell, 1977).

On a day well below the lethal temperature for *C. maenas*, the crabs displayed a behavioural preference for shaded over unshaded habitat. Distances in the arena were potentially shorter than experienced in the wild and the level of thermal stress could increase the degree of risk perceived by the crab to search for more favourable habitats. Although it is

possible that the individuals tested also chose the shaded areas, which were covered, as refuge, this behaviour is also consistent with avoidance of physiological stress. Elsewhere in its introduced range, *C. maenas* has been observed to display behavioural responses to emersion that minimize physiological stress (Simonik and Henry, 2014). By spending periods of emersion under seaweeds and rocks, where temperatures were half and humidities double those of exposed surfaces, crabs were able to behaviourally mitigate the desiccation, hypercapnia and thermal stress that otherwise occurred (Simonik and Henry, 2014). Although the crab may also avoid physiological stress by remaining in subtidal waters, these typically have higher densities of large predators that can exert top-down control on *C. maenas* (Hunt and Yamada, 2003; de Rivera et al., 2005; Jensen et al., 2007). Furthermore, there may be trade-offs to remaining in less stressful environments, for example reduced access to prey resources. In our study, which also sampled at multiple elevations of unvegetated habitat, *C. maenas* abundance did not increase with decreasing elevation. Our study did not investigate the role of prey availability in contributing to the high abundance of *C. maenas* in mangroves. *C. maenas* is a generalist consumer of invertebrates (Ropes, 1968), and is known to consume Sydney rock oysters (*Saccostrea glomerata*; M.J. Bishop unpublished data) and pygmy mussels (*Xenostrobus securis*; Veiga et al., 2011) each of which form dense aggregations attached to and between grey mangrove pneumatophores (Chapman and Tolhurst, 2004; Bishop et al., 2012). It is likely that the locally high availability of prey also contributes to the crab's use of mangroves.

There is growing evidence that foundation species, in modifying local environmental conditions, may not only facilitate native species but also non-native species (Altieri et al., 2010). Our study provides evidence for an association between the non-native crab *C. maenas* and mangroves in eastern Australia. Further studies are now needed to confirm the nature of the association.

Acknowledgements

We thank D. Bateman, T. Cloatre and M. Gralow for assistance with fieldwork. T.M. Glasby, B.K. Kelaher, M.A. Coleman and an anonymous reviewer provided helpful comments that improved the quality of the manuscript. This study was funded by an Australian Research Council (ARC) Linkage Grant to M.J.B. with industry partners, the NSW Department of

Primary Industries, Sapphire Coast Marine Discovery Centre, Southern River Catchment Authority and Bateman's Marine Park. This work was performed during a Ph.D. candidature at Macquarie University. C.J.G was in receipt of an Australian Postgraduate Award Industry (APAI) and an International Macquarie Research Excellence Scholarship (IMQRES).

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CHAPTER 5

Putative predators of *Carcinus maenas* in eastern Australia

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Abstract

Following the arrival of non-native species into suitable environments, interactions with native biota can influence non-native species establishment, proliferation and spread. The European shore crab, *Carcinus maenas*, is a global invader for which top-down control by predators has been implicated in limiting range expansion in its North American east coast range. Little is known, however, about predators of this non-native species within its Australian distribution. We conducted quarterly trapping over two years in 10 Australian estuaries to assess relationships between the abundance of *C. maenas* and native predators. Feeding assays in aquaria between *C. maenas* and putative predators, identified from negative associations in occurrence, assessed the ability of putative predators to recognise *C. maenas* as prey. Six taxa were negatively correlated with *C. maenas* abundance and capable of eating the non-native crab. These were the Blue Swimmer Crab (*Portunus pelagicus*), octopods (*Octopus* spp.), Leatherjackets (Monocanthidae), Yellowfin Bream (*Acanthopagrus australis*), Toadfish (Tetraodontidae) and the Eastern Fiddler Ray (*Trygonorrhina fasciata*). *Octopus* spp. were rare in and Blue Swimmer Crabs absent from mangroves, the habitat in which *C. maenas* was most abundant. A tethering experiment confirmed that *Octopus* spp. prey on *C. maenas* in the field, with greater rates of mortality of the crab close to than away from *Octopus* spp. lairs. Overall, our study indicates that a diverse assemblage of predators may prey upon *C. maenas* in south-eastern Australia.

Further studies assessing relative rates of predation by these predators on *C. maenas* are needed to assess whether they are exerting significant top-down control.

Introduction

The transportation of non-indigenous species (NIS) has increased exponentially with the growth of world trade (Ruiz et al. 1997; Mack et al. 2000). At any one time, more than 7,000 species are being transported by commercial shipping to novel environments (Rilov and Crooks 2009). Although only 10% of all species translocations result in successful introductions, and only 10% proliferate and spread (Williamson and Fitter 1996), successfully invading NIS can cause billions of dollars of damage (Pimentel et al. 2005). Hence, a key concern of ecologists is to identify the factors that determine when and where NIS establish, proliferate and spread.

The match between the biology of the introduced species and the abiotic and biotic components of novel environments are broadly acknowledged to determine post arrival establishment, proliferation and spread of NIS (Carlton 1996; Sakai et al. 2001). Initially, abiotic factors act as a filter for organisms whose physiological tolerances do not match the new environment (Sakai et al. 2001). Subsequently biotic characteristics of the recipient community such as its diversity, and hence availability of empty niches, abundance of predators and parasitic species determine 'biotic resistance', the capacity of the native community to resist invasion (Elton 1958; Olyarnik et al. 2009). In some novel environments, NIS experience release from coevolved relationships with competitors, predators and parasites, such that they can grow rapidly, reach larger sizes and high population densities (Grosholz and Ruiz 1996; Torchin et al. 2001; Colautti et al. 2004). In other areas, abundant generalist predators provide significant top-down control of NIS (Thresher 1997; Byers and Noonburg 2003).

Biotic resistance has been hypothesised to play a role in contributing to the spatially variable establishment, proliferation and spread of the global invader, the European shore crab, *Carcinus maenas* (Hunt and Yamada 2003; de Rivera et al. 2005; Jensen et al. 2007). *Carcinus maenas* is an epibenthic omnivore, native to northwest Europe and North Africa (Broekhuysen 1936; Naylor 1962; Crothers 1967), that has established non-indigenous

populations on the Atlantic and Pacific coasts of North America, Japan, South Africa and southern Australia (Carlton and Cohen 2003; Thresher et al. 2003). The broad salinity and temperature tolerances of *C. maenas*, and its biphasic life cycle, which includes planktonic larval and benthic stages, have facilitated its translocation by shipping (Carlton and Cohen 2003). Although *C. maenas* has established and rapidly spread from its point of introduction along several coastlines (Carlton and Cohen 2003; de Rivera et al. 2007), its proliferation and spread has been less than predicted from its life history and physiological tolerances (Carlton and Cohen 2003; Thresher et al. 2003; Hampton and Griffiths 2007).

Carcinus maenas is susceptible to predation during its benthic and pelagic lifecycle phases, both within its native and introduced ranges (Crothers 1968; Klassen and Locke 2007). Along the east coast of the USA, predation by the native portunid crab *Callinectes sapidus* alone or in combination with other factors is thought to have limited the southward range expansion of the crab (de Rivera et al. 2005). Large Rock Crabs (*Cancer irroratus*), Jonah Crabs (*Cancer borealis*) and American Lobster (*Homarus americanus*) have been hypothesised to contribute to the non-native crab's largely intertidal distribution along this coastline by preying upon and competing with *C. maenas* in subtidal areas (Donahue et al. 2009; League-Pike and Shulman 2009), although *C. maenas* can out-compete and prey upon juvenile *H. americanus* (Rossong et al. 2006; Williams et al. 2006). On the West Coast of North America, aggressive Rock Crabs (*Cancer* spp.) have been identified as predators of *C. maenas* that have the potential to reduce the NIS abundance and spread (Hunt and Yamada 2003; Jensen et al. 2007).

In Australia, *C. maenas* was first recorded in Port Phillip Bay, Victoria in 1900 (Fulton and Grant 1900, 1902), and has subsequently established populations in Victoria, South Australia, New South Wales (NSW) and Tasmania (Thresher et al. 2003). Yet, despite predictions that the crab would expand its range northwards to southern Queensland (Latitude = 28°S, Carlton and Cohen 2003; Summerson et al. 2007; Compton et al. 2010), persistent populations appear to be limited to Batemans Bay, NSW, and south at latitudes below 35.7°S (C.J. Garside and T.M. Glasby, unpublished data). Even south of this point, and despite high genetic connectivity of the population along the east coast (Burden et al. 2014), the occupation of many estuaries by *C. maenas* is highly ephemeral and crabs remain largely confined to intertidal habitats, in particular, mangroves (Chapter 2). By contrast, in Tasmania the distribution and abundance of *C. maenas* has rapidly expanded following its introduction

in 1993 (Gardner et al. 1994). Previously, it has been suggested that the relatively slow range expansion of *C. maenas* in south-eastern Australia, compared to Tasmania, may be due to top-down control by predators only present on the mainland (Thresher 1997). For example, the Blue Swimmer Crab, *Portunus pelagicus* (a confamilial of *C. sapidus* which preys upon *C. maenas* in the eastern USA; de Rivera et al. 2005), is found on mainland Australia but not in Tasmania.

A number of native species along the east Australian coast might serve as predators of *C. maenas* (Zeidler 1997). Here, as a first step in ascertaining whether south-east Australian ecological communities may exhibit some biotic resistance to *C. maenas*, we identify potential predators of *C. maenas* in south-eastern Australia, with abundances that are negatively correlated with the abundance of the non-native crab, and that are demonstrated through laboratory feeding trials to be capable of feeding on *C. maenas*. We also conducted a tethering experiment in a key habitat of one predator, the octopus, to assess whether in the field this predator consumes the non-native crab.

Methods

Trapping survey

Relationships between the abundance of *C. maenas* and of native biota were assessed through eight quarterly trapping surveys over two years, from December 2010 through to September 2012, in 10 estuaries along the south coast of New South Wales (NSW), Australia (Fig. 1). We deliberately trapped only in estuaries for which there are records of *C. maenas* (C.J. Garside and T.M. Galsby, unpublished data) as our focus was on establishing the potential role of native predators and competitors in influencing *C. maenas* abundance.

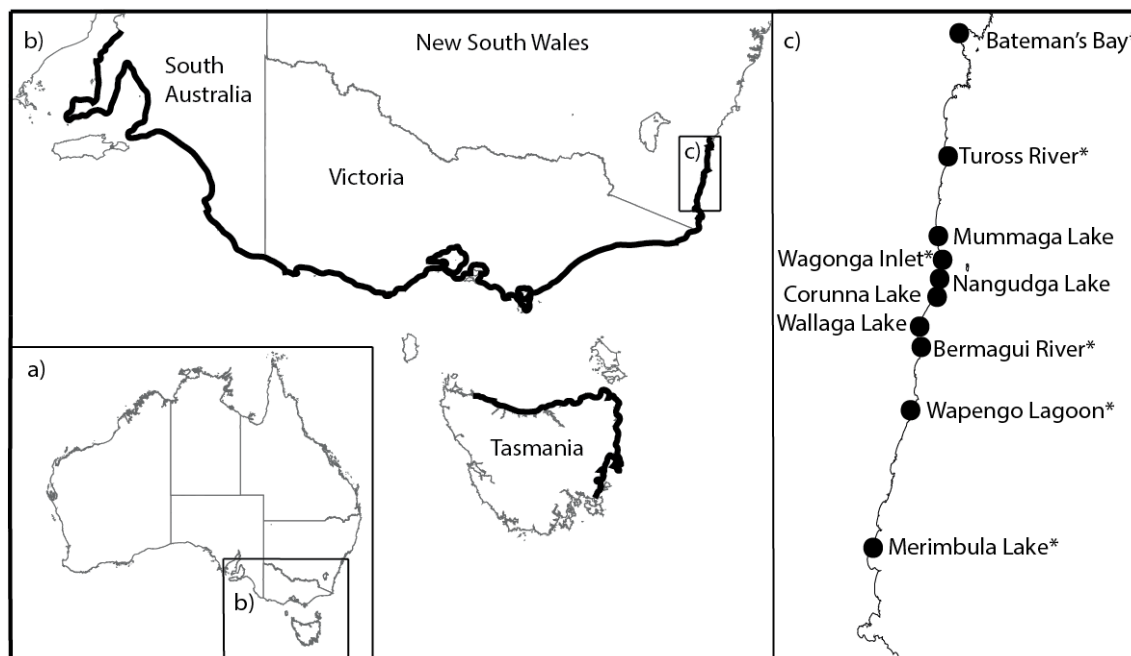


Figure 1. Map of Australia (a) showing the present distribution of *Carcinus maenas* (bold line) in Australia (b) and the estuaries surveyed (c). * denotes estuaries in which five habitats were sampled (mangrove, oyster, seagrass, saltmarsh and unvegetated sediments). In all others, three habitats (seagrass, saltmarsh and unvegetated sediment) were sampled.

In each estuary, on each date, ten replicate traps were deployed in each of (1) saltmarsh (dominated by *Sarcocornia* spp., *Sporobolus* spp., and *Juncus* spp.), (2) seagrass (a mixture of *Posidonia australis*, *Zostera* spp., and *Halophila* spp.), (3) unvegetated rock or sand habitat near the mouth of the estuary and, where present, (4) mangroves (*A. marina*) and (5) oyster lease/oyster reef (mixed beds of *S. glomerata* and *C. gigas*), to give a total of 30–50 traps per estuary (see Fig. 1). This design ensured good spatial and habitat coverage within each estuary, such that relationships between *C. maenas* and potential predators could be established across the full range of their densities. Traps in seagrass and oyster habitats were set in subtidal areas (with the exception of Nangudga where only intertidal seagrass habitat was present), in water < 3 m deep, and traps in mangroves were in the intertidal zone. The traps in saltmarsh and in unvegetated habitats were intertidal when the estuaries, many of which were Intermittently Closed and Open Lakes or Lagoons (ICOLLs), had entrances that were open, but subtidal (< 1 m depth) when closed estuary entrances prevented a tide.

The traps were 62 × 42 × 20 cm yabby traps, covered with 1.3 cm square plastic netting, and with an opening at either end, extending the width of the trap, and situated at the apex of

two inward facing 45° panels. Traps of this design are commonly used for censusing *C. maenas* in Australian estuaries (Hewitt and Martin 2001; Thresher et al. 2003) and not only target *C. maenas*, but also capture a diversity of vertebrate and invertebrate species as by-catch. Although they may underestimate the abundance of large mobile predators, traps of similar design have previously been used to estimate abundances of other benthic species (Hunt and Yamada 2003). Traps were weighted with half a clay fired brick, baited with a single fresh 8–15 cm Pilchard (*Sardinops neopilchardus*) housed in a perforated bait-bag of black 1.3 cm plastic mesh, and deployed in either the early morning or late afternoon, for a period of 22–24 hrs. Pilot studies in an area with high densities of *C. maenas* showed no significant difference in catch between traps deployed for 12 or 24 hrs (ANOVA, $F_{1,6} = 0.47$, $p = 0.517$), or for traps set during the morning or evening (ANOVA, $F_{1,1} = 0.98$, $p = 0.504$). All animals in each trap were identified and enumerated at the end of the deployment period.

Feeding trials

To ascertain which of the species caught in the traps might prey upon *C. maenas* during the benthic phase of its life history, a literature search and feeding trials were performed. The literature search: (1) determined the feeding guild of native species encountered in traps; and (2) identified any previous records of these species predating or damaging benthic invertebrates. The feeding trials, at the Merimbula Aquarium, NSW, tested the capability of predators, identified in the traps and determined from the literature to be capable of feeding on benthic invertebrates, to damage or consume *C. maenas*. Additionally, we conducted feeding trials on three larger predators, Snapper (*Pagrus auratus*), Giant Cuttlefish (*Sepia apama*) and Eastern Blue Groper (*Achoerodus viridis*) common in embayments along the south-east Australian coast, and overlapping in their distribution and habitat utilization with *C. maenas*, which due to their size may have been excluded from our traps.

Feeding trials were conducted in aquaria. The size of tank was commensurate with predator size ranging from 0.6 × 0.35 × 0.4 m (e.g. Blue Swimmer Crabs, Toadfish) to 5.75 × 4 × 2.2 m (e.g. Snapper, Eastern Blue Groper). Sediment was not added to tanks because our goal was to assess the capability of the predators to consume *C. maenas* independent of habitat. The temperature in the tanks ranged from 16–22°C and salinity from 32–35, matching the range of conditions across which *C. maenas* and potential predators were collected. All tanks

experienced natural light cycles. In the trials, small (carapace width: < 3 cm), medium (carapace width: 3–5 cm), or large (carapace width: > 5–8 cm) *C. maenas*, collected from intertidal and shallow subtidal waters of Merimbula Lake (36.89°S, 149.92°E) and Fisheries Creek, NSW (37.11°S, 149.92°E) were offered individually to wild-caught predators (see Table 1 for a list) that had been acclimated to the tank for 1 week or more and fed *ad libitum* up to 24hrs immediately prior to each trial. We observed whether each size class of crab was consumed by the predator over a 12 hr period. Trials were repeated with replicate individuals of each predator species until either a crab had been preyed upon by a particular size class of predator, indicating its capability of consuming *C. maenas*, or 20 individuals of the predator species had been tested with no evidence of predation (recorded as a negative result for that species; Table 1).

Table 1. Species caught in traps that were identified from the literature to either prey upon *Carcinus maenas* (C) or other similar-sized benthic crustaceans (B) and on which feeding trials were run. ✓ = predation on *C. maenas* observed; X = the trial was conducted but no evidence of predation on *C. maenas* observed; n/a= trial not conducted.

Species	Citation for predation on <i>C. maenas</i> or on other benthic crustaceans	Type of literature evidence	Caught by traps	Size range of predators in trial (cm)^	Size of <i>C. maenas</i> consumed within aquarium feeding trials			Number of trials before predation occurred		
					< 3 cm	3-5 cm	> 5 cm	< 3 cm	3-5 cm	> 5 cm
Blue Swimmer Crab (<i>Portunus pelagicus</i>)	De Lestang et al. 2000	B	Y	15	✓	✓	n/a	1	1	n/a
Mud Crab (<i>Scylla serrata</i>)	Hill 1976, 1979	B	Y	14-19	✓	✓	✓	1	1	1
Leatherjacket (Monocanthidae spp.)	Bell et al. 1978	B	Y	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Yellowfin Bream (<i>Acanthopagrus australis</i>)	Morton et al. 1987	B	Y	400-450	✓	✓	n/a	1	1	n/a
Toadfish (Tetraodontidae spp.)	Bell et al. 1984	B	Y	5-12	X	n/a	n/a	did not consume		
Flathead (Platycephalidae spp.)	Hadwen et al. 2007	B	Y	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Snapper (<i>Pagrus auratus</i>)	Colman 1972	B	N	40-45	✓	✓	✓	1	1	1
Eastern Blue Groper (<i>Achoerodus viridis</i>)	Thomson 1959	B	N	75-125	✓	✓	✓	1	1	1
Eel (<i>Anguilla</i> spp.)	Baker 2010	B	Y	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Moray Eel (<i>Gymnothorax prasinus</i>)	Russell 1983	B	Y	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Southern Conger Eel (<i>Conger verreauxi</i>)	Russell 1983*	B	Y	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Stingaree (<i>Urolophus</i> spp.)	Bulman et al. 2001	B	Y	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Eastern Fiddler Ray (<i>Trygonorrhina fasciata</i>)	Marshall et al. 2007	B	Y	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Giant Cuttlefish (<i>Sepia apama</i>)	Guerra 1985*	B	N	50	✓	✓	✓	1	1	1
Octopus (<i>Octopus tetricus</i>)	Mather 1991a	B	Y	50	✓	✓	✓	1	1	1

*cites congener species; ^Size of predators were measured as total length for fish and cephalopods and carapace width at the widest anterolateral spines for crabs.

Tethering experiment

To test the hypothesis that *Octopus* spp., one of the taxa negatively correlated with the abundance *C. maenas*, would consume *C. maenas* in the field, a tethering experiment was performed in Merimbula Lake (36.89°S, 149.92°E) in October–December 2013. In the event of *Octopus* spp. predation on *C. maenas*, we expected greater mortality of crabs close to the lairs of *Octopus* spp. (see; Mather 1991a) than further away. Twenty-five lairs of *Octopus* spp. were identified in water < 1 m deep at low tide based on the observation of a shell-lined hole. The presence of an octopus at each lair was confirmed either visually or by inserting a plastic pole into the lair which would be grabbed by the octopus if it was present. At each lair, a single tethered crab was deployed within 1 m from the entrance and another 5 m away, using a paired design. Within each pair, crabs were deployed at similar depth and in the same habitat type.

Tethered crabs were 56 ± 1 mm (SE) in carapace width (between 5th anterolateral spines), undamaged at the time of deployment, and had been collected from Merimbula Lake during the previous 24 hrs. Crabs were tethered using a 35 cm long piece of nylon uncoated 49 strand (7 × 7), 14 kg test wire, glued (using Selleys® Tarzan's grip® shock-proof super glue) to the carapace of each crab using a 3.2 mm diameter zinc-plated washer centrally positioned between the 4th and 5th walking legs. The nylon tethers were then attached to 15 cm long steel landscape garden pins, anchored in the sediment. Tethered crabs could still bury and, in pilot studies, remained attached to their tethers in the absence of predation.

To test the hypothesis that a greater proportion of crabs close to than away from each octopus lair would be preyed upon, we made observations of the crab for 5 min after deployment, then checked the tether after 24 hours. Crabs were noted as alive and undamaged, alive and damaged (e.g. missing chelae), dead and undamaged, dead and damaged (with the site of damage noted or indicated by a piece of the carapace still attached to the tether), or missing.

Statistical analyses

We used Spearman's rank correlations to test for negative relationships between the abundance of *C. maenas* and the abundance of native predators that were identified by feeding trials and the literature to be capable of consuming *C. maenas* and were present in more than one estuary. To correct for the inflated Type I error associated with conducting

multiple comparisons, p -values were interpreted following Bonferroni corrections. Because the abundance of *C. maenas* per trap was typically very low and many predators are mobile and forage over much larger scales than the individual trap, relationships were assessed at three scales: (1) traps (with analyses only run for putative predators with abundances of > 50 individuals across the eight surveys, and following removal of data points that represented joint absences between *C. maenas* and the predator); (2) estuaries (using catch per unit effort; CPUE) and; (3) surveys (using CPUE averaged across whole surveys, $n = 8$), the latter of which assessed whether the abundance of *C. maenas* was negatively correlated with seasonal variation in the abundance of predators.

For those native predators that were negatively correlated with the non-native crab at the scale of traps, we ran permutational analyses of variance (PERMANOVA Primer6; Anderson et al. 2008) to assess differences in their abundance among habitat types. PERMANOVAs for *C. maenas* are not reported here, as patterns of *C. maenas* distribution among habitats have previously been described in Chapter 2. PERMANOVA uses permutations to partition the variation among individual and interaction terms using a dissimilarity matrix as the data input (in this instance Euclidean distance matrices). However, unlike ANOVAs, PERMANOVAs make no assumptions about the underlying distribution of the variables (Anderson 2005). Two sets of PERMANOVAs were run, each with the factors habitat (fixed), estuary (random) and sampling date (random). The first included the four estuaries in which only three of the habitat types were present (seagrass, saltmarsh and unvegetated). The second set of PERMANOVAs included the six estuaries where all five habitat types were present (unvegetated, seagrass, mangrove, saltmarsh and oyster). The analyses used traps as replicates ($n = 10$). Where significant treatment effects were demonstrated (at $\alpha = 0.05$), the PERMANOVA analyses were followed by pair-wise post-hoc tests to assess levels of treatments that differed.

For the tethering experiment, a significant difference between the proportion of crabs surviving at each of the two distances from octopus lairs was assessed using chi-squared tests.

Results

Trapping survey

Of the 3360 traps deployed, 38 were lost, stolen or tampered with, leaving 3322 that could be included in the analyses. In total, across the eight surveys, the traps caught 268 *C. maenas* as well as another 1838 animals representing 52 species. Nineteen percent of crabs showed signs of damage (missing appendages, regenerated claws, carapace damage). Of the damaged crabs, 25% had both claws missing, 40% of had one claw missing, 18% had claws that were regenerating from damage, 8% had a missing walking leg, 2% had damage to their carapace and the remaining 8% had a combination of types of damage.

Analysis of data from the survey revealed that at the scale of traps, the abundance of *C. maenas* was negatively correlated with the abundance of the Blue Swimmer Crab (*Portunus pelagicus*), Leatherjackets (species of Monocanthidae), Yellowfin Bream (*Acanthopagrus australis*), Toadfish (species of Tetraodontidae), the Eastern Fiddler Ray (*Trygonorrhina fasciata*) and Octopus (*Octopus* spp.; Fig. 2). At the scale of estuary and survey only the catch per unit effort (CPUE) of *Octopus* spp. was significantly negatively correlated with the CPUE of *C. maenas* (Table 2; Fig. 3). All other correlations were statistically non-significant following Bonferroni correction (Table 2).

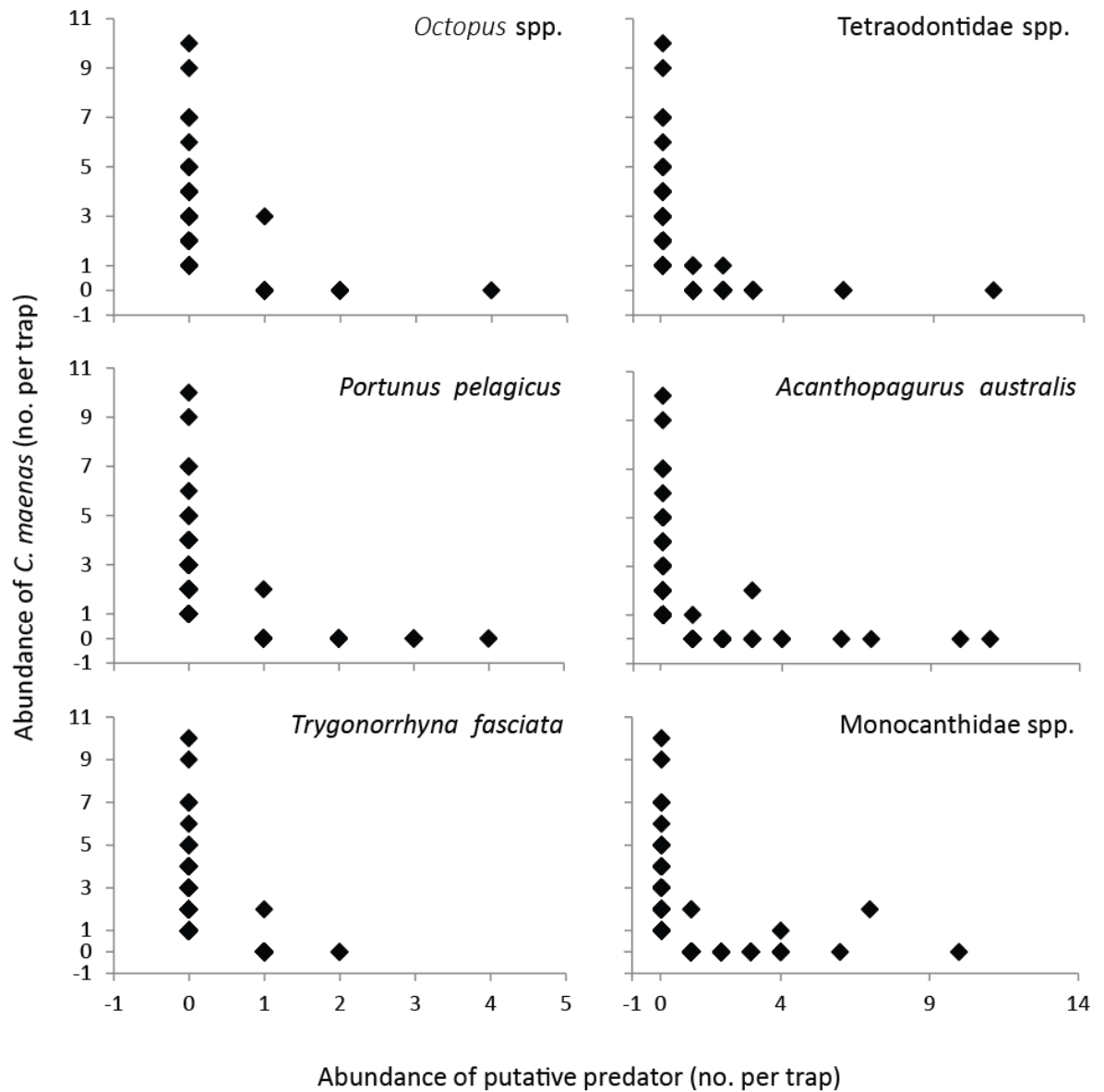


Figure 2. The relationship between the total abundance per trap of *Carcinus maenas* and each of *Octopus* spp. (Octopus; $n = 224$), Tetraodontidae spp. (Toadfish; $n = 255$), *Portunus pelagicus* (Blue Swimmer Crabs; $n = 186$), *Acanthopagrus australis* (Yellowfin Bream; $n = 236$), *Trygonorrhina fasciata* (Eastern Fiddler Ray; $n = 180$), and Monocanthidae spp. (Leatherjackets; $n = 244$), across all habitats and surveys following removal of data points that represented joint absences between *C. maenas* and the predator. Each diamond represents a replicate trap, with some overlap in points.

Table 2. The potential predators of benthic phase (1st instar–adult) *Carcinus maenas* detected in traps, their total catch across the 8 surveys of 10 estuaries, and their correlation with *C. maenas* at the scale of trap ($n= 186\text{--}255$), estuary ($n= 10$ for each of the 8 surveys), and survey ($n= 8$). Analyses at the scale of estuaries and surveys used catch per unit effort in the correlations. Following Bonferroni corrections for repeated tests, results are interpreted as significant at $\alpha= 0.008$ (trap) and $\alpha= 0.005$ (survey and estuary) and are highlighted in bold

Species	Total	Spearman's ρ (trap)	p -value (trap)	Spearman's ρ (estuary)	p -value (estuary)	Spearman's ρ (survey)	p -value (survey)
Blue Swimmer Crab (<i>Portunus pelagicus</i>)	79	-0.804	0.001	0.158	0.162	-0.527	0.180
Mud Crab (<i>Scylla serrata</i>)	7	n/a	n/a	-0.083	0.466	-0.130	0.758
Leatherjacket (Monacanthidae spp.)	183	-0.845	0.001	0.082	0.471	-0.262	0.531
Yellowfin Bream (<i>Acanthopagrus australis</i>)	170	-0.865	0.001	-0.297	0.008	-0.415	0.307
Toadfish (Tetraodontidae spp.)	197	-0.875	0.001	-0.046	0.683	-0.120	0.778
Flathead (Platycephalidae spp.)	13	n/a	n/a	-0.149	0.187	-0.198	0.639
Eel (<i>Anguilla spp.</i>)	25	n/a	n/a	-0.247	0.027	-0.647	0.083
Moray Eel (<i>Gymnothorax prasinus</i>)	19	n/a	n/a	-0.103	0.365	-0.355	0.388
Southern Conger Eel (<i>Conger verreauxi</i>)	4	n/a	n/a	-0.56	0.623	0.000	1.000
Stingaree (<i>Urolophus</i> spp.)	8	n/a	n/a	-0.050	0.658	-0.643	0.086
Eastern Fiddler Ray (<i>Trygonorrhina fasciata</i>)	51	-0.513	0.001	-0.0.98	0.385	-0.503	0.204
Octopus (<i>Octopus</i> spp.)	106	-0.876	0.001	-0.408	0.001	-0.922	0.001

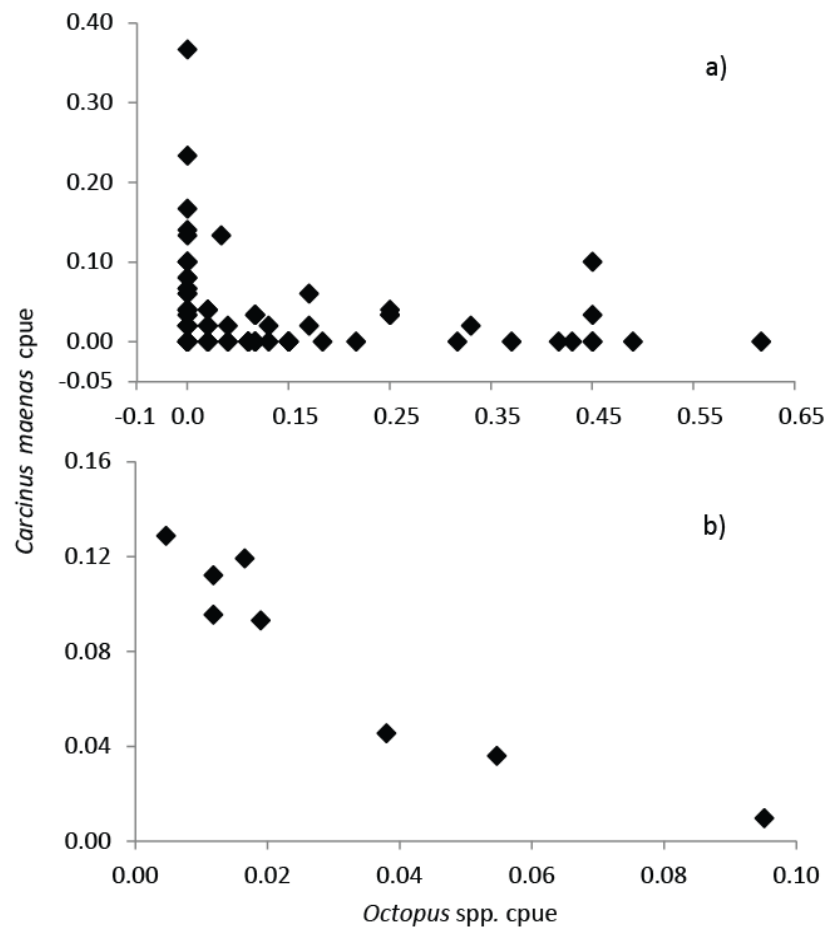


Figure 3. The relationship between the catch per unit effort (CPUE) of *Carcinus maenas* and *Octopus* spp. at the scale of a) estuary (CPUE was calculated for each of the 10 estuaries surveyed at a given time point, with a point for each of the eight times each estuary was surveyed, $n = 80$) and b) survey (using CPUE averaged across whole surveys, $n = 8$).

Analyses of this data set comparing the abundance of *C. maenas* among habitat types have previously been reported (Chapter 2) and revealed that the crab was more abundant in mangroves, than in seagrass, saltmarsh, oysters, or unvegetated rock/sand (Fig. 4a). Among the other habitat types, the crab did not display temporally persistent or spatially consistent patterns of difference (Chapter 2; Fig. 4a).

Among the four estuaries in which only the three habitats, seagrass, saltmarsh and unvegetated sand/rock were surveyed, Eastern Fiddler Rays were absent. Each of the five other taxa that were negatively correlated with *C. maenas* (i.e. Blue Swimmer Crabs, Leatherjackets, Yellowfin Bream, Toadfish, and Octopus), each displayed no significant interaction between habitat and survey date and/or estuary, allowing interpretation of main effects of habitat (PERMANOVA: non-sig. interactions with Habitat, $p > 0.05$). Blue Swimmer

Crabs (PERMANOVA: $Pseudo-F_{2,855} = 3.09$, $p = 0.031$) and Leatherjackets (PERMANOVA: $Pseudo-F_{2,855} = 2.80$, $p = 0.027$) each displayed significant differences among the three habitats. There was a non-significant trend towards Blue Swimmer Crabs being more abundant in seagrass than the unvegetated habitat, while Leatherjackets were more abundant in the unvegetated and seagrass habitats than the saltmarsh habitat (post-hoc tests $p < 0.05$). Yellowfin Bream, Toadfish and *Octopus* spp. showed no main significant effects of habitat (post-hoc tests $p > 0.05$).

Among the six estuaries in which all five habitat types were present, habitat utilization by the Blue Swimmer Crab (PERMANOVA, Survey $\times$ Estuary $\times$ Habitat interaction: $Pseudo-F_{140,2135} = 1.42$, $p = 0.009$), *Octopus* spp. (Survey $\times$ Estuary $\times$ Habitat: $Pseudo-F_{140,2135} = 1.34$, $p = 0.007$), Leatherjackets (Survey $\times$ Estuary $\times$ Habitat: $Pseudo-F_{140,2135} = 1.39$, $p = 0.006$), Toadfish (Survey $\times$ Estuary $\times$ Habitat: $Pseudo-F_{140,2135} = 1.80$, $p = 0.001$) and Eastern Fiddler Rays (Survey $\times$ Estuary $\times$ Habitat: $Pseudo-F_{140,2135} = 1.48$, $p = 0.001$) differed among surveys and among estuaries. By contrast, Yellowfin Bream did not differ in abundance among habitats, in any of the surveys or within any of the estuaries (PERMANOVA: Survey $\times$ Estuary $\times$ Habitat, non-sig. interactions or main effects, $p > 0.05$). At all sampling times, and in every estuary, the Blue Swimmer Crab was notably absent from mangroves and Eastern Fiddler Rays were absent from saltmarsh (Fig. 4). *Octopus* spp. were absent from mangrove habitats at all but one sampling time (December 2010) and from saltmarsh at all but two sampling times (December 2010, 2011; Fig. 4c). Leatherjackets were only present in saltmarsh at two sampling times, and only within two estuaries (Fig. 4f). Although Toadfish were not caught in all estuaries at every sampling time, where present, this group was more abundant in mangroves than any other habitat (post-hoc tests $p < 0.05$; Fig. 4b). In March 2011, Eastern Fiddler Rays were more abundant in the oyster habitat than elsewhere. Other comparisons of taxon abundances among habitats were not statistically significant (post-hoc tests $p > 0.05$).

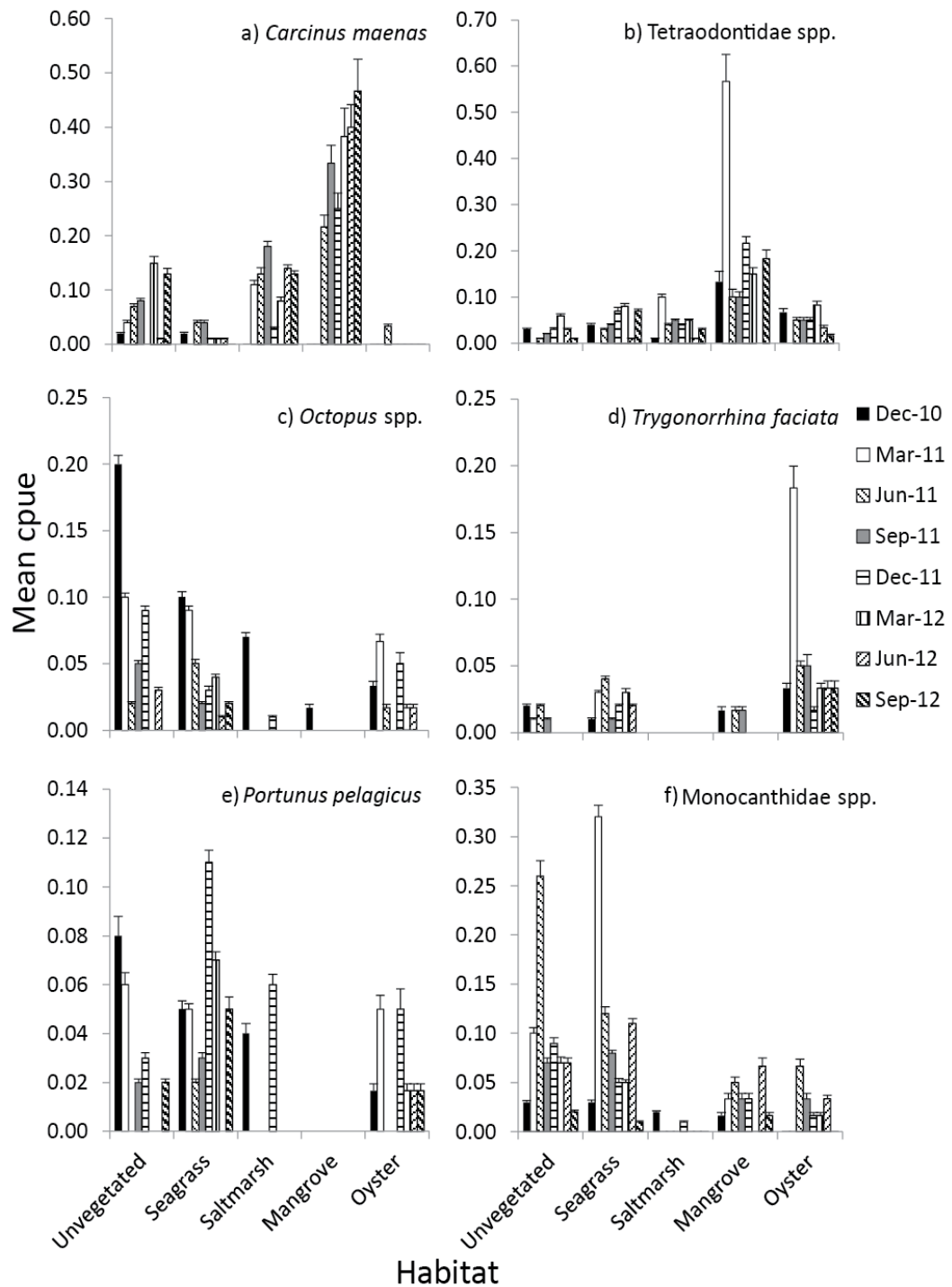


Figure 4. Mean (+SE) catch per unit effort (CPUE) of a) *Carcinus maenas*, b) Tetraodontidae spp. (Toadfish), c) *Octopus* spp. (Octopus), d) *Trygonorrhina fasciata* (Eastern Fiddler Ray), e) *Portunus pelagicus* (Blue Swimmer Crabs), and f) Monacanthidae spp. (Leatherjackets) across estuaries in which the five habitat types were sampled. $n = 6$ replicate estuaries for oyster and mangrove habitats and $n = 10$ for unvegetated, seagrass and saltmarsh habitats.

Feeding trials

Literature searches revealed that of the species caught in traps, 12 were potential predators of *C. maenas*, either documented to feed on *C. maenas* or other benthic crustaceans (Table 1). Aquarium feeding trials were performed with five of these, with all but the Toadfish consuming *C. maenas*. In addition, another three species (the Eastern Blue Groper, the Giant Cuttlefish and Snapper) that were not targeted by the traps, but that are common benthic-feeders along the south-east coast of Australia, were identified by feeding trials to be capable of consuming *C. maenas* (Table 1).

Tethering experiment

All except one of the *C. maenas* tethered 1 m from an octopus lair were dead after 24 hrs, with only the carapace remaining on the tether. Of the 25 *Octopus* spp., we visually observed 16 eating *C. maenas* tethered within 1 m of a lair in the first 5 min of deployment. By contrast, all of the crabs deployed 5 m from the octopus lair were alive and undamaged at the end of the 24 hr period. Hence, the proportional mortality of *C. maenas* was significantly greater close to than away from the Octopus lairs ($\chi^2 = 46.153$, $df = 1$, $p = 0.001$).

Discussion

It has been suggested that the relatively slow range expansion of *Carcinus maenas* in south-eastern mainland Australia, relative to other parts of the world, may be due to top-down control by predators (Thresher 1997, Hunt and Yamada 2003, de Rivera et al. 2005, Jensen et al. 2007). As a first step in providing the information needed to test this hypothesis, this investigation identified predators native to eastern Australia with the capacity to exert top-down pressure on the non-native crab, *C. maenas*. Using trapping surveys, we identified six groups of predators that displayed negative correlations with the abundance of *C. maenas* at the scale of traps, one of which (*Octopus* spp.) also displayed negative correlations among estuaries when averaged across traps, and survey dates when averaged across all of the estuaries. Furthermore, in aquarium feeding trials we identified another three species, not caught by the traps but common in south-eastern Australian waters, which were able to identify *C. maenas* as a prey item and consume the non-indigenous crab. Although not

assessed by this study, predation by birds and terrestrial species may also influence abundances of the crab (see Crothers 1968; Klassen and Locke 2007).

Of the species targeted by our trapping survey, *Octopus* spp. displayed the strongest negative correlation with *C. maenas*, both within traps, among estuaries, and temporally across surveys. Of the five habitats surveyed, *Octopus* spp. were least abundant in mangroves, the habitat in which we have previously observed *C. maenas* abundances to be greatest (Chapter 2). In aquarium feeding trials, *Octopus tetricus*, consumed a wide range of size classes of *C. maenas*. Furthermore, in a field tethering experiment we directly observed *Octopus* spp. feeding on *C. maenas* within 1 m from their lairs. The high rate of predation among *C. maenas* close to *Octopus* lairs likely reflected not only their proximity to this predator, but also may have been influenced by the greater difficulty that the crabs had in burying to escape predators in the sediments close to lairs that had a high percentage of shell hash.

Although our tethering experiment detected only predation of *C. maenas* in close proximity to *Octopus* lairs, our design did not assess whether crabs further from lairs were preyed upon over periods > 24 hr or when more proximal prey items were not available. Octopuses often make nightly migrations to and from their lairs to search for food (Mather 1991a,b). Over longer periods *Octopus* spp. may influence the distribution and abundance of *C. maenas* over broader areas. The field survey found that the abundance of *Octopus* spp. was also negatively correlated with that of *C. maenas* at the larger scale of estuaries. Hence, either *Octopus* spp. are found in very high densities in some estuaries and their abundance was greatly underestimated here, or over longer time periods *Octopus* spp. influence *C. maenas* over larger scales than metres.

Although octopods could not be identified to species by this catch-and-release study, it is likely that most were *Octopus tetricus*. Although *O. tetricus* is most common from southern Queensland to NSW (Anderson 1997), warming waters have facilitated range expansion to Victoria and the northeast coast of Tasmania (Madin et al. 2012). In Tasmania, which is farther south than the distributional limit of mangroves, *C. maenas* are most abundant near the mouths of estuaries in shallow soft sediments of low to moderate wave energy, although smaller numbers of the crab are also found in unvegetated silts and sands and in sediments vegetated with seagrass or saltmarsh (Thresher et al. 2003). Hence, Tasmanian populations

of *C. maenas* are likely to come into contact with expanding *Octopus* distributions. If predation by *O. tetricus* on *C. maenas* is sufficient to influence abundance, there may be ramifications of the predator's ongoing range expansion for the Tasmanian *C. maenas* population that, historically, would not have encountered this predator.

The Blue Swimmer Crab, *Portunus pelagicus*, was another species displaying a negative correlation with *C. maenas* at the scale of traps and determined to prey on *C. maenas*. A confamilial of *P. pelagicus*, the Blue Crab *Callinectes sapidus*, also consumes *C. maenas* and has been implicated in limiting the southern extent of the *C. maenas* invasion on the east coast of North America (de Rivera et al. 2005). *Portunus pelagicus* was notably absent from mangroves, where *C. maenas* was most abundant. Previously, Tanner (2007) found that juvenile *P. pelagicus* buried into sediments less in the presence of adult *C. maenas* than adult conspecifics, suggesting that the non-native crab may represent less of a predation threat to *P. pelagicus* than conspecifics. Further investigations are needed to determine the biological interactions of these two species. The Blue Swimmer Crab is among those species that are locally abundant along the coast of mainland Australia, but are notably absent from Tasmania where *C. maenas* has rapidly proliferated and spread (Walton et al. 2002, Thresher et al. 2003).

Three groups of benthic feeding fishes, the Leatherjackets (Monacanthidae spp.), Yellowfin Bream (*Acanthopagrus australis*) and Toadfish (Tetraodontidae spp.) were also among the predators that displayed negative correlations with *C. maenas* at the scale of trap. In aquarium trials, large Yellowfin Bream 400–450 mm were found to consume small *C. maenas* up to 50 mm in carapace width. It is unclear whether Leatherjackets, which are known to include invertebrates in their diet (Bell et al. 1984), might also be capable of consuming small *C. maenas*, as they were not tested. Toadfish, which regularly consume estuarine crabs (e.g. *Heloecius cordiformis*, B.P. Kelaher, pers. obs.) were most abundant in mangroves out of the five habitats examined, but in feeding trials did not consume *C. maenas*. As our feeding trials only considered predation on crabs greater than 20mm in carapace width (CW), it remains possible that toadfish may be able to consume first instar *C. maenas*, but this would need to be tested. In a previous study we found greater predation on tethered *C. maenas* inside versus just outside of mangrove forests (Chapter 4), which may be consistent with an effect of Toadfish. The persistence of crabs within mangrove forests

suggests that any predation by Toadfish is insufficient to exert top-down control on *C. maenas*.

Our traps, which were designed to catch crabs and small benthic predators, trapped relatively few Stingarees and Eels (Table 2). Consequently, the abundance of these species was likely to be underestimated by our study, and only Eastern Fiddler Rays (*T. fasciata*) displayed a significant negative correlation with *C. maenas* at the scale of traps. Aquarium experiments, indicated that several other species, Snapper (*P. auratus*), Eastern Blue Groper (*A. viridis*) and Giant Cuttlefish (*S. apama*), that were not targeted by our traps, may also be capable of exerting top-down pressure on *C. maenas*. Hence, it is likely that a much larger predator community than that identified by the present study exerts some predation pressure on *C. maenas* in eastern Australia. As our traps used an attractant (the odour of bait) to draw in predators, it is likely that their catches came from a relatively large area (tens of meters). The same method was used to sample *C. maenas*, the co-occurrence of species in traps indicates that these species were occupying similar areas. Although use of unbaited traps would have provided a better estimate of crabs and predators in particular habitats, it was not considered a viable method here due to the very low abundances of *C. maenas* and the high probability that these would not have been sampled without use of an attractant.

Our study did not explicitly consider how the ability of predators to consume *C. maenas* co-varied with predator size, or with habitat type. All predators used in our experiments were mature individuals, of at least average size. With the exception of Octopus, our feeding trials were based entirely within the laboratory. It is possible that juveniles and sub-adults would not be able to consume all size classes, if any, of the crabs. Furthermore, it is possible that in the field, predators may choose other prey items over *C. maenas*, or *C. maenas* may be able to escape predation. Additional experiments would be required to confirm rates of predation in the field. In identifying species which are capable of consuming *C. maenas* across at least part of their size range, our study identifies taxa that should be the focus of future studies considering top-down control of *C. maenas* in Australia.

Lower rates of predation on *C. maenas* in mangroves than in other habitats may have explained the higher abundance of the non-indigenous crab in the mangroves. Blue Swimmer Crabs were absent and Octopus notably rare in mangroves and Toadfish, although

abundant, were not demonstrated by this study to be capable of consuming *C. maenas* > 20 mm. As well as mangroves containing low densities of key predators, the pneumatophores of mangroves provide a structurally complex habitat that may decrease the foraging efficiency of finfish predators (Warren 1990, Primavera 1997, Smith and Hindell 2005). It is possible that other factors explained the high abundance of *C. maenas* in mangroves; for example, high densities of prey items (e.g. Nagelkerken et al. 2008) or a shaded environment which enables *C. maenas*, which in Australia is towards the upper limits of its thermal range (< 0 to 35°C; Eriksson and Edlund 1977, Hidalgo et al. 2005), to tolerate hot Australian summers.

It is likely that multiple factors have contributed to the slow spread of *C. maenas* northwards along the east Australian coastline. First, the direction of the crab's spread is against the prevailing direction of the East Australian Current such that transport northwards is primarily via cyclonic and anticyclonic eddies in summer and autumn (Roughan et al. 2011). Second, warm temperatures that are approaching the upper thermal limit of *C. maenas* may limit survival and, hence, establishment of the crab in northern NSW and southern Queensland. Nevertheless, the results of this study raise the possibility that consumption by native predators is also contributing to the crab's slow northwards spread. The capacity of native predators to limit the distribution and abundance of *C. maenas* has been demonstrated elsewhere in *C. maenas*' invasive range (Hunt and Yamada 2003, de Rivera et al. 2005, Jensen et al. 2007). Given the capacity for diverse predator assemblages to act on *C. maenas*, future studies examining the interactive (additive, synergistic or antagonistic) effects on the invasive crab of competing native predators would provide important insights into the relative importance of top-down mechanisms in regulating *C. maenas*' distribution and abundance.

Acknowledgements

We thank D. Bateman, B. Coates, M. Li, M. McMaster, J. Rowland, J. Smith, R. Lopes, C. Sperry and L. Stone for assistance with fieldwork. We also thank M. McMaster and A. Daly at the Merimbula Aquarium for their help, use of facilities and animals. Dr Culum Brown (Macquarie University) and Mark McGrouther (Australian History Museum) helped with fish identification. T.M. Glasby and two anonymous reviewers provided helpful comments that

improved the quality of the manuscript. This study was funded by an Australian Research Council (ARC) Linkage Grant (to M.J. Bishop, T.M. Glasby, M.A. Coleman and B.P. Kelaher) with industry partners, the NSW Department of Primary Industries, Sapphire Coast Marine Discovery Centre, Southern River Catchment Management Authority and the Bateman's Marine Park. This work was performed during a Ph.D. candidature at Macquarie University. C. Garside was in receipt of an Australian Postgraduate Award Industry (APAI) and an International Macquarie Research Excellence Scholarship (IMQRES). Ethics approval was granted under the Macquarie University's Animal Ethics Committee (2011/056–3; Appendix 2).

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CHAPTER 6

General discussion

Although previous studies have documented the distribution of *C. maenas* in New South Wales (NSW) (Hewitt and Martin 1996, Hewitt and Martin 2001, Pollard and Rankin 2003) little was known about the environmental correlates and controls of its distribution and abundance. This study, which conducted surveys in 14 NSW estuaries and manipulative experiments in a subset of these, not only provides enhanced understanding of the distribution of *C. maenas* in southern NSW, but also enhances our understanding of several factors that influence this. This is useful information for managers trying to determine the impact of the current invasion and strategies to prevent future proliferation and spread of this non-native species.

The *C. maenas* control plan developed for Australia requires that the impacts of the marine pest be evaluated for a specific region and specific management strategies be implemented such that their benefits offset their costs (Australian Government 2008). Although our study did not directly quantify *C. maenas* impact, the low abundances detected in this study supports the view that the mainland of Australia has experienced less economic and environmental damage from the invader than Tasmania, which was invaded some 90 years after mainland Australia (Thresher 1997). The trapping survey, reported in Chapter 2, found that in NSW the abundance of *C. maenas* is presently patchy in space and time, and that even where present, abundances are low. Recruitment (Chapter 3), abiotic conditions (Chapter 2), habitat suitability (Chapter 4) and local predator assemblages (Chapter 5) likely contribute to the patchy distribution and abundance of *C. maenas*. Knowing the ecology of *C. maenas* in its introduced range and the determinants that control its distribution is essential for appropriate implementation of management strategies.

The distribution of *C. maenas* in NSW

Our survey of 14 NSW estuaries provided the first record of *C. maenas* in the estuaries Mummaga Lake and Tuross River and confirmed the presence of the crab in 8 others. Our survey which extended as far north as Durras Lake (35.63°S, 150.30°E), detected *C. maenas* no further north than Bateman's Bay (35.74°S, 150.25°E). Previous studies have provided

records of *C. maenas* further north in Kyeemagh, Botany Bay (33.57°S; Ah Yong 2005) and although several of these records have been one-off sightings, it is possible that *C. maenas* is distributed further to the north.

Within our study region, the entrance morphology of the Intermittently Closed and Open Lakes and Lagoons (ICOLLs) that dominate the NSW coast was an important determinant of *C. maenas* distribution. It was shown that the time an estuary remained open was an important predictor of *C. maenas* presence, with the crab notably absent from estuaries with entrances that remained closed for >60% of our study. The frequency of entrance opening influenced the presence of *C. maenas* by directly determining the opportunities for recruitment from the external, coastal environment. Closed entrances provide few colonisation opportunities, with the absence of crabs likely a direct consequence of the closed sand bar restricting colonisation. During periods of entrance closure, the presence of *C. maenas* is, presumably, dependent on crabs having successfully recruited to the estuary during previous periods when the entrance was open and subsequently tolerating the shifting environmental conditions. When estuaries remain closed the number of estuaries along the coastline that can be colonised by *C. maenas* is not only reduced, but the distance between open estuaries and hence the distance *C. maenas* must travel to disperse from one estuary to the next increases.

Within estuaries with crabs, *C. maenas* varied in abundance among habitats but the crab was present in all five habitats surveyed: mangroves, seagrass, saltmarsh, oyster, and unvegetated sand/rock. A pilot study failed to detect *C. maenas* at the back of estuaries, adjacent to freshwater inputs. This could have been because salinities in these areas were below those tolerated by *C. maenas* or because these areas supported a suite of species with the ability to consume or compete with *C. maenas*. Alternatively, the hydrodynamics of estuaries may prevent the transport of *C. maenas* megalopae to these areas.

This study did not consider the occupation by the crab of habitats outside of estuaries. It is possible that due to *C. maenas*' plasticity and wide environmental tolerances, populations could exist along the NSW coast outside of estuaries (one individual has been noted, M. McMaster, pers. comm.). However, based on previous studies, it is likely *C. maenas* are less abundant on the outer coast due to hydrodynamic forces (Grosholz and Ruiz 1996, Hampton

and Griffiths 2007). Future studies are needed to confirm the presence of *C. maenas* on the outer coast and to what depth they are found.

Factors influencing the abundance of *C. maenas* in NSW

Studies investigating the proliferation of non-native species often focus on competition and predation in determining spatial patterns, overlooking the role of foundation species in facilitating native biodiversity. Our study considered the role of both positive and negative interactions in influencing the abundance of *C. maenas* at the scale of estuaries, habitats and traps.

Among estuaries where *C. maenas* was detected there was considerable spatial variability in abundance (Chapter 2). There was no significant linear relationship with the proportion of time that each estuary entrance was open, although abundance was positively correlated with salinity, which in turn is influenced not only by whether an estuary is open or closed to the ocean and has tidal exchange, but also on freshwater runoff. Instead, estuaries with a history of opening and closing naturally had, collectively, a higher Catch Per Unit Effort (CPUE) than the permanently open estuaries. As predator assemblages are generally more diverse in open than closed estuaries (Jones and West 2005, Hadwen et al. 2007), we hypothesised that differences in top-down control may account for the greater crab abundance in the ICOLLs than in the permanently open estuaries. Previously, it has been suggested that the relatively slow range expansion of *C. maenas* in south-eastern Australia, as compared to Tasmania, may be due to top-down control by predators only present on the mainland (Thresher 1997).

The capacity of native predators to limit the distribution and abundance of *C. maenas* has been demonstrated elsewhere in *C. maenas*' invasive range (Hunt and Yamada 2003, de Rivera et al. 2005, Jensen et al. 2007). In our trapping surveys, six groups of predators that have been either observed to directly consume *C. maenas* or are known from the literature to consume benthic crustaceans displayed negative correlations with the abundance of *C. maenas* at the scale of traps, estuaries and/or survey dates, when averaged across all estuaries. In aquarium feeding trials, we identified another three species, not sampled by the traps but common in south-eastern Australian waters that were able to identify *C.*

maenas as a prey item and consume the non-indigenous crab. Of the species sampled in our trapping survey, *Octopus* spp. displayed the strongest negative correlation with *C. maenas*, both within traps, among estuaries, and temporally across surveys, suggesting the capacity for *Octopus* to influence the distribution and abundance of *C. maenas* at a range of scales. The blue swimmer crab, *Portunus pelagicus*, shown to predate *C. maenas*, displayed a negative correlation at the scale of traps. A confamilial species of *P. pelagicus*, the blue crab *Callinectes sapidus*, also consumes *C. maenas* and has been implicated in limiting the southern extent of the *C. maenas* invasion on the east coast of North America (de Rivera et al. 2005).

Within estuaries, our trapping survey consistently identified more *C. maenas* in mangrove forests than in any of the other four habitat types (oyster, saltmarsh, seagrass, bare sand/rock near the entrance) that were sampled. Furthermore, targeted sampling revealed greater crab abundances under the mangrove canopy than in adjacent unvegetated habitat of similar tidal elevation, or lower on the shore. The mangrove canopy provides shading that may enable *C. maenas* to persist in environments where the temperature of unshaded substrates approaches or exceeds the maximum temperature the crab can tolerate (Chapter 4). It may also benefit the crab by facilitating abundant bivalve prey resources, for example the oysters and mussels that attach to pneumatophores (Nagelkerken et al. 2008) and by influencing predator assemblages and their foraging efficiency (Sheridan and Hays 2003), although in this study we found greater predation on *C. maenas* under the mangrove canopy than in adjacent unvegetated habitats. *P. pelagicus*, a species with which *C. maenas* was negatively correlated, was notably absent from mangroves (Chapter 5). As abundance was low in this habitat (Chapter 2) prey availability was not a contributing factor. However, if *C. maenas* exhibit a population increase prey may become depleted and may limit reproductive outputs. Further investigations are needed to determine the biological interactions between *C. maenas* and *P. pelagicus*.

Recruitment

Our recruitment study and cohort analysis indicate that in south-eastern Australia, *C. maenas* megalopae are primarily settling in the late Austral winter to early spring (primarily August to October). This contrasts with the reproductive phenology of *C. maenas* at higher latitudes in its native range, where settlement primarily occurs in autumn (Crothers 1967,

Audet et al. 2008) and also the phenology of native Australian crabs, which in this study primarily recruited in autumn. The different seasonal pattern of *C. maenas* recruitment seen between the present study and many of the European studies presumably reflects the milder Australian climate triggering reproduction at a different time of year. The milder temperatures in NSW enable *C. maenas* to hatch larvae year round as hatching can start when water temperatures exceed 10°C. We only detected successful recruits in the late winter and early spring (Chapter 3) indicating the reproductive cycle is happening during the coldest times of the year. Although some of the cohort patterns show, once in four years, recruitment later in the year (summer–spring), these are less likely as water temperatures reach their annual peak in the autumn and survivorship of *C. maenas* larvae decreases at higher temperatures (Sprung 2001, de Rivera et al. 2007a). The phenology of settling in the autumn has the potential to benefit *C. maenas* by increased by minimizing temporal overlap in recruitment with native crabs, which may serve as competitors and allowing temperature-sensitive stages in the reproductive cycle to occur outside of summer temperature extremes. Nevertheless, it remains possible that native crabs could predate on the newly settled megalopae of *C. maenas*.

We found greater recruitment to bags of structural habitat than to recruitment bags that were empty. When crab counts were summed across the study, bags with live oysters collectively contained the greatest overall number of *C. maenas*. Hence, our study reinforces the view that translocation of cultured oysters may potentially be a mechanism of *C. maenas* spread (Thresher et al. 2003). Oyster farming is the largest aquaculture industry in NSW and translocation among estuaries is common. Oyster farmers in Queensland depend on spat (juvenile oysters) grown in NSW and some restrictions are in place on where it can be moved. If not carefully managed, this anthropogenic dispersive mechanism could see the crab greatly increase its range over a short period of time as has occurred along the west coast of North America (de Rivera et al. 2007a).

Implications for management

Under the National System for the Prevention and Management of Marine Pest Incursions established marine invasive species that cause significant impacts should receive ongoing management and control (Australian Government 2008). The Australian government

through the National Introduced Marine Pests Coordination Group (NIMPCG) have determined that the European green crab *C. maenas* should be considered a National Control Plan (NCP) species. The plans developed for these species are meant to establish a nationally agreed species-specific response with guidance on the development of strategies to reduce impacts and minimise spread in the future. Decisions to act can be independent by state but using cross-jurisdictional resources requires agreement among them.

The entrances of more than half of the Intermittently Closed and Open Lakes or Lagoons in NSW are artificially opened to mitigate flooding effects (Haines et al. 2006). This study suggests that artificially opening or permanently opening an estuary to the ocean through means of a training wall may increase the probability of *C. maenas* invasion relative to closed estuaries. It appears estuaries that remain predominantly closed may experience a high degree of natural resistance to invasion. Hence the benefits of artificially opening estuaries may be offset by the potential cost of enhanced biological invasion (West and West 2007). Haines et al. (2006) advocate conducting a thorough environmental impact assessment prior to excavating an estuarine entrance. We suggest that such assessments should include consideration of the effects of ICOLL entrance opening on species invasions. When artificially opening a closed estuary is necessary, this would best be performed in the months from February–May in order to reduce the chance of the estuary being invaded by new *C. maenas* recruits. If *C. maenas* is already present in an estuary, an option to intentionally close the estuary to eradicate the pest may outweigh the benefits of having the estuary open. However, the needs of other species (i.e. anadromous or catadromous) should not be overlooked.

The preliminary evidence provided by our study for negative relationships between native predators and the abundance of *C. maenas* suggests that the maintenance of low abundances of *C. maenas* along the coast of the Australian mainland may be contingent upon careful management of predator populations, many of which are the target of commercial fisheries. For example, the blue swimmer crab, a predator of *C. maenas* is targeted by a commercial fishery worth \$22 million (AUD) (Flood et al. 2012, EconSearch 2013). Commercial catches of this species have declined in recent years, for unknown reasons, and in some estuaries the fishing mortality exceeds natural mortality (Rowling et al. 2010). Such declines in native predator abundance from overfishing or from disease might

potentially enable *C. maenas* to get an upper hand, if it is freed from competition or top-down control.

Our finding that *C. maenas* recruitment primarily occurs in the late Austral winter to spring suggests that efforts to detect zoea or megalopae in the water column would be best focused at this time of year. New environmental DNA (eDNA) techniques can screen water samples for the presence of species for which a sequence is available (Jerde et al. 2011), although the approach cannot distinguish between the presence of live specimens and dead remains. It may be possible to relate the strength of the signal from the water sample to the abundance of the animal in the field, although ground-truthing of plankton concentrations with the eDNA signal would be required. This information could change the way cryptic and emerging invaders are detected and the level of intervention needed to reduce the potential of aquatic invasions.

Management of invasive species includes prevention, early detection, immediate response, eradication and subsequent control. The preferred and most economical option is prevention. Should prevention fail, early detection through surveillance is the best chance for any eradication efforts (Australian Government 2008). Due to the high spatial and temporal variability in *C. maenas* abundance, and the generally low abundances of the non-native crab, comprehensive trapping efforts would be required to track the status of ephemeral New South Wales populations. Our results suggest that surveys aimed at early detection of *C. maenas* successfully recruiting to adult size classes would be best placed to focus on trapping in mangroves. If trapping for *C. maenas* in estuaries where mangroves are absent, traps may be best placed next to structure of saltmarsh or woody debris. If detected, eradication would always be the preferred option in the discovery of a non-indigenous species (NIS), but this needs special consideration in regard to the economic and ecological costs. All of these options are further compounded by the open nature of the marine environment and the knowledge whether the species is cryptic (Carlton 1996).

There has been a successful eradication of one marine invasive species in Australia, the black striped mussel (*Mytilopsis sallei*) from Darwin (Bax et al. 2001). To our knowledge there have been no successful eradications of invasive populations of *C. maenas* to date. Trapping programmes have shown success in reducing the adult populations in Bodega Bay California

(de Rivera et al. 2007b) and Twofold Bay Eden, NSW (Pollard and Rankin 2003) both of which have not eradicated the species.

Similarities and differences between the Australian and North American *C. maenas* invasions

Lessons about global invasion may be learnt by comparing invasion patterns between different parts of the world. In North America, there has been much work done on the invasion of *C. maenas* along the east and west coastlines (Grosholz and Ruiz 1995, Carlton and Cohen 2003, Klassen and Locke 2007, Darling and Tepolt 2008, Tepolt et al. 2009, Yamada and Kosro 2010), which might provide an interesting comparison to the east Australian *C. maenas* invasion.

As compared to the east and west coasts of North America, the rate of *C. maenas* range expansion in Australia has been slow (see Table 1, General Introduction). Coastal lakes and lagoons are present on the south-east coast of Australia, and the south west coast of North America, but are uncommon on the east coast of North America, although the latter has large numbers of permanently open estuaries. The present study suggests that the periodic closure of coastal lagoons may limit recruitment opportunities by *C. maenas* (Chapter 2). Furthermore, closure of lagoon entrances would act to increase the distance of coastline *C. maenas* must travel before reaching a coastal inlet. Interestingly, on the west coast of North America, there has been less range expansion to the south, where coastal lagoons are present, than to the north. For example, from San Francisco Bay, California *C. maenas* spread south to Elkhorn Slough, approximately 125km but the northern expansion has been >2000 km (Chapter 1, Grosholz and Ruiz 1996, Yamada and Kosro 2010). It is possible that the dynamics of coastal lagoons slow spread of the invader. Along stretches of the North American coastline where distances between estuaries were large, the rate of range expansion was accelerated during strong El Niño periods (Yamada and Kosro 2010), indicating that outside of these times, the distance between estuaries may be a limiting factor.

In North America range expansion has predominantly been in a northward direction, towards a cooler climate. As a cold temperate species, *C. maenas* may be experiencing a

slower rate of spread in Australia, due to some of its range expansion occurring northwards into sub-optimal, warmer, abiotic conditions. The association found by our study between *C. maenas* and the shaded environment of mangroves, despite the high risk of predation in this habitat, suggests that on the east coast of Australia, *C. maenas* may be temperature-stressed, especially towards the northern limit of its present distribution. Nevertheless, further research would be needed to demonstrate temperature stress and a role for mangroves in alleviating this.

On each continent, range expansion has been linked to natural (Yamada and Kosro 2010) and anthropogenic mechanisms (Aquaculture; Thresher et al. 2003, Recreational boating; Darbyson et al. 2009, Coastal vessel traffic; Blakeslee et al. 2010). The present study provides evidence of an association between *C. maenas* and oyster aquaculture on the east Australian coast, with the non-native crab recruiting to oyster bags on an aquaculture lease. The greater human population densities in the USA may, however, provide greater opportunity for translocation by these secondary vectors.

The catch rates of *C. maenas* in North America, like those for Australia, appear highly variable among locations. Although some of this variability may arise from differences in trapping methods among targeted research efforts and broad-scale monitoring programs, a large component is likely to reflect spatio-temporal variation in crab populations. In the present study, the CPUE for southern NSW was 0.06 crabs per trap (Chapter 2). Previous estimates from studies within Australia specifically targeting habitat most likely to be invaded range between 1.8 for NSW, 3 for South Australia and Victoria and 7.4 for Tasmania (Thresher et al. 2003). On the west coast of America a CPUE as high as 21.3 has been reported for Bodega Harbour, California, although, after 66 days of trapping for controlling density, the CPUE was reduced to 1.4 (de Rivera et al. 2007b). In Yaquina Bay, Oregon CPUE was 0.398 (Hunt and Yamada 2003). On the West coast of British Columbia an explorative trapping investigation had a CPUE of 0.26 (Gillespie et al. 2007). In Willapa Bay, Oregon CPUE was slightly lower than our study <0.015, but used passive pitfall traps rather than baited traps (McDonald et al. 2006). On the east coast of Canada in Prince Edward Island CPUE was as high as 32.5 but averaged 8.32 for the island (Mc Niven et al. 2013). In the Bras d'Or Lakes in Nova Scotia the CPUE was found to be 6.71 (Vercaemer et al. 2011). On each continent, it appears that spatial variation in the community of native predators and competitors may influence the abundance of *C. maenas*. There is experimental evidence that biotic resistance

may be limiting the proliferation of *C. maenas* on the east and west coasts of North America (Hunt and Yamada 2003, de Rivera et al. 2005).

Future research needs

This study was performed during a strong La Niña event, which produced the two wettest years recorded in Australian history (Bureau of Meteorology 2012). During this period, several ICOLLs were open for the first time in many years, and in estuaries that remained closed salinities were generally low. As we found a significant positive correlation between salinity and *C. maenas* abundance, it is possible that during drier conditions, when salinities are generally higher, abundances of the non-native crab may increase. Further, it is possible that the hypersaline conditions that can develop following significant evaporation from ICOLLs closed during hot, dry summers might challenge the salinity tolerances of this euryhaline species. Following observations of salinities in Tilba Lake, NSW reaching more than 70‰, dead *C. maenas* were found on the shore (M. A. Coleman, unpublished data). By repeating surveys of *C. maenas* during the more dominant El Niño events, we may gain a better understanding of the current range *C. maenas* occupy in NSW. Further, we could test the hypothesis that following closure of ICOLLs, abundances of *C. maenas* decrease due to an absence of recruitment to replace mortality. To date, no research has been done to determine whether *C. maenas* can reproduce in predominantly closed estuaries.

Compared to the invasion rate seen on both sides of North America the spread of *C. maenas* in Australia has been slow (Chapter 1). This does not appear to be due to low connectivity – to the contrary, genetic connectivity of the non-native crab appears high along the east Australian coast (Burden et al. 2014). Very little work has been done on the role of biotic resistance of the receiving communities of slowing this spread. This thesis did find negative correlations for specific predator species and the abundance of *C. maenas* on a trap scale, but would be improved by gathering predator abundance data using other methods. More laboratory and manipulative field studies are needed to ascertain the effect of native predators on the abundance and habitat utilisation of *C. maenas*. Rates of predation of native predators on *C. maenas* were not examined in this study and would help determine the population sizes needed of individuals to limit the spread of *C. maenas*.

In mangroves, where *C. maenas* might reach locally high abundances, it will be important to ascertain whether *C. maenas* is impacting native fauna, such as the bivalves (e.g. oysters, mussels, clams) on which they prey. Very few investigations have looked at the impact of *C. maenas* on local fauna on the mainland of Australia. Investigations in Tasmania have shown that predation by *C. maenas* on *Katelysia scalarina* has been predicted to have a significant negative economic impact on this fishery (Walton et al. 2002). It is likely that there are some direct and indirect impacts to native communities of *C. maenas*, although given the crab's low abundance, these may be weak. Baseline surveys, such as those being conducted by the NSW Department of Primary Industries, in estuaries where *C. maenas* is not yet present may be useful for ascertaining impacts, if they are invaded in the future.

Conclusion

Carcinus maenas is one of the world's worst invasive species (Lowe et al. 2000). Its impacts from invasion around the world have been well documented (Le Roux et al. 1990, Cohen et al. 1995, Grosholz and Ruiz 1995, 1996, McDonald et al. 2001, Klassen and Locke 2007, Murray et al. 2007, Garbary et al. 2013). Although *C. maenas* has been present in Australia for over a century (Fulton and Grant 1900, 1902), it appears that to date the crab's abundance, and hence impact, remains low, particularly along the mainland coast. In Tasmania, *C. maenas* has been predicted to significantly impact the venerid clam *Katelysia scalarina* (Walton et al. 2002) and has induced morphological changes in a predatory whelk *Haustrum vinosum* (Freeman et al. 2013). The information generated by this thesis on correlates and controls of the distribution and abundance of *C. maenas* in eastern Australia will assist in the continued effective implementation of the National Control Plan for *C. maenas* and the individual state's surveillance monitoring and response initiatives. *Carcinus maenas*' history of ecological impacts, persistent populations and plasticity, requires ongoing monitoring of its distribution, abundance and impacts in Australia to ensure that the National Control Plan continues to be effective in minimising the impacts of this global invader.

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Appendix 2. Ethics approval certificate.



**MACQUARIE
UNIVERSITY**

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2011/056-3 **Date of Expiry:** 14 December 2013

Full Approval Duration: 18 December 2011 to 14 December 2014 (36 months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) and will only be renewed upon receipt of a satisfactory Progress Report before expiry.

Principal Investigator:
Dr Melanie Bishop
Biological Sciences
Macquarie University NSW 2109
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Associate Investigator:
Clifford Garside 0406 036 236

In case of emergency, please contact: Animal Welfare Officer: 9850 7758 / 0439 497 383
or the Principal Investigator / Associate Investigator named above

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: Management of coastal lakes to minimise invasion

Type of animal research: 7: Research – Environmental Study

Aims of the project: The aims of this research are to test the hypothesis that there is a greater abundance of predatory invertebrates and vertebrates in permanently open than mostly open lakes, (2) that in lakes where there are more predators, there is a greater rate of mortality of crabs, and (3) demonstrating which species of predator are capable of consuming *C. maenas* (and across what size ranges) through aquarium experiments and underwater videoling.

Surgical Procedures category: 3 (Minor Conscious Intervention)

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Sex	Weight	Age		Source
The following are the most likely types of species to be caught: Dusky flathead (<i>Platycephalus fuscus</i>), Yellowfin bream (<i>Acanthopagrus australis</i>), Trevally (<i>Pseudocaranx dentex</i>), Sand whiting (<i>Sillago ciliata</i>), Snapper (<i>Pagrus auratus</i>), Tarwhine (<i>Rhabdosargus sarba</i>), Trumpeter whiting (<i>Sillago maculata</i>), Slimy mackerel (<i>Scomber australasicus</i>), Kingfish (<i>Seriola lalandi</i>) Yellowtail (<i>Trachurus novaezelandiae</i>), Large tooth flounder (<i>Pseudorhombus asius</i>), leatherjacket (<i>Meuschenia spp</i>), Damselfish (<i>Pomacentrus australis</i>), Eastern smooth boxfish (<i>Anoplocapros inermis</i>), Red gumard (<i>Chelidonicichthys kumu</i>) – all of various size and age, Maui octopus (<i>Octopus maorum</i>), Common octopus (<i>octopus tetricus</i>), Squid (<i>Loligo</i> , <i>Sepioteuthis</i> , <i>Nototodarus spp.</i>) and cuttlefish (<i>Sepia spp.</i>)						
Capture and Release				TOTAL	550	Wild

Location of research:

Location	address
Several estuaries along the southern NSW coast from the southern extent of Nadgee Lake to Smiths Lake in the north. These include: Nadgee Lake, Wonboyn Lake, Two fold Bay, Pambula Lake, Merimbula Lake, Wallagoot Lake, Nelson Lagoon, Wapengo Lake, Barragoot Lake, Bermagui River, Wallaga Lake, Corunna Lake, Nangudge Lake, Wagonga Inlet, Mummaga Lake, Tuross River, Coila Lake, Clyde River, Durras Lake, Jervis Bay, Sydney Harbour, and Smiths Lake.	N/A

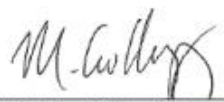
Amendments approved by the AEC since initial approval:

- 1) Addition of Cephalopods (Feb 2012)

Conditions of Approval:

- 1) Seine/Fyke netting component of capture and release approved December 2011
- 2) Capture and retention of fish approved with further documentation provided February 2012.

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.



Prof Michael Gillings (Chair, Animal Ethics Committee)

Approval Date: 6 December 2012