Muresk Institute of Agriculture

Behavioural responses of Australian freshwater crayfish (*Cherax tenuimanus* and *Cherax albidus*) to water-borne odours

Shaun Gareth Height

This thesis is presented for the degree of
Doctor of Philosophy
of
Curtin University of Technology

November 2008

Behavioural responses of Australian freshwater crayfish (*Cherax tenuimanus* and *Cherax albidus*) to water-borne odours

Shaun Height

Executive summary

Interactions between non-native yabbies (*Cherax albidus*) and indigenous marron (*Cherax tenuimanus*) in the south-west of Western Australia are not well understood. While there is abundant evidence to suggest that invasive freshwater crayfish are detrimental to native species, the nature and degree of impact on marron populations by exotic yabbies remains unclear. Researchers have hypothesized that invasive species make faster and more appropriate use of information about their environment than native species. This greater behavioural plasticity can result in displacement of indigenous species, successful colonisation by invaders, and subsequent disturbance to natural ecosystems and representative biodiversity.

The research presented in this thesis examines the behavioural responses of an indigenous crayfish (*C. tenuimanus*) and an invasive crayfish (*C. albidus*) to water-borne odours derived from food, alarm sources and finfish predators. This study was undertaken to assist in the understanding of predatory and competitive interactions between indigenous and non-indigenous crayfish and fish predators, with particular relevance to Western Australia. Predation and competition are major forces influencing community structure in ecosystems; therefore knowledge of competitive and predatory interactions will be of benefit when considering future translocation policies.

Behavioural trials were conducted in two culture systems (54 L aquaria and a 70,000 L mesocosm), where marron and yabbies were exposed to a range of water-borne odours from finfish predators (silver perch and Murray cod), with and without competition from conspecific and heterospecific crayfish. A number of variables likely to influence crayfish behaviour were investigated: strength of chemical odour; crayfish size, gender, diurnal and nocturnal activity patterns; predator size; prior-residence; suitable habitat/shelter; and feed availability.

A key innovation in this research was the high replication in the aquarium-based observation trials using a Latin Cube design, which resulted in greater statistical strength and lower variability. More importantly, this research deviated from the tradition of exclusively using the 'individual crayfish' approach for odour-detection experiments and tested these results in a 70,000 L communal observation tank. This was an important development in crayfish behavioural experimentation, particularly as several key findings from the individual crayfish approach were confirmed in a multi-species environment.

Results from this study supported the hypothesis that invasive crayfish species make more appropriate use of a wider range of information about their environment than native crayfish species. Yabbies were found to possess behavioural characteristics not present in marron, such as clearer behavioural modifications to food and heterospecific odour, and cautionary behaviour in the presence of odour from a finfish predator. During simulated daylight conditions, marron displayed behaviours conducive to predation that were not present in yabbies, including less time spent in shelter and more time spent in locomotory activity. However, during specialised night-time observational studies developed during this research, these differences were not evident. This would not seem to be an unusual result, given that crayfish naturally forage at night and become more active; however, it may have important implications for future behavioural studies of crayfish, indicating a bias associated with day-time approaches. Crayfish size also played a role in behavioural modifications to water-borne odours. Larger marron displayed clearer changes in behaviour and were more responsive to heterospecific alarm odour than juveniles. Furthermore, juveniles of both species were more active than adults and sub-adults.

The expansion of the yabby population into Western Australian habitats occupied by marron has been facilitated through translocation for aquaculture, and biological characteristics of the species, some of which are typical of other invasive crayfish species including: tolerance of a variety of conditions; rapid growth; early sexual maturity; burrowing to escape drought and predation; capable of multiple spawns in a

growth season; and aggressiveness. Another characteristic of invasive crayfish species also shared by yabbies, as supported by the results of this study, is high behavioural plasticity.

Although marron do not share the same level of behavioural plasticity found in yabbies, their larger body size increases their success in competitive interactions. The comparatively smaller body size of yabbies may be the major factor limiting their population expansion in the presence of marron, especially in water-bodies where shelter is a limited resource.

Marron are an important endemic species in Western Australia, but their conservation is threatened by competition and predation from exotic species. The research presented in this thesis indicates that invasive yabbies are more receptive to chemical stimuli and better equipped to respond to predation risk than marron. This information will be of benefit when considering future translocation policy in Western Australia and highlights the need for a cautious approach to species introductions.

This thesis is dedicated to my grandparents

Derrick and Joan Beale

And my parents

Maurice and Gloria Height

STATEMENT OF SOURCES

DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Shaun Height **10 November 2008**

STATEMENT OF ACCESS TO THE THESIS

I, the undersigned, the author of this thesis, understand that Curtin University of Technology, Perth, Western Australia, will make it available for use within the University library and allow access to users in other approved libraries. All users consulting this thesis have to sign the following statement:

"In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author, and to make proper written acknowledgment for any assistance which I have obtained from it."

Beyond this, I do not wish to place any restrictions on access to this thesis.

Shaun Height **10 November 2008**

Acknowledgements

Many thanks to the staff and fellow research students at Curtin that have helped me over the years; Simon Longbottom and Dr Jane Fewtrell for technical support; Kerri Rioux for making things happen; Assoc. Prof. Ravi Fotedar for timely comments and perspective; Jane McNeil, Cameron White, Laurence Cross, David Tate and Tom Colvin for help conducting experiments; and Laura Woodings for excellent assistance during the mesocosm trial and showing up for all those early starts.

Special thanks to; Simon Boyd for technical assistance and advice; Blair Marsh for ideas and help with early trials; and to Dr Prayadt Wangpen for support, friendship and laughs during the long days in 'the dungeon'.

I feel very fortunate to have been under the guidance of Assoc. Prof. Glen Whisson during this research; you have given me unreserved support, understanding during tough-times and provided excellent opportunities to travel and gain knowledge from Astacologists from abroad. Special thanks also to Tania Whisson for comments on the final draft.

To my friends and family: thanks for your words of encouragement and all of the good-times, laughs and welcome stress relief over the years; Kay and Ray for help (and car sales) along the way; Dad and Yvonne for support and advice; and to Nanna and Grandpop for instilling a love and respect of aquatic life in me from an early age (and Grandpop for the 'education').

Finally I would like to thank my partner Emma for her endless patience, love and support, words cannot express what this has meant to me!

Contents

List of tables

List of figures

List of plates

Publications arising from this research

1. Height, S.G. and Whisson, G.J. 2006. Behavioural responses of Australian freshwater crayfish (*Cherax cainii* and *Cherax albidus*) to exotic fish odour. *Australian Journal of Zoology* 54: 399-407.

2. Height, S.G., Marsh, B. and Whisson, G.J. 2006. The influence of gender, size, life-stage and prior residence on shelter acquisition by marron (*Cherax tenuimanus*) and yabbies (*Cherax albidus*). *Freshwater Crayfish* 15: 79-86.

3. Height, S.G. and Whisson, G.J. 2008. Food odour detection by Australian freshwater crayfish (*Cherax tenuimanus* and *Cherax albidus*). *Behaviour* (submitted).

4. Height, S.G. and Whisson, G.J. Influence of predator size on the behavioural responses of *Cherax albidus* to predator odour. *Crustaceana* (under prep.)

5. Height, S.G. and Whisson, G.J. Behavioural responses of juvenile and adult Australian freshwater crayfish (*Cherax tenuimanus* and *Cherax albidus*) to alarm odours at night. *Journal of Crustacean Biology* (under prep.)

6. Height, S.G. and Whisson, G.J. Shelter utilisation by Australian freshwater crayfish (*Cherax tenuimanus* and *Cherax albidus*) under threat from finfish predators. *Oecologia* (under prep.)

Chapter one

Introduction

1.0 Introduction

1.1 Global species invasions

Biological invasions are defined as the introduction, establishment and spread of species outside their native range (Richardson and Pyšek 2006), and are second only to habitat loss as a cause of species endangerment and extinction (Lowe et al. 2004). The first intentional species introductions occurred with human migration (Lowe et al. 2004). Along with the development of the modern world and globalisation came an increase in the number of species being transported beyond their natural range (Kolar and Lodge 2001; Lowe et al. 2004). Human activities such as agriculture, aquaculture, recreation and transportation, promote both the intentional and accidental spread of species (Kolar and Lodge 2001). Although some of these introductions may be beneficial, many have resulted in devastating economic impacts and are a major threat to biodiversity and ecosystem function (Wilcove et al. 1998; Sala et al. 2000). In recent times, research efforts to understand the ecological and economic costs of biological invasions have increased (Pimentel et al. 2000, 2005; Prentis et al. 2008), and some authors predict that management of alien and translocated species may be one of the biggest challenges that conservation biologists face in coming decades (Harris and Battaglene 1990; Harris 2003; Lintermans 2004).

1.2 Aquatic species translocations

At its broadest definition, the translocation of aquatic organisms encompasses any assisted movement of an organism beyond its accepted distribution (Ministerial Council on Forestry, Fisheries and Aquaculture 1999). Aquatic organisms have been deliberately translocated on a global scale for aquaculture and fisheries enhancement (Coy 1979; Lawrence and Jones 2002), and unintentionally in ballast water and hull fouling of ships (Renata and RavishankarRenata 2006; Gherardi and Acquistapace 2007) and accidental release of legally imported aquarium species (Lowe et al. 2004).

One of the most notorious aquatic translocations known is the introduction of Nile perch (*Lates niloticus*) to Lake Victoria, Africa, in 1954 (Lowe et al. 2004). Perch were introduced to the lake to counteract the drastic drop in native fish stocks caused by over-fishing. Through predation and resource competition with native species, Nile perch have now contributed to the extinction of over 200 native species (Reinthal and Kling 1997; Lowe et al. 2004).

1.3 Australian aquatic species translocations

Aquatic species have been translocated into and within Australia since the mid-1800s (Clements 1988). Initially these introductions occurred for the purposes of fisheries enhancement (Allen et al. 2002) during an era when government policy was not as comprehensive as it is today. Current national legislation, designed to conserve the integrity of indigenous native species, is predominantly concerned with the impacts of introduced exotic species (Koehn 2004). Australian aquatic species translocations include exotic species introduced to Australia from abroad (e.g. redfin perch *Perca fluviatilis;* brown trout *Salmo trutta*; and rainbow trout *Oncorhynchus mykiss*) and the introduction of native species to new habitats (e.g. Murray cod *Maccullochella peelii peelii*; and golden perch *Macquaria ambigua*). To date, 77 native fish species have been translocated within Australia (Hannon 2008) and 10 introduced fish species have become established in the inland waters of Western Australia (Morgan et al. 2004a). Although some of these species translocations have been deliberate, others have been accidental or illegally conducted (Morgan et al. 2002).

The translocation of aquatic species in Australia has resulted in significant social and economic impacts, both positive and negative. For example, translocation has created viable recreational fisheries in many areas where the indigenous native fish fauna are not of sufficient size to be of interest to anglers (Astbury 2004), and allowed the establishment of aquaculture industries for species outside their natural range (Hannon 2008). However, translocation has also resulted in many negative impacts on native species. One well-known example is the reduced biodiversity of the Murray-

Darling Basin due to the explosion of the exotic carp (*Cyprinus carpio)* population (Harris and Gehrke 1997; Koehn et al. 2000).

1.4 Ecosystems of south-western Australia

The diverse aquatic habitats of south-western Australia are characterised by long dry summers and cool wet winters, and are known for their highly endemic freshwater fish and decapod crustacean fauna (Morgan et al. 1998; Morgan 2004). South-West ecosystems have a long history of species introductions dating back to the 1860s. Consequently, the unique fauna of this region is threatened by competition and predation from exotic species, along with habitat loss through land clearing (increasing salinity) and eutrophication (Morgan 2004).

1.5 Translocation policy in Western Australia

In the past, translocations of aquatic organisms into Western Australia were only permitted on two conditions. Firstly, introduced animals were subject to a period of quarantine to confirm their disease-free status. Secondly, animals were only permitted to be stocked into impounded waters (Thorne and Brayford 2000). However, recent focus on the environmental sensitivities surrounding aquatic translocation has led to the development of a risk assessment process by Fisheries Western Australia (FWA) and the Environmental Protection Authority. This process was designed to facilitate the environmental sustainability of commercial aquaculture and stock enhancement for recreational fishing, whilst providing the appropriate levels of protection required to conserve native flora and fauna (FWA 2002a). A key requirement of a risk management approach to aquatic translocation control is access to quantifiable evidence regarding known and perceived risks associated with a particular translocation decision.

Given the history of aquatic translocations into Western Australia, there is an obvious need to assess the current status and effects of these previous translocations, as a basis for informing future management and policy decisions associated with similar actions.

1.6 History of species translocations in Western Australia

In the 200 years since European colonisation, many exotic plant and animal species have been introduced to Australia, including a long list of aquatic organisms (Morrissy and Cassells 1992; Allen et al. 2002; FWA 2002a). The first finfish were released in the south-east corner of Australia during the 1860s and 1870s, with further introductions in Western Australia shortly after (Allen et al. 2002; FWA 2002a). As a result, exotic fish and crustaceans have existed in Western Australia for over 100 years, originally being translocated for the purposes of enhancing recreational fishing (Allen et al. 2002), stocking farm dams (Morrissy and Cassells 1992), and more recently, commercial aquaculture (FWA 2002b; 2003). However in some cases, specimens have escaped from man-made impoundments and established self-sustaining populations in the wild. One such example is the invasive freshwater crayfish *Cherax albidus*, commonly known as the 'yabby' (Morrissy and Cassells 1992; FWA 2000; Gherardi et al. 2002a). Other species of concern include redfin perch, trout, silver perch, Murray cod and golden perch.

1.6.1 Yabbies

The yabby is native to the eastern states of Australia and was first introduced to farm dams in the Wheatbelt region of southern Western Australia in 1932 (Morrissy and Cassells 1992). Although now farmed commercially in Western Australia (Lawrence and Jones 2002), yabbies have become a cause for concern due to their progressive invasion into natural water-bodies in the South-West (Morrissy and Cassells 1992; Whisson 2003; Beatty 2006). Currently, the Fisheries Department of Western Australia has imposed restrictions on farming, and further translocation of the species on the

west side of a 'boundary' extending from Perth to Albany (Figure 1.1) on the south coast (Lawrence and Morrissy 2000).

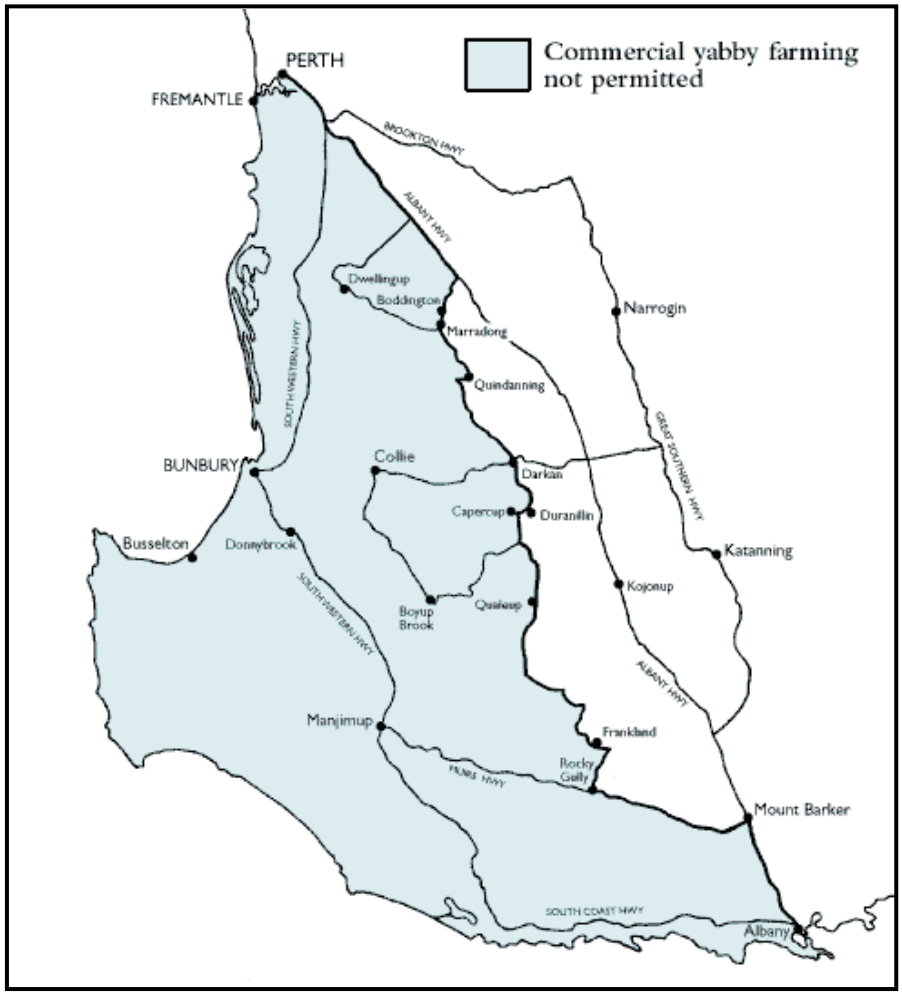

Figure 1.1 Area of south-western Australia where commercial yabby farming is not permitted (shaded)

1.6.2 Redfin perch

Another example of an infamous aquatic translocation is the European or redfin perch (*Perca fluviatilis*), stocked into Western Australian waterways in 1892 and during the early 1900s (Weatherley 1977; Coy 1979). Redfin perch are a freshwater percid

species native to European and north Asian temperate waters (Merrick and Schmida 1984). Biological attributes such as rapid growth, high fecundity, broad environmental and habitat tolerances, and voracious predatory behaviour (Appleberg and Odelström 1988; Blake and Hart 1995; Morgan et al. 2002) contribute to the ability of redfin perch to invade natural waterways (Hutchinson 1991). Upon entering a new environment, redfin perch populations tend to expand until the food supply becomes exhausted, resulting in the stunted growth of individuals (FWA 2002a). Currently, redfin perch are classed as an aquatic pest in Western Australia. Recreational anglers are asked not to return specimens to the water following capture, because their diet includes both native and introduced fish and crustaceans, most notably, the iconic marron *Cherax tenuimanus* (Pen and Potter 1992; Morgan et al. 2002; Molony and Bird 2005). In fact, redfin perch prey so heavily on marron that it has been suggested they inflict more damage on marron populations than recreational fishing (Morgan et al. 2002). The persistence of marron populations in waters occupied by redfin perch is attributed to the fact that marron can grow too large for perch to consume (maximum weight 2 kg; Coy 1979) (Morgan et al. 2002).

1.6.3 Trout

Brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) are further examples of exotic fish predators present in the South-West. Although their preference is for lotic habitats (Allen et al. 2002), these salmonids were first introduced to Western Australia's cool south-western streams, albeit somewhat unsuccessfully, in the late 1870s (FWA 2002a). Although initial survival was poor, the establishment of a Government hatchery for stock replenishment eventually resulted in significant increases in individuals in the wild (Allen et al. 2002; FWA 2002a). However, few self-sustaining stocks of trout have become established in Western Australia and the recreational trout fishery relies on an annual stocking program (FWA 2002a). Within South-West ecosystems trout consume fish, insects, aquatic snails, amphibians and decapod crustaceans (including marron, koonacs *Cherax plebejus* and gilgies *Cherax quinquecarinatus*) (Jenkins 1952; Pusey and Morrison 1989; Henderson 2000). The

potential for trout to impact on important native species, such as marron, has been acknowledged (Astbury 2004). However, the presence of trout appears to limit the proliferation of feral redfin perch in South-West ecosystems through competition and predation (Astbury 2004).

1.6.4 Silver perch

Silver perch (*Bidyanus bidyanus*) is a freshwater fish native to the Murray-Darling river system in eastern Australia, first translocated into Western Australian inland farm dams in 1950 (Lawrence 1995). Although now farmed commercially in Western Australia (Whisson 2000), silver perch remain under tight regulation and are to be kept in impounded waters (Thorne and Brayford 2000) because they are known to consume freshwater crayfish, including marron and yabbies (Whisson 2000; Storer 2005). Silver perch were recently found in the Swan coastal plain, presumably as a result of escape from aquaculture facilities; it is not known if this species is capable of reproduction in this habitat (FWA 2003; Morgan et al. 2004a).

1.6.5 Murray cod and golden perch

Murray cod (*Maccullochella peelii peelii*) and golden perch (*Macquaria ambigua*) are native to the Murray-Darling river system and are currently under consideration for translocation into Western Australia for recreational stocking and aquaculture (FWA 2003).

Murray cod are one of the world's largest freshwater fish species (Rowland 1989) and an aggressive and territorial apex predator known to consume everything from freshwater crayfish and fish species to frogs, turtles, water birds and terrestrial animals such as possums, mice and snakes (Lake 1978; Rowland 1988; McDowall 1996; Ebner 2006). Early attempts to introduce Murray cod to Western Australia were made in the late 1800s (Morrissy 1970; Coy 1979; Rowland 1989), but the species is not known to currently exist in native habitats.

Golden perch were stocked into the Swan-Avon River in Western Australia in the late 1800s (Coy 1979), and a number of specimens were recently captured in the Swan River (Lawrence 1993; FWA 2003). Golden perch can tolerate a range of habitats, temperatures and salinities (Langdon 1987) and have been known to spawn in impounded waters (Rowland 1983; Merrick and Schmida 1984). The few studies that have examined the diet of golden perch in their native habitat found that adults prey heavily on other fish species and freshwater crayfish; specifically, the Murray River crayfish *Euastacus armatus* (Merrick and Schmida 1984). Golden perch hunt for prey both during the day and at night (Merrick and Schmida 1984), and are therefore able to predate on nocturnal species such as crayfish.

The biological characteristics of Murray cod and golden perch make both species a serious predatory threat to native species in Western Australia. Morgan et al. (2002) outlined serious concern for marron populations in the South-West should these species escape or be stocked into waters occupied by marron; Murray cod and golden perch can both attain sizes that would be capable of ingesting even the largest of marron.

1.6.6 Other aquatic translocations into Western Australia

Several Australian species have faced translocations within and into Western Australia (WA), such as marron (*Cherax tenuimanus* [WA range extended]), barramundi (*Lates calcarifer* [WA range extended]), black bream (*Acanthopagrus butcheri* [WA range extended]) and redclaw (*Cherax quadricarinatus* [introduced to the Kimberley region of WA from Queensland]) (Lawrence 1993; Thorne and Brayford 2000; FWA 2002a; Doupé et al. 2004). A common factor driving many of these translocations is economic gain through commercial aquaculture production (Thorne and Brayford 2000; FWA 2002a). There are numerous other species translocations that have occurred within and into Western Australia, however their status falls beyond the scope of this thesis.

1.7 Thesis rationale

In the south-west of Australia, interspecific interactions between non-native yabbies and indigenous marron are not well understood. Yabby populations have expanded into Western Australian habitats occupied by marron as a result of translocation for aquaculture (Molony et al. 2002; Whisson 2003; Beatty et al. 2005b). While there is abundant evidence to suggest that invasive freshwater crayfish are detrimental to native species (Gherardi and Holdich 1999; Gherardi et al. 2002b), the nature and degree of impact on marron populations by exotic yabbies is unclear and the subject of current debate. Given the detrimental impacts of invasive freshwater crayfish on native species in other parts of the world, there is an urgent need to gain further information regarding interactions between marron and yabbies.

One poorly understood aspect is the behavioural plasticity of these species. Some researchers have hypothesized that invasive species make faster and more appropriate use of information about their environment than native species. This greater behavioural plasticity can result in displacement of indigenous species and successful colonisation of invaders (Gherardi et al. 2002b).

This research examines the behavioural responses of indigenous marron and invasive yabbies, to water-borne odours from food, alarm sources and finfish predators. This study was undertaken to assist in the clarification and documentation of interactions between indigenous and non-indigenous crayfish and to assess their behaviour in the presence of predation risk. Predation and competition are major forces influencing community structure in aquatic ecosystems, therefore knowledge of competitive and predatory interactions will be of benefit when considering future translocation policy in Western Australia.

1.8 General aim of research

To examine the behavioural responses of an indigenous crayfish, *Cherax tenuimanus* (marron), and an invasive crayfish, *Cherax albidus* (yabby), present in Western Australia, to water-borne odours from food, alarm sources, and finfish predators.

1.8.1 Specific objectives

i. To compare the behavioural responses of marron and yabbies to different concentrations of food odour;

ii. To determine if crayfish size influences the behavioural response of marron and yabbies to silver perch odour;

iii. To investigate the effect of silver perch size on behavioural responses of yabbies to silver perch odour;

iv. To determine if crayfish size influences the behavioural response of marron and yabbies to alarm odour;

v. To assess differences in behavioural modifications of marron and yabbies presented with food and alarm odours a) during the day, and b) during the night;

vi. To evaluate the influence of crayfish size, prior residence and food availability on shelter utilisation by marron and yabbies under predation threat;

vii. To investigate modifications to experimental protocols for future crayfish behavioural studies;

viii. To discuss thesis findings in the context of invasive species management and translocation in Western Australia.

Chapter two

Literature review

2.0 Literature review

2.1 Introduction

The movement of aquatic organisms outside their natural range not only affects the distribution of the translocated species, but also the dynamics and biodiversity of the invaded community (Gherardi and Holdich 1999). Since the late 1800s a number of aquatic species have been introduced to the fragile ecosystems of south-western Australia, the effects of which are not well understood. Of particular concern is the impact of exotic species on native marron (*Cherax tenuimanus*) populations. Marron become target prey for introduced finfish predators like rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and redfin perch (*Perca fluviatilis*). Marron also compete for resources with another freshwater crayfish native to the eastern states of Australia - the congeneric yabby (*Cherax albidus*). The distribution of yabbies in Western Australia has progressively increased since their introduction and the species is classed as invasive (Morrissy and Cassells 1992; Beatty et al. 2005b).

This review investigates some of the ecological ramifications of past translocations involving native and exotic freshwater crayfish species, and the characteristics and factors that contribute to successful invasions, with emphasis on chemical communication and behaviour in freshwater crayfish.

2.2 Crayfish behaviour

Researching animal behaviour is a difficult task because it requires accurate interpretation and understanding of an organism's actions based on observation (Gherardi 2002). In the case of aquatic animals, observing them in natural habitats without creating a disturbance is extremely difficult and in many cases impossible. Studies on crayfish behaviour have been conducted under both laboratory and field conditions, focussing on activity patterns (DeCoursey 1983; Cukerzis 1988; Barbaresi

and Gherardi 2001), feeding behaviour (Caine 1975; Dunham et al. 1997), habitat selection (Partridge 1978; Mundahl and Benton 1990; Gherardi et al. 2000b), movement (Merkle 1969; Guan and Wiles 1997), the use of shelters and burrows (Westin and Gydemo 1988; Hobbs 1991; Martin and Moore 2007), reproductive behaviour (Snedden 1990; Villanelli and Gherardi 1998), juvenile behaviour (Ameyaw-Akumfi 1976; Holdich and Reeve 1988); agonistic interactions (Capelli 1975; Huner and Barr 1984; Zulandt et al. 2008); and the influence of predators (Lima and Bednekoff 1999; Nyström 2005).

Crayfish behaviour is influenced by biological and non-biological information that individuals receive about their environment. Information comes from sources such as food, conspecific and heterospecific crayfish, predators and prey. Therefore to study crayfish behaviour and the factors that influence that behaviour, it is necessary to understand the modes of communication they utilise.

2.2.1 Communication

Aquatic animals send and receive information based on their physical and chemical status (Lodge and Hill 1994; Lonsdale et al. 1998). Communication between animals within the aquatic environment can involve tactile (Herberholz et al. 2004), hydrodynamic (Blake and Hart 1995; Bouma and Hazlett 2001), visual (Blake and Hart 1993b; Hazlett and McLay 2000), and chemical cues (Appelberg et al. 1993; Hazlett and Schoolmaster 1998). In laboratory studies researchers often isolate these cues to gain an understanding of their influence on crayfish behaviour (e.g. Shave et al. 1994; Hazlett 2000). However, in a natural habitat, communication and behaviour are influenced by a combination of biological and environmental elements (White et al. 1995; Pecor and Hazlett 2006b) and rarely is crayfish behaviour based on information from one source alone (Aquiloni and Gherardi 2008). Of particular interest to the research presented in this thesis, are the mechanisms of communication amongst crayfish and those between crayfish and fish predators.

2.2.2 Tactile communication

Tactile communication, or physical interaction, occurs during aggressive interactions between crayfish and in encounters between predator and prey. Crayfish fight over resources such as food (Ahvenharju and Ruohonen 2007), shelter (Blank and Figler 1996), and mating partners (Ameyaw-Akumfi 1976; Villanelli and Gherardi 1998). Numerous studies have found that crayfish size is the most important factor with respect to the outcome of these interactions (Bovbjerg 1956; Vorburger and Ribi 1999; Wangpen 2005). In predator-prey relationships, crayfish use tactile stimuli to identify predation risk (Hazlett 1999; Herberholz et al. 2004). Physical contact from a predator often results in crayfish invoking the 'tail flip' response as a means of evasion (Webb 1979; Vogt 2002). Crayfish responses to tactile cues can be heightened in the presence of other stimuli such as chemical cues (Bouwma and Hazlett 2001).

2.2.3 Hydrodynamic cues

Hydrodynamic receptors (Figure 2.1) comprise thousands of hairs spread over the body and appendages of crayfish forming dispersed sensory arrays (Thomas 1970). These hairs detect water vibrations, or 'hydrodynamic stimuli', providing crayfish with important information on the direction of water flow, and the presence and movement of conspecifics, predators and prey (Vogt 2002).

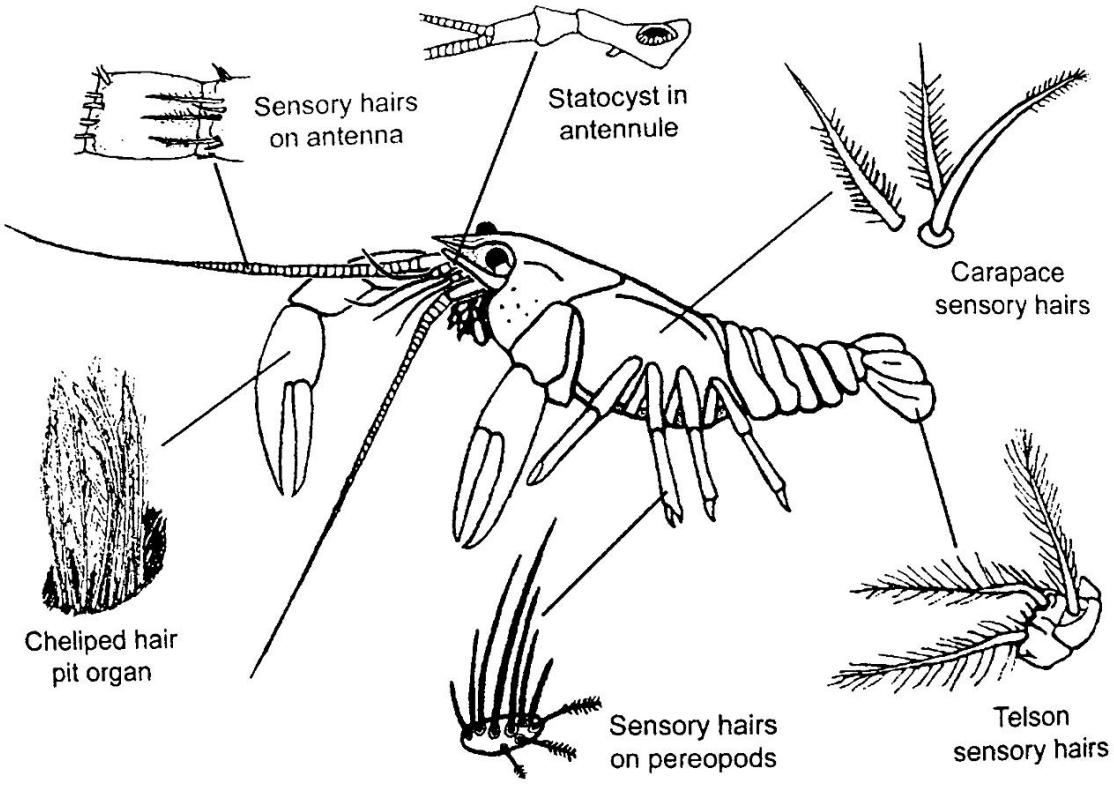

Figure 2.1 Hydrodynamic receptors in crayfish

Source: Breithaupt and Tautz 1990, in: Holdich 2002, p 136.

2.2.4 Visual communication

Visual cues play a major role in predator-prey relationships between fish and crayfish (Disler and Smirnov 1977; Wine and Krasne 1982; Blake and Hart 1993b). Fish predators generally rely on visual contact when hunting prey (Nyström 2002). It has been well documented that the presence of a fish predator causes changes in crayfish behaviour (Stein and Magnuson 1976; Blake and Hart 1993b), ultimately affecting crayfish feeding and growth (Lima 1998; Nyström 2005).

Blake and Hart (1993b) physically isolated crayfish (*Pacifastacus leniusculus*) and a fish predator, permitting only visual contact. They found that crayfish altered their behaviour in response to visual cues from both eels (*Anguilla anguilla*) and redfin perch. Blake and Hart (1993b) further noted that crayfish behaviour appeared to be influenced more by visual contact with eels, than with perch, speculating that this may have been a consequence of the greater activity of the eels. In contrast to these results, Appelberg et al. (1993) reported that given only visual cues, crayfish (*Astacus astacus*) did not respond to fish predators. However, Appelberg et al. (1993) also noted that the lack of response by crayfish may have been a result of both the experimental design, and the behaviour of the fish in captivity.

Other studies on the interactions between fish predators and crayfish (Appelberg and Odelström 1988; Mather and Stein 1993; Spanier et al. 1998) generally fail to distinguish between visual and chemical communication, making it difficult to determine the extent to which either of these forms of communication affects crayfish behaviour. However, a study by Bouma and Hazlett (2001) indicated that crayfish behaviour in response to stimuli from a predator is based on the integration of tactile, visual and chemical cues.

2.2.5 Chemical communication

Crayfish respond to an array of chemical signals within their habitat such as food odours (Ameyaw-Akumfi 1977; Grasso and Basil 2002), sex pheromones (Ameyaw-Akumfi 1977; Hazlett 1985a), disturbance pheromones (Thorp and Ammerman 1978; Hazlett 1985b; 1989; 1990), alarm odours (Hazlett 1994a; Mitchell & Hazlett 1996; Gherardi et al. 2002a) and predator odours (Appelberg *et al*. 1993; Blake & Hart 1993; Hazlett and Schoolmaster 1998).

The chemoreceptors of crayfish are mainly concentrated on the antennule, oral appendages and pereopods (Holdich and Reeve 1988; Hazlett 1990). Receptors on the antennule (‘aesthetascs’, Plate 2.1) are used in olfaction and sensing odours from

distant sources such as predator and alarm odours (Vogt 2002). The receptors on the mouthparts and pereopods perceive molecules by direct contact with the source (‘gustation’), typically sensing food quality (Holdich and Reeve 1988).

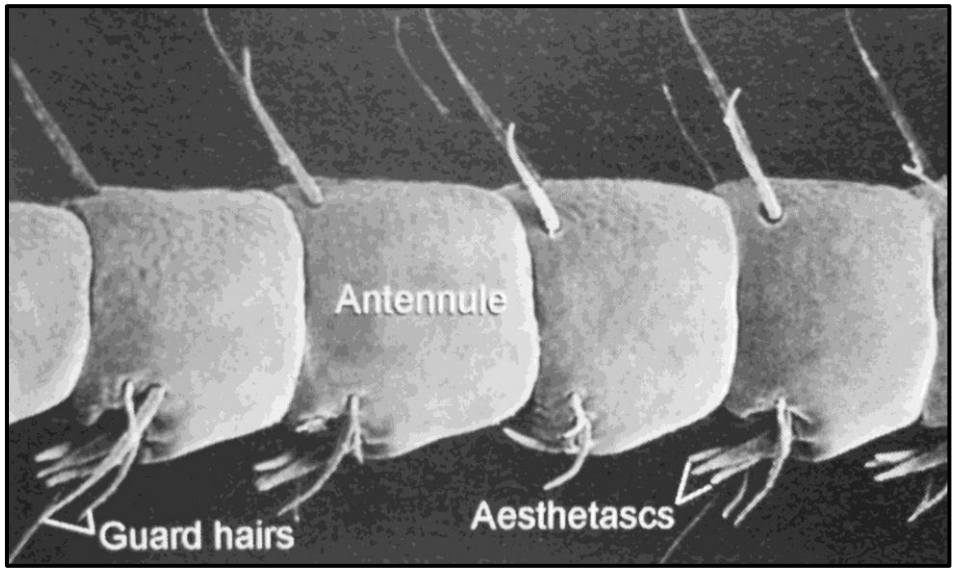

Plate 2.1 Crayfish antennule showing chemosensory hairs (aesthetascs)

Source: Sandeman and Sandeman 1996, in: Holdich 2002, p 137.

2.2.5.1 Disturbance pheromones

When crayfish are subjected to sources of stress they release chemicals known as ‘disturbance pheromones’ (Hazlett 1989). However, these chemicals are not detected by all species. For example, Hazlett (1985b) determined that *Orconectes virilis, Orconectes rusticus* and *Orconectes propinquus* released chemicals when they were disturbed: *O. virilis* responded to disturbance pheromones produced by *O. rusticus* and *O. propinquus* by exhibiting behavioural modifications, whilst *O. rusticus* and *O. propinquus* did not display any response. Hazlett (1985b) also noted that disturbance pheromones were not specific to particular stressors exposed to a crayfish, nor were they species specific.

2.2.5.2 Alarm odours

In contrast to disturbance pheromones (produced when an organism is not physically harmed, eg. Hazlett 1989; 1990) alarm odours are the chemicals released by an organism when its skin or exoskeleton is broken during predation or other life-threatening events (Hazlett 1994a; Mitchell and Hazlett 1996). Acquistapace et al. (2005) proposed that in *P. clarkii*, alarm substances are peptides involved in the hemolymph clotting process.

Some crayfish species display behavioural modifications, or 'alarm response', after detection of alarm substances. Hazlett (1994a) demonstrated the alarm response in *O. virilis* involved adopting the intermediate posture and ceasing movement, speculating this may be an indication the animal was no longer in a relaxed state but rather on alert for potential danger. The response to alarm odour varies amongst species. Some crayfish do not react to alarm odours (eg. *O. propinquus*, Hazlett 1994a), whilst others react not only to conspecific alarm odour (odour from a damaged individual of the same species), but also heterospecific alarm odour (odour from a damaged individual of another species) (Hazlett 1994a; Hazlett et al. 2003).

The release of alarm odour usually coincides with a predation event. Therefore it would be advantageous for a crayfish to modify its behaviour to such stimuli released from both conspecific and heterospecific crayfish (Hazlett 1994a; 2000). Researchers have reported that invasive crayfish such as *O. rusticus*, *Orconectes limosus* and *Procambarus clarkii* utilise alarm odour from a broader range of crayfish species than do displaced native crayfish such as *Austropotomobius pallipes* and *O. propinquus* (Hazlett 2000; Hazlett et al. 2003). In Western Australia, Gherardi et al. (2002a) found that the invasive yabby (*C. albidus*) responds to conspecific and heterospecific (*C. tenuimanus*) odours.

2.2.5.3 Predator odours

Freshwater crayfish are exposed to a wide range of predator types including aquatic invertebrates, amphibians, reptiles, birds and mammals (Foster and Slater 1995). However, the single most influential predator on crayfish with respect to effects on abundance, behaviour and growth, is predatory fish (Nyström 2002). The primary mechanism by which fish hunt is visual detection of prey, therefore chemical recognition of these predators by crayfish would be an asset during times of poor visibility (Vogt 2002).

Crayfish can recognise chemical cues from a range of predators including fish (Hazlett 1990; Willman et al. 1994; Appelberg et al. 1993), eels (Blake and Hart 1993b; 1995) and turtles (Hazlett 1998). Crayfish can also distinguish between odours released from different species. For example, *O. virilis* responded by means of behavioural modification to odour from a predatory turtle (*Chelydra serpentina*), yet did not respond to odour from a non-predatory turtle (*Chrysemys picta*) (Hazlett 1998). Similarly, Appelberg et al. (1993) reported that juvenile *Astacus astacus* could not only distinguish between chemical cues released from several fish species, but also displayed stronger responses to a starved versus a satiated predator. Although the fish species used in that trial co-exist with *A. astacus*, crayfish used had no prior exposure to them, causing the authors to speculate that detection of predatory fish by *A. astacus* was a genetically based adaptation.

2.3 Factors influencing crayfish behaviour

The behaviour of freshwater crayfish is influenced by a complex interaction of biotic and abiotic factors, however, many of these fall outside the scope of this thesis (see Section 2.2). Of particular relevance to this research is the influence of predators, crayfish size and life-stage, and resource availability, on crayfish behaviour. These factors are interrelated and, together, play a role in shaping crayfish behaviour.

2.3.1 Predation risk

The trade-off between foraging and predator avoidance is well documented for crayfish (Stein 1977; Pecor and Hazlett 2006a). The acquisition of resources such as food, shelter and mates, usually requires movement by crayfish. However, movement increases the exposure of crayfish to visual predators such as finfish (Werner and Anholt 1993; Nyström 2002). Therefore resource acquisition and predation avoidance are two conflicting demands by prey animals and the particular trade-off made can be influenced by other factors. For example, Hazlett (2003) found that the invasive *O. rusticus* was less motivated by hunger to forage in the presence of predation risk than native *O. virilis*.

Several studies have reported that the presence of a fish predator is detrimental to crayfish foraging and growth (Appelberg and Odelström 1988; Nyström 2005). Furthermore, some authors have speculated that the nocturnal foraging patterns of crayfish are an adaptation to avoid predators that hunt using their vision (Flint 1977; Hamrin 1987). However, there is no scientific evidence to support this assertion and a number of influencing factors may be responsible, such as crayfish matching the nocturnal habits of its prey (Gherardi 2002).

2.3.2 Crayfish size and life-stage

Body size strongly influences the resource holding potential of an individual (Momot and Leering 1986) and the outcome of aggressive interactions between crayfish (Söderback 1995; Martin and Moore 2007). Vorburger and Ribi (1999) examined aggression and competition for shelter between native *Austropotamobius torrentium* and introduced *P. leniusculus*. Neither species was inherently dominant in aggressive interactions, but dominance was strongly size-dependent, favouring the larger species, *P. leniusculus*. Likewise, Nakata and Goshima (2003) reported that the body size advantage of exotic *P. leniusculus* resulted in successful shelter acquisition over

native *Cambaroides japonicus* in Japan. Inferiority in competition for shelter may lead to increased predation risk, and contribute to species displacement (DiDonato and Lodge 1993; Guiasu and Dunham 1999).

Some authors have reported that ovigerous females can be highly aggressive (Mason 1977; Figler et al. 1997), suggesting that this life-stage is more successful than others in acquiring shelter (Figler et al. 2005). Studies investigating the behaviour of burrowing crayfish species found that female *C. albidus* and *P. clarkii* were more abundant in burrows than males (Correia and Ferreira 1995; Lawrence et al. 2002). Furthermore, the authors suggested that this type of reproductive strategy would increase the chances of juvenile survival.

2.3.3 Resource availability

Crayfish behaviour can be affected by a limited supply of resources such as food and shelter (Gherardi 2002). In the case of limited food, Hazlett et al. (2003) found that starved *O. virilis* were more willing to forage in the presence of a predatory threat than non-starved crayfish. Other researchers have reported that when resources are limited, large dominant crayfish can exclude smaller inferior crayfish from preferred habitat and food sources (Abrahamsson 1966; Momot 1993). Shelter is an important and often limited resource for crayfish (Bovbjerg 1970; Hobbs 1991), providing protection against predation and cannibalism (Lodge and Hill 1994; Figler et al. 1999). Therefore, exclusion from shelter can result in greater exposure to predators and consequently, crayfish mortality. Juvenile crayfish can be more susceptible to shelter exclusion and predation than adults, due to their size disadvantage in competitive interactions (Vorburger and Ribi 1999), and the size-selective nature of finfish predators (Stein 1977).

2.4 Invasive crayfish species

Species invasions can result in substantial loss of biodiversity due to competitive interactions, predation and associated introduction of diseases and parasites (Diamond and Case 1986; Horwitz 1990; Vitousek et al. 1996). During the twentieth century the distribution of freshwater crayfish species and their composition were subject to major changes as a result of crayfish introductions throughout the world (Hobbs et al. 1989; Nyström 2002). The omnivorous nature of freshwater crayfish allows them to occupy many trophic levels, therefore introductions of exotic crayfish species can result in significant changes to the ecology and structure of food-webs in recipient ecosystems (Lodge et al. 1994; Momot 1995; Nyström et al. 1999). Several crayfish species around the world are now threatened or have become extinct, whilst others are widespread and becoming more abundant (Allan and Flecker 1993; Gherardi and Holdich 1999; Nyström 2002).

The ecological implications of crayfish translocation were generally not considered in the past, only in recent times have these impacts been studied (Lodge and Hill 1994; Lewis 2002). There is abundant evidence that invasive crayfish are detrimental to native flora (Lodge and Lorman 1987; Nyström and Strand 1996) and fauna (Lodge et al. 1994; Parkyn et al. 1997; McCarthy et al. 2006), particularly native crayfish (Capelli 1982; Taugbøl and Skurdal 1999). Displacement of native freshwater crayfish by invasive crayfish species has been documented in the United States of America (USA) (Capelli 1982; Butler and Stein 1985; Lodge et al. 2000), Europe (Söderback 1995; Gherardi and Holdich 1999), Australia (Austin and Ryan 2002; Whisson 2003) and Asia (Nakata et al. 2002; Nakata and Goshima 2003).

Once exotic species become established in new aquatic habitats their presence is often considered permanent; eradication is usually difficult, if not impossible (Horwitz 1990; Lodge et al. 1998). Attempted crayfish eradication and control measures have included the use of insecticides (Laurent 1995; Holdich et al. 1999) mechanical

removal (Pöckl 2002; Hein et al. 2007), and fish predation (Frutiger and Müller 2002; Hein et al. 2007).

To date, major crayfish invasions around the world include: *Procambarus clarkii* in Europe, Africa and the USA; *Pacifastacus leniusculus* in the USA and Europe; *Orconectes limosus* in Europe; *Orconectes rusticus* in north America; *Astacus leptodactylus* in Europe; and *Cherax destructor* in Africa and Asia. (Holdich 1999).

2.4.1 Characteristics of invasive crayfish species

There are many factors that may contribute to the invasive success of a freshwater crayfish species, such as the ecology and species composition of the recipient ecosystem, and the biological characteristics of the invader. However, understanding the mechanisms behind successful invasion, colonization and displacement is often difficult due to the complex interaction of multiple factors, such as: competitive exclusion (Bovbjerg 1970); differential predation susceptibility (Butler and Stein 1985); reproductive interference (Garvey and Stein 1993); and crayfish size (Figler et al. 1999). Notwithstanding this, researchers have found that invasive crayfish are typically r-selected species whose characteristics include the ability to reproduce at an early age or size, a high spawning frequency, high fecundity, rapid growth and disease resistance (Lindqvist and Huner 1999; Evans and Edgerton 2001). Conversely, displaced native crayfish are usually k-selected species, characterised by low fecundity, slow growth, a long lifecycle and high susceptibility to disease (Lindqvist and Huner 1999; Gherardi et al. 2002b). Additional characteristics reported in invasive crayfish include: tolerance of a range of environments and physicochemical conditions (Huner and Lindqvist 1995; Holdich et al. 1997; Horwitz and Knott 1995); burrowing behaviour (Barbaresi et al. 2004; Beatty et al. 2005b); and high behavioural plasticity (Hazlett et al. 2003; Gherardi et al. 2002a).

2.4.1.1 Behavioural plasticity

It is evident that the extent to which crayfish utilise information about their environment varies significantly among species. Some crayfish species readily respond to a broad range of chemical cues about their environment, whilst others fail to utilise such information. Researchers have hypothesised that the ability to respond to a wide array of chemical information is a factor underlying the invasion success of a species (Hazlett 2000; Hazlett et al. 2002). This includes the ability of an invader to make faster and more appropriate use of a broader range of information such as cues associated with an increased risk of predation (e.g. alarm and predator odours). Recent studies comparing behavioural characteristics of native and invasive species have supported the general hypothesis that invasive species have a greater capacity for behavioural plasticity than the native species they are displacing (Hazlett 2000; Gherardi et al. 2002a). Hazlett et al. (2003) suggested that behavioural plasticity should be greater in species that inhabit a large range of habitats, or in species that have evolved in regions of high biodiversity (e.g. *O. rusticus*, *O. limosus* and *P. clarkii*) because such species would have had evolutionary opportunities to profit from a wide array of chemical information.

Invasive crayfish have also demonstrated greater behavioural plasticity than native species in their ability to learn and remember an association between different predation-risk cues. Hazlett et al. (2002) trained both native and invasive crayfish to form an association between a novel cue (goldfish odour) and predation risk, by exposing crayfish to novel odour and conspecific alarm odour simultaneously. When later exposed to novel odour only, the invasive *O. rusticus* and *P. clarkii* remembered the association longer than did native *O. virilis* and *A. pallipes*. Efficient learning about predation-risk cues would be a competitive advantage to crayfish when entering new habitats.

In Western Australia, the behavioural plasticity of invasive yabbies and native marron requires further investigation. Yabbies appear to better utilise heterospecific alarm odour and predator odour than marron (Gherardi et al. 2002a; Height 2002). However, the affect of variables such as crayfish size, resource availability, and nocturnal activity patterns on crayfish behavioural responses to chemical stimuli are unknown.

2.5 Marron and yabbies in south-western Australia

2.5.1 Marron

Marron (*C. tenuimanus*) are native to the permanent rivers in the forested, high rainfall areas of south-western Australia (Lawrence and Jones 2002) and are the third largest freshwater crayfish species in the world (Austin and Knott 1996), capable of growing to 2 kg and a carapace length of 200 mm (Coy 1979). Marron require high dissolved oxygen levels (> 6 mg/L) and a water temperature of 24^{o}C for optimum growth and are generally around two years old when they reach sexual maturity (Lawrence and Jones 2002). Marron display traits of both a K- and *r*-selected species (Beatty et al. 2005a). For example, K-selected traits of marron include: they inhabit permanent aquatic systems (Austin and Knott 1996); have a long, synchronised brooding period (Beatty et al. 2003); and grow to a large maximum size (Coy 1979), yet marron brood in summer, a characteristic typical of an *r*-selected species (Beatty et al. 2003, 2005a). The aquaculture potential of the species has resulted in recent translocation of marron within Western Australia, Australia, and overseas (Morrissy et al. 1990; Lawrence and Jones 2002). Marron are omnivorous scavengers (Mills et al. 1994) and a non-burrowing species (Morrissy 1992; Beatty et al. 2003), unlike other native congeneric crayfish (the koonac, *Cherax plebejus*; and gilgie, *Cherax quinquecarinatus*) present in Western Australia (Beatty et al. 2005a). Marron have essentially evolved in the absence of a large piscivorous predator (Morgan et al. 1998; Pen 1999), with the exception of freshwater cobbler (*Tandanus bostocki*), found only in select South-West water-bodies (Morgan et al. 1998).

The nomenclature for marron is currently under debate. Austin and Ryan (2002) applied the name *Cherax cainii* to the widespread species of marron, and *Cherax tenuimanus* to an isolated species of marron found only in the Margaret River. However, Molony et al. (2006) challenged this classification (International Commission on Zoological Nomenclature case number 3267) and proposed that the name *Cherax cainii* be set aside and neotypes designated for both species to maintain the accustomed usage of the name *Cherax tenuimanus.* Whilst this case remains in dispute, marron used for the research presented in this thesis have been referred to as *Cherax tenuimanus,* and are not the species found in the Margaret River.

2.5.2 Yabbies

Yabbies (*C. albidus*) are native to central and eastern Australian, their distribution in Western Australia has expanded significantly since their initial introduction to a farm dam at Narembeen in 1932 (Jasinka et al. 1993; Molony and Bird 2002; Beatty et al. 2005b). Yabbies can attain a maximum size of 220 g (Lawrence and Jones 2002) and are known to compete with native crayfish, particularly marron, for resources (Molony et al. 2002; Beatty 2006). Although marron and yabbies have similar environmental requirements (Morrissy et al. 1990), yabbies appear to possess a number of competitive advantages over marron, including: a younger age at sexual maturity (< 1 year), capable of multiple spawns (Lawrence and Jones 2002; Beatty 2005b); more aggressive behaviour (Morrissy et al. 1990; Mills et al. 1994); the ability to burrow and survive in ephemeral habitats (Beatty et al. 2005b); tolerance of lower dissolved oxygen levels (< 1 mg/L) and higher temperatures (28^{o}C optimum) (Morris and Callaghan 1998; Lawrence and Jones 2002); and higher behavioural plasticity (Gherardi et al. 2002a; Height and Whisson 2006). Thus, like other invasive freshwater crayfish species, yabbies show the characteristics of an *r*-selected species (Lawrence et al. 2002; Beatty et al. 2005b).

Chapter three

Experimental systems, materials and protocols

This chapter describes the recirculating systems used to hold animals and the experimental systems used to conduct research trials reported in this thesis. The crayfish behaviour recording protocol used in Chapters 4, 5, 6 and 7 is detailed in this chapter.

3.0 Experimental systems, materials and protocols

3.1 Experimental systems

3.1.1 Aquarium-based trials

All aquarium-based experiments (Chapters 4 - 7) were conducted under controlled light conditions in a purpose-built research room at the Curtin Aquatic Research Laboratories (CARL) located at Technology Park, Bentley (31.98^{o}S, 115.88^{o}E), Perth, Western Australia. This room (9.7 m length, 4.4 m width, 2.5 m height) was specifically designed for behavioural research and thus contains no windows, allowing researchers to control light conditions, air flow and temperature.

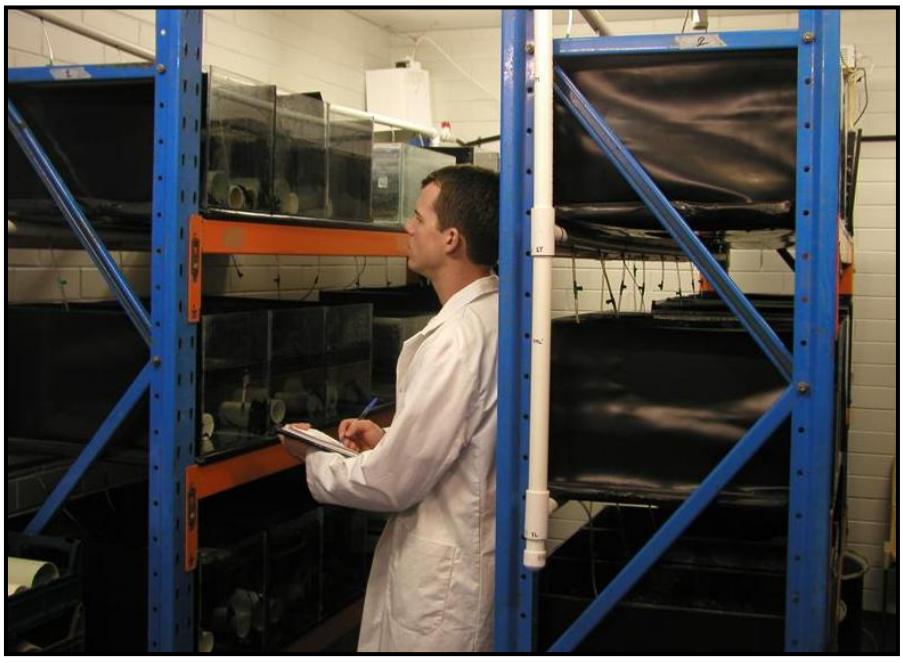

Plate 3.1 Glass aquariums used for crayfish behavioural trials (Chapters 4 - 7)

Fifty four glass aquariums (60 x 30 cm bottom, 40 cm depth) were located on three parallel stands (Plate 3.1). Each stand held six aquariums on each of its three shelves. Each aquarium had a capacity of 72 L and was visually isolated with a black plastic sheet to minimize disturbances to crayfish, except for one end, which was left uncovered for observation (Plate 3.2). During trials, each aquarium was filled with 25 L of dechlorinated tapwater, constantly aerated and supplied with a piece of polyvinyl chloride pipe (PVC) for shelter (length 20 cm, diameter 7.5 cm, unless specified otherwise). Lighting was provided via twelve 40 W fluorescent lights which were fixed to the ceiling and connected to a timer.

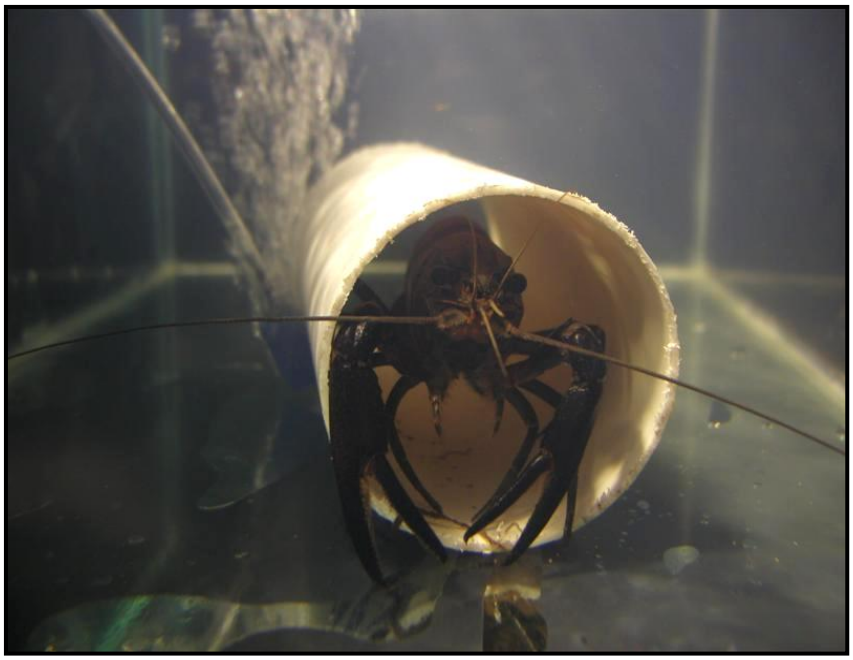

Plate 3.2 View inside an aquarium used for crayfish behavioural trials

3.1.2 Mesocosm-based trial

The oblong shaped reinforced concrete mesocosm (Plate 3.3) used for the trial described in Chapter 8 is located at CARL, dimensions are outlined in Table 3.1. Water was pumped by a 2.15 kW (Waterco© Ltd) pump through a 1000 µm prefilter located on the bottom of the mesocosm and then circulated through a sandfilter (Enduro© EN 850) and a cylindrical biofilter (2.2 m height, 0.9 m diameter, filled with bioballs) before returning it to the mesocosm via a 90 mm PVC pipe. Water circulation operated for 24 h/day and the flow rate from the biofilter was 100 L/min, giving a retention time in the mesocosm of approximately 10 hours. A 230 W submersible pump (PondMate™) circulated water from the mesocosm through a 30 W UV sterilizer (Unicorn UViFlow™ VF9) at a rate of 15 L/min.

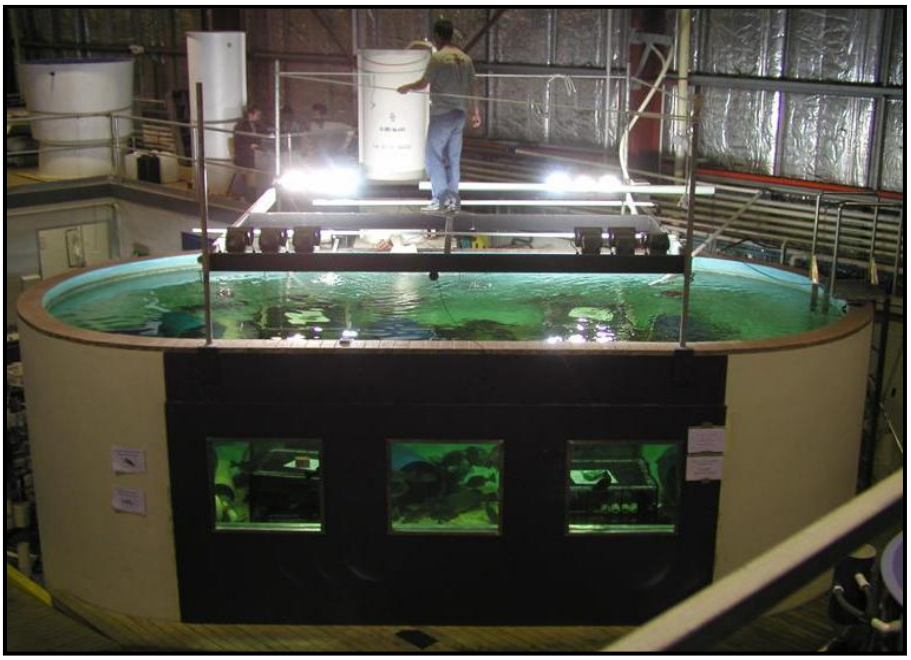

Plate 3.3 The mesocosm located at CARL

Table 3.1 Mesocosm dimensions

total length	8 m
total width	4 m
wall thickness	235 mm
average tank depth	2.9 m
surface area	24 m^2
effective water volume	69 600 L

Aeration was supplied using a diffused air system connected to the CARL mainline, which is powered by a 7.5 kW 3-phase blower (Nash-ELMO™) that operates at 36 kPa for 24 h/day. The diffused air system comprised two lengths of perforated 19 mm polypipe situated lengthwise along the base of the mesocosm approximately 1 m apart, and these were connected to a 50 mm supply line that was plumbed into the CARL mainline. Substrate comprised 10-12 mm quartz gravel spread evenly over the base of the mesocosm at an average depth of 300 mm. Lighting was provided by four banks of three 500 W halogen lights attached to a rectangular metal frame suspended 900 mm above the mesocosm.

3.1.2.1 Submersible cage design

The mesocosm trial (Chapter 8) used four submersible square mesh cages (Plate 3.4) with specifications described in Table 3.2.

Table 3.2 Submersible cage design

length	1 m
width	1 m
depth	1 m
effective volume	1 m^3
mesh size	8 x 8 mm

Cages were constructed using extruded polypropylene, UV-stabilised, oyster mesh (Nylex® Corporation Pty Ltd) panels attached to a rigid 20 mm PVC pipe frame using 150 mm cable ties. The top panel of each cage was hinged at one end using 150 mm cable ties to allow access inside, and had two 200 mm slits cut into it that ran perpendicular to each other. These slits permitted a length of 90 mm PVC pipe to be inserted into the cage for crayfish deployment (described further in Section 8.2.3). A 1 kg lead weight was attached to each corner of the base of each cage using 150 mm cable ties so that the cages were kept submerged and anchored in position during the trial.

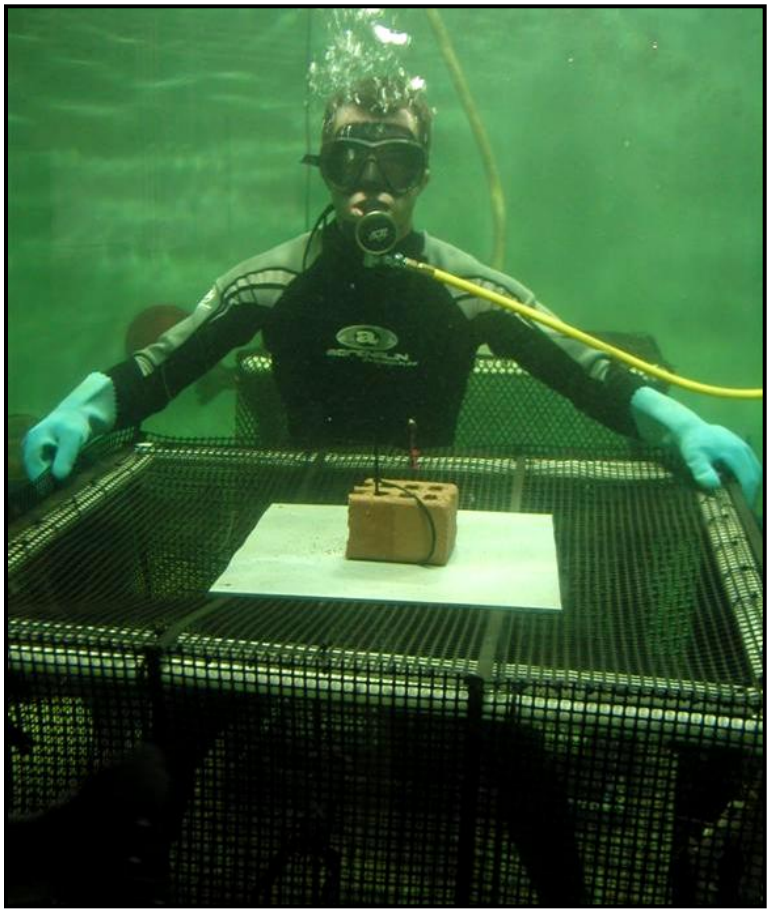

Plate 3.4 1m^3 submersible cage used in the mesocosm trial at CARL (Chapter 8)

3.2 Crayfish and fish holding systems

3.2.1 Crayfish holding systems

Marron and yabbies used in all trials were maintained in 'species only' tanks in two separate culture systems; a small recirculating system located in the research room described in Section 3.1.1, and a large recirculating system located in the main room at CARL. The small and/or large recirculating systems were used to house crayfish according to the number of individuals required for each experiment.

3.2.1.1 Small recirculating system

Nine circular plastic tanks (Plate 3.5) with dimensions described in Table 3.3 were used to hold crayfish.

Table 3.3 Tank specifications for the small recirculating system

internal diameter	0.49 m
average tank depth	0.42 m
surface area	0.75 m^2
effective water volume	300 L

A 0.75 kW pump (Onga™ Pty Ltd) took water from a 370 L sump and circulated it through a 2 μm cartridge filter before a PVC T-piece directed half of the pump output to the nine tanks, and the other half through a 30 W UV sterilizer (Unicorn UViFlow™ VF9) and then two 180 L cylindrical biofilters (filled with bioballs). Water from the tanks and biofilters was gravity-fed back to the sump. Flow rate into each tank was 10 L/min and recirculation ran constantly, giving a retention time in each tank of approximately 30 minutes. Each tank had a 40 mm central stand-pipe (covered with 5 mm mesh) and was constantly aerated using an airstone connected to a 19 mm polypipe that was plumbed into the 50 mm CARL mainline (described in Section 3.1.2). Approximately 10% of the

system volume was exchanged weekly, or as required, determined by water quality monitoring. Lengths of PVC pipe (< 250 mm) and/or bundles of nylon mesh were placed in each tank for crayfish to shelter in, the size and type of shelter was dependent on resident crayfish in the tank at any given time.

Plate 3.5 Small recirculating system used to hold crayfish for behavioural trials at CARL

3.2.1.2 Large recirculating system

CARL contains three independent recirculating systems. Each system consists of three circular fibreglass tanks (Plate 3.6, with dimensions outlined in Table 3.4) and two 16 m^3 below ground sumps. Following is a description of one of these systems, which was used to hold crayfish for the experiments detailed in this thesis. A 2.37 kW pump (Waterco Hydrostorm™ 300+) took water from a 16 m^3 below ground bio-sump (containing 5 m^3 of bio-balls) and delivered it to each tank via a 40 mm PVC pipe.

Water flowed out of each tank in two ways; by spilling into an overflow box and then a swirl-separator attached to the side of each tank, or, through an outlet at the bottom of the tank into the swirl-separator via a 40 mm tube. Particulate waste accumulated in the swirl-separator was released (as required) via a valve and ran to waste. Clean water from the swirl-separator was gravity fed back into a 100 mm mainline and then into a 62 µm drum filter (Aquasonic® RDF100) before flowing over the bio-balls and back into the below ground sump. Each tank was aerated via two airstones secured to the 40 mm PVC central standpipe using 150 mm cable ties. The airstones were connected to the CARL mainline using 5 mm tubing.

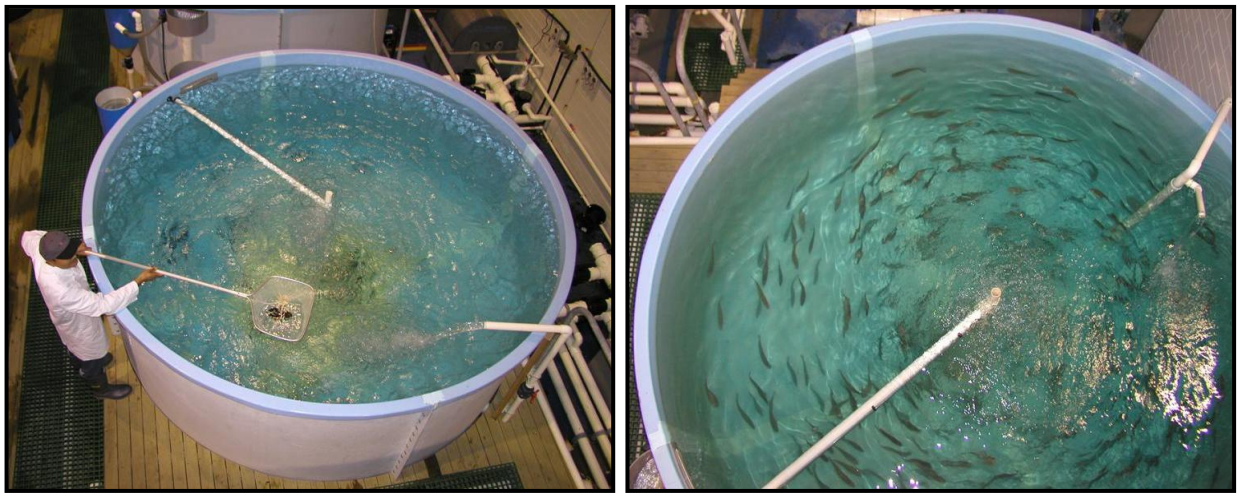

Plate 3.6 Fibreglass tanks used to hold crayfish and fish at CARL

Table 3.4 Tank specifications for the large recirculating system

internal diameter	3.32 m
average tank depth	1.6 m
surface area	8.66 m^2
effective water volume	13 850 L

3.2.2 Fish holding system

Silver perch used in the trials described in Chapters 5 and 6 were kept in a recirculating system identical to that described in Section 3.2.1.2. As previously mentioned, there are three of these independent recirculating systems operating at CARL.

3.3 Crayfish behaviour recording protocol

Crayfish behavioural observations described in Chapters 4 - 7 were made in accordance with protocols established by Hazlett (1994a). The reaction time (in seconds) and the percentage of time (for the 3 minute test period) spent in each behaviour and posture were recorded.

3.3.1 Reaction time

Reaction time is the time passed (in seconds) before a change in crayfish behaviour or posture is noticed.

3.3.2 Crayfish behaviour and posture

After exposure to control water or test solutions crayfish behaviours and postures were observed and recorded according to the protocol described in Table 3.5.

Table 3.5 Crayfish behaviours and postures* recorded during aquarium-based trials (Chapters 4 – 7)

behaviour	**description**
in shelter	
locomotion	movement of the ambulatory legs
feeding movements	
scraping	crayfish moved maxillipeds and/or scraped the substratum with chelipeds and periopods
sham feeding	crayfish brought chelipeds and periopods to the mouthparts as shown during feeding
searching	crayfish were searching with their chelae
antennae	flicking movements of antennae
antennule	flicking movements of antennule
posture	**description**
lowered	body is in contact with the substratum, the chelipeds drawn in towards the body, and the tail fan curled under the abdomen
intermediate	body is held just off the substratum, the tips of the chelae lightly touching the substratum, and the tail fan nearly perpendicular to the substratum
raised	body is elevated off the substratum, the chelipeds held off the substratum and parallel to it or higher, and the abdomen or tail fan extended

*originally described by Hazlett (1994a)

3.4 Statistical analysis and data presentation

Details of the statistical analysis are provided in the relevant section of each chapter. All numerical data are presented as mean ± standard error unless stated otherwise.

3.5 Water quality monitoring

Water quality was monitored during all trials. The frequency of measurements is detailed in the relevant sections of Chapters 4 – 8. Dissolved oxygen and temperature were taken using a YSI© 550A handheld meter; salinity was measured using an Atago® S-10E refractometer; pH and conductivity were measured using a TPS© WP – 80 handheld meter; and nitrite, nitrate and ammonia were measured using a Windaus Winlab® LF 2400 Photometer. Water quality data for all trials are contained in Appendix 2.

Chapter four

Food odour detection by marron and yabbies

This chapter examines the behavioural responses of marron and yabbies to six different concentrations of food odour in a laboratory trial. This study provides baseline data on the feeding responses of crayfish that will be used for comparisons with future laboratory trials in this thesis.

4.0 Food odour detection by marron and yabbies

4.1 Introduction

Water-borne cues mediate a variety of ecological interactions in the aquatic environment, making the ability to detect and utilize chemical information a significant evolutionary advantage. This is particularly the case for nocturnal species such as freshwater crayfish that are able to detect and respond to chemical stimuli from many sources including food (Holdich and Reeve 1988; Steele et al. 1999), conspecific and heterospecific crayfish (Hazlett 1985a, 1985b, 1989, 2000; Gherardi et al. 2002a; Aquiloni and Gherardi 2008) and predators (Blake and Hart 1993b; Hazlett and Schoolmaster 1998; Height and Whisson 2006). The behavioural response of crayfish to food odour not only depends on the source of the cue and its efficiency as an attractant (Taugbol et al. 1997; Corotto et al. 2007; Volpe et al. 2008), but also other prevailing factors, such as predation risk (Blake and Hart 1993b; Hazlett and Schoolmaster 1998; Height and Whisson 2006) and hydrodynamics of the water-body (Keller et al. 2001; Tomba et al. 2001; Pecor and Hazlett 2006b). The behavioural response is further affected by a crayfish's detection capability, which is of particular interest to ecologists studying invasive species and the mechanisms underlying their competitive exclusion of native species (Acquistapace et al. 2003; Corkum and Belanger 2007). Characteristics suggested as contributing to the successful establishment of invasive species within native crayfish territories include: tolerance of a variety of environmental conditions (Huner and Lindqvist 1995; Holdich et al. 1997); rapid growth and high fecundity (Morrissy 1990; Huner 2001); disease resistance (Evans and Edgerton 2001); and high behavioural plasticity (Hazlett 2000; Gherardi et al. 2002a; Hazlett et al. 2003).

Behavioural plasticity is a broad term referring to the ability of an animal to process information about its environment (Gherardi et al. 2002a; Height and Whisson 2006), with a high level implying a higher-order processing capability resulting in increased

survival and improved ecological performance (Gherardi et al. 2002b; Hazlett et al. 2002, 2003; Height and Whisson 2006). Previous studies comparing the responses of native and invasive crayfish to chemical cues have found that invasive crayfish respond to a broader range of cues and display different behavioural modifications than native species (Hazlett 2000; Gherardi et al. 2002a). However, the sensitivity of crayfish to varying concentrations of water-borne stimuli remains unclear. Some crayfish chemoreceptors can respond to only a few molecules of substance (McMahon 2002), but the affect of stimuli concentration on crayfish behaviour is poorly understood. An increased understanding of odour detection capabilities in invasive and non-invasive crayfish species could assist in the development of strategies for managing nuisance crayfish outbreaks and associated detrimental impacts on native ecosystems.

Marron (*Cherax tenuimanus*) are a large freshwater crayfish native to the permanent rivers and streams in south-western Australia. Yabbies (*Cherax albidus*) are native to the eastern states of Australia and were first introduced to Western Australia in 1932 to stock farm dams (Morrissy and Cassells 1992). Although yabbies are now commercially farmed in Western Australia, they are considered invasive and compete with native marron for resources (Gherardi et al. 2002a; Height and Whisson 2006).

The aim of this study was to compare behavioural responses of a freshwater crayfish native to Western Australia (*C. tenuimanus*) and an invasive crayfish (*C. albidus*) to different concentrations of food odour in a laboratory environment.

4.2 Materials and methods

4.2.1 Site and experiment system

This trial was conducted using the glass aquaria at the Curtin Aquatic Research Laboratories (described in Section 3.1.1), located at Bentley, Western Australia.

4.2.2 Experimental animals

Marron used in the trial were sourced from a commercial producer at Manjimup (34.23^{o}S, 116.13^{o}E), Western Australia, and averaged 97.47 ± 0.66 g (n = 54; 27 male, 27 female). Yabbies were sourced from a commercial supplier at Kukerin (33.18^{o}S, 118.08^{o}E), Western Australia, and averaged 46.06 ± 1.14 g (n = 54; 27 male, 27 female). Crayfish were kept in species only tanks at CARL in the crayfish holding systems described in Section 3.2.1 for one month prior to the trial and fed commercial crayfish pellets (Glen Forrest Stockfeeders™ Pty Ltd, Appendix 5). Crayfish were not fed during the trial or acclimation period. Lighting was provided via a 12 h : 12 h, light : dark cycle.

4.2.3 Preparation of food solution

The food solution was prepared by macerating 50 g of commercial crayfish pellets in 400 mL of dechlorinated tap water and filtering with coarse filter paper (Plate 4.1). The ratio of food to water was the same as that used in similar behavioural trials conducted with marron and yabbies (eg. Gherardi et al*.* 2002a; Height and Whisson 2006). Following suggestions by Hazlett (1994a, 1994b), food solutions were prepared fresh daily.

Plate 4.1 Preparation of the food solution

4.2.4 Experimental design

Two separate experiments were conducted, one using marron and one using yabbies. Each experiment ran for two days and comprised six treatment groups as described in Table 4.1. A 10–1000 µL adjustable pipette (Eppendorf® Pty Ltd) and 2 mL disposable syringes (Terumo® Corporation) were used to measure test solutions.

Each treatment was replicated nine times daily (18 replicates total). Treatments were allocated to crayfish tanks according to a Latin Cube design (Federer 1991) (Figure 4.1). The purpose of this was to randomise the effect of the existing variation in light intensity and temperature, between tanks on different tiers, on the experimental results. Crayfish behavioural observations were recorded for 54 tanks each day.

Table 4.1 Treatment description for food odour detection trial

treatment	composition		effective food odour concentration
	water	food odour	(ppm)
Control	2.5 mL	-	**-**
Treatment one (T1)	2.4 mL	0.1 mL	4
Treatment two (T2)	2.35 mL	0.15 mL	6
Treatment three (T3)	2.2 mL	0.3 mL	12
Treatment four (T4)	1.875 mL	0.625 mL	25
Treatment five (T5)	1.25 mL	1.25 mL	50
Treatment six (T6)	-	2.5 mL	100

Each treatment was replicated 18 times

4.2.5 Experimental procedure

The experimental procedure was adapted from those used by Hazlett (1994a) and Gherardi et al. (2002a). A single crayfish was placed into each of the 54 aquaria (60 x 30 cm bottom, described in Section 3.1.1) containing 25 L of dechlorinated tap water and left to stand for 24 h prior to receiving a test solution. Each aquarium was aerated, contained a piece of PVC pipe for shelter (length 20 cm, diameter 7.5 cm) and was visually isolated with a black plastic sheet to minimise disturbances to crayfish. After 24 h acclimation, crayfish were observed for two 3 minute time periods during which observations were recorded every 15 seconds: (i) a 3 minute control period following the addition of 2.5 mL of control water (dechlorinated water) and, immediately afterwards, (ii) a 3 minute period following the addition of the test solution. The addition of control water always preceded the test solution.

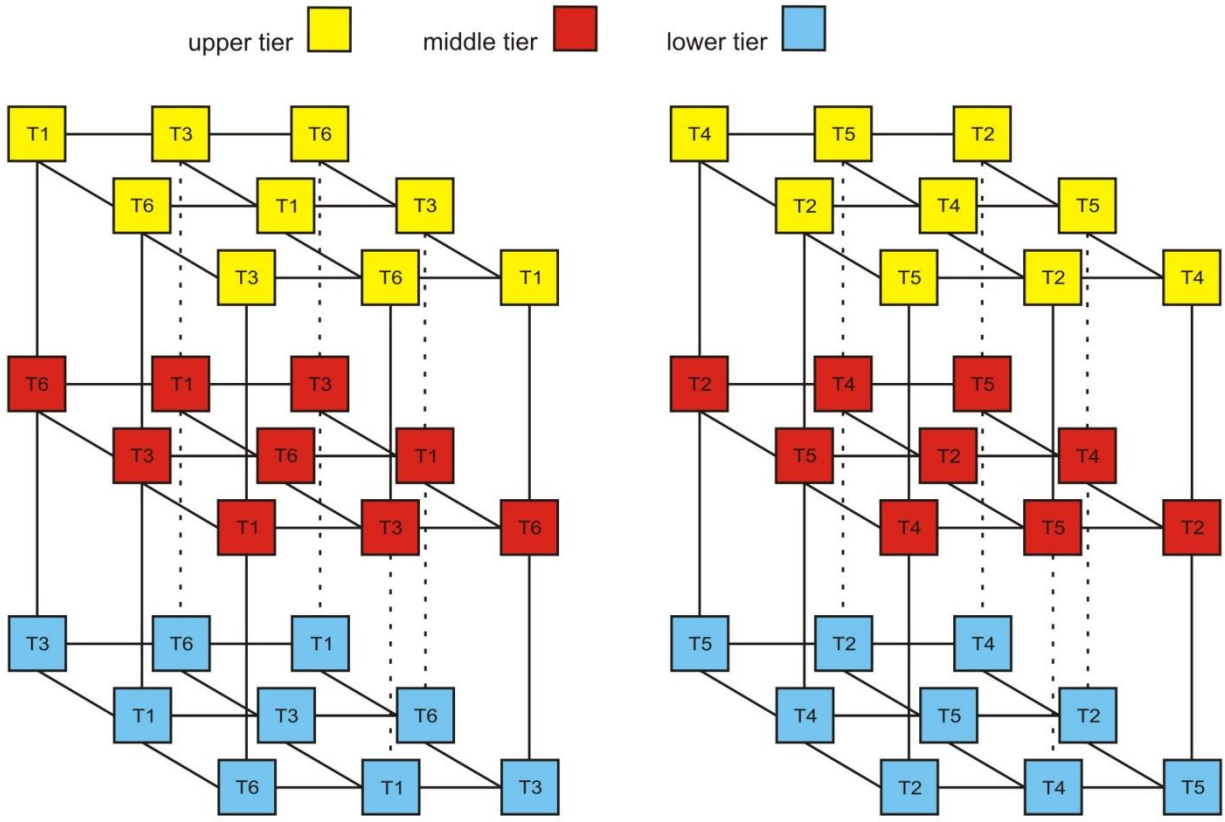

Figure 4.1 Treatment allocation for the food odour detection trial using a Latin Cube design

Test solutions comprised: (T1) 0.1 mL food odour + 2.4 mL water, (T2) 0.15 mL food odour + 2.35 mL water, (T3) 0.3 mL food odour + 2.2 mL water, (T4) 0.625 mL food odour + 1.875 mL water, (T5) 1.25 mL food odour + 1.25 mL water, and (T6) 2.5 mL food odour. Test species were marron and yabbies.

To minimise disturbances to crayfish, control water and test solutions were injected discretely (via a syringe) into the corner of the aquarium farthest from the animal. Each crayfish received one of the six test solutions per day. On the second day of the experiment, the treatment allocation used in latin cube 1 was applied to latin cube 2, and *vice versa*. Therefore each crayfish received two of the six treatments over a two day period, and each treatment was replicated eighteen times. Crayfish behaviours and postures were recorded as described in Section 3.3. After crayfish behavioural observations were completed, crayfish were removed and each aquarium was drained and thoroughly cleaned to eliminate any residual odour that may influence crayfish

behaviour to solutions tested the following day. Aquariums were then refilled using dechlorinated tap water and crayfish were returned and left for 24 h before subsequent testing.

4.2.6 Water quality monitoring

Total ammonia, salinity, pH, nitrite, nitrate, temperature, dissolved oxygen and conductivity were recorded daily for three randomly selected crayfish tanks from each treatment group for both days of the two trials. Water quality parameters remained within normal limits for marron and yabbies (Lawrence and Jones 2002). A summary of these results is provided in Appendix 2; Table 1.

4.2.7 Statistical analysis

Background differences during control periods for marron and yabbies were compared using the Wilcoxon-Mann-Whitney test (Z for large samples, Morgan et al. 2004b). Mean reaction time and the mean percentage of time spent inside shelter, in locomotion, while feeding, cleaning, flicking antennae/antennule and climbing were compared, as recorded for each crayfish during control periods. Comparisons between control and test periods for the reaction time and the percentage of time spent in each behaviour and posture were made using the Wilcoxon signed ranks test (Siegel and Castellan 1988).

The magnitude of change (i.e. the difference in absolute values) in reaction time and percentage of time spent in the different postures and behaviours were calculated for each individual between the control water and test solutions. This calculation was necessary because species displayed background differences in behaviour. The direction of change was either positive or negative if values in the presence of a test solution were higher or lower than those recorded during the control periods respectively. Comparisons among the test solutions within a species were completed using Friedman two-way analysis of variance by ranks (χ^2) and the Sign test to

determine significant differences between test solutions (Morgan et al. 2004b). The responses of marron and yabbies to the same test solution were compared using the Wilcoxon-Mann-Whitney test ($\angle$ for large samples, Morgan et al. 2004b). All numerical data are presented as mean ± standard error unless stated otherwise. Extensive statistical data summary tables are provided in Appendix 3; Tables 1.1, 1.2, 2.1 and 2.2.

4.3 Results

4.3.1 Reaction time

No significant differences were found in the reaction times of marron and yabbies to control water when compared within or between species ($P > 0.05$). Reaction times were not significantly different between species for any test solution ($P > 0.05$). Within species, marron reacted faster to all test solutions except T1, when compared to control water ($P < 0.05$; Figure 4.2; Appendix 3, Table 1.1). Among the test solutions, marron did not react faster to any one solution, with the exception of T5 (45.83 ± 13.64 s), which was faster than T1 (93.89 ± 15.87 s, $P < 0.05$).

Yabbies reacted faster to all of the test solutions than control water ($P < 0.05$), and reacted faster to the strongest cue (T6) than to any other test solution ($P < 0.05$; Figure 4.2).

4.3.2 Interspecific differences in response to control water

Marron were more active than yabbies, spending more time moving, displaying raised and intermediate postures, exhibiting feeding behaviour, and flicking their antennae and antennules than yabbies ($P < 0.01$, $n = 108$, except for cleaning and antennule flicking, $P < 0.05$; Table 4.2; Figure 4.3). Conversely, yabbies were dormant in their behaviour, spending more time inside the shelter in lowered posture ($P < 0.01$, $n = 108$).

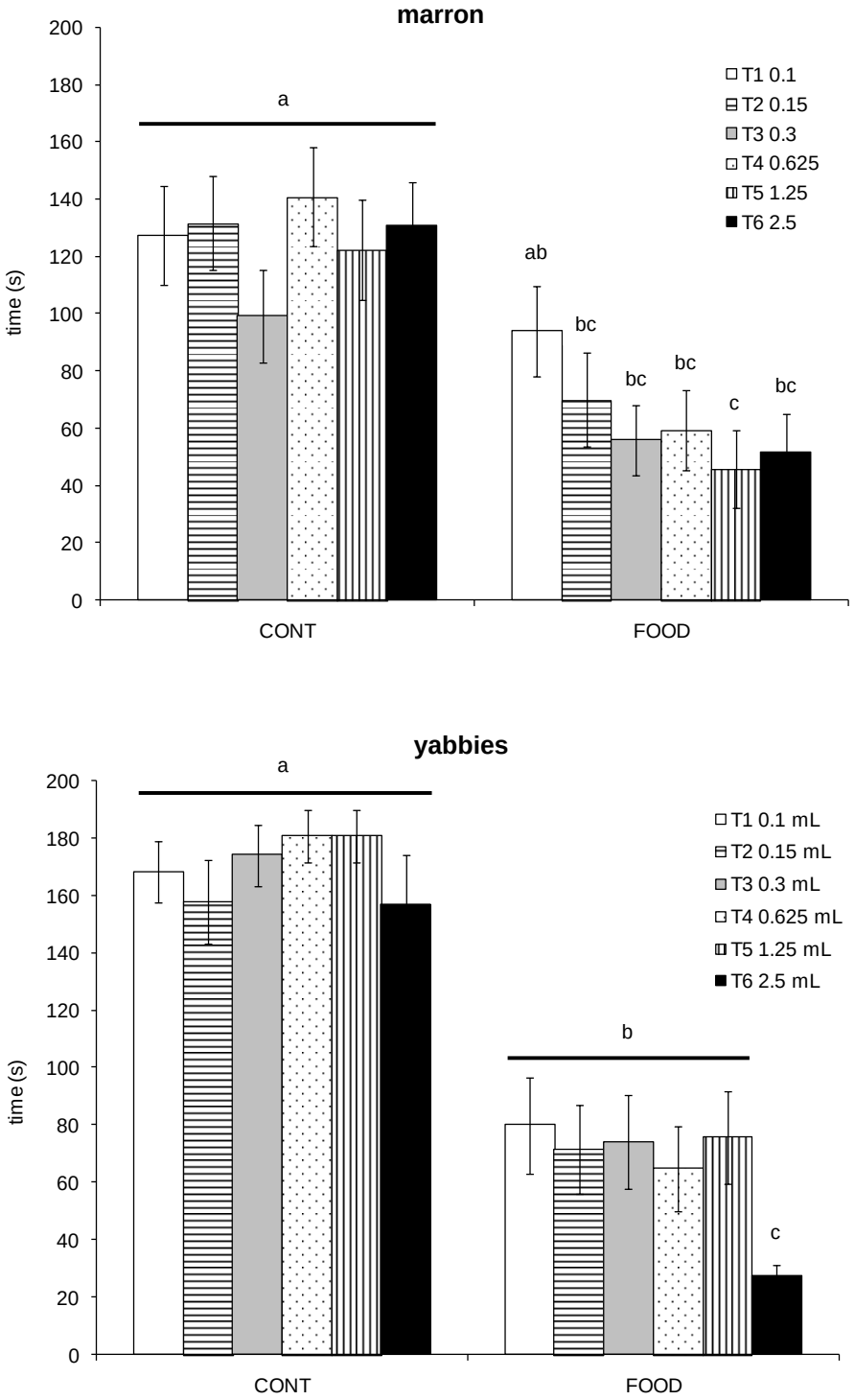

Figure 4.2 Marron and yabby reaction times to control water and food odour

Test solutions comprised: (T1) 0.1 mL food odour + 2.4 mL water, (T2) 0.15 mL food odour + 2.35 mL water, (T3) 0.3 mL food odour + 2.2 mL water, (T4) 0.625 mL food odour + 1.875 mL water, (T5) 1.25 mL food odour + 1.25 mL water, and (T6) 2.5 mL food odour. Statistical tests for significance were made using the Wilcoxon signed ranks test. Different letters denote significant differences at $P < 0.05$. Error bars are means ± standard error.

Table 4.2 Marron and yabby reaction time (s), behaviours and postures (% time) during the control period

	marron	yabby	*Z*	P - value
reaction time	125.42 (16.69)	169.81 (11.98)	-1.354	0.176
in shelter	1.35 (1.02)	67.17 (4.48)	-10.357	0.000**
locomotion	8.26 (1.43)	3.42 (1.29)	-3.750	0.000**
raised posture	7.98 (2.30)	1.64 (1.12)	-3.570	0.000**
intermediate posture	38.18 (4.34)	14.60 (3.19)	-4.018	0.000**
lowered posture	52.92 (4.60)	83.76 (3.38)	-4.816	0.000**
feeding	8.40 (1.46)	1.07 (0.45)	-5.021	0.000**
cleaning	3.35 (1.10)	1.00 (0.56)	-2.531	0.011*
antennae flicking	10.75 (1.94)	2.14 (0.83)	-4.830	0.000**
antennule flicking	23.58 (2.48)	15.31 (1.93)	-2.501	0.012*

Values are means ± standard error. Statistical tests for significance were made using the Wilcoxon-Mann-Whitney test. *significantly different at P < 0.05, **significantly different at P < 0.01

4.3.3 Differences between control water and test solutions

4.3.3.1 Marron

Marron increased feeding behaviour in the presence of each of the six test solutions (P < 0.01). Antennae flicking increased in the presence of T3 and T6 (P < 0.01). Antennule flicking increased in the presence of T5 and T6 (P < 0.05).

4.3.3.2 Yabbies

Yabbies increased feeding behaviour in the presence of each test solution (P < 0.01), along with the amount of time spent moving (P < 0.05, except T6; P > 0.05). Time spent flicking antennae and antennules increased in the presence of each test solution (P < 0.05) along with time spent displaying intermediate posture however, this was significant only for T1, T4, T5 and T6 (P < 0.01). Yabbies spent more time displaying lowered posture during the control period than in the presence of any of the six test solutions (P < 0.05, except T3; P > 0.05). Time spent in the shelter decreased in the presence of T1 and T2 (P < 0.05).

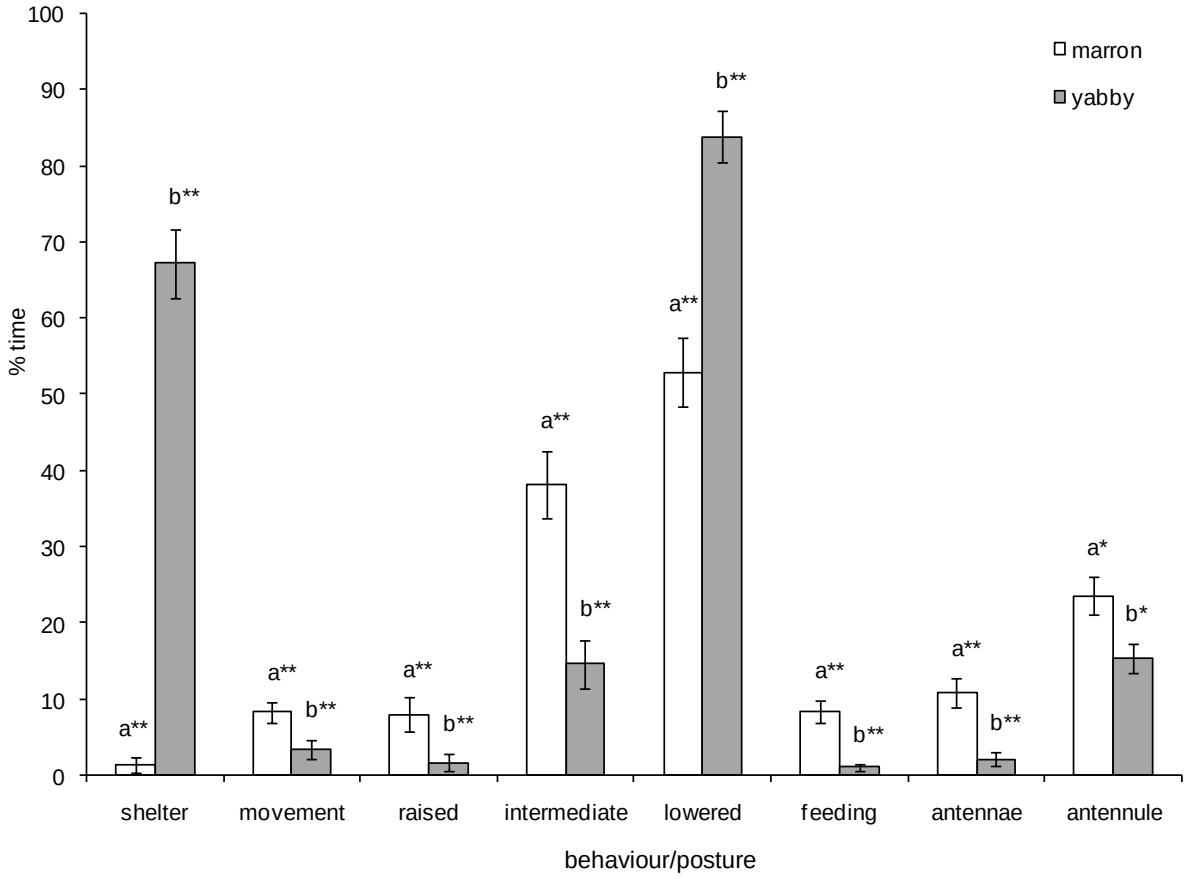

Figure 4.3 Differences between marron and yabbies in response to control water

Comparison between species for the same behaviour/posture used Wilcoxon-Mann-Whitney test (*Z*, n = 108). Values for the same behaviour/posture with different letters are significantly different at *P < 0.05, **P < 0.01. Error bars are means ± standard error.

4.3.4 Intraspecific comparison among test solutions

Marron and yabbies responded to the six food solutions with different levels of feeding intensity (marron: χ^2 = 11.453, d.f. = 5, P < 0.05; yabbies: χ^2 = 19.899, d.f. = 5, P < 0.01, Table 4.3). Yabbies displayed the strongest feeding response to the most concentrated food solution (P < 0.05; Table 4.4). Marron failed to demonstrate any clear differences in feeding behaviour between the most diluted and most concentrated cues. Excluding feeding behaviour, no other differences in behaviour or posture were found for marron and yabbies to the six food solutions (P > 0.05).

Table 4.3 Intraspecific differences in behaviours and postures (% time) among six food solutions

	marron	**yabby**
in shelter	2.176	5.446
locomotion	0.798	2.908
raised posture	4.814	2.000
intermediate posture	3.652	5.490
lowered posture	4.108	5.517
feeding	11.453*	19.899**
cleaning	3.372	7.857
antennae flicking	3.759	8.071
antennule flicking	3.440	5.025

Values are Friedman test statistic (χ^2 , d.f. = 5). Test solutions comprised: (T1) 0.1 mL food odour + 2.4 mL water, (T2) 0.15 mL food odour + 2.35 mL water, (T3) 0.3 mL food odour + 2.2 mL water, (T4) 0.625 mL food odour + 1.875 mL water, (T5) 1.25 mL food odour + 1.25 mL water, and (T6) 2.5 mL food odour. *P < 0.05, **P < 0.01.

Table 4.4 Intraspecific comparison of marron and yabby feeding responses (% time) to six food solutions

	T1 (0.1mL)	**T2 (0.15mL)**	**T3 (0.3mL)**	**T4 (0.625mL)**	**T5 (1.25mL)**	**T6 (2.5mL)**
marron	30.77 ± 7.79^{a}	39.74 ± 7.99^{a}	38.03 ± 7.05^{ab}	35.04 ± 7.26^{a}	44.02 ± 8.34^{a}	60.26 ± 7.55^{ac}
yabby	34.62 ± 8.07^{a}	44.87 ± 7.49^{a}	26.50 ± 6.53^{ab}	50.00 ± 8.09^{ac}	35.90 ± 8.16^{a}	66.24 ± 7.12^{d}

Values are treatment means (% time) ± standard error. Test solutions comprised: (T1) 0.1 mL food odour + 2.4 mL water, (T2) 0.15 mL food odour + 2.35 mL water, (T3) 0.3 mL food odour + 2.2 mL water, (T4) 0.625 mL food odour + 1.875 mL water, (T5) 1.25 mL food odour + 1.25 mL water, and (T6) 2.5 mL food odour. Values in any one row not followed by the same superscript are significantly different at P < 0.05. Statistical tests for significance among test solutions within species used Friedman two-way analysis of variance by ranks (χ^2) and the Sign test for post-hoc analysis.

4.3.5 Interspecific comparisons

Marron displayed raised posture more often than yabbies in the presence of T5 (P < 0.05) and spent a longer duration in intermediate posture and flicking their antennae in the presence of T3 (P < 0.05). Yabbies spent more time inside the shelter than marron in the presence of T2, T4 and T6 (P < 0.05).

4.4 Discussion

4.4.1 Background differences between marron and yabbies

Through-out this trial, marron and yabbies displayed significant differences in background behaviour (behaviour in the presence of control water), supporting similar results obtained for these species in other studies (Gherardi et al. 2002a; Height and Whisson 2006). Marron were more active than yabbies, spending more time in locomotion and feeding, and less time in lowered posture. Conversely, yabbies were dormant in their behaviour, spending more time inside the shelter in lowered posture. In a natural habitat, this behaviour by marron would increase the chance of locating food resources; however, they would be left more exposed to predators. From an evolutionary perspective, marron have been the dominant invertebrate in their natural environment and have prevailed in habitats that are relatively predator-free (Morrissy 1997; Morgan et al. 1998; Allen et al. 2002). Conversely, yabbies have evolved in a predator-rich environment and can burrow to escape drought, becoming inactive for long periods of time (Morrissy and Cassells 1992).

Evolving in the absence of predators may have led marron to be less cautious than yabbies, which, in a laboratory situation at least, results in a greater proportion of time utilising shelter. The dormant behaviour of yabbies observed in this trial may be an extension of their inactivity in burrows in the wild, presumably to conserve energy over extended periods of time. However, in a natural habitat, shelter is typically a limited resource (Bovbjerg 1970) and it is likely that shelter use would be influenced by other factors including competition from conspecific crayfish, crayfish size, habitat type and complexity, presence of macrophytes, and water depth and quality (Vorburger and Ribi 1999; Nakata and Goshima 2003; Height et al. 2006).

4.4.2 Crayfish responses to food odour

Interestingly, both species of crayfish exhibited a behavioural response to all concentrations of food odour tested. In a pre-trial test to determine final odour concentrations for the present trial, responses indicated that 0.1 mL of food odour may be approaching the detection limit for both species, or the critical volume of stimulus required to elicit a feeding response in marron and yabbies (Height, unpublished). Further testing of lower concentrations of food odour is required to accurately determine the detection limits of marron and yabbies. McMahon (2002) reported that some crayfish chemoreceptors can respond to only a few molecules of stimulant; however, when presented with formulated feeds, crayfish can display slow and intermittent feeding responses (Volpe et al. 2008). This is problematic, particularly in a culture situation where the ration may be left untouched for several hours, resulting in valuable nutrient loss through leaching (Marchetti et al. 1999). It is possible that in a laboratory environment, such as that used in the current study, crayfish are more sensitive to chemical stimuli than they would be in nature. For example, in the laboratory extraneous sources of variation were eliminated to focus on the affect of cue concentration on crayfish behavioural responses, these sources include other chemical stimuli, and the presence of conspecifics and predators. In a natural habitat it is most likely that these factors would significantly influence crayfish behaviour.

Other researchers have investigated behavioural responses of crayfish to various concentrations of food stimuli (eg. Kreider and Watts 1998; Corotto et al. 2007; Volpe et al. 2008); however, comparisons with this study are impractical due to differences in recording techniques and the methodology employed. For example, Kreider and Watts (1998) found that the feeding responses of *Procambarus clarkii* to soybean meal solution decreased as the cue was diluted. However, that study used a series of ordinally ranked behaviours to determine the crayfish response level to food.

Yabbies displayed stronger, clearer behavioural modifications to the test solutions. Marron did not react faster to the lowest concentration of food odour (0.1 mL) when compared to control water. In contrast, yabbies reacted faster to all of the test solutions than to control water. Further, yabbies reacted faster to the highest odour concentration (2.5 mL) than to any other test solution. These results indicate that yabbies utilised the food cue more efficiently (e.g. faster reaction times to all test solutions than to control water) and appropriately (e.g. fastest reaction time to the strongest cue). Results from the dye test (Appendix 1) indicated that it took 2.5 mL of solution 30 ± 0.71 s to mix uniformly throughout the aquarium, and test solutions less than 0.625 mL a significantly longer period of time. In light of this, the reaction time of yabbies to 2.5 mL of food odour was immediate (27.5 ± 3.69 s). By comparison, the response by marron was delayed (51.67 ± 13.24 s).

Stronger, clearer behavioural modifications to chemical stimuli by invasive crayfish (when compared to native crayfish), have been observed by other researchers (Gherardi et al. 2002a; Acquistapace et al. 2004; Height and Whisson 2006). However, none of those studies examined the effect of cue concentration on behavioural responses of crayfish. In the present study, in addition to increased feeding behaviour, yabbies also increased movement, antennae and antennule flicking and decreased time spent in lowered posture for each test solution. These differences can be attributed to the background behavioural differences between marron and yabbies during the control periods. Yabbies were less active than marron and spent more time inside the shelter in lowered posture. However, once yabbies detected the presence of a feeding stimulant, they became aroused and commenced searching/feeding behaviours. Although the duration of time spent executing feeding behaviours was similar to that of marron, the increase in feeding behaviour was more noticeable due to their prior inactivity/dormancy; i.e., magnitude of change values for yabbies were greater than for marron. Yabbies also displayed a more intense feeding response to the most concentrated food solution than to any other solution, supporting previous documentation of appropriate use of chemical information by this species

(Gherardi et al. 2002a; Height and Whisson 2006) and other invasive crayfish species including *Procambarus clarkii*, *Orconectes limosus* and *Orconectes rusticus* (Hazlett 2003; Hazlett et al. 2003). In comparison, marron failed to demonstrate any clear differences in feeding behaviour between the most diluted and most concentrated cues.

4.4.3 Concluding remarks and recommendations

Results of this study provide information on behavioural modifications of marron and yabbies to different concentrations of food odour in a laboratory situation. Both species responded to all of the test solutions as feeding stimulants, therefore a future recommendation is for further testing to determine the lower detection limits for food odour by marron and yabbies. Another recommendation is to investigate the influence of hydrodynamics on chemical ecology for these species. The influence of hydrodynamics on detection of chemical stimuli by decapod crustaceans has been investigated by other researchers (eg. Weissburg and Zimmer-Faust 1994; Keller et al. 2001; Hazlett et al. 2006), but was outside the scope of this study. Different flow conditions can influence chemical ecology in crayfish (Moore and Grills 1999; Hazlett et al. 2006; Pecor and Hazlett 2006b), and because marron and yabbies are found in both lentic and lotic habitats, it is recommended that their behavioural responses to chemical stimuli are tested under both conditions. Under the present conditions, yabbies utilised the cues more efficiently and appropriately, as has been found in other invasive crayfish species in response to chemical stimuli. These results indicate that within the native-invasive crayfish dichotomy, yabbies possess behavioural attributes that are not shared by marron, but are present in other invasive crayfish species.

Chapter five

Influence of body size on behavioural responses of crayfish to predator odour

---

This chapter compares the behavioural responses of three sizes of marron and yabbies to odour from a finfish predator, silver perch. It follows an earlier trial conducted by Height and Whisson (2006) which examined the responses of marron and yabbies to odour from cobbler and redfin perch. This trial was conducted to determine if crayfish responses to predator odour vary with their size and life-stage.

5.0 Influence of body-size on behavioural responses of crayfish to predator odour

5.1 Introduction

Over the last decade many studies have attempted to investigate responses of indigenous and non-indigenous crayfish to chemical stimuli in an effort to better understand the factors contributing to invasion success (Hazlett and Schoolmaster 1998; Bouwma and Hazlett 2000; Acquistapace et al 2005; Hazlett et al. 2006). Researchers have found that invasive crayfish species make more appropriate use of a wider range of information from their environment, and display greater behavioural plasticity than native species they are displacing (Hazlett 2000; Hazlett et al. 2002).

Following their introduction in 1932 the distribution of yabbies (*Cherax albidus*) has progressively increased throughout Western Australia (WA) with the species now known to compete with native marron (*Cherax tenuimanus*) (Morrissy and Cassells 1992; Whisson 2003; Beatty 2006). Notwithstanding this, little is known about interactions between marron and yabbies. Given the detrimental impacts of invasive freshwater crayfish on native species in other parts of the world (Gherardi and Holdich 1999) there is an urgent need to gain further information regarding interactions between marron and yabbies. One poorly understood aspect is the behavioural plasticity of these species, which is highly relevant considering the range of exotic finfish predators that are established, and those that are being considered, for introduction to ecosystems in the south-west of WA.

Silver perch (*Bidyanus bidyanus*) are native to the eastern states of Australia (Lake 1971; Rowland and Kearney 1992) and have been stocked into farm dams in Western Australia since the 1950s (Lawrence 1995). Recently, this species was discovered in the Swan coastal plain, presumably due to escape from an aquaculture facility

(Morgan et al. 2004a). Silver perch are known to consume freshwater crayfish (Whisson 1997, 2000; Storer 2005), and thus represent a threat to native marron.

Previous studies have found that some crayfish species are able to detect and respond to chemical cues from predators (Appelberg et al. 1993; Blake and Hart 1993a; Shave et al. 1994). However, this ability appears to vary significantly between species and also depend on the characteristics of the predator (Appelberg et al. 1993; Shave et al. 1994). Crayfish size may also influence detection capabilities; for example, Hazlett and Schoolmaster (1998) reported different behavioural modifications to predator odour in juvenile and adult *Orconectes virilis.*

The aim of this trial was to investigate if crayfish size influences the behavioural responses of marron and yabbies to odour from a fish predator. Height and Whisson (2006) found that marron did not respond to odour from native cobbler (*Tandanus bostocki*) or exotic redfin perch (*Perca fluviatilis*), but yabbies displayed responses to both predator odours. The present study examines the behavioural responses of three size grades of marron and yabbies to silver perch odour to determine if crayfish responses vary with life-stage.

5.2 Materials and methods

5.2.1 Site and experiment system

This trial was conducted using the glass aquaria at the Curtin Aquatic Research Laboratory (CARL) (described in Section 3.1.1), located at Bentley, Western Australia. Lighting was provided via a 12 h : 12 h, light : dark cycle for the duration of the trial.

5.2.2 Experimental animals

5.2.2.1 Crayfish

Marron used in the trial were sourced from a commercial producer at Manjimup (34.23^{o}S, 116.13^{o}E), and yabbies from a commercial supplier at Kukerin (33.18^{o}S, 118.08^{o}E), Western Australia. Size and weight data for the three size grades (small, medium and large; n = 18 for each grade, male : female ratio = 1 : 1) of both species is provided in Table 5.1. Crayfish were kept in 'species only' tanks at CARL in the crayfish holding systems described in Section 3.2.1 for one month prior to the trial and fed commercial crayfish pellets (Glen Forrest Stockfeeders™ Pty Ltd, Appendix 5). Crayfish were not fed during the trial, or the 24 h acclimation period preceding the trial.

5.2.2.2 Silver perch

Silver perch were sourced from a commercial hatchery at Parkerville, Western Australia (31.87^{o}S, 116.14^{o}E), and stocked into a recirculating system described in Section 3.2.2 three months prior to commencement of the trial. Perch were fed commercial silver perch pellets (Glen Forrest Stockfeeders™ Pty Ltd, Appendix 5) daily. Twenty four hours prior to the trial 19 perch weighing 148.64 ± 11.13 g (mean ± standard error) each were moved into a 300 L tank in the recirculating system described in Section 3.2.1.1. The tank was aerated and covered with black plastic. Perch were not fed for the duration of the trial to prevent water fouling.

Table 5.1 Size and weight data (mean ± standard error) for small, medium and large marron and yabbies used in the predator odour trial

		carapace length (mm)	weight (g)
small	*marron*	25.61 ± 0.42	5.22 ± 0.26
	yabby	14.14 ± 0.37	4.46 ± 0.33
medium	*marron*	54.90 ± 0.84	46.13 ± 1.92
	yabby	23.22 ± 0.50	14.02 ± 0.82
large	*marron*	86.65 ± 0.81	183.42 ± 4.07
	yabby	39.37 ± 0.79	58.04 ± 2.75

Each size grade comprised 18 crayfish (9 males and 9 females). No significant differences were found for carapace length or weight between males and females for each grade within species.

5.2.3 Preparation of test solutions

Two test solutions were used for the trials: food odour (FOOD), and perch odour (PRED), described in Table 5.2. Dechlorinated tap water was used as the control (CONT).

Table 5.2 Treatment description for predator odour trial

Treatment	Description
Control (CONT)	20 mL water
Food odour (FOOD)	10 mL FOOD + 10 mL water
Predator odour (PRED)	10 mL PRED + 10 mL FOOD

Each treatment was replicated 18 times.

Food solution was always added simultaneously with predator odour because crayfish detection of predation risk cues is usually more discernible when feeding behaviour, displayed by crayfish in the presence of food odour alone, is suppressed (Acquistapace et al. 2004). Following suggestions of Hazlett (1994a, 1994b), all test solutions were prepared fresh daily.

5.2.3.1 Food solution

The food solution was prepared as described in Section 4.2.3.

5.2.3.2 Perch odour solution

One hour prior to recording behavioural observations each day, water recirculation to the perch holding tank was shut-off. Water was then siphoned from the tank until 120 L remained, providing an effective density of 2824.17 ± 78.59 g perch/120 L tank water (23.54 g/L). The purpose of this was to increase the fish density whilst retaining acceptable water quality in the absence of filtration. The perch tank was covered with black plastic and provided constant aeration. Perch odour solution was derived from 10 mL of water drawn discretely into a syringe (Terumo® Corporation Pty Ltd) from the perch holding tank. Perch odour was always taken from the holding tank immediately prior to discharge into a crayfish tank. Following the completion of crayfish behavioural observations each day, water recirculation to the perch tank resumed and any faeces were vacuumed from the tank.

5.2.4 Experimental design

Each trial ran for three days including the 24 h acclimation period preceding the trial (described in Section 5.2.5). On day one, 27 marron and 27 yabbies (nine each of small, medium and large for both species) were distributed into aquariums according to two latin cube designs (Federer 1991) (Figure 5.1). The purpose of this was to randomise the effect of the existing variation in light intensity and temperature, between tanks on different tiers, on the experimental results.

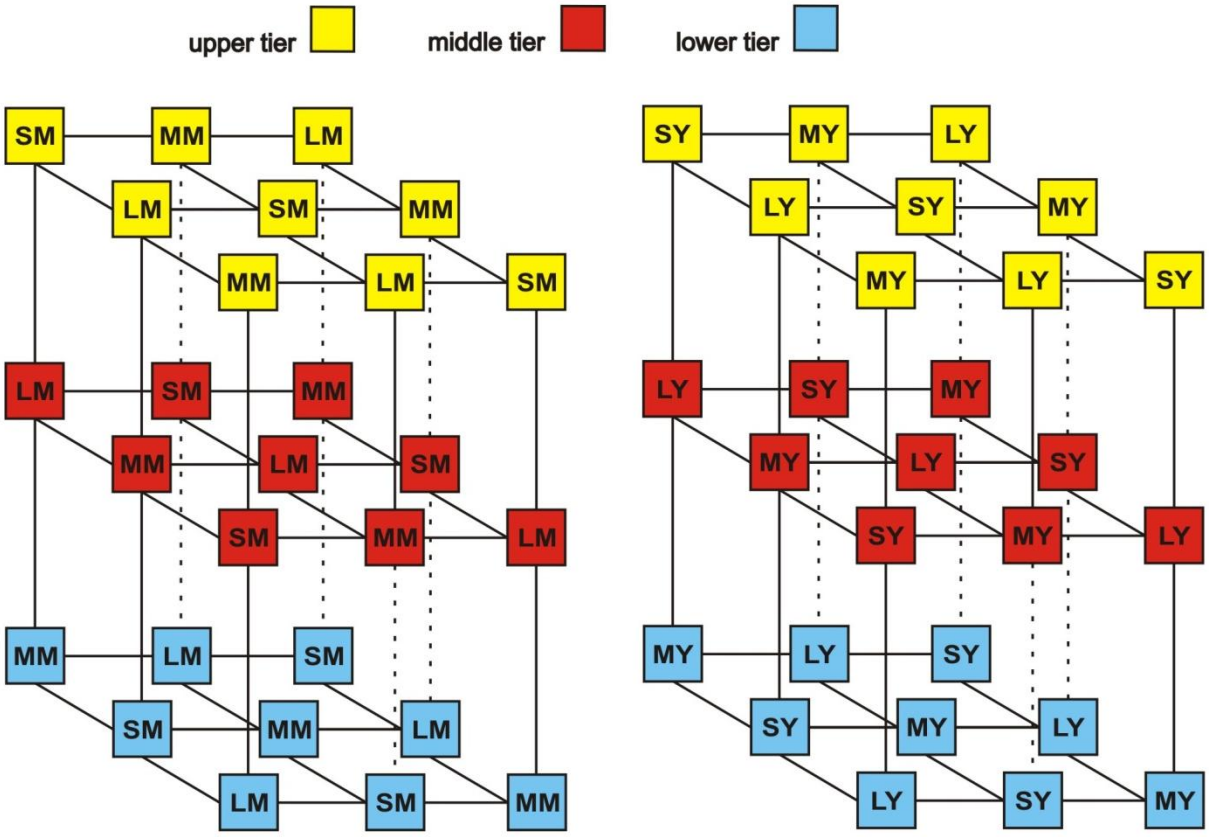

Figure 5.1 Distribution of crayfish in aquariums for the predator odour trial using a Latin Cube design.

Crayfish test groups (n = 18) comprised: (SM) small marron, (MM) medium marron, (LM) large marron, (SY) small yabbies, (MY) medium yabbies, and (LY) large yabbies. Test solutions were: (CONT) 20 mL water, (FOOD) 10 mL water + 10 mL food odour, (PRED) 10 mL food odour + 10 mL perch odour.

5.2.5 Experimental procedure

The experimental procedure was adapted from those used by Hazlett (1994a) and Gherardi et al. (2002a). A single crayfish was placed into each of the 54 aquaria (60 x 30 cm bottom, described in Section 3.1.1) containing 25 L of dechlorinated tap water and left for 24 h acclimation. Each aquarium was aerated, contained a piece of PVC pipe for shelter (length 20 cm, diameter 7.5 cm) and was visually isolated with a black plastic sheet to minimise disturbances to crayfish. After 24 h acclimation, crayfish were observed for two 3 minute time periods during which observations were recorded every 15 seconds: (i) a 3 minute control period following the addition of 20 mL of

control water (dechlorinated water) and, immediately afterwards, (ii) a 3 minute period following the addition of the test solution (20 mL). Each crayfish randomly received one of the two test solutions (FOOD or PRED) per day so that after two consecutive days, each individual had received both test solutions. The addition of control water always preceded the test solution. To minimise disturbances to crayfish, control water and test solutions were squirted discretely via a syringe into the corner of the aquarium farthest from the animal. Crayfish behaviours and postures were recorded as described in Section 3.3. After crayfish behavioural observations were completed, crayfish were removed and each aquarium was drained and thoroughly cleaned to eliminate any residual odour that may influence crayfish behaviour to solutions tested the following day. Aquariums were then refilled using dechlorinated tap water and crayfish were returned and left for 24 h before subsequent testing. On day three after each individual had received both test solutions, crayfish were returned to holding tanks (See section 3.2.1). One week later, fresh individuals (27 marron and 27 yabbies, nine each of small, medium and large) were allocated to aquariums as described in Section 5.2.4, and the experiment was repeated. Therefore, at the completion of both trials, behavioural observations had been recorded for 54 marron and 54 yabbies. Perch were fed during the one week rest period between trials.

5.2.6 Water quality monitoring

Total ammonia, salinity, pH, nitrite, nitrate, temperature, dissolved oxygen and conductivity were recorded daily for crayfish tanks and the perch tank during both trials. Water quality parameters remained within normal limits for marron and yabbies (Lawrence and Jones 2002); and silver perch (Rowland 1995). A summary of these results is provided in Appendix 2; Table 2.

5.2.7 Statistical analysis

Background differences during control periods for marron and yabbies were compared using the Wilcoxon-Mann-Whitney test (Z for large samples). Mean reaction time and the mean percentage of time spent inside shelter, in locomotion, while feeding, and flicking antennae and antennule were compared, as recorded for each crayfish during control periods. Comparisons between control and test periods for the reaction time and the percentage of time spent in each behaviour and posture were made using the Wilcoxon signed ranks test (Z).

The magnitude of change (i.e. the difference in absolute values) in reaction time and percentage of time spent in the different postures and behaviours were calculated for each individual between the control water and test solutions. This calculation was necessary because species and size grades displayed background differences in behaviour. The direction of change was either positive or negative if values in the presence of a test solution were higher or lower than those recorded during the control periods respectively. Intraspecific comparisons among the test solutions within a size grade were made using Wilcoxon signed ranks test. Intraspecific comparisons to the same test solution between size grades were made using the Kruskal-Wallis test. The responses of marron and yabbies to the same test solution were compared using the Wilcoxon-Mann-Whitney test (Z for large samples). All numerical data are presented as mean ± standard error unless stated otherwise. Extensive statistical data summary tables are provided in Appendix 3; Tables 3, 4 and 5.

5.3 Results

5.3.1 Intraspecific differences in response to control water

5.3.1.1 Marron

During the two control periods small marron had the fastest reaction time (115.03 ± 12.54 s; Table 5.3; $P < 0.05$). Marron behaviour was similar across the three size grades, but large marron spent the least time utilising the shelter (3.67 ± 2.78% of the 3 minute control period; $P < 0.05$).

Table 5.3 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) during control periods

	small		medium		large	
	marron	**yabby**	**marron**	**yabby**	**marron**	**yabby**
reaction time	115.03 ± 12.54^{a}	156.67 ± 11.87^{bc}	145.71 ± 10.36^{b}	132.50 ± 15.28^{ab}	122.47 ± 11.23^{ab}	168.33 ± 7.22^{c}
in shelter	33.97 ± 7.74^{a}	69.44 ± 7.59^{bc}	33.55 ± 7.95^{a}	50.21 ± 7.84^{ab}	3.67 ± 2.78^{d}	75.00 ± 7.32^{c}
locomotion	14.74 ± 4.71^{a}	10.26 ± 4.08^{a}	6.84 ± 2.47^{a}	19.02 ± 4.67^{a}	8.33 ± 2.57^{a}	1.71 ± 1.07^{b}
raised posture	21.79 ± 6.58^{a}	0.00 ± 0.00^{b}	20.09 ± 6.46^{a}	3.21 ± 1.97^{bc}	13.68 ± 5.48^{ac}	0 ± 0^{b}
intermediate posture	33.76 ± 7.65^{ab}	17.95 ± 5.79^{bc}	32.69 ± 7.52^{ab}	50.43 ± 7.78^{a}	51.28 ± 7.93^{a}	5.56 ± 3.32^{c}
lowered posture	44.44 ± 8.40^{a}	79.27 ± 6.20^{b}	47.22 ± 8.27^{a}	46.37 ± 7.95^{a}	35.04 ± 7.76^{a}	91.67 ± 4.23^{b}
feeding	4.27 ± 1.52^{a}	1.28 ± 0.94^{b}	1.71 ± 0.76^{ab}	0.85 ± 0.51^{bc}	3.21 ± 1.16^{ac}	1.28 ± 1.28^{b}
antennae flicking	14.96 ± 4.61^{ab}	7.69 ± 3.29^{ac}	6.41 ± 2.18^{ac}	19.23 ± 4.38^{b}	9.19 ± 2.16^{b}	2.99 ± 1.54^{c}
antennule flicking	32.91 ± 4.47^{a}	22.86 ± 3.65^{b}	32.26 ± 3.81^{ab}	34.40 ± 5.23^{ab}	34.83 ± 3.10^{a}	25.00 ± 3.77^{b}

Values in any one row not followed by the superscript are significantly difference at $P < 0.05$. Differences in control periods compared by Wilcoxon-Mann-Whitney test (n = 36).

5.3.1.2 Yabbies

Large yabbies were distinctly the least active, spending the most time inside the shelter (75 ± 7.32%) in lowered posture (91.67 ± 4.23%) and the least time in locomotion (1.71 ± 1.07%; $P < 0.05$; Table 5.3).

5.3.2 Interspecific differences in response to control water

Yabbies were less active than marron, spending more time inside the shelter and in lowered posture. Large yabbies spent less time moving than any other group (1.71 ± 1.07%; $P < 0.05$; Table 5.3).

5.3.3 Differences between control water and test solutions

5.3.3.1 Reaction time

Small, medium and large marron and yabbies reacted faster to each of the test solutions compared to control water ($P < 0.01$).

5.3.3.2 Marron

Small marron increased feeding behaviour in the presence of food odour and predator odour ($P < 0.01$), and locomotion, antennae and antennule flicking in the presence of predator odour only ($P < 0.05$). Although small marron spent more time in locomotion in the presence of food odour (23.08 ± 6.55%) than for predator odour (17.09 ± 5.55%), due to the large proportion of time spent moving in the control period (25.21 ± 8.32%), the magnitude of change between control water and food odour was not significant ($P > 0.05$).

Medium and large marron displayed similar behavioural modifications to the test solutions, increasing feeding behaviour, locomotion, antennae and antennule flicking after exposure to food odour and predator odour ($P < 0.05$).

5.3.3.3 Yabbies

Small, medium and large yabbies responded similarly to food odour and predator odour, increasing feeding behaviour, locomotion, antennae and antennule flicking and reducing time spent in shelter and lowered posture ($P < 0.05$).

5.3.4 Intraspecific comparison among test solutions

5.3.4.1 Reaction time

No significant differences were found for reaction times between food odour and predator odour for any size class of marron and yabbies ($P > 0.05$).

5.3.4.2 Marron

No significant differences were found in the behavioural responses of small and large marron to food odour and predator odour (Table 5.4). Medium marron displayed greater increases in locomotion, intermediate posture, antennae and antennule flicking in the presence of food odour compared to predator odour ($P < 0.05$).

5.3.4.3 Yabbies

Small, medium and large yabbies displayed similar behavioural modifications to food odour and predator odour (Table 5.5). The only significant difference found in responses to the two solutions was for time displaying raised posture in the presence of food odour by medium yabbies ($P < 0.05$; Table 5.5).

Table 5.4 Differences in the magnitude of change between control water and test solutions for marron reaction times (s), behaviours and postures (% time)

	small	**medium**	**large**
reaction time	0.821	0.779	0.903
in shelter	0.944	1.483	1.000
locomotion	0.701	2.235* FOOD > PRED	0.901
raised posture	0.704	0.170	0.000
intermediate posture	1.496	2.563* FOOD > PRED	1.037
lowered posture	1.473	1.730	1.297
feeding	0.911	1.495	0.153
antennae flicking	0.736	2.966** FOOD > PRED	0.854
antennule flicking	0.805	2.507* FOOD > PRED	1.925

Values are the Wilcoxon signed ranks test statistic (Z). Test solutions ranked in decreasing order of magnitude of change. Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (PRED) 10 mL perch odour + 10 mL food odour. *P < 0.05, **P < 0.01.

Table 5.5 Differences in the magnitude of change between control water and test solutions for yabby reaction times (s), behaviours and postures (% time)

	small	**medium**	**large**
reaction time	1.034	0.486	1.526
in shelter	0.371	0.938	1.669
locomotion	0.692	1.019	1.543
raised posture	1.414	2.150* FOOD > PRED	1.214
intermediate posture	1.825	0.567	1.019
lowered posture	1.481	0.913	1.962
feeding	0.024	0.393	0.119
antennae flicking	0.342	0.569	1.802
antennule flicking	0.521	0.687	1.639

Values are the Wilcoxon signed ranks test statistic (Z). Test solutions ranked in decreasing order of magnitude of change. Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (PRED) 10 mL perch odour + 10 mL food odour. *P < 0.05, **P < 0.01.

5.3.5 Intraspecific comparison to the same test solution

5.3.5.1 Marron

Large marron displayed a stronger feeding response to food odour and predator odour than small and medium marron ($P < 0.01$; Table 5.6; Figure 5.2) and responded with greater intensity to predator odour than small and medium marron ($P < 0.01$).

Table 5.6 Intraspecific differences for the magnitude of change between control water and test solutions for marron reaction times (s), behaviours and postures (% time)

	FOOD	**PRED**
reaction time	3.514	3.624
in shelter	2.246	1.020
locomotion	2.275	11.531** L > S = M
raised posture	0.700	1.216
intermediate posture	5.548	14.841** L = S > M
lowered posture	7.691* M = L > S	10.642** L > S = M
feeding	19.565** L > S = M	16.586** L > S = M
antennae flicking	3.657	22.037** L > S > M
antennule flicking	2.118	8.954* L > M

Values are the Kruskal-Wallis test statistic (H, d.f. = 2). Post-hoc analysis used Mann-Whitney U test. Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (PRED) 10 mL perch odour + 10 mL food odour. S = small marron; M = medium marron; L = large marron. Size grades ranked in decreasing order of magnitude of change.*P < 0.05, **P < 0.01.

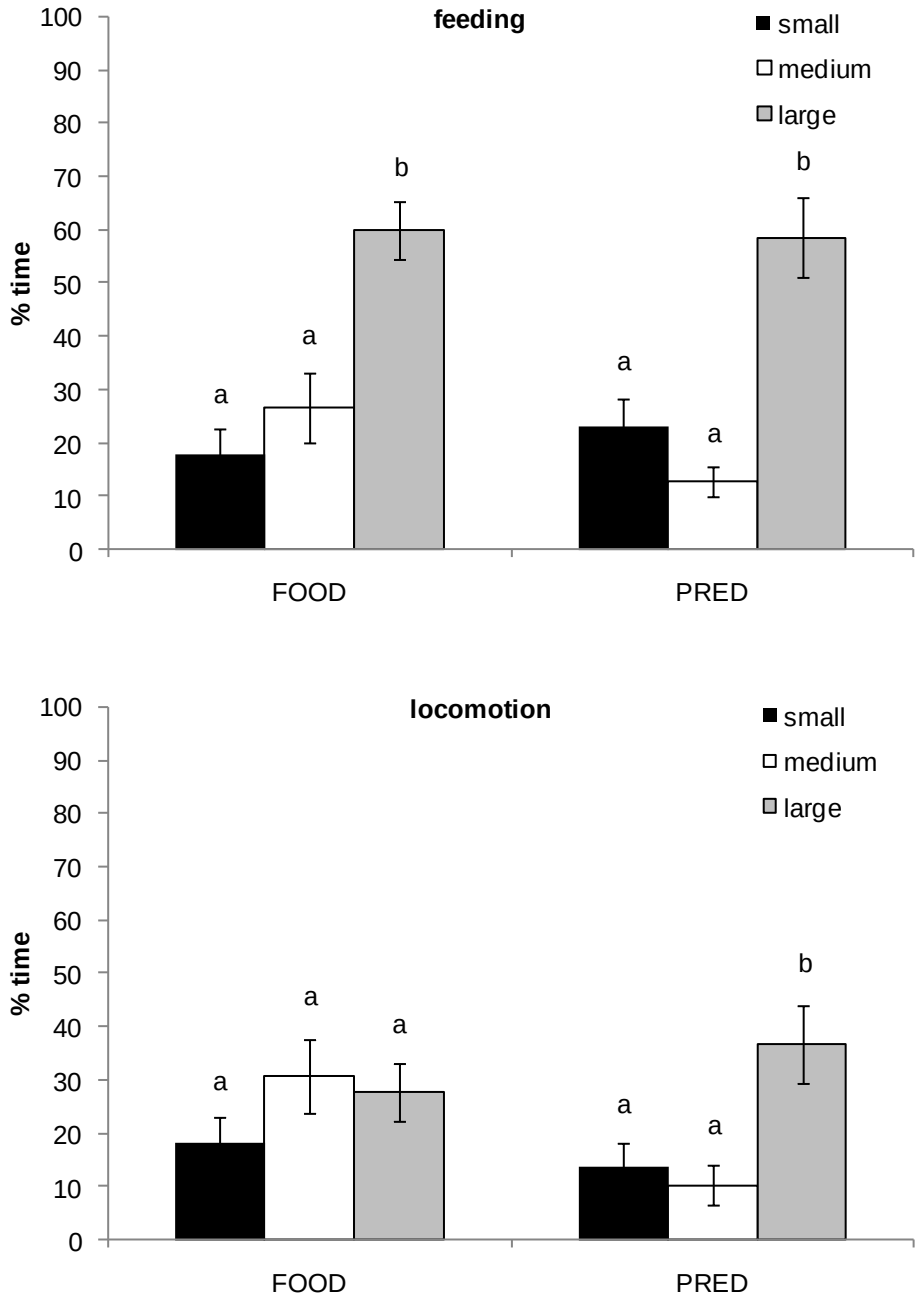

Figure 5.2 Differences in the magnitude of change between control water and test solutions for marron

Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water, and (PRED) 10 mL perch odour + 10 mL water. Comparisons between magnitude of change (the absolute difference between values recorded during control and test periods) used the Kruskal-Wallis test. Different letters for the same test solution denote significant differences between size classes at $P < 0.05$. Error bars are means ± standard error.

5.3.5.2 Yabbies

Small, medium and large yabbies displayed similar behavioural modifications to food odour and predator odour (Table 5.7; Figure 5.3).

Table 5.7 Intraspecific differences for the magnitude of change between control water and test solutions for yabby reaction time (s), behaviours and postures (% time)

	FOOD	PRED
reaction time	3.616	3.602
in shelter	2.402	1.923
locomotion	4.606	2.446
raised posture	13.981** M > L = S	1.851
intermediate posture	0.241	3.557
lowered posture	2.015	2.638
feeding	0.413	0.757
antennae flicking	3.794	0.428
antennule flicking	0.805	1.187

Values are the Kruskal-Wallis test statistic (H, d.f. = 2). Post-hoc analysis used Mann-Whitney U test. Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (PRED) 10 mL perch odour + 10 mL food odour. S = small yabby; M = medium yabby; L = large yabby. Size grades ranked in decreasing order of magnitude of change.*P < 0.05, **P < 0.01.

5.3.6 Interspecific comparisons

Small marron were the most active group during the control period and by comparison, small yabbies were relatively inactive. This difference resulted in greater magnitude of change values for small yabbies for reaction time and most behaviours and postures (Table 5.8).

A similar trend was evident for medium and large marron and yabbies. That is, yabbies were less active than marron during control periods resulting in greater magnitude of change values due to their higher activity in response to the test solutions.

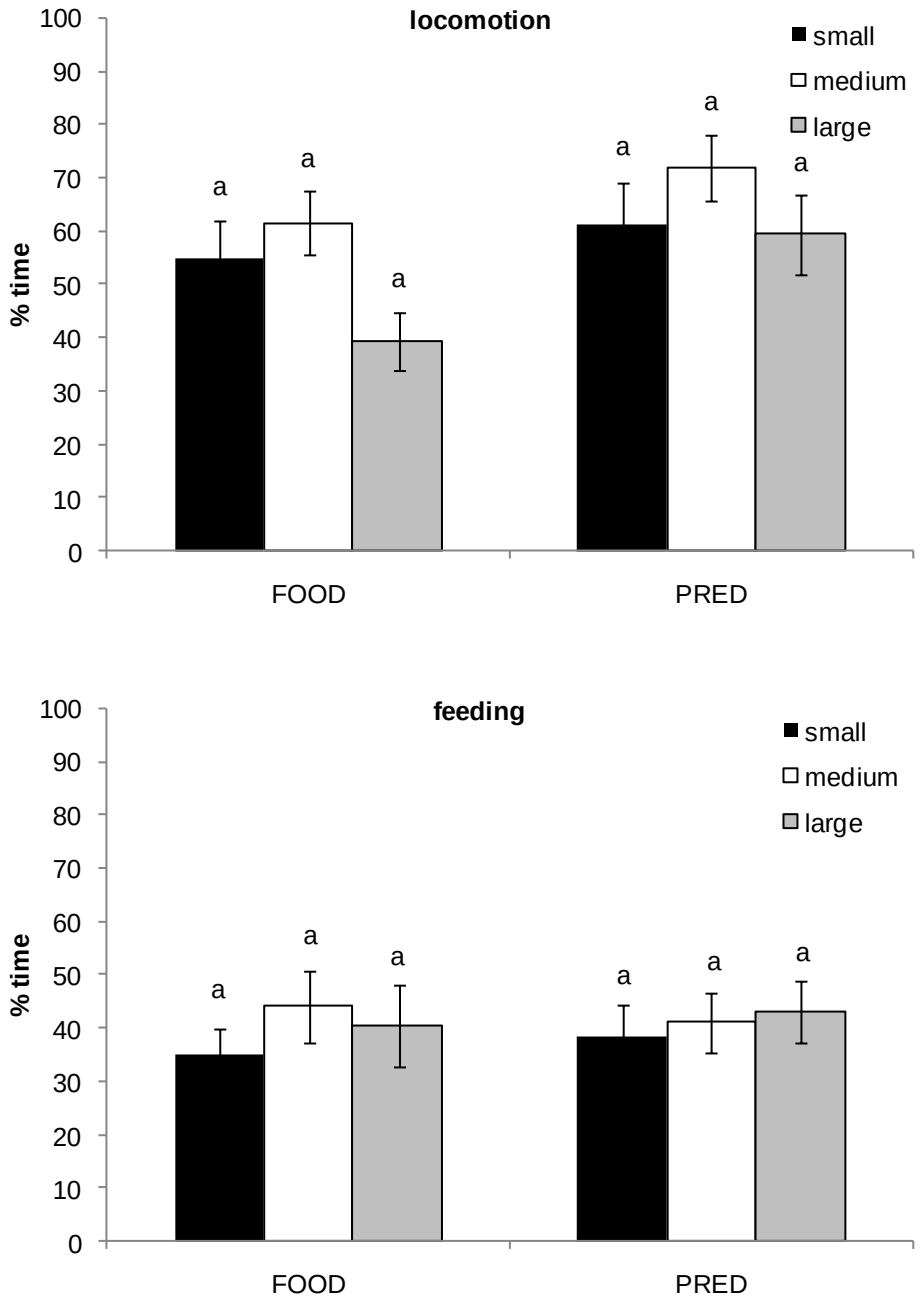

Figure 5.3 Differences in the magnitude of change between control water and test solutions for yabbies

Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water, and (PRED) 10 mL perch odour + 10 mL water. Comparisons between magnitude of change (the absolute difference between values recorded during control and test periods) used the Kruskal-Wallis test. No significant differences existed between size classes for the same test solution, or between test solutions for the same crayfish size grade. Error bars are means ± standard error.

Table 5.8 Interspecific differences between marron and yabbies for the magnitude of change between control water and test solutions for reaction time (s), behaviours and postures (% time)

		FOOD	PRED
	reaction time	2.122** SY > SM	2.231** SY > SM
small	in shelter	1.964	2.949** SY > SM
marron	locomotion	3.484** SY > SM	3.866** SY > SM
	raised posture	2.087* SM > SY	1.466
versus	intermediate posture	2.623** SY > SM	2.246* SY > SM
	lowered posture	3.865** SY > SM	3.441** SY > SM
small	feeding	2.223* SY > SM	2.002* SY > SM
yabbies	antennae flicking	4.083** SY > SM	4.103** SY > SM
	antennule flicking	3.377** SY > SM	4.636** SY > SM
	reaction time	1.019	0.934
medium	in shelter	2.335* MY > MM	3.589** MY > MM
marron	locomotion	2.973** MY > MM	4.737** MY > MM
	raised posture	1.132	0.457
versus	intermediate posture	0.080	3.327** MY > MM
	lowered posture	0.338	3.198** MY > MM
medium	feeding	1.840	3.716** MY > MM
yabbies	antennae flicking	3.006** MY > MM	4.507** MY > MM
	antennule flicking	1.290	3.909** MY > MM
	reaction time	1.274	2.115* LY > LM
large	in shelter	2.106* LY > LM	4.308** LY > LM
marron	locomotion	0.894	2.081* LY > LM
	raised posture	2.060* LM > LY	0.853
versus	intermediate posture	0.784	0.874
	lowered posture	2.472* LY > LM	2.527* LY > LM
large	feeding	1.732	1.909
yabbies	antennae flicking	1.403	1.976* LY > LM
	antennule flicking	3.597** LY > LM	3.276** LY > LM

Values are the Wilcoxon-Mann-Whitney test statistic (Z). Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (PRED) 10 mL perch odour + 10 mL food odour. SM = small marron, SY = small yabby; MM = medium marron, MY = medium yabby; LM = large marron, LY = large yabby. Species ranked in decreasing order of magnitude of change. *P < 0.05, **P < 0.01.

5.4 Discussion

The behavioural responses of marron and yabbies to odour from a potential finfish predator were first investigated by Height and Whisson (2006). Experimental crayfish used in that study had an average carapace length 70 mm (marron) and 52 mm (yabbies), and bodyweight of 90 g (marron) and 45 g (yabbies). The present trial used different size grades of crayfish, but the same experimental design, facilitating comparisons between the two studies.

5.4.1 Responses to perch and food odour

Results of the present trial did not indicate a behavioural response of marron or yabbies to silver perch odour. In fact, all crayfish showed no difference in their behavioural modifications when presented with food or when presented with food plus perch odour. While this was an unexpected outcome for yabbies, the result for marron follows Height and Whisson's (2006) study where the indigenous crayfish did not detect odours from native or exotic fish predators. There are a number of possible explanations for this result.

Firstly, the behavioural response to food odour may have dominated over the response to perch odour. Other studies have reported that when two different sources of stimulus are presented to crayfish, behaviour appropriate to one input can dominate over responses appropriate to the other (Hazlett 1994a, 1999). Hazlett (1999) found that when presented with food and snapping turtle (*Chelydra serpentina*) odour, *O. virilis* displayed responses that more closely resembled those to food alone, than those shown to predator odour when presented alone. Further, Hazlett (1999) reported that alarm odour inhibited feeding behaviour more than predator odour, but simultaneous exposure to alarm and predator odour resulted in a stronger inhibition of feeding behaviour than either alarm or predator odour alone. The feeding response displayed by yabbies to perch odour in the present trial is surprising considering odour from *Tandanus bostocki* and *Perca fluviatilis* inhibited feeding behaviour in Height and

Whisson's (2006) study. This suggests that the responses of yabbies to predator odour are highly variable and further investigation is warranted; particularly, the effect of cue strength on the behavioural response.

Another explanation for the feeding response by marron and yabbies to perch odour is a lack of prior experience with perch. Hazlett and Schoolmaster (1998) found that juvenile *O. virilis* reared in tanks did not respond to predator odour due to inexperience, but experienced adults from the wild did. Yabbies used in the present trial were reared in aquaculture ponds and had not previously encountered silver perch, however, so were the yabbies used by Height and Whisson (2006) which responded to predator odour. Although marron used in this study were reared in aquaculture ponds, marron used by Height and Whisson (2006) were from a natural population and did not respond to predator odour either, suggesting that marron do not display behavioural modifications to predator odour.

As expected, food odour elicited feeding-related behaviours in both marron and yabbies, supporting previous findings (Gherardi et al. 2002a; Height and Whisson 2006). Both species increased feeding behaviour, locomotion, antennae and antennule flicking, providing clear evidence that crayfish detected chemical stimuli. Other authors have reported that upon detection of food odours crayfish increase feeding behaviour and locomotory movements (Hazlett 1994a; Moore and Grills 1999; Keller et al. 2001). The behavioural responses of marron and yabbies to food odour in this study were similar to other studies (Height and Whisson 2006; this thesis, Chapter 4).

5.4.2 Influence of body-size

The influence of body-size on the responses of crayfish to chemical cues has not been widely investigated. In this study, all sizes of yabbies displayed similar responses to food and perch odour. However, large marron showed a greater intensity in the strength of their responses to food and predator odour than small and medium marron.

The reason for this result is unclear, though may be due to the greater life experience of older individuals; larger marron may be more efficient at identifying and responding to food cues than smaller crayfish.

5.4.3 Interspecific differences

Yabbies, which are an invasive species, exhibited a greater response to the test odours than did the native marron. Similar findings have been previously documented for these species (Gherardi et al. 2002a; Height and Whisson 2006; this thesis, Chapter 4) and other pairs of native/invasive crayfish species (Hazlett 2000; Hazlett et al. 2003; Acquistapace et al. 2004). In the present study, this finding may be partly attributed to the differences in activity between marron and yabbies during control periods; yabbies were less active than marron, resulting in greater magnitude of change values between control and test solutions. A more likely explanation, is that yabbies are more receptive to chemical stimuli than marron, a result supported by previous studies (Gherardi et al. 2002a; Height and Whisson 2006; this thesis, Chapter 4).

5.4.4 Concluding remarks and recommendations

Results of the present trial did not indicate a behavioural response of marron or yabbies to silver perch odour. This result was not surprising for marron (e.g. Height and Whisson 2006), but was an unexpected outcome for yabbies, demonstrating the variable nature of crayfish responses to predator odour documented by other authors (Appelberg et al. 1993; Hazlett 1994a; Shave et al. 1994; Hazlett and Schoolmaster 1998; Hazlett et al. 2002). While body-size did not influence behavioural responses of yabbies, large marron responded with greater intensity to test solutions than smaller conspecific crayfish, likely due to greater life experience. However, the feeding responses displayed by yabbies were more intense than those displayed by marron, supporting previous research (Chapter 4).

Further investigation is required to address the contrasting results from this study and Height and Whisson's (2006) previous study where yabbies responded to predator odour. Specifically, marron and yabbies should be examined to determine if they are capable of developing a learned association between a novel odour and predation risk, as has been documented in other crayfish species (Hazlett 1999; Hazlett et al. 2002).

Chapter six

Influence of predator size on crayfish behavioural responses to predator odour

---

The preceding chapter investigated the effect of crayfish size on the behavioural response to predator odour. This chapter follows with an examination of the influence of predator size on crayfish behavioural responses to predator odour. Marron were not used in this trial due to a lack of response to fish odour in previous studies.

6.0 Influence of predator size on crayfish behavioural responses to predator odour

6.1 Introduction

The extent to which the size of a crayfish or predator influence the behaviour of crayfish to chemical cues is not reported in the literature. The previous chapter of this thesis investigated size-dependent responses of crayfish to predator odour. This chapter follows with an examination of the influence of predator size on crayfish behavioural responses to predator cues.

The ability of crayfish to detect and respond to predator odour varies between crayfish species, and according to the characteristics of the predator (Hazlett and Schoolmaster 1998). Some authors have suggested that crayfish may possess an inherent ability to chemically detect co-occurring predators, and can subsequently minimise predation risk, presumably due to shared evolutionary history (Appelberg et al. 1993; Shave et al. 1994). Height and Whisson (2006) investigated the behavioural responses of marron (*Cherax tenuimanus*) and yabbies (*Cherax albidus*) to odour from native (*Tandanus bostocki*) and exotic (*Perca fluviatilis*) predatory fish species present in Western Australia. Marron did not respond to odour from either species as a predation-risk cue. In contrast, both fish odours inhibited feeding behaviour in yabbies, demonstrating the behavioural plasticity of this species. Other studies comparing behaviour of native and invasive crayfish species have supported the general hypothesis that invasive crayfish display higher behavioural plasticity than native species they are displacing (Hazlett 2000; Hazlett et al. 2002, 2003). This assertion appears to apply to marron and yabbies in Western Australia; yabbies respond to a wider range of information about their environment than do marron (Gherardi et al. 2002a; Height and Whisson 2006).

The inability of marron to respond to chemical stimuli from fish predators is an obvious disadvantage considering the presence of exotic fish predators in habitats occupied by marron (Morgan et al 2002, 2004a). Silver perch (*Bidyanus bidyanus*) are native to the eastern states of Australia and have been introduced to Western Australia for aquaculture (Lawrence 1995). The translocation of silver perch into and within Western Australia remains under tight regulation (Thorne and Brayford 2000), yet recently, the species was captured in the Swan River (Morgan et al. 2004a), presumably as a result of escape from an aquaculture facility. Silver perch are known to prey on crayfish (Whisson 2000). Like most fish species, silver perch are gape-limited predators (Stein and Magnuson 1976; DiDonato and Lodge 1993; Elvira et al. 1996), and as fish size increases, so does the size of prey that can be consumed (Whisson 2000).

There is evidence that crayfish size can influence their behavioural response to chemical stimuli (Hazlett and Schoolmaster 1998). However, it is not known if predator size influences the behavioural response of crayfish; i.e. does the chemical nature of fish odour (and the behavioural responses of crayfish) change with the physiology of the animal? The aim of this trial was to determine if the size of a fish predator affects the behavioural response of yabbies to predator odour. Due to a lack of response to fish odour in earlier studies, marron were not used in the present investigation (Storer 2005; Height and Whisson 2006; this thesis, Chapter 5). Conversely, yabbies have previously demonstrated responses to fish odour (Height and Whisson 2006), and greater behavioural plasticity in response to chemical stimuli than native marron (Gherardi et al. 2002a).

6.2 Materials and methods

6.2.1 Site and experiment system

This trial was conducted using the glass aquaria at the Curtin Aquatic Research Laboratories (CARL) (described in Section 3.1.1), located at Bentley, Western Australia. Lighting was provided via a 12 h : 12 h, light : dark cycle for the duration of the trial.

6.2.2 Experimental animals

6.2.2.1 Yabbies

Eighteen yabbies (nine males and nine females, average weight 47.26 ± 2.41 g, average carapace length 45.13 ± 0.91 mm) were sourced from a farm dam near Narrogin (32.97°S, 117.23°E), Western Australia, and transferred to holding tanks at CARL (described in Section 3.2.1.2) one week prior to the trial. Crayfish were fed commercial crayfish pellets (Glen Forrest Stockfeeders™ Pty Ltd, Appendix 5) prior to commencement of the trial, but were not fed during the trial or the preceding 24 h acclimation period.

6.2.2.2 Silver perch

Thirty silver perch from three size grades: small (151.93 ± 1.75 g, n = 10), medium (202.51 ± 1.24 g, n = 10) and large (254.31 ± 1.49 g, n = 10) were sourced from a commercial hatchery at Parkerville, Western Australia (31.87^{o}S, 116.14^{o}E), and stocked into three 200 L holding tanks (572 mm diameter, 850 mm height) at CARL one week prior to commencement of the trial. Perch were fed commercial silver perch pellets (Glen Forrest Stockfeeders™ Pty Ltd, Appendix 5) daily, but were not fed during the trial to prevent water fouling. Each tank was aerated and covered with black

plastic to minimise disturbance to fish. Filtration was provided individually to each tank by a Hagen© Aquaclear™ 300 filter.

6.2.3 Preparation of test solutions

Four test solutions were used for the trials: food odour (FOOD), and perch odour from small (PREDS), medium (PREDM) and large perch (PREDL), described in Table 6.1. Dechlorinated tap water was used as the control (CONT). PREDS, PREDM and PREDL contained water taken from one of three perch holding tanks containing small, medium or large perch respectively (further described in Section 6.2.3.2).

Table 6.1 Treatment description for predator size trial

Treatment	Description
Control (CONT)	20 mL water
Food odour (FOOD)	10 mL FOOD + 10 mL water
Small predator odour (PREDS)	10 mL PREDS + 10 mL FOOD
Medium predator odour (PREDM)	10 mL PREDM + 10 mL FOOD
Large predator odour (PREDL)	10 mL PREDL + 10 mL FOOD

PREDS, PREDM and PREDL comprised water taken from tanks holding small, medium or large perch respectively.

Food solution was always added simultaneously with predator odour because crayfish detection of predation risk cues is usually more discernible when feeding behaviour, displayed by crayfish in the presence of food odour alone, is suppressed (Acquistapace et al. 2004). Following suggestions of Hazlett (1994a, 1994b), all test solutions were prepared fresh daily.

6.2.3.1 Food solution

The food solution was prepared as described in Section 4.2.3.

6.2.3.2 Perch odour solution

One hour prior to commencing behavioural observations each day, the Aquaclear™ 300 filter connected to each perch tank was switched off to eliminate any effect that biological filtration may have on chemical cues from perch. Water was then siphoned from each tank until the required volume of water remained (Table 6.2). This ensured homogeneity of perch mass (g) to water (L) ratio (effective density), between small, medium and large perch tanks. This ratio followed that used in similar crayfish behavioural trials (Height and Whisson 2006; this thesis, Chapter 5). The black plastic tank cover was then placed back over the tank with the aeration system left running.

Table 6.2 Effective densities used for perch odour solutions

perch grade (treatment)	weight (g)	CV (%)	number	biomass (g)	tank volume (L)	effective density (g/ L)
small (PREDS)	151.93 ± 1.75	3.64	10	1538	75	20.51
medium (PREDM)	202.51 ± 1.24	1.94	10	2061	100	20.61
large (PREDL)	254.31 ± 1.49	1.85	10	2502	125	20.02

Tank volume (L) was the volume of water remaining in each perch tank after siphoning, to provide the same effective perch density in each tank. All perch tanks contained 200 L when full. CV = coefficient of variation

Perch odour solution comprised 10 mL of water drawn discretely into a syringe (Terumo® Corporation Pty Ltd) from a perch holding tank containing small, medium or large perch. Perch odour was always taken from the holding tank immediately prior to discharge into a crayfish tank. Following the completion of crayfish behavioural observations each day, filtration to each tank was switched-on, faeces (if present) were vacuumed from the tank, and then tanks were re-filled with dechlorinated water.

6.2.4 Experimental design and procedure

The experimental procedure was adapted from those used by Hazlett (1994a) and Gherardi et al. (2002a). The trial ran for three consecutive days. On day one, a single yabby was placed into each of the 18 aquaria (60 x 30 cm bottom, described in Section 3.1.1) containing 25 L of dechlorinated tap water and left for 24 h acclimation. Each aquarium was aerated, contained a piece of PVC pipe for shelter (length 20 cm, diameter 7.5 cm) and was visually isolated with a black plastic sheet to minimise disturbances to crayfish. After 24 h acclimation, crayfish were observed for two 5 minute time periods during which observations were recorded every 15 seconds: (i) a 5 minute control period following the addition of 20 mL of control water (dechlorinated water) and, immediately afterwards, (ii) a 5 minute period following the addition of the test solution (20 mL). Each yabby randomly received FOOD or one of the three perch odour solutions on day two of the trial and vice versa on day three, so at the end of day three, each yabby had received FOOD and one of the three PRED solutions. The addition of control water always preceded the test solution. To minimise disturbances to crayfish, control water and test solutions were squirted discretely via a syringe (Terumo® Corporation Pty Ltd) into the corner of the aquarium farthest from the animal. Crayfish behaviours and postures were recorded as described in Section 3.3.

After crayfish behavioural observations were completed, crayfish were removed and each aquarium was drained and thoroughly cleaned to eliminate any residual odour that may influence crayfish behaviour to solutions tested the following day. Aquariums were then refilled using dechlorinated tap water and crayfish were returned and left for 24 h before subsequent testing. PREDS, PREDM and PREDL were allocated to crayfish tanks (three males and three females) according to two Latin square designs depicted in Figure 6.1, using the middle tier of aquariums (see Section 3.1.1). Thus, six yabbies received PREDS, six yabbies received PREDM and six yabbies received PREDL, and all crayfish received FOOD.

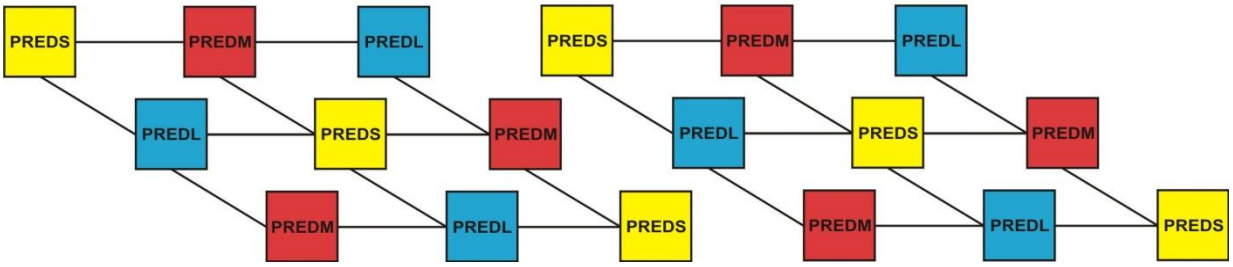

Figure 6.1 Perch odour allocation to crayfish tanks for the predator size trial

PREDS = small perch odour; PREDM = medium perch odour; PREDL = large perch odour.

6.2.5 Water quality monitoring

Total ammonia, salinity, pH, nitrite, nitrate, temperature, dissolved oxygen and conductivity were recorded daily for yabby and perch tanks. Water quality parameters remained within normal limits for yabbies (Lawrence and Jones 2002) and silver perch (Rowland 1995). A summary of these results is provided in Appendix 2; Table 3.

6.2.6 Statistical analysis

Comparisons between control and test periods for the reaction time and the percentage of time spent in each behaviour and posture were made using the Wilcoxon signed ranks test (Z).

The magnitude of change (i.e. the difference in absolute values) in reaction time and percentage of time spent in the different postures and behaviours were calculated for each individual between the control water and test solutions. The direction of change was either positive or negative if values in the presence of a test solution were higher or lower than those recorded during the control periods respectively. Comparisons between food and perch odours within groups were completed using the Wilcoxon

signed ranks test. Comparisons between the three perch solutions were made using Friedman two-way ANOVA. Extensive statistical summary tables are provided in Appendix 3; Tables 6, 7 and 8.

6.3 Results

6.3.1 Differences between control water and test solutions

Yabbies flicked their antennules more in the presence of the test solutions than control water ($P < 0.05$). All yabbies increased feeding behaviour in the presence of FOOD ($P < 0.05$, Figure 6.2). In response to perch odour, feeding behaviour increased in the presence of PREDS ($P < 0.05$), but not PREDM and PREDL ($P > 0.05$). Yabbies reacted faster to FOOD and PREDS ($P < 0.05$), and spent more time in intermediate posture after exposure to PREDS ($P < 0.05$). Tables 9, 10 and 11 in Appendix 3 contain mean values (± standard error) of the reaction time (s), behaviours and postures (% time), of yabbies in the presence of FOOD, PREDS, PREDM and PREDL.

6.3.2 Comparison between test solutions within groups

PREDS was more successful than FOOD in eliciting feeding behaviour and antennae flicking ($P < 0.05$, Table 6.3). No significant differences were found in the amount of time displaying feeding behaviours for FOOD and PREDM or PREDL ($P > 0.05$), but yabbies increased locomotion more to FOOD than PREDM ($P < 0.05$).

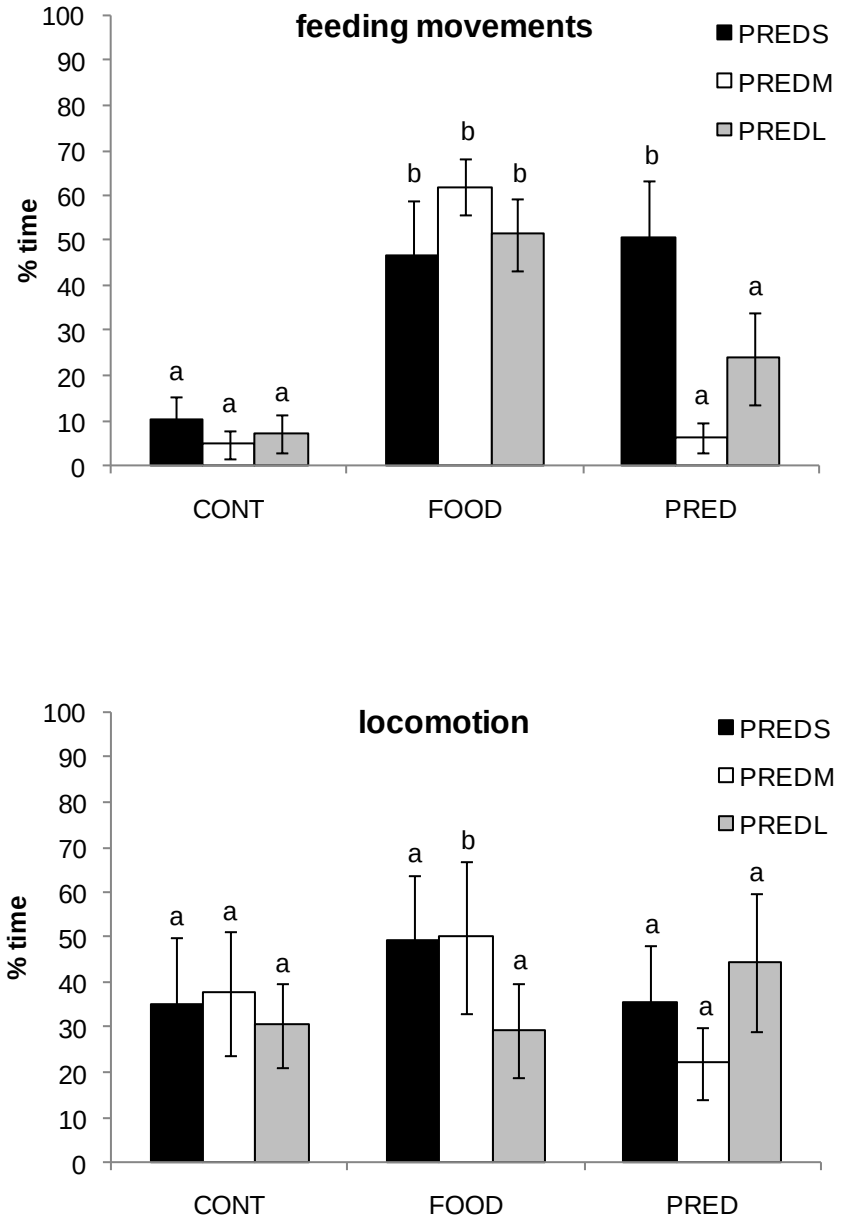

Figure 6.2 Behavioural responses of yabbies to control water and test solutions

Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water, (PREDS) 10 mL small perch odour + 10 mL food odour, (PREDM) 10 mL medium perch odour + 10 mL food odour water, (PREDL) 10 mL large perch odour + 10 mL food odour. Statistical tests for significance were made using the Wilcoxon signed ranks test. Different letters for the same series denote significant differences between control water and test solutions at $P < 0.05$. Error bars are means ± standard error.

Table 6.3 Differences in the magnitude of change between control water and test solutions for yabby reaction time (s), behaviours and postures (% time)

	small perch	**medium perch**	**large perch**
reaction time	0.106	0.108	0.674
in shelter	0.447	0.816	1.633
locomotion	1.084	2.207* FOOD > PREDM	1.577
raised posture	1.461	1.604	1.625
intermediate posture	1.826	1.577	0.105
lowered posture	0.948	0.542	2.023* FOOD > PREDL
feeding	2.023* PREDS > FOOD	0.406	1.826
antennae flicking	2.023* PREDS > FOOD	0.365	1.289
antennule flicking	1.363	1.153	2.032* PREDL > FOOD

Values are the Wilcoxon signed ranks test statistic (Z). Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (PREDS) 10 mL small perch odour + 10 mL food odour; (PREDM) 10 mL medium perch odour + 10 mL food odour; (PREDL) 10 mL large perch odour + 10 mL food odour. Test solutions ranked in decreasing order of magnitude of change. *P < 0.05.

6.3.3 Comparison between perch odour solutions

No significant changes were found in the magnitude of change among PREDS, PREDM and PREDL for any behaviour or posture (P > 0.05, d.f. = 2).

6.4 Discussion

6.4.1 Responses to food odour

Yabbies clearly responded to chemical stimuli, displaying increased use of the antennules, the primary olfactory device in freshwater crayfish (Tierney and Atema 1988; Oh and Dunham 1991; Grasso and Basil 2002). Food odour was successful at eliciting feeding related behaviours in yabbies, concurring with other studies on this species (Gherardi et al. 2002a; Height and Whisson 2006; this thesis, Chapters 4 and 5). Interestingly, yabbies that received PREDM were the only group that increased locomotion in the presence of food odour. Increases in feeding behaviour and locomotion are two well documented behavioural modifications by crayfish upon detection of chemical cues associated with potential food sources (Tierney and Atema

1988; Kreider and Watts 1998; Acquistapace et al. 2004). Yabbies used in the trials documented in Chapters 4 and 5 of this thesis all increased feeding behaviour and locomotion in the presence of food odour. The percentage of time yabbies spent in locomotion after exposure to food odour in the present trial was comparable to (and in some cases higher than) previous trials. However, the result was not significant in this trial due to the high activity observed in the control period. The dormant behaviour observed in yabbies during control periods, described in previous chapters, was not observed in this trial. One influencing factor may have been the longer acclimation period received by yabbies used in previous trials (one month), compared to that of the present trial (one week), when crayfish were first placed in CARL holding tanks (Section 3.2.1.2). Notwithstanding this, all crayfish received the same 24 h acclimation in the experimental system before the trial commenced (Described at Section 6.2.4).

6.4.2 Influence of predator size on crayfish behavioural response

Yabbies displayed a combination of feeding and alarm responses to perch odour. Detection of predation risk substances by crayfish may result in protective behavioural modifications, such as avoidance of areas of potential danger, ceasing or reducing activity, and increasing the use of shelter and watchful posture (Appelberg et al. 1993; Blake and Hart 1993b; Hazlett 1994a; Shave et al. 1994; Hazlett and Schoolmaster 1998). In the current trial, yabbies did not reduce locomotory movements to PREDS, PREDM or PREDL. However, PREDM and PREDL inhibited feeding behaviour. This combination of feeding and alarm response, appears appropriate, but not specific, to food and perch odour when presented simultaneously to crayfish. In the case of PREDS, yabbies showed a clear feeding response. In fact, when the magnitude of change values were compared, yabbies displayed a stronger feeding response to PREDS than to food odour (see Table 6.3).

The ability of crayfish to detect and respond to predator odour varies between species and according to the characteristics of the predator. Appelberg et al. (1993) reported an inherent ability in *Astacus astacus* to detect and minimise predation risk from co-

occurring fish species. *A. astacus* increased shelter use to odour from four predatory fish species, but did not respond to chemical stimuli from a non-predatory fish tested. Further, *A. astacus* demonstrated stronger responses to a starved predator than to a non-starved one. Blake and Hart (1993b) found that *Pacifastacus leniusculus* exhibits predator avoidance behaviour to perch odour. Shave et al. (1994) found that *Paranephrops zealandicus* responded to chemical cues in the skin mucus of native predatory eels (*Anguilla dieffenbachii*), but not introduced trout (*Salmo trutta*). Hazlett and Schoolmaster (1998) reported variation in the ability of four sympatric species of cambarid crayfish to detect and respond to odour from a common predator. Adult *Orconectes virilis* responded to snapping turtle (*Chelydra serpentina*) odour, but juveniles only displayed a response after snapping turtle odour was paired with conspecific alarm odour. *Orconectes propinquus* did not display any response to predator odour.

Significant work has been undertaken in an attempt to explain the variability of crayfish responses to chemical stimuli. The responses displayed by yabbies to perch odour in this study may be due to a number of factors. Firstly, the strong feeding response to PREDS may be because smaller perch do not produce the same odour compound as the larger perch sizes used, or they do not produce odour of the same intensity. Alternatively, yabbies may have detected perch odour and not associated it with predation risk, and instead responded to PREDS as a food source. Furthermore, Storer (2005) provided evidence that contradicted this claim, reporting that yabbies did not display feeding behaviour when presented with perch cues alone, and not in combination with food cues. However, Storer (2005) used different methodologies to the present study; crayfish and perch were placed in a single aquarium partitioned by permeated opaque glass to allow exchange of chemical cues. The inhibited feeding response of yabbies to PREDM and PREDL in the present study was not complimented with a reduction in locomotion, suggesting a combination of feeding and alarm responses from crayfish. The explanation for this result, compared to that for PREDS remains unclear, and warrants further testing of crayfish responses to odour from different sized predators.

6.4.3 Concluding remarks and recommendations

Behavioural modification by yabbies in the presence of predator odour has been previously documented (Height and Whisson 2006), but appears to be highly variable, and not as clear as those made in the presence of conspecific and heterospecific alarm odour (Gherardi et al. 2002a). The aim of the present trial was to determine if predator size influences the behavioural modifications of yabbies to predator odour. Although crayfish behavioural responses to predator odour have received some attention from researchers in the past, this study is the first known investigation on the affect of predator size on crayfish behavioural responses to their odour.

Two important results follow; firstly, yabbies displayed different behavioural responses to chemical cues from small, medium and large perch. Secondly, these findings provide evidence that yabbies can differentiate between chemical cues pertaining to food and those pertaining to perch, supporting previous studies (Storer 2005; Height and Whisson 2006). However, the basis for that differentiation requires further elucidation. Yabbies may associate perch cues with predation risk, or may be displaying cautionary behaviour in the presence of an unknown odour, i.e. higher behavioural plasticity. The lack of response to small perch odour may be a result of physiological differences between this size grade and the two larger perch sizes tested, or, another example of variability in yabby responses to predator cues (e.g. Chapter 5).

Results from this study identify the need to further examine the behavioural responses of yabbies to odour from a range of predator sizes to elucidate the variability in crayfish responses observed in this trial, and in previous research (Chapter 5). The three size grades of silver perch used in this trial (small, 151.93 g; medium, 202.51 g; and large, 254.31 g) were selected due to their low variation in size (see CV, Table 6.2). Ideally, a wider range of perch sizes would have been used; perhaps more reflective of the life stages of this species. Juvenile (< 25g), sub-adult (~250g) and mature (> 1kg) silver perch are recommended for similar trials in the future.

Additional recommendations for future studies include:

- compare behavioural modifications of yabbies from a naturalised population, and a monoculture environment, to predator odour. Hazlett et al. (2002) and Acquistapace et al. (2003) have demonstrated that crayfish are able to learn about cues associated with predation-risk. Crayfish from a natural population may have had the opportunity to 'learn' as a result of greater exposure to predation risk than individuals from a monoculture environment;
- investigate the effect of fluctuations in odour concentration, for both food and predator odour, on the responses of crayfish. For example, when two conflicting cues are presented to crayfish simultaneously, it is possible for responses appropriate to one cue to dominate over responses appropriate to the other; i.e. feeding versus alarm response (Hazlett 1999);
- determine if the responses of marron and yabbies to predation risk cues are influenced by their hunger status. Hazlett (2003) found that invasive *Orconectes rusticus* were less motivated by hunger to feed in the presence of predation risk cues than native *O. virilis*;
- determine the chemical compounds present in silver perch odour and develop a method to quantify them.

Chapter seven

Influence of crayfish size on behavioural response to alarm odour at night

---

This chapter investigated the behavioural responses of marron and yabbies to alarm odour. It follows an earlier study conducted by Gherardi et al. (2002) but with two notable differences: (i) the behaviour of three sizes of marron and yabbies were examined, and (ii) crayfish behaviour was observed at night using night-vision equipment. The results of this trial are compared with similar research on marron and yabbies, the differences in crayfish behaviour between day and night-time are discussed.

7.0 Influence of crayfish size on behavioural response to alarm odours at night

7.1 Introduction

Crayfish are generally considered nocturnal animals (Morrissy and Caputi 1981; Nyström 2005). This activity pattern is considered adaptive, and one where crayfish are either matching the habits of their prey (Gherardi 2002), or minimising predation risk from visual predators (Flint 1977; Maitland and Campbell 1992).

Predatory fish species are recognised as the most influential predator with respect to crayfish growth, survival and distribution (Nyström 2002; Nyström et al. 2006). Many fish exhibit size-selective predation, preferring juvenile crayfish over adults (Stein and Magnuson 1976; Whisson 2000; Olsson et al. 2006). Therefore chemical recognition of predators, or an event indicative of predation risk, would be an asset to crayfish, conferring an advantage, particularly when visibility is poor (Vogt 2002; Height and Whisson 2006).

Previous studies have determined that some crayfish species can detect chemical cues from predators (Appelberg et al. 1993; Hazlett and Schoolmaster 1998), and alarm cues from conspecific and heterospecific crayfish (Bouwma and Hazlett 2001; Gherardi et al. 2002a). Alarm cues are thought to be contained in the hemolymph of crayfish (e.g. *Procambarus clarkii*, Acquistapace et al. 2005), and are released as the animal is crushed or injured, as can occur during predation (Hazlett 1994a).

Detection and utilisation of chemical information varies among crayfish species. A number of studies have compared behavioural responses of pairs of native and invasive crayfish species to chemical cues (e.g. Hazlett 2000; Hazlett et al. 2003; Acquistapace et al. 2004). These researchers have concluded that invasive crayfish display a higher level of behavioural plasticity, and use a broader range of predation

risk cues than the native species they are displacing. In Western Australia, native marron (*Cherax tenuimanus*) are facing competition from invasive yabbies (*Cherax albidus*). Previous studies comparing behaviour of these species in response to chemical stimuli have found (i) yabbies respond similarly to heterospecific alarm odour and conspecific alarm odour (Gherardi et al. 2002a), and (ii) yabbies respond to exotic predator odour (Height and Whisson 2006). These studies and others have examined crayfish behavioural responses in the laboratory during daylight hours, largely owing to difficulties in recording crayfish behaviour in the dark. Notwithstanding this, crayfish are nocturnal, and given that chemical, water-borne communication would be of obvious importance to the survival of a nocturnal aquatic animal, a logical life history question is – are similar behavioural responses observable at night time? Futher, do observable differences in behaviour exist between different crayfish size classes?

Size-dependent responses of crayfish to chemical cues have not been widely investigated; however, yabbies reach sexual maturity at a smaller size than marron, and marron grow much larger than yabbies (Lawrence and Jones 2002). Juveniles and adults of both species are likely to display different behavioural modifications to chemical stimuli. Extensive research has been conducted on the responses of *P. clarkii* and *Orconectes* sp. crayfish to chemical stimuli (e.g. Hazlett 1994a; Hazlett et al. 2003; Acquistapace et al. 2004). However, with the exception of Hazlett and Schoolmaster (1998), no study has examined the differences in behaviour of adult and juvenile crayfish to chemical stimuli whilst keeping other variables constant. This issue may have received little attention in *P. clarkii* and *Orconectes* sp. crayfish due to the small difference in size between juvenile and adult crayfish, and the short lifespan of these species; e.g. 4 years maximum for *P. clarkii* (Huner 2002); 3-4 years average for *Orconectes* sp. crayfish (Momot 1988). Interestingly, Hazlett and Schoolmaster (1998) found that juvenile and adult *Orconectes virilis* responded differently to chemical stimuli, demonstrating the ability of this species to learn about predation-risk cues as they age. In the case of marron and yabbies, body-size is highly relevant, due to the large size difference between juvenile and adult crayfish, and because marron can

grow larger than can be consumed by any aquatic predator currently present in their natural habitat (see Morgan et al. 2002).

The aim of this study was to compare behavioural responses of marron and yabbies to alarm odour at night, and to determine if crayfish size influences behavioural modifications under these conditions.

7.2 Materials and methods

7.2.1 Site and experiment system

This trial was conducted using the glass aquaria at the Curtin Aquatic Research Laboratory (CARL, Section 3.1.1), located in Bentley, Western Australia. Lighting was provided via a 12 h : 12 h, light : dark cycle for the duration of the trial. All crayfish behavioural observations were undertaken a minimum of three hours following the commencement of the dark period using 1st Generation ATN Viper night-vision goggles (Plate 7.1).

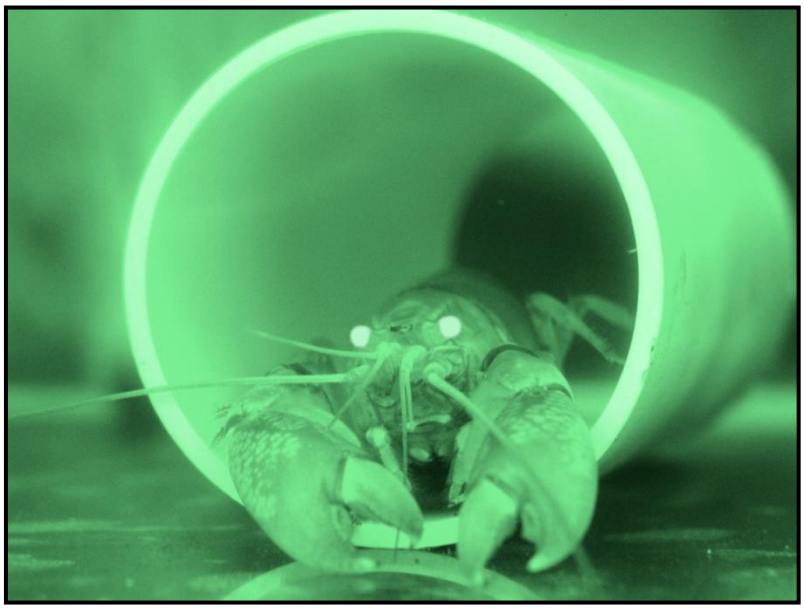

Plate 7.1 Yabby observed in shelter using night-vision goggles

7.2.2 Experimental animals

Marron used in the trial were sourced from a commercial producer at Manjimup (34.23°S, 116.13°E), and yabbies from a commercial supplier at Kukerin (33.18°S, 118.08°E), Western Australia. Size and weight data for the three size classes (small, medium and large; n = 18 for each class, male : female ratio = 50 : 50) of both species is provided in Table 7.1. Crayfish were kept in 'species only' tanks at CARL in the crayfish holding systems described in Section 3.2.1 for one month prior to the trial and fed commercial crayfish pellets (Glen Forrest Stockfeeders™, Appendix 5). Crayfish were not fed during the trial, or the acclimation period preceding the trial.

Table 7.1 Size and weight data (mean ± s.e.) for small, medium and large marron and yabbies used in the alarm odour night trial

		carapace length (mm)	**weight (g)**
small	*marron*	24.44 ± 0.37	5.12 ± 0.17
	yabby	13.97 ± 0.31	4.48 ± 0.35
medium	*marron*	53.81 ± 0.76	45.55 ± 1.55
	yabby	23.18 ± 0.53	13.86 ± 0.91
large	*marron*	85.83 ± 0.90	182.00 ± 3.73
	yabby	39.19 ± 0.78	57.85 ± 2.49

No significant differences were found for carapace length or weight between males and females for each size class within species.

7.2.3 Preparation of test solutions

Three test solutions were used for the trials: food odour (FOOD), conspecific alarm odour (CONS), and heterospecific alarm odour (HETE), described in Table 7.2. Dechlorinated tap water was used as the control (CONT).

Table 7.2 Experimental treatment for alarm odour night trial

Treatment	Description
Control (CONT)	20 mL water
Food odour (FOOD)	10 mL FOOD + 10 mL water
Conspecific alarm odour (CONS)	10 mL CONS + 10 mL FOOD
Heterospecific alarm odour (HETE)	10 mL HETE + 10 mL FOOD

Each treatment was replicated 18 times.

Food solution was always added in conjunction with conspecific and heterospecific odours because crayfish detection of alarm odours is usually more discernible when feeding behaviour, displayed by crayfish in the presence of food odour alone, is subjugated (Aquistapace *et al*. 2004). Following suggestions by Hazlett (1994a, 1994b), all test solutions were prepared fresh daily.

7.2.3.1 Food solution

The food solution was prepared as described in Section 4.2.3.

7.2.3.2 Conspecific and heterospecifc solutions

Conspecific and heterospecific alarm odour solutions were prepared by macerating a 45 - 50 g male conspecific or heterospecific crayfish respectively, then thoroughly mixing the pieces in 400 mL of dechlorinated tap water and filtering with Whatman® grade 4, 20 – 25 µm coarse filter paper.

7.2.4 Experimental design

Two experiments were conducted: marron were used as the test species in one experiment, and yabbies in the other. Each experiment lasted 3 days (crayfish

behaviour was recorded from 18 tanks each day), including the 24 h acclimation period (see Section 7.2.5) before the first test solution was introduced to each tank. Small, medium and large crayfish were allocated to aquariums according to two Latin Cube designs (Federer 1991; Figure 7.1), which minimised any variation in light intensity and temperature between tanks on different tiers.

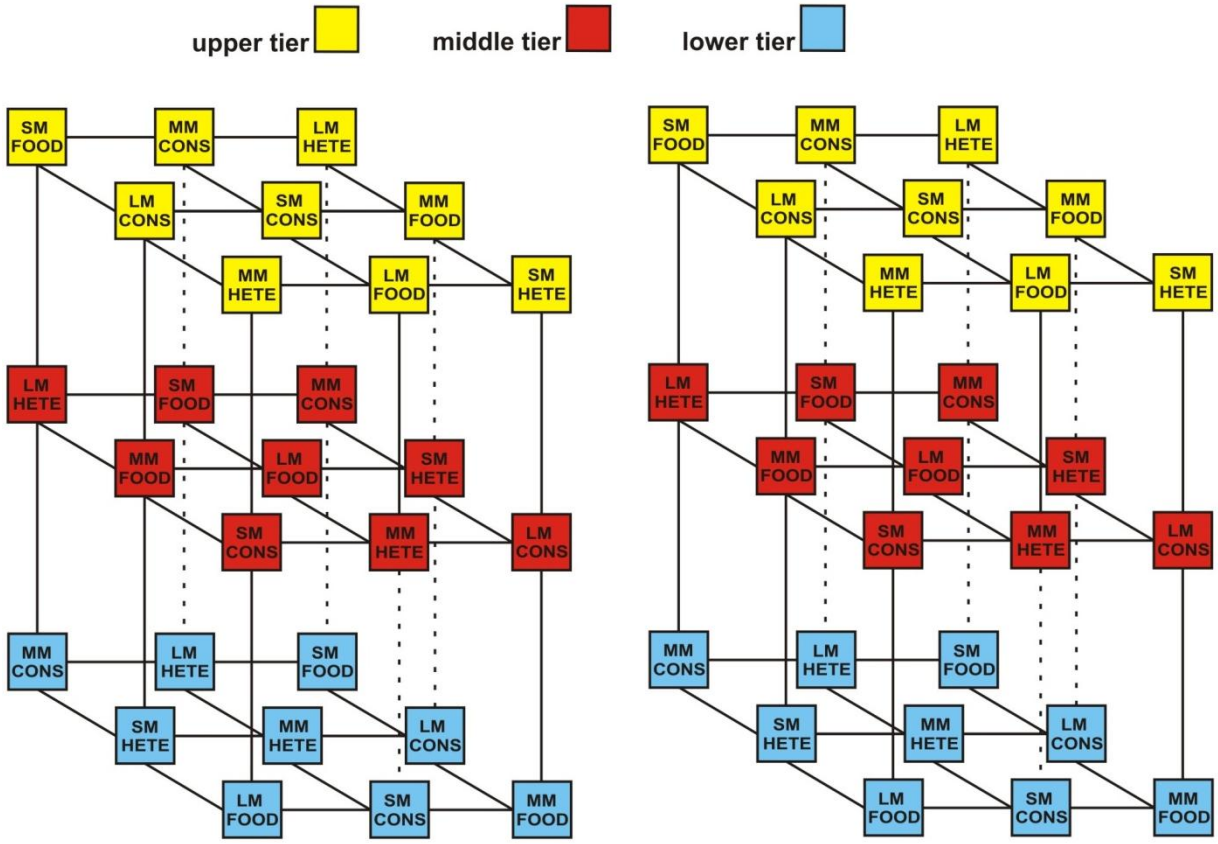

Figure 7.1 Treatment allocation to aquariums for the alarm odour trial using a Latin Cube design

Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (CONS) 10 mL conspecific odour + 10 mL food odour; (HETE) 10 mL heterospecific odour + 10 mL food odour. Crayfish were: (SM) small marron; (MM) medium marron; (LM) large marron. Marron were replaced with small, medium and large yabbies in the second experiment.

7.2.5 Experimental procedure

The experimental procedure was adapted from Hazlett (1994a) and Gherardi et al. (2002a). A single crayfish was placed into each of the 54 aquaria (60 x 30 cm bottom, described in Section 3.1.1) containing 25 L of dechlorinated tap water and left for 24 h acclimation. Each aquarium was aerated, contained a piece of PVC pipe for shelter (length 20 cm, diameter 7.5 cm) and was visually isolated with a black plastic sheet to minimise disturbances to crayfish. After 24 h acclimation, crayfish were observed for two 3 minute time periods during which observations were recorded every 15 seconds: (i) a 3 minute control period following the addition of 20 mL of control water (dechlorinated water) and, immediately afterwards, (ii) a 3 minute period following the addition of the test solution (20 mL). Each crayfish received one of the three test solutions (FOOD, CONS or HETE) per day so that after three consecutive days, each individual had received all three test solutions. The addition of control water always preceded the test solution. To minimise disturbances to crayfish, control water and test solutions were injected discretely via syringe (Terumo® Corporation) into the corner of the aquarium farthest from the animal. Crayfish behaviours and postures were recorded as described in Section 3.3. After crayfish behavioural observations were completed, crayfish were removed and each aquarium was drained and thoroughly cleaned to eliminate any residual odour that may influence crayfish behaviour to solutions tested the following day. Aquariums were then refilled using dechlorinated tap water and crayfish were returned and left for 24 h before subsequent testing.

7.2.6 Water quality monitoring

Total ammonia, salinity, pH, nitrite, nitrate, temperature, dissolved oxygen and conductivity were recorded daily for crayfish tanks during both trials. Water quality parameters remained within normal limits for marron and yabbies (Lawrence and Jones 2002). A summary of these results is provided in Appendix 2; Table 4.

7.2.7 Statistical analysis

Background differences during control periods for marron and yabbies were compared using the Wilcoxon-Mann-Whitney test (Z for large samples). Mean reaction time and the mean percentage of time spent inside shelter, in locomotion, while feeding, and flicking antennae and antennules were compared, as recorded for each crayfish during control periods. Comparisons between control and test periods for the reaction time and the percentage of time spent in each behaviour and posture were made using the Wilcoxon signed ranks test (Z).

The magnitude of change (i.e. the difference in absolute values) in reaction time and percentage of time spent in the different postures and behaviours were calculated for each individual between the control water and test solutions. This calculation was necessary because species and size classes displayed background differences in behaviour. The direction of change was either positive or negative if values in the presence of a test solution were higher or lower than those recorded during the control periods respectively. Intraspecific comparisons among the test solutions within a size class were completed using Friedman two-way analysis of variance by ranks (F_r) and the Sign test to determine significant differences between test solutions (Selvanathan et al. 2000). Intraspecific comparisons to the same test solution between size classes were completed using the Kruskal-Wallis test (H) and the Mann-Whitney U test post-hoc to determine significant differences between sizes (Siegel and Castellan 1988). The responses of marron and yabbies to the same test solution were compared using the Wilcoxon-Mann-Whitney test (Z for large samples). Extensive statistical data tables are displayed in Appendix 3; Tables 9, 10 and 11.

7.3 Results

7.3.1 Intraspecific differences in response to control water

7.3.1.1 Marron

During the three control periods, the reaction time (mean seconds ± standard error) was significantly different between small, medium and large marron (small; 90 ± 10.41 < large; 128.06 ± 10.35 < medium; 160.28 ± 8.54, $P < 0.05$, Table 7.3). Small marron were more frequently observed displaying feeding behaviour than medium and large marron ($P < 0.01$). Large marron flicked their antennules more frequently than small and medium marron (large > small > medium, $P < 0.05$).

Table 7.3 Inter- and intraspecific differences in mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) during control periods

	small		medium		large	
	marron	**yabby**	**marron**	**yabby**	**marron**	**yabby**
reaction time	90 ± 10.41^{a}	102.78 ± 9.70^{ab}	160.28 ± 8.54^{c}	143.33 ± 9.28^{cd}	128.06 ± 10.35^{bde}	133.61 ± 9.99^{ce}
in shelter	27.78 ± 5.61^{ad}	0.71 ± 0.58^{b}	64.81 ± 6.30^{c}	35.33 ± 6.03^{ad}	35.19 ± 6.09^{a}	40.17 ± 6.68^{d}
locomotion	32.76 ± 4.36^{a}	53.13 ± 3.70^{b}	9.69 ± 3.14^{c}	17.09 ± 3.62^{cd}	26.35 ± 4.43^{ad}	20.66 ± 3.14^{d}
raised posture	44.30 ± 6.02^{a}	33.05 ± 3.65^{a}	12.96 ± 4.32^{b}	15.53 ± 4.06^{b}	13.53 ± 3.83^{b}	11.11 ± 2.58^{b}
intermediate posture	45.58 ± 5.55^{a}	54.56 ± 3.89^{a}	46.87 ± 6.12^{a}	46.58 ± 5.98^{a}	50.14 ± 5.40^{a}	47.86 ± 5.27^{a}
lowered posture	10.11 ± 3.39^{a}	12.39 ± 3.39^{b}	40.17 ± 6.12^{c}	37.89 ± 6.28^{bc}	39.17 ± 5.71^{c}	40.88 ± 5.76^{c}
feeding	11.25 ± 2.22^{a}	8.97 ± 2.07^{a}	1.71 ± 0.97^{b}	8.55 ± 2.34^{ac}	1.85 ± 0.61^{b}	4.56 ± 1.57^{bc}
antennae flicking	36.47 ± 3.69^{a}	51.85 ± 3.46^{b}	15.95 ± 3.07^{c}	25.64 ± 3.50^{d}	30.91 ± 4.02^{ad}	29.06 ± 2.93^{ad}
antennule flicking	31.48 ± 2.93^{a}	57.98 ± 2.76^{b}	21.79 ± 2.97^{c}	46.87 ± 2.77^{d}	41.74 ± 2.84^{d}	45.58 ± 3.40^{d}

Values in any one row not followed by the superscript are significantly difference at $P < 0.05$. Differences in control periods compared by Wilcoxon-Mann-Whitney test (n = 54 for each size class).

7.3.1.2 Yabbies

Small yabbies showed the fastest reaction to CONT (small; 102.78 ± 9.70 < medium; 143.33 ± 9.28 = large; 133.61 ± 9.99, $P < 0.05$, Table 7.3), and were more active than medium and large yabbies, spending less time in shelter and more time in locomotion, displaying raised posture and flicking antennae and antennules ($P < 0.01$). Small

yabbies also spent more time displaying feeding behaviour than large yabbies ($P < 0.05$).

7.3.2 Interspecific differences in response to control water

Small yabbies were the most active crayfish, spending the least time in shelter and lowered posture and more time in locomotion and flicking antennae and antennules than any other group ($P < 0.05$). Medium marron utilised the shelter more frequently than any other crayfish ($P < 0.05$).

7.3.3 Differences between control water and test solutions

7.3.3.1 Reaction time

All crayfish reacted faster to the test solutions than to control water ($P < 0.05$; Appendix 3).

7.3.3.2 Marron

Small marron increased feeding behaviour in the presence of all three test solutions ($P < 0.05$), and demonstrated the greatest behavioural modifications (with respect to the control) in the presence of FOOD; increasing time in locomotion, displaying raised posture and flicking antennae ($P < 0.05$). In the presence of CONS and HETE, the only behavioural modification evident was an increase in time spent displaying feeding behaviour ($P < 0.05$).

Medium marron increased the amount of time spent moving, displaying feeding behaviour and flicking antennae for FOOD, CONS and HETE ($P < 0.05$), with respect to each control period. In the presence of FOOD, medium marron spent more time in raised posture ($P < 0.05$). After exposure to CONS or HETE, medium marron also spent less time in shelter and flicked their antennules more frequently ($P < 0.05$). In

the presence of CONS, medium marron increased time spent in intermediate posture ($P < 0.05$).

The only behavioural modification demonstrated by large marron (with respect to the control period) in the presence of CONS, was an increase in feeding behaviours ($P < 0.05$). After exposure to FOOD or HETE, in addition to an increase in the time spent displaying feeding movements, large marron spent more time in locomotion and flicking antennules ($P < 0.05$). In the presence of FOOD, large marron also increased time spent in raised posture ($P < 0.01$). In the presence of HETE, large marron increased time spent in intermediate posture and flicking antennules ($P < 0.05$).

7.3.3.3 Yabbies

Small yabbies increased feeding behaviour and the time spent flicking antennae and antennules after exposure to FOOD, CONS or HETE, with respect to the control periods ($P < 0.05$). In the presence of FOOD, small yabbies spent more time in locomotion and lowered posture ($P < 0.05$). Small yabbies spent more time in shelter and moving in intermediate posture in the presence of HETE ($P < 0.05$).

After exposure to FOOD or HETE, medium yabbies spent more time moving, displaying feeding movements and flicking antennae and antennules with respect to control periods ($P < 0.05$). Further, in the presence of FOOD, medium yabbies spent less time in shelter and more time in intermediate posture ($P < 0.05$), and in the presence of HETE, medium yabbies increased time displaying raised posture ($P < 0.01$). After exposure to CONS, the only behavioural modifications by medium yabbies, were increases in time displaying intermediate posture and antennule flicking ($P < 0.05$).

Exposure to FOOD, CONS or HETE resulted in large yabbies increasing feeding movements and antennae and antennule flicking compared to respective control periods ($P < 0.05$). Furthermore, large yabbies spent more time moving in the

presence of HETE, and increased time spent moving and in raised and intermediate postures, while decreasing time spent in shelter in the presence of FOOD ($P < 0.05$).

7.3.4 Intraspecific comparison among test solutions

7.3.4.1 Reaction time

No significant differences were found for reaction times between FOOD, CONS and HETE for any size class, for marron or yabbies ($P > 0.05$).

7.3.4.2 Marron

CONS inhibited feeding behaviour in all marron ($P < 0.05$; Table 7.4, Figure 7.2). The influence of HETE on feeding behaviour became prevalent as crayfish size increased: small marron showed no response to HETE; medium marron displayed a stronger feeding response to FOOD than to HETE, but the difference was not significant; HETE inhibited feeding behaviour in large marron ($P < 0.01$). In fact, this inhibition was just as strong as for CONS ($\chi^2 = 22.776$; $P < 0.01$).

Table 7.4 Differences in the magnitude of change between control water and test solutions for marron reaction time (s), behaviours and postures (% time)

	small	**medium**	**large**
reaction time	0.346	4.983	5.461
in shelter	3.659	4.979	1.088
locomotion	6.464* FOOD > CONS	1.485	0.875
raised posture	1.433	10.292** FOOD > HETE	1.088
intermediate posture	3.254	0.090	5.485
lowered posture	12.483** FOOD > CONS = HETE	5.839	0.585
feeding	11.853** FOOD = HETE > CONS	6.618* FOOD > CONS	22.776** FOOD > HETE = CONS
antennae flicking	0.824	5.324	5.681
antennule flicking	2.590	4.794	0.623

Values are Friedman two-way analysis of variance test statistic (χ^2, d.f. = 2). Post-hoc analysis used Sign test. Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (CONS) 10 mL conspecific odour + 10 mL food odour; (HETE) 10 mL heterospecific odour + 10 mL food odour. Test solutions ranked in decreasing order of magnitude of change. *P < 0.05, **P < 0.01.

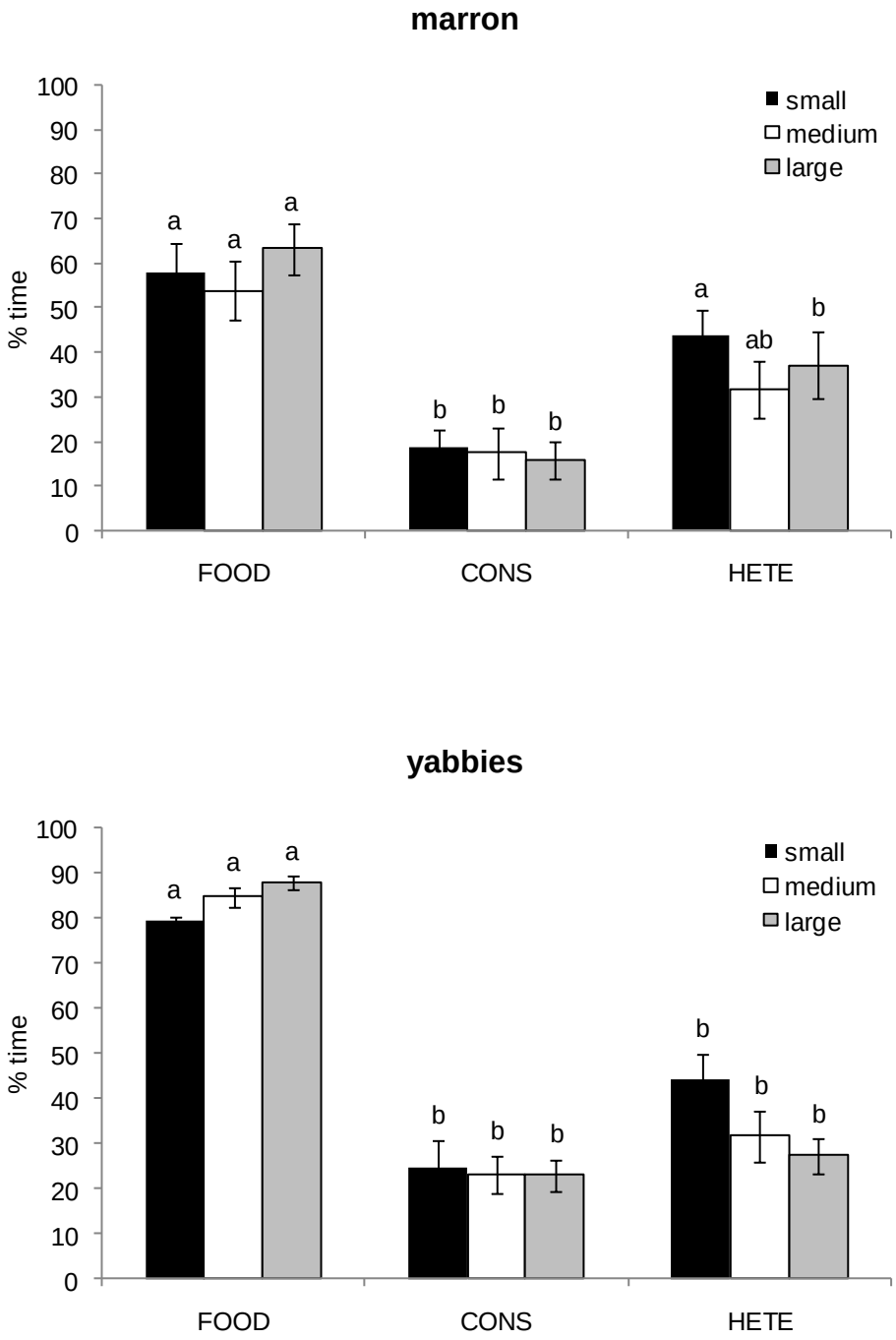

Figure 7.2 Magnitude of change for marron and yabby feeding behaviour

Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water, (CONS) 10 mL conspecific odour + 10 mL food odour, (HETE) 10 mL heterospecific odour + 10 mL food odour. Statistical tests for significance were made using the Wilcoxon signed ranks test. Different letters for the same size class denote significant differences at $P < 0.05$. Error bars are means ± standard error.

7.3.4.3 Yabbies

CONS and HETE inhibited feeding behaviour in small, medium and large yabbies ($P < 0.01$; Table 7.5, Figure 7.2).

Table 7.5 Differences in the magnitude of change between control water and test solutions for yabby reaction time (s), behaviours and postures (% time)

	small	**medium**	**large**
reaction time	1.333	4.619	2.652
in shelter	10.129** CONS = HETE > FOOD	0.745	5.091
locomotion	6.451* HETE > FOOD	2.203	1.507
raised posture	0.094	3.206	8.951* FOOD > HETE
intermediate posture	0.029	4.457	1.743
lowered posture	2.308	7.309* FOOD > HETE	5.645
feeding	25.768** FOOD > HETE = CONS	27.912** FOOD > HETE = CONS	27.111** FOOD > HETE = CONS
antennae flicking	4.423	4.522	10.866** FOOD = HETE > CONS
antennule flicking	9.761** HETE > FOOD	3.029	5.072

Values are Friedman two-way analysis of variance test statistic (χ^2, d.f. = 2). Post-hoc analysis used Sign test. Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (CONS) 10 mL conspecific odour + 10 mL food odour; (HETE) 10 mL heterospecific odour + 10 mL food odour. Test solutions ranked in decreasing order of magnitude of change. *P < 0.05, **P < 0.01.

7.3.5 Intraspecific comparison to the same test solution

7.3.5.1 Marron

Small, medium and large marron did not display any differences in feeding behaviour or locomotion for FOOD, CONS or HETE ($P > 0.05$; d.f. = 2, Table 7.6).

Table 7.6 Intraspecific differences for the magnitude of change between control water and test solutions for marron reaction time (s), behaviours and postures (% time)

	FOOD	CONS	HETE
reaction time	1.688	10.783** M = L > S	2.802
in shelter	4.198	0.848	0.475
locomotion	2.763	2.323	0.118
raised posture	6.391* M > L	10.878** S > L = M	5.517
intermediate posture	3.497	0.382	4.073
lowered posture	6.622* M > S	12.739** M = L > S	16.841** L = M > S
feeding	1.146	0.736	1.983
antennae flicking	0.241	0.061	1.952
antennule flicking	0.604	3.580	8.141* M > S = L

Values are Kruskal-Wallis test statistic (H, d.f. = 2). Post-hoc analysis used Mann-Whitney U test. Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (CONS) 10 mL conspecific odour + 10 mL food odour; (HETE) 10 mL heterospecific odour + 10 mL food odour. S = small marron; M = medium marron; L = large marron. Size classes ranked in decreasing order of magnitude of change. *P < 0.05, **P < 0.01.

7.3.5.2 Yabbies

Compared to small yabbies, medium and large yabbies showed a stronger feeding response, and a greater increase in locomotion to FOOD ($P < 0.01$, d.f. = 2, Table 7.7. However, these differences are attributed to the greater activity of small yabbies during control periods. No differences existed between sizes for feeding behaviour and locomotion for CONS and HETE ($P > 0.05$).

Table 7.7 Intraspecific differences for the magnitude of change between control water and test solutions for yabby reaction time (s), behaviours and postures (% time)

	FOOD	CONS	HETE
reaction time	3.12	6.567* L > S	2.449
in shelter	13.709** L = M > S	0.700	3.086
locomotion	11.485** L = M > S	0.632	0.561
raised posture	5.155	0.050	4.043
intermediate posture	10.336** M > S = L	0.910	1.595
lowered posture	6.507* L > S	0.856	12.795** L > S = M
feeding	13.244** L = M > S	0.210	4.983
antennae flicking	20.891** M = L > S	0.547	2.362
antennule flicking	14.928** L = M > S	0.774	0.069

Values are Kruskal-Wallis test statistic (H, d.f. = 2). Post-hoc analysis used Mann-Whitney U test. Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (CONS) 10 mL conspecific odour + 10 mL food odour; (HETE) 10 mL heterospecific odour + 10 mL food odour. S = small yabby; M = medium yabby; L = large yabby. Size classes ranked in decreasing order of magnitude of change. *P < 0.05, **P < 0.01.

7.3.6 Interspecific comparisons

Food odour was more successful at eliciting feeding behaviour in yabbies than marron ($P < 0.01$, Table 7.8). No significant differences existed between marron and yabbies for locomotion or feeding movements for CONS and HETE ($P > 0.05$).

Table 7.8 Differences between marron and yabbies for the magnitude of change between control water and test solutions for reaction time (s), behaviours and postures (% time)

		FOOD	CONS	HETE
	reaction time	0.070	0.477	0.585
small	in shelter	2.088* SM > SY	0.302	1.683
marron	locomotion	2.991** SM > SY	0.350	0.048
	raised posture	0.000	1.053	0.720
versus	intermediate posture	0.770	1.067	1.599
	lowered posture	0.789	2.058* SY > SM	3.131** SY > SM
small	feeding	2.684** SY > SM	0.225	0.289
yabbies	antennae flicking	2.182* SM > SY	0.803	0.975
	antennule flicking	2.802** SM > SY	0.259	0.767
	reaction time	1.693	1.176	2.726** MY > MM
medium	in shelter	1.462	0.505	0.378
marron	locomotion	1.151	0.754	0.286
	raised posture	1.950	2.682** MY > MM	2.960** MY > MM
versus	intermediate posture	1.974* MY > MM	0.367	1.028
	lowered posture	0.637	0.169	1.742
medium	feeding	3.731** MY > MM	1.502	0.479
yabbies	antennae flicking	2.797** MY > MM	0.640	1.701
	antennule flicking	1.535	1.057	2.439** MM > MY
	reaction time	3.581** LM > LY	1.335	0.053
large	in shelter	0.798	1.031	0.739
marron	locomotion	2.010* LY > LM	1.568	0.111
	raised posture	2.272* LY > LM	2.579* LY > LM	0.476
versus	intermediate posture	0.776	1.736	0.383
	lowered posture	2.210* LY > LM	2.295* LM > LY	0.687
large	feeding	3.721** LY > LM	1.731	0.652
yabbies	antennae flicking	1.236	0.304	1.038
	antennule flicking	2.461* LY > LM	0.241	0.608

Values are Wilcoxon-Mann-Whitney test statistic (Z). Test solutions comprised: (FOOD) 10 mL food odour + 10 mL water; (CONS) 10 mL conspecific odour + 10 mL food odour; (HETE) 10 mL heterospecific odour + 10 mL food odour. SM = small marron, SY = small yabby, MM = medium marron, MY = medium yabby, LM = large marron, LY = large yabby. Species ranked in decreasing order of magnitude of change. *P < 0.05, **P < 0.01.

7.4 Discussion

Behavioural responses of marron and yabbies to alarm odours were first investigated by Gherardi et al. (2002a). Marron used in that trial had a cephalothorax length of 45.3 – 64.6 mm and yabbies 35.1 – 54.1 mm. The present trial also investigated behavioural responses of marron and yabbies to alarm odours, using similar methods, but with two notable differences: (i) crayfish were tested in nocturnal conditions, and (ii) three distinct size grades of crayfish were compared, helping to fill an existing gap in knowledge about size-dependent responses of decapod crustaceans to chemical stimuli.

7.4.1 Crayfish behaviour at night

Earlier studies comparing behavioural responses of marron and yabbies to chemical stimuli (Height and Whisson 2006; this thesis, Chapters 4 and 5) found that marron were more active than yabbies during control periods. In both of those studies, crayfish behavioural responses to chemical stimuli were observed during day-time conditions. In this study crayfish were observed using infra-red goggles at night. The dormant behaviour observed in yabbies during day-time conditions (eg. Height and Whisson 2006; this thesis, Chapters 4 and 5) was not observed in nocturnal conditions employed in this trial. In fact, clear background differences previously observed between the two species were not present. During night-time observations, yabbies spent more time foraging and less time utilising the shelter than they did during day-time observations (Chapters 4 and 5). Marron were also more active at night than during the day. This result was not unexpected because crayfish are nocturnal (Hazlett et al. 1979; Bojsen et al. 1998; Nyström 2005); however, it does bring attention to the differences in energy use between marron and yabbies. During day-time hours, and in the absence of any source of stimulus, yabbies appear to conserve energy and minimise predation risk by becoming inactive and utilising shelter. During night-time hours, yabbies were more active, seemingly to forage. Conversely, marron spent more

time foraging and were more exposed to predation risk than yabbies during day-time hours.

The foraging strategy demonstrated by yabbies appears more conducive to survival than that of marron, because yabbies minimise exposure to visual predators during daylight hours, and forage at night when it is safer to do so. However, in a natural habitat, numerous other factors would influence crayfish behaviour and activity patterns, and therefore energy expenditure and growth, such as the presence of predators; habitat type and complexity; crayfish size and lifestage; food availability; and water depth and quality (Gherardi 2002; Werner and Peacor 2003; Nyström 2005; Height et al. 2006).

7.4.2 Size dependent responses of crayfish to control water

Small marron and yabbies were more active than larger individuals, and spent more time displaying feeding behaviours in the absence of food cues (i.e. in response to control water). These results are likely due to the high energy requirements of juvenile crayfish (Svensson 1993); juveniles moult more often than adult crayfish, and feed to build up energy reserves during intermoult periods (Reynolds 2002). Although increasing movement and foraging behaviour maximises the chances of a crayfish finding food (Acquistapace et al. 2004), it comes at the expense of increased exposure to predators, a well documented trade-off for crayfish (Lima and Dill 1990; Werner and Anholt 1993; Pecor and Hazlett 2006a). It follows that juvenile marron and yabbies would be more susceptible to predation than adult crayfish due to their high exposure to risk (i.e. time spent foraging), and because gape-limited fish predators are known to target smaller-sized prey (Werner and Hall 1974; Stein 1977; Kuhlmann et al. 2008). In contrast, larger crayfish are less likely to be consumed by fish predators (Butler and Stein 1985; DiDonato and Lodge 1993; Elvira et al. 1996) and the greater life experience of these individuals may result in them spending less time foraging in the absence of food cues

7.4.3 Crayfish responses to test solutions

Marron and yabbies showed differences in the quality and intensity of their responses to the test solutions; notably, the response of yabbies to conspecific alarm odour. Whilst all marron increased feeding behaviour to food odour, conspecific odour and heterospecific odour, medium yabbies failed to demonstrate an increase. Additionally, all yabbies increased movement to food odour and heterospecific odour, but not conspecific odour. This is a clear indication that yabbies displayed an alarm response to conspecific alarm odour; other studies have demonstrated that crayfish decrease activity, or cease movement, after detection of alarm substances (Hazlett 1994a; 2000).

In contrast to these results, Gherardi et al. (2002a) reported that marron and yabbies increased feeding behaviour, but did not increase movement after exposure to food, conspecific or heterospecific odours. Height and Whisson (2006) reported increased movement in yabbies and increased feeding in marron and yabbies after exposure to food and predator odour. The lack of movement by crayfish in those trials may be due to the day-time hours in which crayfish behaviour was observed. For example, although crayfish increased feeding behaviour to the cues, they may not have increased movement due to the perception of predation-risk. In the present trial marron and yabbies were both more active in the control periods than in previous trials conducted with these species (Gherardi et al. 2002a; Height and Whisson 2006; this thesis, Chapters 4 and 5), using the same experimental protocol. These results provide further evidence of the inherent differences observed in crayfish behaviour between day-time and night-time.

7.4.3.1 Intraspecific differences to test solutions

Food odour was the most successful test odour at eliciting feeding responses in marron and yabbies. However, all yabbies displayed the same intensity of feeding responses to the three test solutions: food odour was always more successful than

conspecific odour and heterospecific odour in eliciting feeding behaviour. No such trend was apparent in marron, though large marron showed a greater reduction in feeding responses to heterospecific odour than small and medium marron. Crayfish used in this trial were sourced from commercial suppliers that cultured either marron or yabbies. Therefore marron would not have had previous experience with yabbies, or their alarm odour, and *vice versa* for yabbies. These results provide tentative evidence suggesting: (i) marron innately associate conspecific alarm odour with predation risk, but display greater caution to heterospecific alarm odour with age, and (ii) yabbies innately associate conspecific and heterospecific alarm odour with predation risk. However, more research needs to be conducted to confirm these assertions; specifically, the difference in responses to heterospecific alarm odour between size grades of marron.

Gherardi et al. (2002a) reported that marron and yabbies displayed a reduction in feeding responses to conspecific odour and heterospecific odour compared to food odour. As a comparison of size, marron used in that trial were similar to medium marron used in this trial, and the yabbies were similar to large yabbies used in this trial. It is not known if marron and yabbies used by Gherardi et al. (2002) had previous interspecific interaction, thus, the possibility of crayfish demonstrating a 'learned response' to heterospecific alarm odour cannot be excluded. Research on the behavioural responses of native and invasive crayfish to chemical stimuli in the United States and Europe is more advanced than for Australian *Cherax* species, and extensive work has been conducted to explain variability among species and populations. Notwithstanding this, Acquistapace et al. (2004) indicated that further research in those areas is still required to explore the innate or learned basis in the recognition of alarm signals in crayfish, as undertaken for amphibians (e.g. Wildy and Blaustein 2001) and fish (e.g. Pfeiffer 1963; Waldman 1982). In the case of marron and yabbies, it is likely that behavioural modifications by crayfish from mixed-species naturalized populations would differ to those of individuals from aquaculture ponds (such as marron and yabbies used in the present study), as documented for *Procambarus acutus acutus* and *P. clarkii* by Acquistapace et al. (2004).

7.4.3.2 Interspecific differences to test solutions

The interspecific comparison revealed that yabbies demonstrated a stronger feeding response to food odour than marron across all size classes. This result is likely due to the greater activity observed in yabbies during night-time when compared to day-time conditions. An earlier study comparing feeding behaviour of marron and yabbies to food odour (Chapter 4) did not find any differences in the intensity of feeding responses between species to six different concentrations of food cues. Similarly, Gherardi et al. (2002a) did not report any differences in feeding responses of marron and yabbies to food odour.

7.4.4 Concluding remarks and recommendations

Results of this study indicate that the behavioural responses of marron and yabbies to alarm odours are influenced by crayfish size and nocturnal conditions. Small crayfish spent more time foraging than larger individuals, likely due to the higher energy requirements of juvenile crayfish. All sizes of marron and yabbies responded to conspecific alarm odour and all yabbies responded to heterospecific alarm odour. However, a key-finding of this study is that heterospecific alarm odour inhibited feeding behaviour in large marron just as strongly as conspecific alarm odour. This result has not previously been reported for marron. In relation to nocturnal conditions, behavioural differences observed between marron and yabbies during control periods in previous trials (Height and Whisson 2006; this thesis, Chapters 4 and 5) were not observed in this trial, because yabbies were more active at night.

Further research on behavioural responses of marron and yabbies to alarm odour is required to elucidate the nature of the responses displayed by marron in this trial. Furthermore, the results of this trial provide circumstantial evidence suggesting that yabbies reared in the absence of aquatic predators innately respond to conspecific alarm odour. It is plausible that this response extends to heterospecific alarm odour

because it is presumably of a similar chemical nature. Alternatively, these responses may be a consequence of the release of alarm odour during acts of cannibalism in an aquaculture environment, where crayfish associate alarm odours with the danger of being cannibalised (Acquistapace et al. 2004). Similarly, marron may innately recognise conspecific alarm odour, but only develop an association between heterospecific alarm odour and predation risk with age and experience, as demonstrated by large marron in this trial. The only true means to identify innate recognition of alarm odours is to test the responses of crayfish that have been reared in isolation without any prior contact with conspecific or heterospecific crayfish.

Results of this trial confirmed that marron and yabbies can detect and respond to conspecific and heterospecific alarm odour, supporting the findings of Gherardi et al. (2002a). However, the response of small and medium marron to heterospecific alarm odour was not as clear as large marron, which displayed a response appropriate for that cue. In contrast, yabbies demonstrated clearer behavioural modifications to chemical stimuli and all sizes or yabbies responded similarly to food, conspecific and heterospecific alarm odour. Specifically, food odour was more successful than conspecific and heterospecific odour in eliciting a feeding response. Further, yabbies did not increase locomotory movement in the presence of conspecific odour, demonstrating a strong alarm response to this cue. These results provide evidence supporting the assertion that invasive crayfish have a greater capacity for behavioural plasticity than native crayfish.

Chapter eight

Shelter utilisation by crayfish under threat from finfish predators

---

The trial described in this chapter deviated from the traditional aquarium-based 'individual crayfish' approach for odour-detection experiments and tested previous results in a 70 000 L communal observation tank (mesocosm). Shelter use by marron and yabbies under predation threat from silver perch and Murray cod was observed using an underwater video camera. Importantly, this trial moved out of the laboratory and took a step towards the natural habitat by observing crayfish behaviour in a semi-natural multispecies environment.

8.0 Shelter utilisation by crayfish under threat from finfish predators

8.1 Introduction

In aquatic ecosystems, invasive species have been identified as one of the greatest threats to freshwater biodiversity and ecosystem function (Lodge et al. 2000). Freshwater crayfish are well-documented as an invasive species, having been translocated by humans frequently over the recent past, mostly for aquaculture ventures (Gherardi and Holdich 1999, Lodge et al. 2000). In Western Australia, competitive interactions between native marron (*Cherax tenuimanus*) and invasive yabbies (*Cherax albidus*) are not well-understood, particularly in the case of shelter acquisition. Shelter is an important limiting resource for crayfish (Bovbjerg 1970), providing protection against predation and cannibalism (Lodge and Hill 1994, Figler et al. 1999). Inferiority in competition for shelter may lead to increased predation risk, and contribute to species displacement (DiDonato and Lodge 1993, Blank and Figler 1996, Guiasu and Dunham 1999).

While there is evidence that invasive freshwater crayfish can be detrimental to native species (Gherardi and Holdich 1999, Gherardi et al. 2002b), it appears that in at least one large water-body in Western Australia, where displacement had previously been documented (Whisson 2003), the incumbent marron population is expanding in the presence of a well-established yabby population (Campbell and Whisson 2002). This is an unexpected occurrence, and important to the current aquatic translocation debate in Western Australia. It identifies the complex nature of the mechanisms underlying displacement and competitive exclusion in mixed crayfish populations, with available habitat and the use of it a key factor in competitive exclusion (Wangpen 2007). Notwithstanding the findings of Campbell and Whisson (2002), the higher plasticity and greater capacity for survival of yabbies over marron has been well documented (Height and Whisson 2006; this thesis, Chapters 4 and 7), which is of

obvious concern to managers of the native fauna in the south-west of Western Australia.

Studies reported earlier in this thesis have investigated the behavioural responses of crayfish to various chemical substances in controlled laboratory conditions (Chapters 4, 5, 6 and 7). This experiment continues to explore the knowledge gained in laboratory trials using a much larger experimental unit (70,000 L mesocosm observation tank) to gain a closer understanding of interactions between marron, yabbies and fish predators in a natural habitat. Specifically, this trial explored the influence of crayfish size, prior residence, food availability and predation pressure on shelter utilisation and selection by marron and yabbies. *Vallisneria* sp. was used as natural shelter in crayfish cages, as used by crayfish farmers (Swannell 1994; Whisson 2000), along with three different sizes of artificial shelter.

Murray cod (*Maccullochella peelii peelii*) and silver perch (*Bidyanus bidyanus*) were used in the study to represent a significant predatory threat to crayfish. Murray cod have not previously been used in investigations in this thesis, but were chosen for this study because they are one of several predatory fish species under consideration for translocation into Western Australia for commercial aquaculture (FWA 2003). Murray cod are one of the world's largest freshwater fish species (Rowland 1989) and a top-order carnivorous predator and known consumer of freshwater crayfish (McDowall 1996; Ebner 2006).

8.1.1 Aim

The aim of this experiment was to investigate shelter utilisation by marron (*Cherax tenuimanus*) and yabbies (*Cherax albidus*) co-stocked with potential finfish predators.

8.1.2 Objectives

The specific objectives of this study were to:

i. determine the influence of crayfish size on shelter utilisation and selection when under threat from finfish predators;

ii. assess the influence of prior residence on shelter acquisition by marron and yabbies when under threat from finfish predators;

iii. determine if the presence of food influences shelter occupation, or survival, of marron and yabbies when under threat from finfish predators;

iv. compare and contrast interspecific differences in shelter utilisation by marron and yabbies under threat from finfish predators.

8.2 Materials and methods

8.2.1 Site and experiment system

This trial was conducted using the 70,000 L mesocosm (described in Section 3.1.2; Plate 3.3) located at the Curtin Aquatic Research Laboratories (CARL), Bentley, Western Australia. Lighting was provided a 12 h : 12 h, light : dark cycle for the duration of the trial. Sunrise and sunset were simulated between 0700 to 0800 and 1800 to 1900 hours, respectively. To simulate sunrise, one 500 W halogen light from each of the four light banks (described in Section 3.1.2; see Figure 8.1) was switched on at 0700, then a second light from each bank was switched on at 0730, and the remaining light was switched on at 0800. Sunset was simulated by reversing this procedure; the first light from each bank was switched off at 1800, the second at 1830, and the remaining light at 1900, so that the mesocosm was in total darkness.

8.2.2 Experimental design

The experiment comprised four treatment groups as described in Table 8.1. Each treatment was replicated four times, once in each of the four cage locations shown in Figure 8.1.

8.2.3 Experimental procedure

Four mesh cages (described in Section 3.1.2.1) were located on the floor of the mesocosm by a diver (Figure 8.1). Each cage contained three stacks of 12 shelters (25 mm, 50 mm and 90 mm) which were held together by 100 mm cable ties and secured to the base of each cage, and a planted pot (465 mm x 220 mm x 175 mm) containing *Vallisneria* sp*.* (Plate 8.1). Prior resident crayfish were added to each cage (as per treatment allocation schedule, Table 8.1) at 1115 h via a 3 m length of 90 mm PVC pipe inserted through the access slit cut into the top mesh panel. After crayfish were distributed to a cage, the PVC pipe was removed and the access slit on the top

of the cage covered with a 350 x 350 mm piece of Perspex™ held in position by a 110 x 110 x 220 mm clay brick. Therefore on day one of the experiment, each cage contained twelve prior resident crayfish (four each of small, medium and large marron or yabbies). Records of shelter occupation by crayfish commenced at 1300 h and were taken every two hours between 0700 and 1900 h for two days. Aggressive interactions between crayfish were also noted.

Table 8.1 Treatment allocation schedule for the mesocosm trial at CARL

Treatment number	**Description**			
	prior resident	**number**	**intruder**	**number**
Treatment one (T1) unfed	small marron	4	small yabby	4
	medium marron	4	medium yabby	4
	large marron	4	large yabby	4
Treatment two (T2) fed	small marron	4	small yabby	4
	medium marron	4	medium yabby	4
	large marron	4	large yabby	4
Treatment three (T3) unfed	small yabby	4	small marron	4
	medium yabby	4	medium marron	4
	large yabby	4	large marron	4
Treatment four (T4) fed	small yabby	4	small marron	4
	medium yabby	4	medium marron	4
	large yabby	4	large marron	4

Final density of crayfish in each cage was 24/m^3

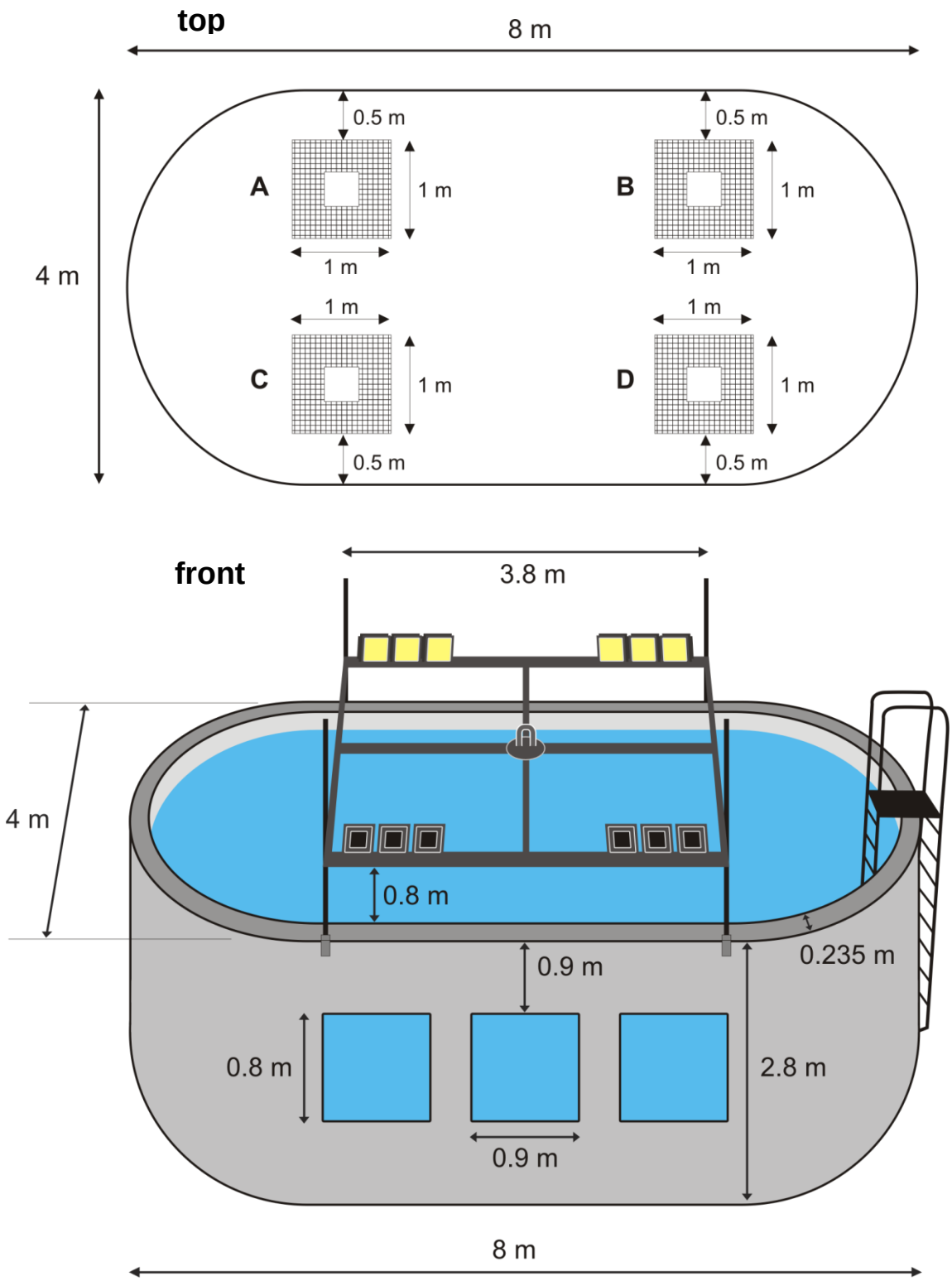

Figure 8.1 Treatment allocation and tank setup for mesocosm trial

A, B, C, D = cage locations on mesocosm floor. Note Perspex™ covers over mesh cages in top view and 4 x 3 light banks fixed above mesocosm in perspective view.

Plate 8.1 Mesh cage used for mesocosm trial at CARL

Shelter sizes were 25 mm, 50 mm, and 90 mm PVC pipe. Note evidence of crayfish feeding on *Vallisneria*.

Fed treatments (T2 and T4) were supplied 20 – 25 g of commercial crayfish pellets (Glen Forrest Stockfeeders™ Pty Ltd, Appendix 5) after the 0700 and 1700 recording periods daily. Crayfish pellets were delivered to cages using a three meter length of PVC pipe to prevent consumption by resident silver perch and Murray cod (see Section 8.2.4.2). Pellets were distributed over the roof panel of the cage and left to sink through the meshes to the bottom of the cage.

After two days, intruding crayfish of a different species were added to each cage (i.e. cages containing marron received yabbies and vice versa) and records of shelter occupation and aggressive interactions continued for another two days. Fed treatments received 40 - 45 g of crayfish pellets after the 0700 and 1700 recording periods daily. Cages were then removed from the mesocosm by a diver and crayfish survival and any loss of appendages were noted. Therefore, at the completion of the

experiment, 28 records of shelter occupation were taken for prior residents (4 days x 7 records taken each day), and 14 records of shelter occupation were taken for intruders. This experimental procedure was then replicated another three times, once in each of the four cage locations shown in Figure 8.1.

8.2.4 Experimental animals

8.2.4.1 Crayfish

Marron used in the trials were sourced from a commercial producer at Manjimup (34.23^{o}S, 116.13^{o}E) and yabbies from a commercial supplier at Kukerin (33.18^{o}S, 118.08^{o}E), Western Australia. Size and weight data for the three size grades (small, medium and large; n = 64 for each grade, male : female ratio = 1 : 1) of both species is provided in Table 8.2. Crayfish were kept in species only tanks at CARL in the crayfish holding systems described in Section 3.2.1 for one month prior to the trials and fed commercial crayfish pellets (Glen Forrest Stockfeeders™, Appendix 5).

Table 8.2 Size and weight data (mean ± standard error) for small, medium and large marron and yabbies used in the mesocosm trial

		carapace length (mm)	**weight (g)**
small	*marron*	68.94 ± 0.50	107.69 ± 0.75
	yabby	39.63 ± 0.42	26.00 ± 0.61
medium	*marron*	73.44 ± 0.68	136.25 ± 1.05
	yabby	46.25 ± 0.21	43.50 ± 0.85
large	*marron*	81.31 ± 0.93	169.56 ± 1.15
	yabby	51.69 ± 0.35	65.38 ± 0.92

One hour prior to stocking crayfish in the mesocosm, three different colours of nail polish were used to paint markings on the chelae and tails of small, medium and large crayfish. These markings were to assist in crayfish identification. To distinguish between males and females, a white dot was also painted on the tail, chelae and carapace of each female.

8.2.4.2 Fish

Approximately 70 silver perch (0.5 – 4 kg) and 10 Murray cod (13 - 16.8 kg) resided in the mesocosm (Plate 8.2) for the duration of the trial. Both species were originally sourced from commercial hatcheries and had been reared in the mesocosm at CARL for over two years. Three 205 L plastic drums used for shelter and spawning sites for the Murray cod were left in the mesocosm during the trial.

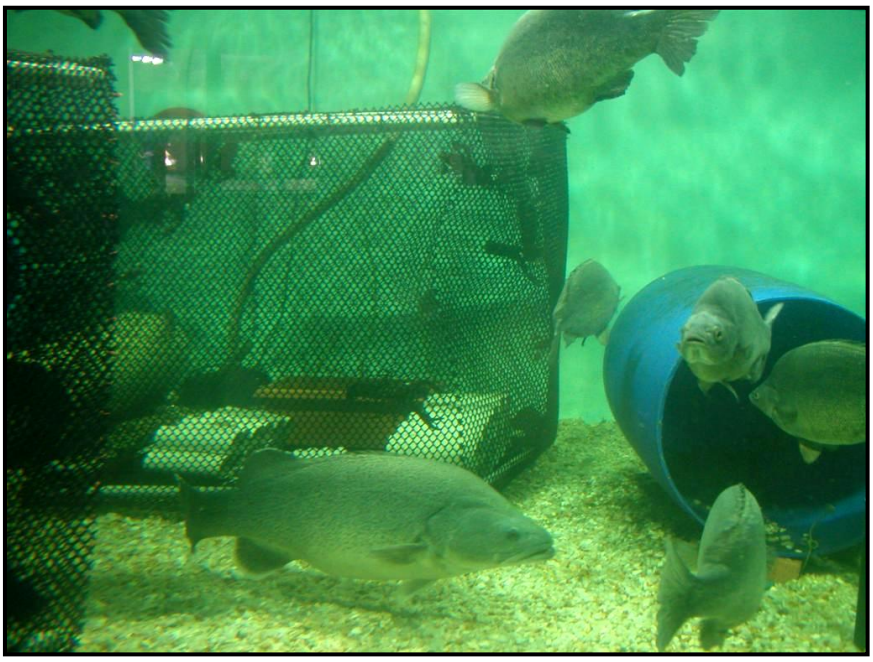

Plate 8.2 Murray cod, silver perch and a cod shelter in the mesocosm at CARL during the trial

8.2.5 Recording procedure

Observations of crayfish shelter occupation were made using a PACOM™ night-vision colour camera fitted with a 3.6 mm lens and infra red ⅓" colour CCD. The camera was attached to a manually operated 4 m boom and connected to a monitor where an observer recorded shelter occupation and any aggressive interactions between crayfish. The boom operator was positioned on the lighting frame above the mesocosm and moved the camera around the perimeter of each cage, following instruction from the observer, so that each bunch of shelters and the *Vallisneria* were inspected for crayfish occupation. It took approximately 30 minutes to record crayfish shelter occupation for all four cages at each recording period.

8.2.6 Water quality monitoring

Total ammonia, salinity, pH, nitrite, nitrate, temperature, dissolved oxygen and conductivity were recorded daily for the mesocosm during each trial. Water quality parameters remained within normal limits for all crayfish and fish (Lawrence and Jones 2002; Rowland 1995; Fisheries Victoria 2008). A summary of these results is provided in Appendix 2; Table 5.

8.2.7 Statistical analysis

Shelter occupation data from this experiment violated the assumptions of normality, therefore nonparametric tests were used in the statistical analysis (Siegel and Castellan 1988; Morgan et al. 2004b). All interspecific comparisons of shelter use between marron and yabbies of the same size and intraspecific comparisons between treatments for crayfish of the same size used the Mann-Whitney test. Comparisons of shelter use by small, medium and large crayfish within treatments used the Kruskal-Wallis test to determine any differences beween size grades and the Mann-Whitney test post-hoc (Morgan et al. 2004b) to rate significant differences between treatment means. Comparisons of shelter occupation between days (one and two versus days three and four) for the same crayfish used the Wilcoxon signed ranks test. Intraspecific comparisons within treatment between shelter types for same size crayfish used the Friedman test and the Wilcoxon signed ranks test post-hoc (Morgan et al. 2004b) to rate significant differences between treatment means. Survival data were analysed using a one-factor ANOVA.

8.3 Results

8.3.1 Background differences in shelter use between marron and yabbies

As prior residents, yabbies occupied the shelter more frequently than marron during the first two days of the experiment (when only prior resident crayfish were present in each cage, $P < 0.05$, Figure 8.2) and after the addition of intruding crayfish (days three and four, $P < 0.05$, Table 8.3). As intruders, yabbies still spent more time in shelter than marron, with this trend strongest in large crayfish ($P < 0.05$).

Table 8.3 Differences in shelter use between marron and yabbies in the presence of fish predators

treatment	**crayfish size**	**Days 3 & 4 (PR + INT)**	
		T1: unfed marron (PR)	T3: unfed yabby (PR)
T1: unfed marron (PR)	small	12.95 ± 3.01[a]	42.86 ± 6.82[b]
versus	medium	30.36 ± 4.87[a]	50.00 ± 5.52[b]
T3: unfed yabby (PR)	large	8.48 ± 4.21[a]	37.95 ± 3.30[b]
		T2: fed marron (PR)	T4: fed yabby (PR)
T2: fed marron (PR)	small	27.23 ± 5.40[a]	49.55 ± 8.94[a]
versus	medium	36.16 ± 5.94[a]	56.70 ± 3.20[b]
T4: fed yabby (PR)	large	8.93 ± 3.24[a]	44.20 ± 2.94[b]
		T3: unfed marron (INT)	T1: unfed yabby (INT)
T3: unfed marron (INT)	small	27.23 ± 8.13[a]	47.77 ± 6.81[a]
versus	medium	27.23 ± 7.30[a]	44.64 ± 5.18[a]
T1: unfed yabby (INT)	large	7.59 ± 1.84[a]	34.48 ± 7.36[b]
		T4: fed marron (INT)	T2: fed yabby (INT)
T4: fed marron (INT)	small	20.54 ± 5.39[a]	51.79 ± 4.58[b]
versus	medium	43.75 ± 4.52[a]	49.55 ± 4.02[a]
T2: fed yabby (INT)	large	4.02 ± 1.71[a]	28.13 ± 6.56[b]

Values are mean time in shelter (%) ± standard error. PR = prior resident; INT = intruder. Interspecific comparison between treatments for same size class used Mann-Whitney test. Values in any row not followed by the same superscript are significantly different at $P < 0.05$.

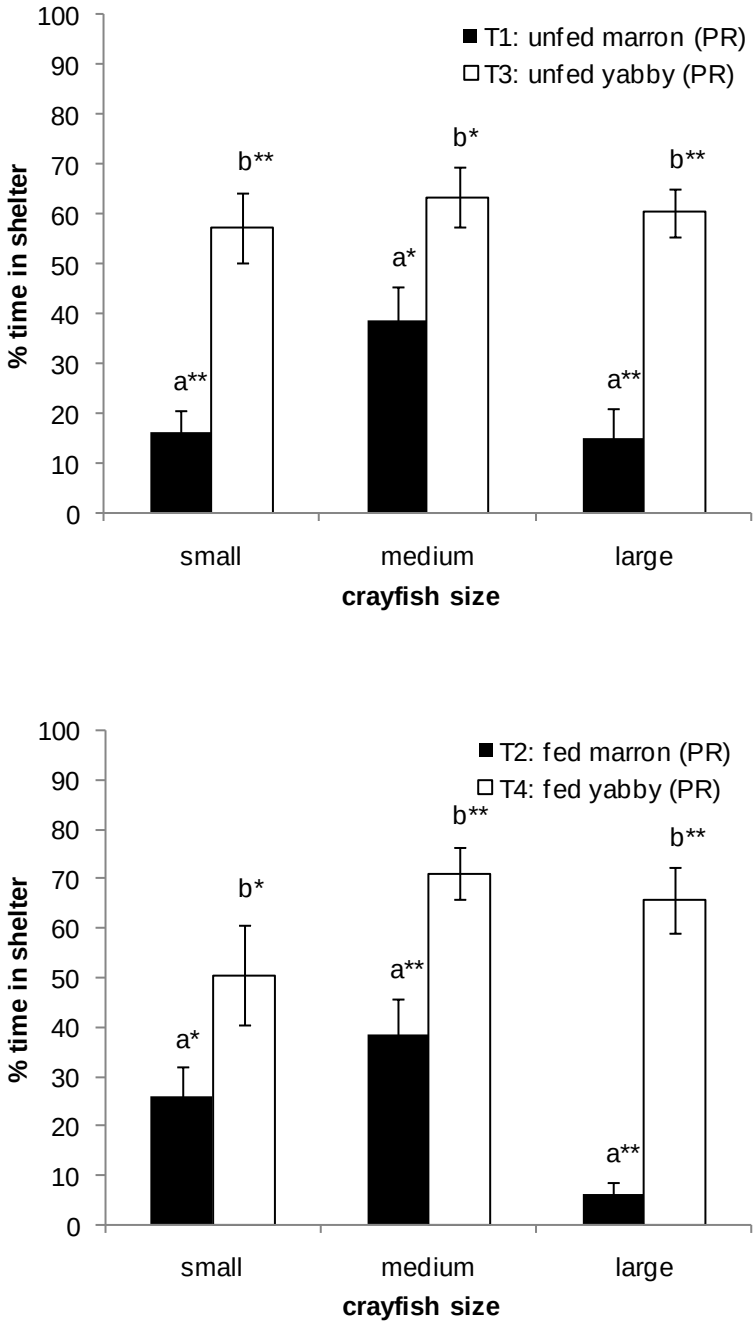

Figure 8.2 Shelter use by prior resident crayfish (day one and two) in the presence of fish predators

Statistical tests for significance were made using the Mann-Whitney test. Different letters for the same size class denote significant differences between species at *P < 0.05, **P < 0.01. Error bars are treatment means ± standard errors.

8.3.2 Influence of crayfish size on shelter use

Crayfish size was not a determining factor in the use of shelter by yabbies ($P > 0.05$, Table 8.4) with the exception of large fed yabbies as intruders ($P < 0.05$, T2). Crayfish size was a determining factor in the use of shelter by marron across all treatments ($P < 0.05$). Regardless of prior resident or intruder status, large marron spent less time in shelter than small and medium marron.

Table 8.4 Intraspecific differences in shelter use by small, medium and large marron and yabbies within treatment groups

treatment	crayfish size	day 1 & 2 (PR)	day 3 & 4 (PR)	day 3 & 4 (INT)
T1	small	16.07 ± 4.63[a]	12.95 ± 3.01[a]	47.77 ± 6.81[a]
unfed marron (PR)	medium	38.39 ± 7.01[b]	30.36 ± 4.87[b]	44.64 ± 5.18[a]
unfed yabby (INT)	large	14.73 ± 6.35[a]	8.48 ± 4.21[a]	34.48 ± 7.36[a]
T2	small	25.89 ± 6.18[a]	27.23 ± 5.40[a]	51.79 ± 4.58[a]
fed marron (PR)	medium	38.39 ± 7.23[a]	36.16 ± 5.94[a]	49.55 ± 4.02[a]
fed yabby (INT)	large	6.25 ± 2.21[b]	8.93 ± 3.24[b]	28.13 ± 6.56[b]
T3	small	57.14 ± 7.08[a]	42.86 ± 6.82[a]	27.23 ± 8.13[ab]
unfed yabby (PR)	medium	63.39 ± 5.95[a]	50.00 ± 5.52[a]	27.23 ± 7.30[b]
unfed marron (INT)	large	60.27 ± 4.84[a]	37.95 ± 3.30[a]	7.59 ± 1.84[a]
T4	small	50.45 ± 10.11[a]	49.55 ± 8.94[a]	20.54 ± 5.39[a]
fed yabby (PR)	medium	70.98 ± 5.20[a]	56.70 ± 3.20[a]	43.75 ± 4.52[b]
fed marron (INT)	large	65.63 ± 6.61[a]	44.20 ± 2.94[a]	4.02 ± 1.71[c]

Values are mean shelter use (%) ± standard error. Intraspecific comparison within treatments between small, medium and large crayfish used Kruskal-Wallis test and Mann-Whitney test post-hoc to determine individual differences between crayfish sizes. Values for each treatment within the same column not followed by the same superscript are significantly different at $P < 0.05$.

8.3.3 Prior resident effect

Shelter occupation by prior resident crayfish generally decreased when intruding crayfish were added to each cage (Table 8.5). However, this result was significant only for unfed large marron ($P < 0.05$, T1), unfed large yabbies ($P < 0.05$, T3) and fed medium yabbies ($P < 0.05$, T4). When compared within species, prior residence did not influence shelter occupation by marron or yabbies ($P > 0.05$, Table 8.6).

Table 8.5 Shelter use by prior resident crayfish alone, and in the presence of intruding crayfish

treatment	crayfish size	day 1 & 2 (PR only)	day 3 & 4 (PR + INT)	direction of change	P-value
T1	small	16.07 ± 4.63	12.95 ± 3.01	-	0.292
unfed marron (PR)	medium	38.39 ± 7.01	30.36 ± 4.87	-	0.262
yabby (INT)	large	14.73 ± 6.35	8.48 ± 4.21	-	0.044*
T2	small	25.89 ± 6.18	27.23 ± 5.40	+	0.623
fed marron (PR)	medium	38.39 ± 7.23	36.16 ± 5.94	-	0.917
yabby (INT)	large	6.25 ± 2.21	8.93 ± 3.24	+	0.491
T3	small	57.14 ± 7.08	42.86 ± 6.82	-	0.206
unfed yabby (PR)	medium	63.39 ± 5.95	50.00 ± 5.52	-	0.058
marron (INT)	large	60.27 ± 4.84	37.95 ± 3.30	-	0.011*
T4	small	50.45 ± 10.11	49.55 ± 8.94	-	0.735
fed yabby (PR)	medium	70.98 ± 5.20	56.70 ± 3.20	-	0.043*
marron (INT)	large	65.63 ± 6.61	44.20 ± 2.94	-	0.050

Values are mean time in shelter (%) ± standard error. PR = prior resident; INT = intruder. Intraspecific comparison of shelter use by PR with and without the presence of INT used Wilcoxon signed ranks test. *Significantly different at $P < 0.05$.

Table 8.6 Intraspecific comparison between prior resident and intruding crayfish

treatment	crayfish size	day 3 & 4		P-value
		T1: marron (PR)	T3: marron (INT)	
T1: unfed marron (PR)	small	12.95 ± 3.01	27.23 ± 8.13	0.328
versus	medium	30.36 ± 4.87	27.23 ± 7.30	0.328
T3: unfed marron (INT)	large	8.48 ± 4.21	7.59 ± 1.84	0.505
		T2: fed marron (PR)	T4: fed marron (INT)	
T2: fed marron (PR)	small	27.23 ± 5.40	20.54 ± 5.39	0.382
versus	medium	36.16 ± 5.94	43.75 ± 4.52	0.645
T4: fed marron (INT)	large	8.93 ± 3.24	4.02 ± 1.71	0.234
		T3: unfed yabby (PR)	T1: unfed yabby (INT)	
T3: unfed yabby (PR)	small	42.86 ± 6.82	47.77 ± 6.81	0.721
versus	medium	50.00 ± 5.52	44.64 ± 5.18	0.442
T1: unfed yabby (INT)	large	37.95 ± 3.30	34.38 ± 7.36	0.721
		T4: fed yabby (PR)	T2: fed yabby (INT)	
T4: fed yabby (PR)	small	49.55 ± 8.94	51.79 ± 4.58	0.645
versus	medium	56.70 ± 3.20	49.55 ± 4.02	0.234
T2: fed yabby (INT)	large	44.20 ± 2.94	28.13 ± 6.56	0.065

Values are mean time in shelter (%) ± standard error. PR = prior resident; INT = intruder. Intraspecific comparison between treatments for PR and INT for same size class used Mann-Whitney test. No significant differences existed at $P < 0.05$.

8.3.4 Fed treatments *versus* unfed treatments

When compared within species, there were no significant differences for shelter occupation between fed and unfed treatments for marron and yabbies ($P > 0.05$, Table 8.7).

Table 8.7 Intraspecific comparison of shelter use between fed and unfed treatments

treatment	crayfish size	day 1 & 2 (PR only)		Days 3 & 4 (PR + INT)	
		T1: unfed marron (PR)	T2: fed marron (PR)	T1: unfed marron (PR)	T2: fed marron (PR)
T1: unfed marron (PR)	small	16.07 ± 4.63[a]	25.89 ± 6.18[a]	12.95 ± 3.01[a]	27.23 ± 5.40[a]
versus	medium	38.39 ± 7.01[a]	38.39 ± 7.23[a]	30.36 ± 4.87[a]	36.16 ± 5.94[a]
T2: fed marron (PR)	large	14.73 ± 6.35[a]	6.25 ± 2.21[a]	8.48 ± 4.21[a]	8.93 ± 3.24[a]
		T3: unfed yabby (PR)	T4: fed yabby (PR)	T3: unfed yabby (PR)	T4: fed yabby (PR)
T3: unfed yabby (PR)	small	57.14 ± 7.08[a]	50.45 ± 10.11[a]	42.86 ± 6.82[a]	49.55 ± 8.94[a]
versus	medium	63.39 ± 5.95[a]	70.98 ± 5.20[a]	50.00 ± 5.52[a]	56.70 ± 3.20[a]
T4: fed yabby (PR)	large	60.27 ± 4.84[a]	65.63 ± 6.61[a]	37.95 ± 3.30[a]	44.20 ± 2.94[a]
				T3: unfed marron (INT)	T4: fed marron (INT)
T3: unfed marron (INT)	small			27.23 ± 8.13[a]	20.54 ± 5.39[a]
versus	medium	n/a		27.23 ± 7.30[a]	43.75 ± 4.52[a]
T4: fed marron (INT)	large			7.59 ± 1.84[a]	4.02 ± 1.71[a]
				T1: unfed yabby (INT)	T2: fed yabby (INT)
T1: unfed yabby (INT)	small			47.77 ± 6.81[a]	51.79 ± 4.58[a]
versus	medium	n/a		44.64 ± 5.18[a]	49.55 ± 4.02[a]
T2: fed yabby (INT)	large			34.48 ± 7.36[a]	28.13 ± 6.56[a]

Values are mean time in shelter (%) ± standard error. PR = prior resident; INT = intruder. Intraspecific comparison between treatments for same size class used Mann-Whitney test. Values in any row for the same two days followed by the same superscript are not significantly different. No significant differences existed at $P < 0.05$.

8.3.5 Crayfish shelter selection

The body size of medium and large marron prevented them from entering the small (25 mm) shelter. Medium marron preferred the large (90 mm) shelters ($P < 0.05$; T2, Table 8.8; T2, Table 8.9). Large marron also utilised the large shelters the most, though this result was not significant because shelter occupation by large marron was infrequent. The presence of yabby intruders did not influence the type of shelter used by marron ($P > 0.05$) with the exception of medium marron spending less time in the large shelters ($P = 0.018$; T1, Table 8.10).

Table 8.8 Shelter selection by prior resident crayfish in the presence of finfish predators

treatment	crayfish size	shelter type			
		Vallisneria	**small**	**medium**	**large**
T1: unfed marron (PR) only	small	0.89 ± 0.52^{a}	0 ± 0^{b}	9.38 ± 4.15^{a}	5.80 ± 1.98^{a}
	medium	8.04 ± 3.05^{a}	0 ± 0^{b}	7.14 ± 4.31^{a}	23.21 ± 5.55^{a}
	large	0.45 ± 0.45^{a}	0 ± 0^{a}	3.13 ± 1.34^{a}	11.16 ± 6.12^{a}
T2: fed marron (PR) only	small	3.13 ± 2.56^{a}	0 ± 0^{a}	11.61 ± 5.72^{a}	11.16 ± 3.67^{a}
	medium	1.34 ± 0.85^{a}	0 ± 0^{a}	5.36 ± 2.82^{a}	31.70 ± 8.06^{b}
	large	1.34 ± 0.85^{a}	0 ± 0^{a}	0.89 ± 0.52^{a}	4.02 ± 1.98^{a}
T3: unfed yabby (PR) only	small	0 ± 0^{a}	14.29 ± 5.50^{ab}	13.84 ± 3.60^{b}	29.02 ± 1.34^{c}
	medium	4.02 ± 1.98^{a}	0.45 ± 0.45^{a}	29.91 ± 2.35^{b}	29.02 ± 6.54^{b}
	large	3.57 ± 1.79^{a}	0.45 ± 0.45^{a}	33.04 ± 5.28^{b}	23.21 ± 5.10^{b}
T4: fed yabby (PR) only	small	0 ± 0^{a}	14.29 ± 7.03^{a}	20.09 ± 8.48^{a}	16.07 ± 3.01^{a}
	medium	8.04 ± 3.96^{a}	0.45 ± 0.45^{a}	25.45 ± 5.12^{b}	37.05 ± 7.62^{b}
	large	0 ± 0^{a}	0 ± 0^{a}	26.34 ± 6.41^{b}	39.29 ± 3.01^{b}

Values are mean time in shelter (%) ± standard error. PR = prior resident; INT = intruder. Intraspecific comparison within treatment between shelter types for same size crayfish used Friedman test and Wilcoxon signed ranks test post-hoc. Values in any row not followed by the same superscript are significantly different at $P < 0.05$.

Medium and large yabbies clearly preferred the medium and large shelter sizes ($P < 0.05$; Table 8.8, Table 8.9) when they were alone, and in the presence of intruding marron. The presence of marron intruders caused a reduction in time spent occupying the large shelters by medium and large yabbies ($P < 0.05$; T3, medium and large yabbies; T4, large yabbies, Table 8.10). In fact, the presence of marron intruders resulted in all yabbies spending less time in the medium and large shelters and more time in the small shelters and *Vallisneria*.

Table 8.9 Shelter selection by prior resident crayfish in the presence of intruding crayfish and finfish predators

treatment	crayfish size	shelter type			
		Vallisneria	**small**	**medium**	**large**
T1	small	0.89 ± 0.89^{a}	0 ± 0^{a}	8.93 ± 3.26^{b}	3.13 ± 2.11^{ab}
unfed marron (PR)	medium	8.93 ± 3.26^{a}	0 ± 0^{b}	11.61 ± 4.16^{a}	9.82 ± 2.13^{a}
with yabby (INT)	large	1.34 ± 1.34^{ab}	0 ± 0^{a}	0.89 ± 0.52^{a}	6.25 ± 3.61^{b}
T2	small	2.23 ± 1.69^{a}	0.89 ± 0.52^{a}	12.50 ± 7.75^{a}	11.61 ± 3.46^{a}
fed marron (PR)	medium	2.68 ± 1.15^{ab}	0 ± 0^{a}	10.27 ± 2.56^{b}	23.21 ± 5.00^{c}
with yabby (INT)	large	2.68 ± 2.68^{ab}	0 ± 0^{a}	0 ± 0^{a}	6.25 ± 1.55^{b}
T3	small	2.23 ± 2.23^{a}	16.07 ± 7.89^{a}	14.73 ± 4.08^{a}	9.82 ± 6.86^{a}
unfed yabby (PR)	medium	5.80 ± 3.60^{a}	3.57 ± 1.93^{a}	26.34 ± 5.94^{b}	14.29 ± 3.86^{ab}
with marron (INT)	large	2.68 ± 1.15^{a}	0.45 ± 0.45^{a}	22.32 ± 6.25^{b}	12.50 ± 3.79^{b}
T4	small	0.89 ± 0.52^{a}	22.77 ± 7.83^{b}	14.29 ± 4.25^{b}	11.61 ± 5.33^{b}
fed yabby (PR)	medium	10.27 ± 2.86^{a}	4.46 ± 1.15^{b}	22.77 ± 2.35^{c}	19.20 ± 3.04^{ac}
with marron (INT)	large	3.57 ± 1.46^{a}	1.34 ± 0.85^{a}	20.98 ± 5.07^{b}	18.30 ± 2.56^{b}

Values are mean time in shelter (%) ± standard error. PR = prior resident; INT = intruder. Intraspecific comparison within treatment between shelter types for same size crayfish used Friedman test and Wilcoxon signed ranks test post-hoc. Values in any row not followed by the same superscript are significantly different at $P < 0.05$.

Table 8.10 Comparison of shelter types occupied by prior resident crayfish alone, and with intruding crayfish

treatment	crayfish size	shelter type	% time in shelter		Direction of change	P-value	treatment	crayfish size	shelter type	% time in shelter		Direction of change	P-value
			PR only	PR with INT						PR only	PR with INT		
T1	small	Vallisneria	0.89 ± 0.52	0.89 ± 0.89		1.000	**T3**	small	Vallisneria	0 ± 0	2.23 ± 2.23	+	0.180
		small	0 ± 0	0 ± 0		1.000			small	14.29 ± 5.50	16.07 ± 7.89	+	1.000
unfed		medium	9.38 ± 4.15	8.93 ± 3.26	-	0.915	**unfed**		medium	13.84 ± 3.60	14.73 ± 4.08	+	0.889
marron		large	5.80 ± 1.98	3.13 ± 2.11	-	0.306	**yabby**		large	29.02 ± 1.34	9.82 ± 6.86	-	0.049*
(PR)	medium	Vallisneria	8.04 ± 3.05	8.93 ± 3.26	+	0.596	**(PR)**	medium	Vallisneria	4.02 ± 1.98	5.80 ± 3.60	+	0.916
		small	0 ± 0	0 ± 0		1.000			small	0.45 ± 0.45	3.57 ± 1.93	+	0.102
		medium	7.14 ± 4.31	11.61 ± 4.16	+	0.173			medium	29.91 ± 2.35	26.34 ± 5.94	-	0.458
		large	23.21 ± 5.55	9.82 ± 2.13	-	0.018*			large	29.02 ± 6.54	14.29 ± 3.86	-	0.017*
unfed	large	Vallisneria	0.45 ± 0.45	1.34 ± 1.34	+	0.655	**unfed**	large	Vallisneria	3.57 ± 1.79	2.68 ± 1.15	-	0.317
yabby		small	0 ± 0	0 ± 0		1.000	**marron**		small	0.45 ± 0.45	0.45 ± 0.45		1.000
(INT)		medium	3.13 ± 1.34	0.89 ± 0.52	-	0.129	**(INT)**		medium	33.04 ± 5.28	22.32 ± 6.25	-	0.046*
		large	11.16 ± 6.12	6.25 ± 3.61	-	0.084			large	23.21 ± 5.10	12.50 ± 3.79	-	0.028*
T2	small	Vallisneria	3.13 ± 2.56	2.23 ± 1.69	-	0.317	**T4**	small	Vallisneria	0 ± 0	0.89 ± 0.52	+	0.157
		small	0 ± 0	0.89 ± 0.52	+	0.157			small	14.29 ± 7.03	22.77 ± 7.83	+	0.183
fed		medium	11.61 ± 5.72	12.50 ± 7.75	+	0.715	**fed**		medium	20.09 ± 8.48	14.29 ± 4.25	-	0.270
marron		large	11.16 ± 3.67	11.61 ± 3.46	+	0.498	**yabby**		large	16.07 ± 3.01	11.61 ± 5.33	-	0.246
(PR)	medium	Vallisneria	1.34 ± 0.85	2.68 ± 1.15	+	0.518	**(PR)**	medium	Vallisneria	8.04 ± 3.96	10.27 ± 2.86	+	0.344
		small	0 ± 0	0 ± 0		1.000			small	0.45 ± 0.45	4.46 ± 1.15	+	0.024*
		medium	5.36 ± 2.82	10.27 ± 2.56	+	0.160			medium	25.45 ± 5.12	22.77 ± 2.35	-	0.398
		large	31.70 ± 8.06	23.21 ± 5.00	-	0.233			large	37.05 ± 7.62	19.20 ± 3.04	-	0.080
fed	large	Vallisneria	1.34 ± 0.85	2.68 ± 2.68	+	0.593	**fed**	large	Vallisneria	0 ± 0	3.57 ± 1.46	+	0.066
yabby		small	0 ± 0	0 ± 0		1.000	**marron**		small	0 ± 0	1.34 ± 0.85	+	0.180
(INT)		medium	0.89 ± 0.52	0 ± 0	-	0.157	**(INT)**		medium	26.34 ± 6.41	20.98 ± 5.07	-	0.446
		large	4.02 ± 1.98	6.25 ± 1.55	+	0.157			large	39.29 ± 3.01	18.30 ± 2.56	-	0.011*

Values are mean time in shelter (%) ± standard error. PR = prior resident; INT = intruder. Intraspecific comparison between PR and PR with INT for each shelter type used Wilcoxon signed ranks test. *P < 0.05.

Yabby intruders spent more time in the medium and large shelters (Table 8.11), but this trend was not as prevalent as when yabbies were prior residents without the presence of heterospecific crayfish (Table 8.8).

Table 8.11 Shelter selection by intruding crayfish in the presence of finfish predators

treatment	crayfish size	shelter type			
		Vallisneria	**small**	**medium**	**large**
T1	small	0.89 ± 0.52^{a}	20.09 ± 11.33^{b}	10.71 ± 3.65^{b}	16.07 ± 5.97^{b}
unfed yabby	medium	7.14 ± 5.41^{a}	2.68 ± 1.55^{b}	16.52 ± 4.80^{a}	18.30 ± 4.46^{a}
(INT) only	large	0.89 ± 0.52^{a}	0 ± 0^{a}	16.96 ± 6.12^{b}	16.52 ± 5.98^{b}
T2	small	0.89 ± 0.52^{a}	36.61 ± 9.52^{b}	8.93 ± 4.06^{a}	5.36 ± 3.65^{a}
fed yabby	medium	8.04 ± 1.55^{a}	1.79 ± 1.79^{b}	25.89 ± 2.36^{c}	13.84 ± 4.27^{ac}
(INT) only	large	2.23 ± 2.23^{ac}	0.45 ± 0.45^{a}	16.52 ± 6.89^{b}	8.93 ± 2.63^{bc}
T3	small	6.25 ± 3.61^{ab}	0.89 ± 0.89^{a}	5.80 ± 2.35^{b}	14.29 ± 6.31^{b}
unfed marron	medium	7.14 ± 4.31^{a}	0 ± 0^{b}	8.48 ± 3.21^{a}	11.61 ± 7.01^{a}
(INT) only	large	0.89 ± 0.52^{ab}	0 ± 0^{a}	1.34 ± 0.85^{a}	5.36 ± 0.73^{b}
T4	small	1.34 ± 0.85^{a}	1.34 ± 0.85^{a}	7.14 ± 1.79^{b}	10.71 ± 4.67^{b}
fed marron	medium	3.57 ± 0.73^{a}	0 ± 0^{b}	18.75 ± 2.78^{ac}	21.43 ± 5.55^{c}
(INT) only	large	0 ± 0^{a}	0 ± 0^{a}	0.89 ± 0.89^{a}	3.13 ± 0.85^{a}

Values are mean time in shelter (%) ± standard error. PR = prior resident; INT = intruder. Intraspecific comparison within treatment between shelter types for same size crayfish used Friedman test and Wilcoxon signed ranks test post-hoc. Values in any row not followed by the same superscript are significantly different at $P < 0.05$.

8.3.6 Survival

Survival was lower in unfed treatments than in fed treatments, and was lowest in small yabbies (Table 8.12), which was attributed to cannibalism from larger crayfish. When cages were removed from the mesocosm to determine survival, in some cases, the only discernible remnants of crayfish were small pieces of chelae. It is not possible to determine if cannibalism occurred as a result of moulting; although no gastroliths were found in the cages, they may have been small enough to fall through the mesh into the mesocosm.

Table 8.12 Mean crayfish survival for the mesocosm experiment conducted at CARL

		T1 unfed marron (PR) unfed yabby (INT)	T2 fed marron (PR) fed yabby (INT)	T3 unfed yabby (PR) unfed marron (INT)	T4 fed yabby (PR) fed marron (INT)
small	*marron*	93.75 ± 6.25	100 ± 0	100 ± 0	100 ± 0
	yabby	81.25 ± 13.15	100 ± 0	75 ± 13.36	87.50 ± 8.18
medium	*marron*	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	yabby	75 ± 16.37	100 ± 0	93.75 ± 6.25	93.75 ± 6.25
large	*marron*	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	yabby	100 ± 0	100 ± 0	100 ± 0	100 ± 0

Values are treatment means ± standard error. No significant differences existed between males and females for each size class ($P > 0.05$). No significant differences were found within or between treatments ($P > 0.05$).

8.3.7 Additional observations

During each recording period general crayfish activity within the cage was observed. Aggressive interactions between crayfish and any other interesting observations were noted. Although these data were not analysed statistically, observations included;

- inter- and intraspecific aggressive interactions between male and female crayfish of all sizes on the cage floor and in/around shelters;
- missing chelae on small crayfish;
- inter- and intraspecific cannibalism;
- intraspecific sharing of shelter between males and females;
- interspecific sharing of shelter;
- crayfish feeding on *Vallisneria* in both fed and unfed treatments (see Plate 8.1);
- two large female marron were berried at the completion of one experiment;
- marron were frequently observed climbing up the sides of the cage and hanging from the roof, this behaviour seldom occurred in yabbies;
- Murray cod and silver perch were frequently observed patrolling around crayfish cages, nipping at crayfish climbing the sides, which in most instances, induced a ‘tail-flip’ response by crayfish.

8.4 Discussion

8.4.1 Shelter utilisation by crayfish

Yabbies in this trial clearly spent more time utilising shelter than marron, a finding that is consistent with results from previous studies (Gherardi et al 2002a; Height and Whisson 2006; this thesis, Chapters 4 and 5). If this situation prevailed in the wild, then marron would be left more exposed to predation than yabbies. This is not surprising as marron have evolved in the relative absence of predators and are a non-burrowing crayfish (Morrissy 1992; Morgan et al. 2002). In contrast, yabbies have evolved in a relatively predator-rich environment and are a burrowing crayfish (Lawrence and Morrissy 2000; Lawrence et al. 2002). It follows that low shelter occupation by marron, reported in several studies, appears to be commensurate with a divergent evolution where the larger cousin was not confronted with many predators and was therefore not required to adapt to survive, in contrast to yabbies. In the present trial, crayfish shelter use was observed in conditions that more closely resemble a natural habitat than in other prior studies, that have largely been conducted in aquariums for ease of observation and odour isolation. Crayfish in the present trial were constantly in the presence of potential finfish predators. Whilst marron do not appear to display behavioural modifications to odour from finfish predators (Height and Whisson 2006), there is no question that the fish biomass present in the mesocosm presented significant visual stimulus to crayfish. Given the presence of a conceivable predatory threat, marron spent a larger proportion of time exposed to the threat than did yabbies. This finding provides strong evidence that in habitats occupied by both marron and yabbies, marron would be more susceptible to predation from finfish predators, even when shelter is abundant.

In Western Australia, marron and yabbies are known to co-exist in water-bodies along with introduced predatory finfish species such as redfin perch (*Perca fluviatilis*) and trout (*Oncorhyncus mykiss* and *Salmo trutta*) (Whisson 2003). A high propensity for shelter occupation by yabbies (Chapters 4, 5 and present study) combined with a

greater level of behavioural plasticity than demonstrated by marron (Height and Whisson 2006) indicate that yabby survival would be high in these habitats along with likely shelter eviction and displacement of native marron. However, the mechanisms that shape crayfish populations are complex and influencing factors such as crayfish body-size, where marron possess a clear advantage over yabbies, are known to play a key role (Vorburger and Ribi 1999; Nakata and Goshima 2003; Height et al. 2006).

8.4.2 Influence of crayfish size on shelter use

Shelter occupation by yabbies was consistently high, irrespective of crayfish size, a result consistent with previous findings (Chapter 5). Shelter use by marron was influenced by crayfish size across all treatment groups; medium marron occupied the shelters most frequently, followed by small and then large marron. This trend is clearly illustrated in Figure 8.1, and suggests that marron have an increasing need for shelter until they reach a certain size, after which, shelter occupation begins to decline. The evolutionary history of marron suggests that shelter occupation is more important during juvenile and sub-adult lifestages, which are preyed on by native fish (e.g. freshwater cobbler, western hardyheads, gobies, mud minnows and pygmy perch; Whisson 2003; Morgan and Beatty 2005), than for adults, which are too large to be consumed by native aquatic predators (Morgan et al. 2002).

Another explanation for low shelter use by large marron is the low behavioural plasticity of this species. Marron do not alter their behaviour in the presence of novel odours (Height and Whisson 2006; this thesis, Chapter 5), and may rely on other cues, such as tactile stimulation, to identify threats. On several occasions Murray cod and silver perch were observed attempting to strike crayfish appendages that ventured outside the cage, which generally induced a tail-flip response from crayfish. In the majority of cases these crayfish were marron - yabbies were rarely observed climbing in the cages. Marron may not respond to predatory threats until the situation becomes critical, such as when tactile stimulation occurs, because they are able to avoid predators through the tail-flip response, utilised by crayfish for rapid escape from

danger (Webb 1979; Bouwma and Hazlett 2001). Similar findings have been documented in other decapods, where prey required strong visual or tactile cues from predators before they demonstrated avoidance responses (Hazlett and McLay 2000; Karplus and Barki 2004).

8.4.3 Food and crayfish survival

Starved crayfish are known to forage when exposed to predators (Svensson 1993; Hazlett 2003) and this may be an explanation for low shelter occupation by large marron. However, this is highly unlikely, because marron in both fed and unfed treatments displayed similar patterns of shelter occupation. Likewise, shelter occupation by yabbies did not differ between fed and unfed treatments.

Although no significant differences existed for crayfish survival between treatments, survival was lower for yabbies in unfed treatments (T1 and T3), particularly for small yabbies. This finding is attributed to cannibalism by larger crayfish, as partially consumed crayfish remnants were found in cages when they were removed from the mesocosm.

8.4.4 Shelter selection by crayfish

Previous studies on shelter preferences of crayfish have identified a correlation between crayfish size and shelter size (Cobb 1969; Wangpen 2007). Wangpen (2007) reported that yabbies preferred to occupy the smallest available shelter relative to their own body size. The results of the present study support this finding to an extent; however, the patterns of shelter selection by yabbies in this trial are not as clear as those reported by Wangpen (2007). This is likely due to differences in experimental design between the two trials. Wangpen (2007) recorded shelter use of yabbies exposed to predator odour in aquariums; crayfish in the present trial were in a communal environment and subject to conceivably greater predation risk. Further,

both Wangpen (2007) and the present trial did not find any correlation between body size and shelter preference for marron.

The presence of marron intruders created a shift in the pattern of shelter selection by yabbies in the present trial. Yabbies spent less time in the medium and large shelters and more time in small shelter and the *Vallisneria* plot. This is an interesting result considering the abundance of medium and large shelters in each cage, and the number of vacant shelters at each recording period. The most likely explanation is that marron were dominant over yabbies due to their size advantage, forcing yabbies to less-favoured habitat. Whisson (2003) suggested that similar mechanisms may be restricting the expansion of the yabby population in some water-bodies in south-western Australia. The advantage of crayfish size in both intra- and interspecific interactions, particularly between native and invasive crayfish, has been documented by a number of researchers (Söderbäck 1995; Vorburger and Ribi 1999; Height et al. 2006). However, the native-invasive crayfish dichotomy is unique in Western Australia because marron possess a body-size advantage over invasive yabbies.

8.4.5 Prior residence

The ‘prior residence effect’ occurs when an initial resident in a geographic area has a social dominance advantage over a subsequent intruder (Braddock 1949). Prior residence did not confer an advantage to crayfish in shelter acquisition in this study. Prior residence has previously been reported as a significant advantage in shelter acquisition and retention between marron and yabbies (Height et al. 2006; Wangpen 2007). In those studies shelter was a limited resource (eg. resident and intruder crayfish were placed in an aquarium with a single shelter), therefore interaction between crayfish was highly likely (Gherardi 2002). Furthermore, Height et al. (2006) reported that crayfish size was more pertinent to shelter acquisition than prior residence, and marron hold an advantage over yabbies in this regard, which may be a major factor limiting population expansion of yabbies in the presence of marron. In the present study, abundant shelter (there were twelve shelters of each size, plus a plot of

Vallisneria) may have diminished the prior residence effect. Although the presence of marron (as both prior residents and intruders) caused a shift in the pattern of shelter selection by yabbies, this influence was weak. In prior resident/intruder models where shelter is a limited resource (i.e. two crayfish, one shelter), the prior resident effect is prominent because submissive crayfish do not gain access to shelter (Nakata and Goshima 2003; Height et al. 2006) and limited availability of crayfish shelter results in clear displays of aggression (Gherardi 2002). Conversely, in the present trial, abundant shelter allowed submissive crayfish to seek shelter elsewhere. For yabbies, this meant an increase in use of the small shelters which were not accessible to marron due to their size.

8.4.6 Concluding remarks

In this trial, shelter occupation by marron and yabbies was observed in a multi-species environment containing potential finfish predators. The results provide evidence that marron are highly susceptible to predation in altered environments due to a lack of shelter utilisation when under threat. There is currently no evidence to support chemical detection or behavioural modification of fish odour by marron (Height and Whisson 2006). There is evidence that marron do respond to visual stimuli; however, they have not been reported as displaying avoidance behaviour (Storer 2005). In the present study, the imposing fish biomass would presumably provide a significant source of chemical and visual stimulus to crayfish. In light of this, marron were only observed exhibiting avoidance behaviour (tail-flipping) upon direct stimulation from finfish, and shelter occupation by large marron was low. Whilst it is not possible to determine from these results whether shelter utilisation by yabbies was due to chemical or visual stimuli from finfish, or due to their underlying nature as a burrowing species, it is clear that in the presence of a predatory threat, yabbies spent more time protected by shelter than did marron. This result provides important evidence - beyond isolated aquarium trials - that yabbies possess higher behavioural plasticity than marron.

Many aquarium-based studies have examined crayfish behaviour in response to chemical stimuli. This trial has taken a significant step toward bridging the knowledge-gap between results gained in laboratory studies and their application in the natural environment. Whilst aquarium-based behavioural studies have found that invasive crayfish species display higher behavioural plasticity than native crayfish, the present trial has confirmed this result, for native marron and invasive yabbies, in a multispecies semi-natural environment.

Further investigations are required to elucidate the complex nature of the mechanisms underlying species displacement and competitive interactions between marron and yabbies in order to better manage native crayfish populations, and in developing strategies to control non-endemic species in natural habitats. Future studies should explore the influence of crayfish lifestage, (i.e. ovigerous females) and limited shelter on competitive interactions between marron and yabbies in a communal environment occupied by fish predators.

Chapter nine

General discussion

This thesis has examined the behavioural responses of marron and yabbies to water-borne odours derived from food, alarm sources and finfish predators. Several trends have been evident in the data sets. This final chapter discusses these points in the context of invasive species management and translocation in Western Australia.

9.0 General discussion

9.1 Introduction

This thesis has examined the behavioural responses of an indigenous crayfish (marron - *Cherax tenuimanus*) and an invasive crayfish (yabby - *Cherax albidus*) present in Western Australia, to water-borne odours derived from food, alarm sources and finfish predators. Behavioural responses of crayfish to various stimuli were studied by isolating individuals in laboratory tanks. While this has been the recent standard for this type of research, two significant improvements in the experimental approach were tested: 1) a higher degree of replication (54 experimental tanks) was implemented to tackle the inherent variability in an approach using individual animals; and 2) observations were made during the night – a commonsense approach when dealing with nocturnal animals.

Further, and perhaps most significantly, this research extended the 'individual crayfish' approach for odour-detection experiments by testing results in a 70,000 L communal observation tank or mesocosm. This was an important development for crayfish behavioural experimentation, particularly as several key findings from the individual crayfish approach were confirmed in a multi-species environment. This is relevant to ecologists studying the behavioural responses of aquatic animals to odours and other dissolved stimuli, as it presents a controlled approach to understanding the complexities of communal systems without the significant limitations of conducting this type of research in the wild.

This research was undertaken to assist understanding of interactions and behavioural differences between indigenous and non-indigenous crayfish in Western Australia. Variables that influence crayfish behaviour were investigated along with crayfish responses to a range of water-borne odours. This final chapter compares the

collective findings with prior research on native and invasive crayfish species and discusses the implications for displacement of native marron in Western Australia.

9.2 Behavioural differences between marron and yabbies

The laboratory trials in this thesis (Chapters 4, 5, and 7) assessed the underlying behavioural differences between marron and yabbies to provide benchmark data for comparison with crayfish behaviour in the presence of chemical stimuli. A number of behavioural differences were evident between the two species.

Marron were more active than yabbies and utilised shelter less frequently during daytime experiments. In a natural habitat, this behaviour by marron would increase the chance of locating food resources; however, they would be left more exposed to predators. From an evolutionary perspective, marron have been the dominant invertebrate in their natural environment and have prevailed in habitats that are relatively predator-free (Morgan et al. 2002), which may have led marron to be less cautious than yabbies. Therefore the introduction of predaceous, non-native finfish such as trout, perch and Murray cod into water-bodies occupied by marron poses a real threat to their survival. Conversely, yabbies have evolved in a predator-rich environment and demonstrate higher shelter utilisation than marron (Chapters 4 and 5); and this is increased when under predation threat (Chapter 8).

9.3 Factors affecting crayfish behaviour

The behavioural responses of marron and yabbies to water-borne odours varied significantly between species. A number of factors that influenced crayfish behaviour were identified, including: crayfish size; time of day (e.g. day versus night); and the origin of the test animals.

9.3.1 Crayfish size

Small marron and yabbies were more active than larger conspecific crayfish (Chapters 5 and 7), concurring with results previously reported for marron (Storer 2005). Juveniles spent more time foraging, likely due to their higher energy requirements, compared to adult crayfish. Although increasing movement and foraging maximises the chances of crayfish finding food (Acquistapace et al. 2004), it also increases predation risk because movement increases crayfish exposure to visual predators, such as fish (Werner and Anholt 1993). The trade-off between foraging and avoiding predation is well documented in crayfish (Werner and Gilliam 1984; Nyström 2002; Pecor and Hazlett 2006a). Predation vulnerability in juvenile crayfish is further compounded by size-selective predation from finfish predators. Several authors have reported that predation intensity increases with decreasing prey size (Stein 1977; Blake and Hart 1993a; Whisson 2000).

Crayfish size influenced shelter utilisation by marron. Large marron clearly spent less time in shelter than smaller conspecific crayfish (Chapters 5 and 8), supporting results from previous research (Storer 2005; Wangpen 2007). This finding is likely due to marron evolving in habitats that are relatively predator free (Morgan et al. 2002). The implications of low shelter occupation by marron will be discussed further in Section 9.5. Shelter use by yabbies was uniform across size grades (Chapters 5 and 8), but lower in juveniles at night (Chapter 7).

9.3.2 Influence of recording time: day vs night

In previous studies comparing behaviour of marron and yabbies, behavioural observations were taken during the day (Gherardi et al. 2002a; Height and Whisson 2006), as done by other researchers in similar crayfish behavioural studies (Hazlett 1994a, 2000; Acquistapace et al. 2004). However, crayfish are most active in the dark (Morrissy and Caputi 1981; Mitchell and Hazlett 1996; Nyström 2005); therefore it would seem commonsense to observe crayfish behaviour at night. In the past, few

studies have adopted this approach, but in more recent times, several ecologists have utilised technologies to observe crayfish in the laboratory and field environment at night (Pecor and Hazlett 2006b; Martin and Moore 2007, 2008).

A major finding of this thesis is that the behavioural differences that existed between marron and yabbies during diurnal hours were not found during night-time observations. That is, yabbies spent just as much time foraging as marron. This finding implies that yabbies conserve energy and minimise their exposure to predation risk from visual predators during daylight, and forage at night when predators are less active (Blake and Hart 1993a). This foraging strategy has been documented in other crayfish species (Flint 1977; Maitland and Campbell 1992; Mitchell and Hazlett 1996) and appears conducive to survival. However, some fish species are known to hunt for prey at night, such as golden perch (Merrick and Schmida 1984), currently under consideration for translocation into Western Australia, and redfin perch (Craig 1978), which are abundant in south-western Australia and predate heavily on marron (Morgan et al. 2002; Molony et al. 2004).

This result has potential ramifications for the relevance of previous trials that have observed crayfish behaviours during daylight hours. Further research is warranted on this subject. The conditioning of aquatic animals by manipulating environmental cues has been successfully applied in aquaculture for out-of-season breeding (e.g. Rowland 1988; Battaglene and Talbot 1992; Rónyai 2007); however, the application of such techniques to behavioural research requires validation. This research has provided evidence that adjusting a crayfish's biological clock to accommodate a researcher's preference for recording time may not be a straight-forward task.

9.3.3 Prior residence

The studies documented in this thesis have not found evidence to suggest that prior residence confers any advantage in shelter acquisition for marron or yabbies when co-stocked (Height et al. 2006, this thesis, Chapter 8). Wangpen (2007) provided

evidence to the contrary, concluding that prior residence was advantageous to crayfish shelter acquisition in aquarium-based laboratory experiments. However, it should be noted that in each of those trials shelter was a limited resource (i.e. resident and intruder crayfish were placed in an aquarium with a single shelter), and there was a lack of size differential between resident and intruder crayfish. These factors, in combination, may have contributed to the prior resident effect, because aggressive interaction between crayfish was imminent due to limited shelter (Gherardi 2002) and the advantage of crayfish size was removed (Figler et al. 1999; Nakata and Goshima 2003). In the study reported in this thesis (Chapter 8), shelter was abundant; therefore the prior residence effect was not prominent. More importantly, Height et al. (2006) reported that crayfish size was of much greater advantage than prior residence in determining shelter contest outcomes between marron and yabbies, supporting similar research on other crayfish species (Figler et al. 1999; Nakata and Goshima 2003).

9.3.4 Origin of crayfish

Marron and yabbies used in all of the experiments documented in this thesis were sourced from aquaculture ponds and were kept in species-only tanks at the Curtin Aquatic Research Laboratories. Therefore crayfish had not previously interacted with other species, and predation risks (excluding cannibalism) were minimised in the culture environment. Acquistapace et al. (2004) suggested that crayfish reared in aquaculture ponds may respond differently to predation risk cues than crayfish from a naturalised population. *Procambarus clarkii* from aquaculture ponds displayed clear feeding-related behaviours in response to conspecific and heterospecific alarm odours (Acquistapace et al. 2004), whereas individuals from a wild population demonstrated an alarm response (Hazlett et al. 2003).

In the study reported in this thesis (Chapter 7), large marron and small, medium and large yabbies displayed an alarm response (suppressed feeding behaviour) to conspecific and heterospecific alarm odours. This result provides evidence that yabbies reared in the absence of aquatic predators (i.e. in aquaculture ponds) innately

respond to conspecific alarm odours. It is possible that this response extends to heterospecific alarm odour because it is presumably of a similar chemical nature. Alternatively, these responses may be a consequence of the release of alarm odour during acts of cannibalism in an aquaculture environment, where crayfish associate alarm odours with the danger of being cannibalised (Acquistapace et al. 2004). Similarly, marron may innately recognise conspecific alarm odour, but only develop a cognitive association between heterospecific alarm odour and predation risk with age and experience (Chapter 7). The ability of marron and yabbies to develop learned associations between chemical substances and predation risk requires further investigation.

9.3.5 Other factors

Numerous variables are likely to affect crayfish behaviour but were outside the scope of the present investigation. These include: health and nutritional status; life stage (e.g. ovigerous females); habitat type and complexity; and water depth, temperature, quality and flow conditions (e.g. lentic versus lotic habitats).

9.4 Detection of water-borne odours by marron and yabbies

Both crayfish species detected and responded to food (Chapter 4), conspecific and heterospecific odours (Chapter 7), confirming results first published by Gherardi et al. (2002a). Strong evidence now exists that marron do not associate fish odour with predation risk (Storer 2005; Height and Whisson 2006; this thesis, Chapter 5). Marron used in experiments in this thesis, and in the study reported by Storer (2005), were sourced from aquaculture ponds, and were exposed to odour from a novel species, silver perch (*Bidyanus bidyanus*). Height and Whisson (2006) observed behavioural responses of marron to odour from an introduced predator (redfin perch, *Perca fluviatilis*) and a native predator (freshwater cobbler, *Tandanus bostocki*). Surprisingly, marron in this experiment did not display alarm responses to either of these species, despite co-existence with predatory cobbler in its natural environment; and more

recently with redfin perch for over one hundred years. Importantly, marron used by Height and Whisson (2006) were wild animals from a water-body containing both species of fish predator (Whisson 2003). This provides evidence that (i) marron do not innately respond to predatory fish odour, and (ii) marron do not develop learned responses to predatory fish odour. Other researchers have reported that crayfish associated a novel odour with predation risk after they were exposed to novel odour and conspecific alarm odour simultaneously (Hazlett et al. 2002; Acquistapace et al. 2003; Pecor and Hazlett 2006a). Consequently, when later presented with the novel odour only, crayfish displayed avoidance behaviours. In the case of marron, the possibility of developing an association between odour from a novel predator and predation risk seems highly unlikely considering marron did not display avoidance responses to odour from a predator they evolved with (Height and Whisson 2006), and the low behavioural plasticity of the species (Gherardi et al. 2002a; Height and Whisson 2006; this thesis, Chapters 4, 5 and 7).

However, there are a number of explanations that may be offered for marron failing to display avoidance responses to fish odour. Predation risk cues such as visual and tactile stimuli were absent in laboratory trials (Storer 2005; Height and Whisson 2006; this thesis, Chapter 5). Previous studies on decapods have found that although odours may be detected, avoidance responses only become apparent when chemical stimuli are presented in conjunction with visual or tactile predator cues (Hazlett and McLay 2000; Karplus and Barki 2004). Further, crayfish can display physiological responses to predatory stress without displaying recognisable changes in behaviour (Schapker et al. 2002). Marron may have been aware of a predatory threat, but not modified their behaviour due to the absence of visual and tactile predator cues (Storer 2005; Height and Whisson 2006; this thesis, Chapter 5). This assertion is supported by the tail-flip response (Webb 1979), a strong predator avoidance strategy which allows crayfish to maintain normal activity until tactile stimuli are engaged (Hazlett 1999; Bouwma and Hazlett 2001).

Yabbies from aquaculture ponds have demonstrated responses to predator odour regardless of prior experience (Height and Whisson 2006; Chapter 6), suggesting either a higher cognitive learning associated with predation risk, or an innate ability to detect chemical stimuli from predators, both characteristics have been found in other crayfish species (Appelberg et al. 1993; Hazlett et al. 2002; Acquistapace et al. 2003; Hazlett 2003). Further, other researchers have demonstrated the ability of invasive crayfish to learn, and associate, novel odours with predation risk, and retain this learned association for extended periods (Hazlett et al. 2002). However, such research on the learning ability of Australian *Cherax* species is yet to be conducted. The responses observed in yabbies may not be because they associate predator odour with predation risk; but rather may be cautionary behaviour in the presence of unidentified stimuli.

9.4.1 Behavioural plasticity

A number of authors have reported invasive crayfish displaying greater behavioural plasticity than native species (Gherardi et al. 2002a; Hazlett et al. 2002, 2003); that is, invasive species make more appropriate use of a wider range of ecological information than native species (Gherardi et al. 2002a; Hazlett et al. 2003). For example, *P. clarkii*, *Orconectes limosus and Orconectes rusticus* make appropriate use of alarm odours from congeneric crayfish, yet native species such as *Orconectes propinquus* and *Austropotomobius pallipes* were only able to utilise alarm odour from heterospecific crayfish (Hazlett 2000; Hazlett et al. 2003). Thus, invasive species can utilise a broader range of cues signalling potential danger than native species. There is speculation that this behavioural attribute, high behavioural plasticity, is an important factor contributing to successful establishment of the invader in a foreign environment (Gherardi et al. 2000b; Hazlett 2000), because when entering new habitats, the ability to utilise cues associated with elevated predation risk confers obvious benefits to survival (Hazlett et al. 2002).

In the case of marron and yabbies, Gherardi et al. (2002a) found that yabbies displayed higher behavioural plasticity than marron in response to heterospecific alarm odour. Research documented in this thesis supported this finding within three different sizes of yabbies (Chapter 7) but with one notable exception: large marron responded just as strongly to heterospecific alarm odour as they did to conspecific alarm odour. Notwithstanding this, compared to marron, yabbies have demonstrated more efficient utilisation of food cues (Chapter 4), detection of predator odour (Height and Whisson 2006; this thesis, Chapter 6), and higher shelter occupation in the presence of predatory threat (Chapter 8). These findings support the hypothesis that invasive species display greater behavioural plasticity than native species. Although the experiments documented in this thesis have found evidence of higher plasticity in yabbies than in marron, further investigations between these species are recommended to assess other aspects of plasticity. Specifically, the ability of marron and yabbies to learn about cues associated with predation risk, and their memory capabilities should be investigated, as undertaken for other pairs of native and invasive crayfish (Hazlett et al. 2002; Acquistapace et al. 2003).

9.4.2 Variability in crayfish behaviour

Crayfish behaviour varies among species (Hazlett and Schoolmaster 1998; Hazlett et al. 2003; Nyström 2005) and among populations (Acquistapace et al. 2004; Pecor and Hazlett 2006b). Variability in the behaviour of yabbies was evident in the experiments documented in this thesis. In the trial reported in Chapter 5, yabbies did not show an observable response to silver perch odour, yet in the following experiment (Chapter 6), perch odour clearly inhibited feeding behaviour. Likewise, Height and Whisson (2006) found that yabbies responded to redfin perch and cobbler odours. All three studies (Height and Whisson 2006; this thesis, Chapter 5 and 6) employed the same experimental protocol and used yabbies from aquaculture ponds that were not experienced with fish predators. Thus, the lack of response from yabbies to silver perch odour in Chapter 5 is difficult to explain and warrants further investigation.

Other researchers have attempted to explain the variability in crayfish responses to chemical stimuli, particularly when crayfish are simultaneously presented with two conflicting stimuli (Bouwma and Hazlett 2001; Acquistapace et al. 2004; Pecor and Hazlett 2006a), such as food and predation risk cues. Under these circumstances, crayfish may display a feeding response, anti-predator response, or a combination of both (Bouwma and Hazlett 2001; Pecor and Hazlett 2006a). Furthermore, the crayfish behavioural response can be influenced by a number of factors, such as hunger (Hazlett 2003), cue concentration (Hazlett 1999), prior experience (Acquistapace et al. 2004) and water-flow conditions (Pecor and Hazlett 2006b). In the trial detailed in Chapter 5, yabby responses to food odour (i.e. feeding behaviour and increased movement) appeared to dominate responses to perch odour (i.e. inhibition of feeding behaviour and decreased movement). Further investigation on the influence of odour concentration on behavioural responses of marron and yabbies is warranted. Whilst Chapter 4 examined feeding responses of marron and yabbies to different concentrations of food odour; to date, no studies have explored the behavioural responses of Australian *Cherax* species when confronted with different concentrations of two conflicting cues (i.e. alarm or predator odour in conjunction with food odour).

9.5 Implications for species displacement

9.5.1 Competitive advantages of yabbies

Yabbies are considered an invasive species in Western Australia (Morrissy and Cassells 1992; Height and Whisson 2006) and threaten native marron populations (Molony et al. 2002). Yabby populations have been recorded in a number of water-bodies in the south-west of Western Australia that are also known to contain marron (Molony and Bird 2002; Whisson 2003). Currently, the impact of yabbies on native marron populations is unclear. Both species have similar environmental requirements (Morrissy et al. 1990), but yabbies appear to possess a number of competitive advantages over marron:

- the ability to reproduce at an early age and size (Lawrence et al. 2002);

- tolerance of higher temperatures and lower dissolved oxygen levels (Lawrence and Jones 2002);
- more aggressive behaviour (Morrissy et al. 1990; Mills et al. 1994);
- the ability to burrow to escape drought (Lawrence et al. 2002); and,
- higher behavioural plasticity (Gherardi et al. 2002a; Height and Whisson 2006; this thesis, Chapters 4, 6, 7 and 8).

The experiments detailed in this thesis focussed on the behavioural responses of marron and yabbies to water-borne odours. Whilst these investigations revealed evidence of higher behavioural plasticity in yabbies (Chapters 4, 6, 7 and 8), the extent to which this ability might facilitate successful invasion is difficult to ascertain, because plasticity is only one of many factors that can contribute to the success of a species in new habitats. Therefore caution should be taken when extrapolating laboratory results to the field; and for this reason, the research in this thesis moved a step closer to the natural environment; crayfish behaviour was observed in a semi-natural multispecies setting to gain results more applicable to the wild (Chapter 8).

9.5.2 Factors Influencing competitive interactions

Although there is evidence that invasive freshwater crayfish can be detrimental to native species (Gherardi and Holdich 1999, Gherardi et al. 2002b), it appears that in some water-bodies in Western Australia, where displacement had previously been documented (Whisson 2003), marron populations are expanding in the presence of well-established yabby populations (Campbell and Whisson 2002), although the reasons are unclear. The mechanisms that influence such competitive interactions and subsequent displacement of crayfish populations are complex, and many influencing factors have been identified:

- the availability of resources (Guiasu and Dunham 1999);
- habitat type and complexity (Molony and Bird 2005); and,
- the presence of predators (Butler and Stein 1985; Nyström 2002; Kuhlmann et al. 2008).

However, one of the main factors that may be limiting the expansion of yabby populations into habitats occupied by marron is the body-size advantage of marron (Height et al. 2006).

Crayfish body-size is perhaps the most important factor in determining the outcome of aggressive interactions between crayfish (Vorburger and Ribi 1999; Nyström 2002; Nakata and Goshima 2003). The size advantage marron possess over yabbies may assist in shelter acquisition and retention during more delicate lifestages such as spawning, moulting, or while females are berried or brooding hatched progeny. In habitats where shelter is limited, competitive exclusion of yabbies by marron would result in yabbies being left more exposed to predation risk. The mesocosm experiment (Chapter 8) provided some evidence that this may actually occur; mortality of small and medium yabbies was considerably higher (although not statistically significant) than of small and medium marron.

9.6 Concluding remarks

This thesis investigated the behavioural responses of native marron and invasive yabbies to food, alarm and predator odours. Over the course of this research, two important advances were made to traditional laboratory-based crayfish behavioural studies:

1. a higher degree of replication was implemented to tackle variability using a Latin Cube design;
2. observations were made at night using specialised night-vision equipment.

Results supported the hypothesis that yabbies possess greater behavioural plasticity than marron, and this was tested in response to odours and predation risk. Most importantly, this key finding was confirmed in a multispecies semi-natural environment (mesocosm).

The low behavioural plasticity of marron is an obvious concern in the context of native species conservation; yabbies are better equipped to process ecological information and subsequently alter their behaviour in the presence of predation risk. This information should be taken into account when considering future aquatic species translocations in Western Australia.

References

References

Abrahamsson, S.A.A. 1966. Dynamics of an isolated population of the crayfish *Astacus astacus* Linné. *Oikos* 17: 96-107.

Acquistapace, P., Calamai, L., Hazlett, B.A. and Gherardi, F. 2005. Source of alarm substances in crayfish and their preliminary chemical characterization. *Canadian Journal of Zoology* 83: 1624-1630.

Acquistapace, P., Daniels, W.H., and Gherardi, F. 2004. Behavioural responses to “alarm odours” in potentially invasive and non-invasive crayfish species from aquaculture ponds. *Behaviour* 141: 691-702.

Acquistapace, A., Hazlett, B.A., and Gherardi, F. 2003. Unsuccessful predation and learning of predator cues by crayfish. *Journal of Crustacean Biology* 23: 364-370.

Ahvenharju, T. and Ruohonen, K. 2007. Agonistic behaviour of signal crayfish (*Pacifastacus leniusculus* Dana) in different social environments: Effect of size heterogeneity on growth and food intake. *Aquaculture* 271: 307–318.

Allan, D.J. and Flecker, A.S. 1993. Biodiversity conservation in running waters: identifying the major factors that threaten destruction of riverine species and ecosystems. *BioScience* 43: 32-43.

Allen, G.R., Midgley, S.H. and Allen, M. 2002. *Field Guide to the Freshwater Fishes of Australia.* Western Australian Museum, Perth.

Ameyaw-Akumfi, C. 1976. Some aspects of the breeding biology of the crayfish. PhD Thesis, University of Michigan, Ann Arbor, Michigan.

Ameyaw-Akumfi, C. 1977. Feeding chemoreceptor sites in the crayfish *Procambarus clarkii* (Girard). *Crustaceana* 33: 259-64.

Appelberg, M. 1990. Population regulation of the crayfish *Astacus astacus* L. after liming an oligotrophic, low-alkaline forest lake. *Limnologica* 21: 319-327.

Appelberg, M. and Odelström, T. 1988. Interaction between European perch (*Perca fluviatilis*) and juvenile *Pacifastacus leniusculus* (Dana) in a pond experiment. *Freshwater Crayfish VII*: 37-45.

Appelberg, M., Söderback, B. and Odelström, T. 1993. Predator detection and perception of predation risk in the crayfish *Astacus astacus. Nordic Journal of Freshwater Research* 68: 55-62.

Aquiloni, L. and Gherardi, F. 2008. Assessing mate size in the red swamp crayfish *Procambarus clarkii*: effects of visual versus chemical stimuli. *Freshwater Biology* 53: 461-469.

Astbury, C. 2004. A draft policy for the translocation of brown trout (S*almo trutta*) and rainbow trout (O*ncorhynchus mykiss*) into and within Western Australia, for the purposes of recreational stocking, domestic stocking, commercial and non-commercial aquaculture. Fisheries Management Paper No. 179. Perth, Western Australia.

Austin, C.M. and Knott, B. 1996. Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in south-western Australia: electrophoretic, morphological and habitat variation. *Australian Journal of Zoology* 44: 223–258.

Austin, C.M. and Ryan, S.G. 2002. Allozyme evidence for a new species of freshwater crayfish of the genus *Cherax* Erichson (Decapoda:Parastacidae) from the south-west of Western Australia. *Invertebrate Systematics* 16: 357–367.

Barbaresi, S. and Gherardi, F. 2001. Daily activity in the white-clawed crayfish, *Austropotamobius pallipes*: a comparison between field and laboratory studies. *Journal of Natural History* 35: 1861-1871.

Barbaresi, S., Santini, G., Tricarico, E. and Gherardi, F. 2004. Ranging behaviour of the invasive crayfish, *Procambarus clarkii* (Girard). *Journal of Natural History* 38: 2821-2832.

Battaglene, S.C. and Talbot, R.B. 1992. Induced spawning and larval rearing of snapper, *Pagrus auratus* (Pisces: Sparidae), from Australian waters. *New Zealand Journal of Marine and Freshwater Research* 26: 179-183.

Beatty, S.J. 2006. The diet and trophic positions of translocated, sympatric populations of *Cherax destructor* and *Cherax cainii* in the Hutt River, Western Australia: evidence of resource overlap. *Marine and Freshwater Research* 57: 825–835.

Beatty, S.J., Morgan, D.L. and Gill, H.S. 2005a. Life history and reproductive biology of the gilgie, *Cherax quinquecarinatus*, A freshwater crayfish endemic to southwestern Australia. *Journal of Crustacean Biology* 25: 251–262.

Beatty, S.J., Morgan, D.L. and Gill, H.S. 2005b. Role of life history strategy in the colonisation of Western Australian aquatic systems by the introduced crayfish *Cherax destructor* Clark, 1936. *Hydrobiologia* 549: 219–237.

Beatty, S.J., Morgan, D.L. and Gill, H.S. 2003. The reproductive biology of the large freshwater crayfish *Cherax cainii* in south-western Australia. *Marine and Freshwater Research* 54: 597–608.

Bechler, D.L., Deng, X. and McDonald, B. 1988. Interspecific communication between sympatric crayfish of the genus *Procambarus* (Decapoda, Astacidae). *Crustaceana* 54: 153-162.

Blake, M.A. and Hart, P.J.B. 1993a. Habitat preferences and survival of juvenile signal crayfish, *Pacifastacus leniusculus* - the influence of water depth, substratum, predatory fish and gravid female crayfish. *Freshwater Crayfish* 9: 318-332.

Blake, M.A. and Hart, P.J.B. 1993b. The behavioural responses of juvenile signal crayfish *Pacifastacus leniusculus* to stimuli from perch and eels. *Freshwater Biology* 29: 89-97.

Blake, M.A. and Hart, P.J.B. 1995. The vulnerability of juvenile signal crayfish to perch and eel predation. *Freshwater Biology* 33: 233-244.

Blank, G.S. and Figler, M.H. 1996. Interspecific shelter competition between the sympatric crayfish species *Procambarus clarkii* (Girard) and *Procambarus zonangulus* (Hobbs and Hobbs). *Journal of Crustacean Biology* 16: 300-309.

Bojsen, B.H., Witthofft, H., Styrishave, B. and Andersen, O. 1998. *In situ* studies on heart rate and locomotor activity in the freshwater crayfish, *Astacus astacus* (L.) in relation to natural fluctuations in temperature and light intensity. *Freshwater Biology* 39: 455-465.

Bouwma, P. and Hazlett, B.A. 2001. Integration of multiple predator cues by the crayfish *Orconectes propinquus. Animal Behaviour* 61: 771-776.

Bovbjerg, R.V. 1956. Some factors affecting aggressive behaviour in crayfish. *Physiological Zoology* 29: 127-136.

Bovbjerg, R.V. 1970. Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). *Ecology* 51: 225-236.

Braddock, J.C. 1949. The effect of prior residence upon dominance in the fish *Platypoecilus maculates*. *Physical Zoology* 22: 161-169.

Breithaupt, T. and Tautz, J. 1990. The sensitivity of crayfish mechanoreceptors to hydrodynamic and acoustic stimuli. In: Holdich, D.M. (ed), *Biology of Freshwater Crayfish* Blackwell Science. Oxford, London, p136.

Butler, M.J. and Stein, R.J. 1985. An analysis of the mechanisms governing species replacements in crayfish. *Oecologia* 66: 168-177.

Caine, E.A. 1975. Feeding and masticatory structures of six species of the crayfish genus *Procambarus* (Decapoda, Astacidea). *Forma et Functio* 8: 49-66.

Campbell, L. and Whisson, G.J. 2002. The changing composition of crayfish at Lake Moyanup, Western Australia: the influence of an invasive congener and three predaceous finfish species (Abstract). Fourteenth Symposium of the International Association of Astacology, Quéretaro, Mexico.

Capelli, G.M. 1975. Distribution, life history, and ecology of crayfish in northern Wisconsin, with emphasis on *Orconectes propinquus* (Girard), PhD Thesis, University of Wisconsin, Madison, Wisconsin.

Carlton, J.T. 1996. Pattern, process, and prediction in marine invasion ecology. *Biological Conservation* 78: 97-106.

Clements, J. 1988. *Salmon at the Antipodes: A history and review of trout, salmon and char and introduced coarse fish in Australasia*. Skipton Eels Pty Ltd.

Cobb, J.S. 1969. The shelter related behaviour of the lobster *Homarus americanus*. *Ecology* 52: 108-115.

Corotto, F.S., McKelvey, M.J., Parvin, E.A., Rogers, J.L. and Williams, J.M. 2007. Behavioural responses of the crayfish *procambarus clarkii* to single chemosensory stimuli. *Journal of Crustacean Biology* 27: 24-29.

Corotto, F.S. and O'Brien, M.R. 2002. Chemosensory stimuli for the walking legs of the crayfish *Procambarus clarkii. Journal of Chemical Ecology* 28: 1117-1130.

Correia, A.M. and Ferreira, O. 1995. Burrowing behaviour of the introduced red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) in Portugal. *Journal of Crustacean Biology* 15: 248-257.

Coy, N.J. 1979. *Freshwater fishing in South-West Australia*, Jabiru Books, Perth.

Craig, J.F. 1978. A study of the food and feeding of perch, *Perca fluviatilis* L., in Windermere. *Freshwater Biology* 8: 59-68.

Cross, T.F. 2000. Genetic implications of translocation and stocking of fish species, with particular reference to Western Australia. *Aquaculture Research* 31: 83-94.

Cukerzis, J.M. 1988. *Astacus astacus* in Europe. In: Holdich, D.M. and Lowery, R.S. (eds), *Freshwater Crayfish: Biology, Management & Exploitation.* Chapman & Hall, London, 309-340.

DeCoursey, P.J. 1983. Biological timing. In: Vernberg, F.J. and Vernberg, W.B. (eds), *Behavior and Ecology. The Biology of Crustacea,* vol. 7. Academic Press, New York, 107-62.

Diamond, J. and Case, T.J. 1986. Overview: introductions, extinctions, exterminations, and invasions. In: Diamond, J. and Case, T.J. (eds), *Community Ecology.* Harper & Row Publishers, New York, 65–79.

DiDonato, G.T. and Lodge, D.M. 1993. Species replacements among *Orconectes* crayfishes in northern Wisconsin lakes: the role of predation by fish. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1484-1488.

Dill, L. M. 1987. Animal decision making and its ecological consequences: the future of aquatic ecology and behavior. *Canadian Journal of Zoology* 65: 803–811.

Disler, N.N and Smirnov, S.A. 1977. Sensory organs of the lateral line canal system in two percids and their importance in behaviour. *Journal of the Fisheries Research of Canada* 34: 1492-1503.

Doupé, R.G., Morgan, D.L., Gill, H.S. and Rowland, A.J. 2004. Introduction of redclaw crayfish *Cherax quadricarinatus* (von Martens) to Lake Kununurra, Ord River, Western Australia: prospects for a 'yabby' in the Kimberley. *Journal of the Royal Society of Western Australia* 87: 187–191.

Dunham, D.W., Ciruna, K.A. and Harvey, H.H. 1997. Chemosensory role of antennules in the behavioral integrations of feeding by the crayfish *Cambarus bartonii. Journal of Crustacean Biology* 17: 27-32.

Ebner, B. 2006. Murray cod an apex predator in the Murray River, Australia. *Ecology of Freshwater Fish* 15: 510–520.

Elvira, B., Nicola, G.G. and Almodovar, A. 1996. Pike and red swamp crayfish: a new case on predator-prey relationship between aliens in central Spain. *Journal of Fish Biology* 48: 437-446.

Evans, L.H. and Edgerton, B.F. 2001. Pathogens, parasites and commensals. In: Holdich D.M. (ed), *Biology of freshwater crayfish*, Blackwell Science, Oxford, London, 377-438.

Federer, W.T. 1991. *Statistics and Society: Data Collection and Interpretation, Second Edition*. Marcel Dekker, New York.

Figler, M.H., Blank, G.S. and Peeke, H.V.S. 2005. Shelter competition between resident male red swamp crayfish *Procambarus clarkii* (Girard), and conspecific intruders varying by sex and reproductive status. *Marine and Freshwater Behavior and Physiology* 34: 237-248.

Figler, M.H., Cheverton, H.M. and Blank, G.S. 1999. Shelter competition in juvenile red swamp crayfish (*Procambarus clarkii*): the influences of sex differences, relative size, and prior residence. *Aquaculture* 178: 63-75.

Fisheries Victoria. 2008. Fishing and Aquaculture, Murray cod. Department of Primary Industries, Fisheries, Victoria. Retrieved: 18 September 2008, from http://www.dpi.vic.gov.au/dpi/nrenfaq.nsf/FID/-064E3723CB9EF7B5CA256C44000E56AD?OpenDocument

Fisheries Western Australia. 2000. Introduced freshwater species in Western Australia. Perth, Western Australia.

Fisheries Western Australia. 2002a. The translocation of brown trout *(Salmo trutta)* and rainbow trout *(Oncorhynchus mykiss)* into and within Western Australia. Fisheries Management Paper No. 156. Perth, Western Australia.

Fisheries Western Australia. 2002b. The introduction and aquaculture of non-endemic species in Western Australia: the 'rotund' yabby *Cherax rotundus* and the all-male hybrid yabby. Fisheries Management Paper No. 159. Perth, Western Australia.

Fisheries Western Australia. 2003. The translocation of Golden Perch, Murray Cod and Australian Bass into and within Western Australia for the purpose of recreational fishing, domestic stocking and commercial and non-commercial aquaculture. Fisheries Management Paper No. 174. Perth, Western Australia.

Flint, R.W. 1977. Seasonal activity, migration and distribution of the crayfish, *Pacifastacus leniusculus*, in Lake Tahoe. *The American Midland Naturalist* 97: 280-292.

Foster, J. and Slater, F.M. 1995. A global review of crayfish predation with observations on the possible loss of *Austropotamobius pallipes* in the Welsh Wye due to crayfish plague. *Freshwater Crayfish* 8: 589-613.

Frutiger, A. and Müller, R. 2002. Controlling unwanted *Procambarus clarkii* populations by fish predation. *Freshwater Crayfish* 13: 309-315.

Garvey, J.E. and Stein, R.A. 1993. Evaluating how chela size influences the invasion potential of an introduced crayfish (*Orconectes rusticus*). *American Midland Naturalist* 129: 172-181.

Garvey, J.E., Rettig, J.E., Stein, R.A., Lodge, D.M. and Klosiewski, S.P. 2003. Scale-dependent associations among fish predation, littoral habitat, and distributions of crayfish species. *Ecology* 84: 3339–3348.

Garvey, J.E., Stein, R.A. and Thomas, H.M. 1994. Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. *Ecology* 75: 532-47.

Gherardi, F. 2002. Behaviour. In: Holdich, D.M. (ed), *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, London, 258-290.

Gherardi, F., Acquistapace, P. and Barbaresi, S. 2000a. The significance of chelae in the agonistic behaviour of the white-clawed crayfish, *Austropotamobius pallipes. Marine and Freshwater Behavior and Physiology* 33: 187-200.

Gherardi, F., Acquistapace, P., Hazlett, B.A. and Whisson, G. 2002a. Behavioural responses to alarm odours in indigenous and non-indigenous crayfish species: a case study from Western Australia. *Marine and Freshwater Research* 53: 93-98.

Gherardi, F. and Acquistapace, P. 2007. Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. *Freshwater Biology* 52: 1249-1259.

Gherardi, F. and Holdich, D.M. 1999. *Crayfish in Europe as Alien Species: how to make the best of a bad situation?*, A.A. Balkema, Rotterdam, Brookfield.

Gherardi, F., Raddi, A., Barbaresi, S. and Salvi, G. 2000b. Life history patterns of the red swamp crayfish *Procambarus clarkii*, in an irrigation ditch in Tuscany, Italy. *Crustacean Issues* 12: 99-108.

Gherardi, F., Smietana, P. and Laurent, P. 2002b. Interactions between non-indigenous and indigenous crayfish species. Roundtable Session 2B. *Bulletin Francais De La Peche Et De La Pisciculture* 367: 899-907.

Giri, T. and Dunham, D.W. 1999. Use of the inner antennule ramus in the localisation of distant food odours by *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae). *Crustaceana* 72: 123-127.

Grasso, F.W. and Basil, J.A. 2002. How lobsters, crayfishes, and crabs locate sources of odour: current perspectives and future directions. *Current Opinion in Neurobiology* 12: 721-727.

Guan, R.Z. and Wiles, P. 1997. The home range of signal crayfish in a British lowland river. *Freshwater Forum* 8: 45-54.

Guiasu, R.C. and Dunham, D.W. 1999. Aggressive interactions between the crayfishes *Cambarus bartonii bartonii* and *C. robustus* (Decapoda: Cambaridae): interspecific and intraspecific contests. *Journal of Crustacean Biology* 19: 131-146.

Hamrin, S. 1987. Seasonal crayfish activity as influenced by fluctuating water levels and presence of a fish predator. *Holarctic Ecology* 10: 45-51.

Hannon, S. 2008. An overview of the impacts of translocated native fish species in Australia. Final Report. Department of the Environment, Water, Heritage and the Arts. Sinclair Knight Merz, Armadale.

Harris, J. 2003. Fish stocking and translocation in the Murray-Darling Basin: issues, benefits and problems. Managing fish translocation and stocking in the Murray-Darling Basin workshop: Statement, recommendations and supporting papers. B. Philips (ed.). Canberra, 25-26 September 2002, WWF-Australia.

Harris, J. H. and Battaglene, S. C. 1990. The introduction and translocation of native freshwater fishes in south-eastern Australia. Introduced and translocated fishes and their ecological effects. Proceedings of the Australian Society for Fish Biology Workshop No. 8. Magnetic Island, Townsville, Queensland 24-25 August 1989, Bureau of Rural Resources. Australian Government Publishing Service, Canberra.

Harris, J.H. and Gehrke, P.C. 1997. Fish and rivers in stress: the NSW rivers survey. New South Wales Fisheries Office of Conservation, Cronulla.

Hartman, A. and O'Neill, D. 1999. Fish predators and conspecifics influence molt location choice by red swamp crayfish, *Procambarus clarkii* (Girard). *Freshwater Crayfish* 12: 244-254.

Hatt, H. and Bauer, U. 1982. Electrophysiological properties of pyridine receptors in the crayfish walking leg. *Journal of Comparative Physiology* 148: 221-224.

Hazlett, B.A. 1985a. Chemical detection of sex and condition in the crayfish *Orconectes virilis. Journal of Chemical Ecology* 11: 181-190.

Hazlett, B.A. 1985b. Disturbance pheromones in the crayfish *Orconectes virilis. Journal of Chemical Ecology* 11: 1695-1711.

Hazlett, B.A. 1989. Additional sources of disturbance pheromone affecting the crayfish *Orconectes virilis. Journal of Chemical Ecology* 15: 381-385.

Hazlett, B.A. 1990. Source and nature of disturbance-chemical system in crayfish. *Journal of Chemical Ecology* 16: 2263-2275.

Hazlett, B.A. 1994a. Alarm responses in the crayfish *Orconectes virilis* and *Orconectes propinquus. Journal of Chemical Ecology* 20: 1525-1535.

Hazlett, B.A. 1994b. Crayfish feeding responses to zebra mussels depend on microorgansims and learning. *Journal of Chemical Ecology* 20: 2623-2630.

Hazlett, B.A. 1999. Responses to multiple chemical cues by the crayfish *Orconectes virilis. Behaviour* 136: 161-177.

Hazlett, B.A. 2000. Information use by an invading species: do invaders respond more to alarm odours than native species? *Biological Invasions* 2: 289-294.

Hazlett, B.A. 2003. The effects of starvation on crayfish responses to alarm odour. *Ethology* 109: 587-592.

Hazlett, B.A., Acquistapace, P. and Gherardi, F. 2002. Differences in memory capabilities in invasive and native crayfish. *Journal of Crustacean Biology* 22: 439-448.

Hazlett, B.A., Acquistapace, P. and Gherardi, F. 2006. Responses of the crayfish *Orconectes virilis* to chemical cues depend upon flow conditions. *Journal of Crustacean Biology* 26: 94-98.

Hazlett, B.A. and McLay, C. 2000. Contingencies in the behaviour of the crab *Heterozius rotundifrons. Animal Behaviour* 59: 965-974.

Hazlett, B.A. and Schoolmaster, D.R. 1998. Responses of cambarid crayfish to predator odour. *Journal of Chemical Ecology* 24: 1757-1770.

Hazlett, B.A., Anderson, F.E., Esman, L.A., Stafford, C. and Munro, E. 1992. Interspecific behavioural ecology of the crayfish *Orconectes rusticus. Journal of Freshwater Ecology* 7: 69-76.

Hazlett, B.A., Burba, B., Gherardi, F. and Acquistapace, P. 2003. Invasive species use a broader range of predation-risk cues than native species. *Biological Invasions* 5: 223-228.

Hazlett, B.A., Rittschof, D. and Ameyaw-Akumfi, C. 1979. Factors affecting the daily movement of the crayfish *Orconectes virilis* (Hagen, 1870) (Decapoda, Cambaridae). *Crustaceana, Supplement* 5: 121-130.

Height, S.G. 2002. Behavioural responses of freshwater crayfish (*Cherax tenuimanus* and *Cherax albidus*) to predatory fish odours. Honours Thesis, Curtin University of Technology, Perth.

Height, S.G. and Whisson, G.J. 2006. Behavioural responses of Australian freshwater crayfish (*Cherax cainii* and *Cherax albidus*) to exotic fish odour. *Australian Journal of Zoology* 54: 399-407.

Height, S.G., Marsh, B., and Whisson, G.J. 2006. The influence of gender, size, life-stage and prior residence on shelter acquisition by marron (*Cherax tenuimanus*) and yabbies (*Cherax albidus*). *Freshwater Crayfish* 15: 79-86.

Hein, C.L., Vander Zanden, M.J. and Magnuson, J.J. 2007. Intensive trapping and increased fish predation cause massive population decline of an invasive crayfish. *Freshwater Biology* 52: 1134–1146.

Henderson, M. 2000. Prey selection by three exotic finfish species in south-west Western Australia with emphasis on native freshwater crayfish. Honours Thesis, Curtin University of Technology, Perth.

Herberholz, J., Sen, M.M. and Edwards, D.H. 2004. Escape behavior and escape circuit activation in juvenile crayfish during prey–predator interactions. *Journal of Experimental Biology* 207: 1855-1863.

Hill, A.M., Sinars, D.M. and Lodge, D.M. 1993. Invasion of an occupied niche by the crayfish *Orconectes rusticus*: potential importance of growth and mortality. *Oecologia* 94: 303-306.

Hobbs, H. 1991. Adaptions and convergence in North American crayfishes. *Freshwater Crayfish* 2: 541-549.

Hobbs, H.H., III, Jass, J.P. and Huner, J.V. 1989. A review of global crayfish introductions with particular emphasis on two north American species (Decapoda, Cambaridae). *Crustaceana* 56: 299-316.

Holdich, D.M. 1999. The negative effects of established introductions. In: Gherardi, F. and Holdich, D.M. (eds), *Crayfish in Europe as Alien Species. How to Make the Best of a Bad Situation?* A.A. Balkema, Rotterdam, 31-48.

Holdich, D.M. (ed) 2002. *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, London.

Holdich, D.M. and Reeve, I.D. 1988. Functional morphology and anatomy. In: Holdich, D.M. and Lowery, R.S. (eds), *Freshwater Crayfish – Biology, Management and Exploitation*, Croom Helm, London, and Timber Press, Portland, 11-51.

Holdich, D.M., Harlioglu, M.M. and Firkins, I. 1997. Salinity adaptations of crayfish in British waters with particular reference to *Austropotamobius pallipes, Astacus leptodactylus* and *Pacifastacus leniusculus*. *Estuarine Coastal Shelf Science* 44: 147-154.

Horwitz, P. 1990. The translocation of freshwater crayfish in Australia: Potential impact, the need for control and global relevance. *Biological Conservation* 54: 291-305.

Horwitz, P. and Knott, B. 1995. The distribution and spread of the yabbie *Cherax destructor* complex in Australia: speculations, hypotheses and the need for research. *Freshwater Crayfish* 10: 81–91.

Howard, R.K. 1988. Fish predators of the western rock lobster (*Panulirus cygnus* George) in a nearshore nursery habitat. *Australian Journal of Marine Freshwater Research* 39: 307-316.

Huner, J.V. 2002. Procambarus. In: Holdich, D.M. (ed), *Biology of freshwater crayfish.* Blackwell Science, Oxford, 541-584.

Huner, J.V. and Barr, J.E. 1984. *Red Swamp Crayfish: Biology and Exploitation.* Lousiana Sea Grant College Program, Center for Wetland Resources, Lousiana State University, Baton Rouge, Lousiana.

Huner, J.V. and Lindqvist, O.V. 1995. Physiological adaptations of freshwater crayfish that permit successful aquacultural enterprises. *American Zoologist* 35: 12-19.

Hutchinson, M.J. 1991. Distribution patterns of redfin perch *Perca fluviatilis* Linnaeus and western pygmy perch *Edelia vittata* Castelnau in the Murray River system Western Australia. *Records of the Western Australian Museum* 15: 295-301.

Jasinska, E.J., Knott, B. and Poulter, N. 1993. Spread of the introduced yabbie, *Cherax* sp. (Crustacea: Decapoda: Parastacidae), beyond the natural range of freshwater crayfishes in Western Australia. *Journal of the Royal Society of Western Australia* 76: 67–69.

Jenkins, C.F.H. 1952. The food of trout in Western Australia. *The West Australian Naturalist* 3: 139-141.

Jones, C. and Ruscoe, I. 1996. *Production technology for redclaw crayfish (Cherax quadricarinatus*), Final report. Project 92/119, Fisheries Research and Development Corporation, Department of Primary Industries, Queensland.

Karplus, I. and Barki, A. 2004. Social control of growth in the redclaw crayfish, *Cherax quadricarinatus*: testing the sensory modalities involved. *Aquaculture* 242: 321–333.

Keller, T.A., Tomba, A.M. and Moore, P.A. 2001. Orientation in complex chemical landscapes: Spatial arrangement of chemical sources influences crayfish food-finding efficiency in artificial streams. *Limnology and Oceanography* 46: 238-247.

Koehn, J. 2004. Priority management actions for alien freshwater fish species in Australia. *New Zealand Journal of Marine and Freshwater Research* 38: 457-472.

Koehn, J., Brumley, A. and Gehrke, P. 2000. Managing the impacts of carp. Bureau of Rural Sciences, Canberra.

Kolar, C.S. and Lodge, D.M. 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* 16: 199-204.

Kreider, J.L. and Watts, S.A. 1998. Behavioural (feeding) responses of the crayfish *Procambarus clarkii* to natural dietary items and common components of formulated crustacean feeds. *Journal of Chemical Ecology* 24: 91-111.

Kuhlmann, M.L., Badylak, S.M. and Carvin, E.L. 2008. Testing the differential predation hypothesis for the invasion of rusty crayfish in a stream community: laboratory and field experiments. *Freshwater Biology* 53: 113-128.

Lake, J.S. 1971. *Freshwater Fishes and Rivers of Australia.* Nelson, Sydney.

Lake, J.S. 1978. *Australian freshwater fishes illustrated: an illustrated field guide*. Nelson, Melbourne.

Lambert, N. 1998. Commercial aquaculture of silver perch', in *Proceedings of the Queensland warmwater aquaculture conference (status and potential)*. Aquaculture Information Technologies, Tarome, Australia, 63-82.

Langdon, J.S. 1987. Active osmoregulation in the Australian bass, *Macquaria novemaculeata* (Steindachner), and the golden perch, *Macquaria ambigua* (Richardson) (Percichthyidae). *Australian Journal of Marine and Freshwater Research* 38: 771-776.

Laurent, P.J. 1988. *Austropotamobius pallipes* and *A. torrentium*, with observations on their interactions with other species in Europe. In: Holdich, D.M. and Lowery, R.S. (eds), Freshwater Crayfish: Management and Exploitation. Croom Helm, London, 341-364.

Lawrence, C. 1993. The introduction and translocation of fish, crustaceans and molluscs in Western Australia. Fisheries Management Paper No. 58. Fisheries Department of Western Australia, Perth, Western Australia.

Lawrence, C. 1995. Silver perch *Bidyanus bidyanus*. Aquaculture WA No. 6, Fisheries Department of Western Australia. Perth, Western Australia.

Lawrence, C.S., Brown, J.I. and Bellanger, J.E. 2002. Morphology and occurrence of yabby (*Cherax albidus* Clark) burrows in Western Australian farm dams. *Freshwater Crayfish* 13: 253-264.

Lawrence, C.S. and Jones, C. 2002. *Cherax.* In: Holdich, D.M. (ed), *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, London, 635-669.

Lawrence, C.S. and Morrissy, N.M. 2000. *Yabby frequently asked questions.* Fisheries Western Australia, Perth.

Lewis, S.D. 2002. *Pacifastacus*. In: Holdich, D.M. (ed), *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, London, 511-540.

Lima, S.L. 1998. Nonlethal effects in the ecology of predator-prey interactions. *BioScience* 48: 25-34.

Lima, S.L. and Bednekoff, P.A. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. American Midland Naturalist 153: 649-659.

Lima, S.L. and Dill, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68: 619–640.

Lindqvist, O.V. and Huner, J.V. 1999. Life history characteristics of crayfish: What makes some of them good colonisers? In: Gherardi, F. and Holdich, D.M. (eds.), Crustacean Issues 11: Crayfish in Europe as Alien Species (How to make the best of a bad situation?) A.A. Balkema, Rotterdam, Netherlands: 23-31.

Lintermans, M. 2004. Human assisted dispersal of alien freshwater fish in Australia. New Zealand Journal of Marine and Freshwater Research 38: 481-501.

Lodge, D.M. and Hill, A.M. 1994. Factors governing species composition, population size, and productivity of cool-water crayfish. *Nordic Journal of Freshwater Research* 69: 111-136.

Lodge, D.M and Lorman, J.G. 1987. Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. *Canadian Journal of Fisheries and Aquatic Sciences* 44: 591-597.

Lodge, D.M., Kershner, M.W., Aloi, J.E. and Covich, A.P. 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. *Ecology* 75: 1265-1281.

Lodge, D., Stein, R.A., Brown, K.M., Covich, A.P., Broenmark, C., Garvey, J.E. and Klosiewski, S.P. 1998. Predicting impact of freshwater exotic species on native biodiversity: challenges in spatial scaling. *Australian Journal of Ecology* 23: 53-67.

Lodge, D.M., Taylor, C.A., Holdich, D.M. and Skurdal, J. 2000. Non-indigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. *Fisheries* 25: 7-20.

Lonsdale, D.J., Frey, M.A., and Snell, T.W. 1998. The role of chemical signals in copepod reproduction. *Journal of Marine Systems* 15: 1-12.

Lowe, S., Browne, M., Boudjelas, S. and De Poorter, M. 2004. *100 of the World's Worst Invasive Alien Species. A selection from the Global Invasive Species Database*. Invasive Species Specialist Group, World Conservation Union, Gland, Switzerland.

Lynas, J., Storey, A., Armstrong, K., Prince, J. and Knott, B. 2006. Invasion by the exotic crayfish, *Cherax destructor* Clark (Parastacidae), into habitats of local crayfish near Perth, Western Australia. *Freshwater Crayfish* 15: 176-188.

Maitland, P.S. and Campbell, R.N. 1992. *Freshwater Fishes*, Harper Collins Publishers, London, UK.

Marchetti, M., Tossani, N., Marchetti, S., and Bauce, G., 1999. Leaching of crystalline and coated vitamins in pelleted and extruded feeds. *Aquaculture* 171: 83–91.

Martin, A.L. and Moore, P.A. 2007. Field observations of agonism in the crayfish, *Orconectes rusticus*: shelter use in a natural environment. *Ethology* 113: 1192-1201.

Martin, A.L. and Moore, P.A. 2008. The influence of dominance on shelter preference and eviction rates in the crayfish, *Orconectes rusticus*. *Ethology* 114: 351-360.

Mather, M.E. and Stein, R.A.S. 1993. Direct and indirect effects of fish predation on the replacement of a native crayfish by an invading congener. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1279-1288.

Mathias, A. and Smith, R.J.F. 1993. Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). *Behavioural Ecology* 4: 260-265.

McCarthy, J.M., Hein, C.L., Olden, J.D. and Vander Zanden, M.J. 2006. Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. *Freshwater Biology* 51: 224–235.

McDowall, R. 1996. *Freshwater fishes of south-eastern Australia*. Sydney, Reed Books.

McMahon, B.R. 2002. Ecology. In: Holdich, D.M. (ed), *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, London, 327-376.

Mead, R. and Currow, N.R. 1983. *Statistical methods in agriculture and experimental biology.* Chapman and Hall, London.

Merkle, E.L. 1969. Home range of crayfish *Orconectes juvenalis. American Midland Naturalist* 81: 228-235.

Merrick, J.R. and Lambert, C.L. 1991. *The yabby, marron and redclaw: production and marketing*. Macarthur Press, Paramatta, Australia.

Merrick, J.R. and Schmida, G.E. 1984. *Australian Freshwater Fishes: Biology and Management.* Griffin, Adelaide.

Mills, B.J., Morrissy, N.M. and Huner, J.V. 1994. Cultivation of freshwater crayfishes in Australia. In: Huner, J.V. (ed), Freshwater crayfish aquaculture in North America, Europe and Australia: families Astacidae, Cambaridae and Parastacidae. Food Products Press, New York, 217-289.

Ministerial Council on Forestry, Fisheries and Aquaculture 1999. National policy for the translocation of live aquatic organisms- issues, principles and guidelines for implemantation. Canberra.

Mitchell, B. and Hazlett, B.A. 1996. Predator avoidance strategies of the crayfish *Orconectes virilis. Crustaceana* 69: 400-412.

Molony, B.W. and Bird, C. 2002. Annual report on the monitoring of the recreational marron fishery 2000, with an analysis of long term data and changes within this fishery. Fisheries Western Australia, Report No. 137, Perth.

Molony, B.W., Bird, C. and Nguyen, V. P. 2004. The relative efficacy of stocking fry or yearling rainbow trout (*Oncorhynchus mykiss*) into a large impoundment dominated by redfin perch (*Perca fluviatilis*) in southwestern Australia. *Marine and Freshwater Research* 55: 781–785.

Molony, B.W., Jones, B., Lawrence, C.S. and Gouteff, V.A. 2006. *Cherax tenuimanus* Smith, 1912 (Crustacea, Decapoda, PARASTACIDAE): proposed conservation of usage of the specific name. *Bulletin of Zoological Nomencalture* 63: 231–235.

Molony, B.W., Morrissy, N.M. and Bird, C. 2002. The West Australian recreational marron fishery (*Cherax tenuimanus* (Smith)): history and future challenges. Freshwater Crayfish 13: 207-220.

Momot, W.T. 1988. *Orconectes* in North America and elsewhere. In: Holdich, D.M. and Lowery, R.S. (eds), *Freshwater Crayfish: Biology, Management and Exploitation*. Croom Helm, London, 262-282.

Momot, W.T. 1993. The role of exploitation in altering the processes regulating crayfish populations. *Freshwater Crayfish* 9: 101-117.

Momot, W.T. 1995. Redefining the role of freshwater crayfish in aquatic ecosystems. *Reviews in Fisheries Science* 3: 33–63.

Momot, W.T. and Leering, G.M. 1986. Aggressive interaction between *Pacifastacus leniusculus* and *Orconectes virilis* under laboratory conditions. *Freshwater Crayfish* 6: 87-93.

Moore, P.A. and Grills, J.L. 1999. Chemical orientation to food by the crayfish *Orconectes rusticus*: influence of hydrodynamics. *Animal Behaviour* 58: 953-963.

Morgan, D.L. 2004. Native freshwater fishes of south-western Australia. Fisheries Department of Western Australia. Perth, Western Australia.

Morgan, D.L. and Beatty, S. 2005. Baseline study on the fish and freshwater crayfish fauna in the Blackwood River and its tributaries receiving discharge from the Yarragadee Aquifer. Center for Fish and Fisheries Research, Murdoch University, Perth, Western Australia.

Morgan D.L., Gill H.S. and Potter I.C. 1998. Distribution, identification and biology of freshwater fishes in southwestern Australia. *Records of the Western Australian Museum Supplement* 56: 1-97.

Morgan, D.L., Gill, H.S., Maddern, M.G. and Beatty, S.J. 2004a. Distribution and impacts of introduced freshwater fishes in Western Australia. *New Zealand Journal of Marine and Freshwater Research.* 38: 511-523.

Morgan, D.L., Hambleton, S.J., Gill, H.S., and Beatty, S.J. 2002. Distribution, biology and likely impacts of the introduced redfin perch (*Perca fluviatilis*) (Percidae) in Western Australia. *Marine and Freshwater Research* 53: 1211-1221.

Morgan, G.A., Leech, N.L., Gloeckner, G.W. and Barrett, K.C. 2004b. *SPSS for Introductory Statistics. Use and Interpretation*. Second Edition. Lawrence Erlbaum Associates Publishers, Mahwah, New Jersey.

Morris, S. and Callaghan, J. 1998. The emersion response of the Australian yabbie *Cherax destructor* to environmental hypoxia and the respiratory and metabolic responses to consequent air-breathing. *Journal of Comparative Physiology* B 168: 389-398.

Morrissy, N.M. 1970. Murray Cod, *Maccullochella macquariensis*, in Western Australia. *The Western Australian Naturalist* 11: 130-135.

Morrissy, N.M. 1988. Marron farming – current industry and research developments in Western Australia. In: Evans, L. and O'Sullivan, D. (eds), Proceedings of the first Australian shellfish aquaculture conference. Curtin University of Technology, 59-72.

Morrissy, N.M. 1990. Optimum and favourable temperatures for growth of *Cherax tenuimanus* (Smith 1912) (Decapoda: Parastacidae). *Australian Journal of Freshwater Research* 41: 735-746.

Morrissy, N.M. 1997. Commercial harvesting from farm dams. Aqua info 13. Fisheries Western Australia, Perth.

Morrissy, N.M. 1992. An introduction to marron and freshwater crayfish farming in Western Australia. Fisheries Western Australia, Perth.

Morrissy, N.M. and Caputi, N. 1981. Use of catchability equation for population estimation of marron *Cherax tenuimanus* (Smith) (Decapoda: Parastacidae). *Australian Journal of Marine and Freshwater Research* 32: 213-225.

Morrissy, N.M. and Cassells, G. 1992. Spread of the introduced yabbie *Cherax albidus*, Clark, 1936 in Western Australia. Fisheries Research Report No. 92, Perth, Western Australia.

Morrissy, N.M., Evans, L.H. and Huner, J.V. 1990. Australian freshwater crayfish: aquaculture species. *World Aquaculture* 21: 113-122.

Mundahl, N.D. and Benton, M.J. 1990. Aspects of the thermal ecology in the rusty crayfish *Orconectes rusticus* (Girard). *Oecologia* 82: 210-216.

Nakata, K. and Goshima, S. 2003. Competition for shelter of preferred sizes between the native crayfish species *Cambaroides japonicus* and the alien crayfish species *Pacifastacus leniusculus* in Japan in relation to prior residence, sex difference and body size. *Journal of Crustacean Biology* 23: 897-907.

Nakata, K., Tanaka, A., Hamano, T. and Kawai, T. 2002. Distribution of the signal crayfish *Pacifastacus leniusculus* in Lake Shikaribetsu, Hokkaido, Japan. *Bulletin of the Higashi Taisetsu Museum of Natural History* 24: 27-34.

Nyström, P. 2002. Ecology. In: Holdich, D.M. (ed), *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, London, 192-235.

Nyström, P. 2005. Non-lethal predator effects on the performance of a native and an exotic crayfish species. *Freshwater Biology* 50: 1938-1949.

Nyström, P. and Strand, J.A. 1996. Grazing by a native and an exotic crayfish on aquatic macrophytes. *Freshwater Biology* 36: 631-646.

Nyström, P., Bronmark, C. and Graneli, W. 1999. Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos* 85: 545-553.

Nyström, P., Stenroth, P., Holmqvist, N., Berglund, O., Larsson, P. and Graneli, W. 2006. Crayfish in lakes and streams: individual and population responses to predation, productivity and substratum availability. *Freshwater Biology* 51: 2096-2113.

Oh, J.W. and Dunham, D.W. 1991. Chemical detection of conspecifics in the crayfish *Procambarus clarkii*: role of antennules. *Journal of Chemical Ecology* 17: 161-166.

Olsson, K., Stenroth, P., Nyström, P., Holmqvist, N., McIntosh, A.R. and Winterbourn, M.J. 2006. Does natural acidity mediate interactions between introduced brown trout, native fish, crayfish and other invertebrates in West Coast New Zealand streams? *Biological Conservation* 130: 265-267.

Ott, L. 1988. *An introduction to statistical methods and data analysis*, 3rd edn. PWS-Kent Publishing Company, USA.

Parkyn S.M., Rabeni C.F. and Collier, K.J. 1997. Effects of crayfish (*Paranephrops planifrons*: Parastacidae) on in-stream processes and benthic faunas: a density manipulation experiment. *New Zealand Journal of Marine and Freshwater Research* 31: 685-692.

Partridge, L. 1978. Habitat selection. In: Krebs, J.R. and Davies, N.B. (eds), *Behavioural Ecology: an Evolutionary Appraoch.* Blackwell Scientific Publications, Oxford, 351-376.

Pecor, K.W. and Hazlett, B.A. 2006a. A test of temporal variation in risk and food stimuli on behavioural tradeoffs in the rusty crayfish *Orconectes rusticus*: risk allocation and stimulus degradation. *Ethology* 112: 230-237.

Pecor, K.W. and Hazlett, B.A. 2006b. The influence of flowing water on the resource pursuit-risk avoidance tradeoff in the crayfish *Orconectes virilis. Ethology* 112: 332-338.

Peeke, H.V.S., Figler, M.H. and Chang, E.S. 1998. Sex differences and prior residence effects in shelter competition in juvenile lobsters, *Homarus americanus* Milne-Edwards. *Journal of Experimental Marine Biology and Ecology* 229: 149-156.

Pen L.J. 1999. *Managing our Rivers. A Guide to the Nature and Management of the Streams of South-West Western Australia.* Water and Rivers Commission, Perth, Australia.

Pen, L.J., Potter, I.C. and Calver, M.C. 1993. Comparisons of the food niches of three native and two introduced fish species in an Australian river. *Environmental Biology of Fishes* 36: 167-182.

Pen, L.J. and Potter, I.C. 1992. Seasonal and size-related changes in the diet of perch, *Perca fluviatilis* L., in the shallows of an Australian river, and their implications for the conservation of indigenous teleosts. *Aquatic Conservation: Marine and Freshwater Ecosystems* 2: 243-253.

Persson, L. and Greenberg, L.A. 1990. Juvenile competitive bottlenecks: the perch (*Perca fluviatilis*)-roach (*Rutilus rutilus*) interaction. *Ecology* 71: 44-56.

Pfeiffer, W. 1963. Alarm substances. *Experientia* 19: 113-168.

Pimentel, D., Lach, L., Zuniga, R. and Morrison, D. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50: 53-65.

Pimentel, D., Zuniga, R. and Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273-288.

Pöckl, M. 2002. Craft program for the conservation of native crayfish species in Austria. *Freshwater Crayfish* 13: 221-232.

Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M. and Lowe, A.J. 2008. Adaptive evolution in invasive species. *Trends in Plant Science* 13: 288-294.

Pusey, B.J. and Morrison, P.F. 1989. The diet of rainbow trout in Wungong Dam, Western Australia. *The Western Australian Naturalist* 18: 37-40.

Rabeni, C.F. 1992. Trophic linkage between stream centrachids and their crayfish prey. *Canadian Journal of Fisheries and Aquatic Science* 49: 1714-1721.

Renata, C. and RavishankarRenata, T. 2006. Quantification of risks of alien species introductions associated with ballast water discharge in the Gulf of St. Lawrence. *Biological Invasions* 8: 25-44.

Rónyai, A. 2007. Induced out-of-season tank spawning and stripping of pike perch (Sander lucioperca L.). *Aquaculture Research* 38: 1144-1151.

Rowland, S.J. 1983. The hormone-induced ovulation and spawning of the Australian freshwater fish golden perch, *Macquaria ambigua* (Richardson) (Percichthyidae). *Aquaculture* 35: 221-238.

Rowland, S.J. 1988. Hormone-induced spawning of the Australian freshwater fish Murray cod, *Maccullochella peeli* (Mitchell) (Percichthyidae). *Aquaculture* 70: 371-389.

Rowland, S.J. 1989. Aspects of the history and fishery of the Murray cod, *Maccullochella peeli* (Mitchell) (Percichthyidae). *Proceedings of the Linnaen Society of New South Wales* 111: 201-213.

Rowland, S. 1995. Water quality in the intensive pond culture of silver perch. In: Rowland, S. and Bryant, C. (eds), Silver perch culture. Proceedings of silver perch aquaculture workshops, Grafton and Narrandera, April 1994. Austasia Aquaculture, Hobart, Australia, 51-56.

Rowland, S.J. and Kearney, R.E. 1992. *The prospects for the commercial aquaculture of silver perch (Bidyanus bidyanus).* NSW Fisheries.

Reinthal, P.N. and Kling, G.W. 1997. Exotic species, trophic interactions, and ecosystem dynamics: a case study of Lake Victoria. In: Strouder, D.J. *et al.,* (eds) *Theory and Application in Fish Feeding and Ecology.* University of South Carolina Press, 295-313.

Reynolds, J.D. 2002. Growth and Reproduction. In: Holdich, D.M. (ed) *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, London, 152-191.

Richardson, D.M. and Pyšek, P. 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography* 30: 409-431.

Sala, O.E., Stuart Chapin, F., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, L., Sykes, M.T., Walker, B.H., Walker, M. and Wall., D.H. 2000. Global biodiversity scenarios for the year 2100. *Science* 287: 1770-1774.

Sandeman, R.E. and Sandeman, D.C. 1996. Pre- and postembryonic development, growth and turnover of olfactory receptor neurons in crayfish antennules. In: Holdich, D.M. (ed) *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, London, p 137.

Schapker, H., Breithaupt, T., Shuranova, Z., Burmistrov, Y. and Copper, R. 2002. Heart and ventilator measures in crayfish during environmental disturbances and social interactions. *Comparative Biochemistry and Physiology* Part A 131: 397-407.

Seiler, S.M. and Turner, A.M. 2004. Growth and population size of crayfish in headwater streams: individual- and higher-level consequences of acidification. *Freshwater Biology* 49: 870-881.

Selvanathan, A., Selvanathan, S., Keller, G. and Warrack, B. 2000. *Australian Business Statistics* 2nd edn. Nelson Thompson Learning, Melbourne.

Shave, C.R., Townsend, C.R. and Crowl, T.A. 1994. Anti-predator behaviours of a freshwater crayfish (*Paranephrops zealandicus*) to a native and an introduced predator. *New Zealand Journal of Ecology* 18: 1-10.

Siegel, S. and Castellan, N.J. Jr. 1988. Non parametric statistics for the behavioural sciences, 2nd edn. McGraw-Hill Book Company.

Snedden, W.A. 1990. Determinants of male mating success in the temperate crayfish *Orconectes rusticus*: chela size and sperm competition. *Behaviour* 115: 101-113.

Söderbäck, B. 1994. Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia* 100: 229-235.

Söderbäck, B. 1995. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms. *Freshwater Biology* 33: 291-304

Spanier, E., McKenzie, T.P., Cobb, J.S. and Clancy, M. 1998. Behaviour of juvenile American lobsters, *Homarus americanus*, under predation risk. *Marine Biology* 130: 397-406.

Steele, C., Skinner, C., Steele, C., Alberstadt, P. and Mathewson, C. 1999. Organization of chemically activated food search behavior in *Procambarus clarkii* Girard and *Orconectes rusticus* Girard crayfishes. *Biological Bulletin* 196: 295-302.

Stein, R.A. 1977. Selective predation, optimal foraging, and the predator-prey interaction between fish and crayfish. *Ecology* 58: 1237-1253.

Stein, R.A. and Magnusson, J.J. 1976. Behavioural responses of crayfish to a fish predator. *Ecology* 57: 751-761.

Storer, T.J. 2005. Ethology and production of freshwater crayfish in aquatic polysystems in Western Australia. PhD Thesis, Curtin University of Technology, Perth, Western Australia.

Svensson, M. 1993. Predation by perch, *Perca fluviatilis* (L), and roach, *Rutilus rutilus* (L), on juvenile noble crayfish *Astacus Astacus* (L), in pond experiments. *Freshwater Crayfish* 9: 333-314.

Swannell, T. 1994. A Guide to Marron Farming. Self-published, Western Australia.

Taugbøl, T. and Skurdal, J. 1999. The future of native crayfish in Europe: how to make the best of a bad situation. In: Gherardi, F. and Holdich, D.M. (eds), *Crayfish in Europe as Alien Species. How to Make the Best of a Bad Situation?* A.A. Balkema, Rotterdam, 271-279.

Taugbøl, T., Skurdal, J., Burba, A., Munoz, C. and Saez-Royuela, M. 1997. A test of crayfish predatory and nonpredatory fish species as bait in crayfish traps. *Fisheries Management and Ecology* 4: 127-134.

Taylor, C.A. 2002. Taxonomy and conservation of native crayfish stocks. In: Holdich, D.M. (ed) *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, 236-257.

Thomas, W.J. 1970. The setae of Austropotamobius pallipes (Crustacea: Astacidae). *Journal of Zoology* 160: 91-142.

Thorne, T. and Brayford, H. 2000. The aquaculture of non-endemic species in Western Australia, Silver Perch (*Bidyanus bidyanus*). Fisheries management paper no. 145, Perth, Western Australia.

Thorp, J.H. and Ammerman, K.S. 1978. Chemical communication and agonism in the crayfish, *Procambarus acutus acutus. American Midland Naturalist* 100: 471-474.

Tierney, A.J. and Atema, J. 1988. Behavioural responses of crayfish (*Orconectes virilis* and *Orconectes rusticus*) to chemical feeding stimulants. *Journal of Chemical Ecology* 14: 123-133.

Tierney, A.J. and Atema, J. 1986. Effects of acidification on the behavioural response of crayfishes (*Orconectes virilis* and *Procambarus acutus*) to chemical stimuli. *Aquatic Toxicology* 9: 1-11.

Tomba, A.M., Keller, T.A. and Moore, P.A. 2001. Foraging in complex odour landscapes: chemical orientation strategies during stimulation by conflicting chemical cues. *Journal of the North American Benthological Society* 20: 211-222.

Verhoef, G.D. and Austin, C.M. 1999. Combined effects of shelter and density on the growth and survival of juveniles of the Australian freshwater crayfish, *Cherax destructor* Clark; Part 2. *Aquaculture* 170: 49-57.

Villanelli, F. and Gherardi, F. 1998. Breeding in the crayfish, *Austropotamobius pallipes*: mating patterns, mate choice and intermale competition. *Freshwater Biology* 40: 305-315.

Vitousek, P.M., D'Antonio, C.M., Zloope, L.L. and Westbrooks, R. 1996. Biological invasions as global environmental change. *American Scientist* 84: 468-478.

Vogt, G. 2002. Functional anatomy. In: Holdich, D.M. (ed) *Biology of Freshwater Crayfish.* Blackwell Science, Oxford, 53-151.

Volpe, M.G., Monetta, M., Di Stasio, M. and Paolucci, M. 2008. Rheological behaviour of polysaccharide based pellets for crayfish feeding tested on growth in the crayfish *Cherax albidus. Aquaculture* 274: 339-346.

Vorburger, C. and Ribi, G. 1999. Aggression and competition for shelter between a native and an introduced crayfish in Europe. *Freshwater Biology* 42: 111-119.

Wahle, R.A. 1992. Body-size dependent anti-predator mechanisms of the American lobster. *Oikos* 65: 751-761.

Wahle, R.A. and Steneck, R.S. 1992. Habitat restrictions in early benthic life: Experiments on habitat selection and in situ predation with the American lobster. *Journal of Experimental Marine Biology and Ecology* 157: 91-114.

Waldman, B. 1982. Quantitative and developmental analyses of the alarm reaction in the zebra danio *Brachydanio rerio*. *Copeia* 1:1-9.

Wangpen, P. 2007. The role of shelter in Australian freshwater crayfish polysystems. PhD Thesis, Curtin University of Technology, Western Australia.

Weatherley, A.H. 1977. *Perca fluviatilis* in Australia: zoogeographic expression of a life cycle in relation to an environment. *Journal of the Fisheries Research Board of Canada* 34: 1464-1466.

Webb, P.W. 1979. Mechanics of escape responses in crayfish (Orconectes virilis). *Journal of Experimental Biology* 79: 245-263.

Weissburg, M.J. and Zimmer-Faust. 1994. Odour plumes and how blue crabs use them in finding prey. *Journal of Experimental Biology* 197: 349-375.

Werner, E.E. and Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *The American Midland Naturalist* 142: 242-272.

Werner, E.E. and Gilliam, J.F. 1984. The ontogentic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics* 15: 393-425.

Werner, E.E. and Hall, D.J. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). *Ecology* 69: 1352-1366.

Werner, E.E. and Peacor, S.D. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84: 1083-1100.

Westin, L. and Gydemo, R. 1998. The locomotor activity of juvenile noble crayfish (*Astacus astacus*) and the effects of shelter availability. *Aquaculture* 68: 361-367.

Whisson, G. 2003. Human impacts on marron populations in the South-West. Natural Heritage Trust, Project Final Report. Project No. 983284. Perth.

Whisson, G.J. 1997. Investigating polyculture of marron, *Cherax tenuimanus*, with silver perch, *Bidyanus bidyanus*, and an aquatic macrophyte, *Vallisneria* sp. *Freshwater Crayfish* 11: 577-586.

Whisson, G.J. 2000. Investigation into the commercial polyculture of *Cherax tenuimanus* and *Bidyanus bidyanus*. PhD Thesis, Curtin University of Technology, Western Australia.

White, C.S., Lambert, D.M. and Foster, S.P. 1995. Chemical signals and the recognition concept. In: Lambert, D.M. and Spencer H.G. (eds) *Speciation and the Recognition Concept: Theory and Application.* The Johns Hopkins University Press, Baltimore, MD, 301-326.

Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A. and Losos, E. 1998. Quantifying threats to imperiled species in the United States. *BioScience* 48: 607-615.

Wildy, E.L. and Blaustein, A.R. 2001. Learned recognition of intraspecific predators in larval long-toed salamanders *Ambystoma macrodactylum*. *Ethology* 107: 479-493.

Willman, E.J., Hill, A.M. and Lodge, D.M. 1994. Response of three crayfish congeners (*Orconectes sp*.) to odours of fish carrion and live predatory fish. *The American Midland Naturalist* 132: 44-51.

Zulandt Schneider, R.A. and Moore, P.A. 2000. Urine as a source of conspecific disturbance signals in the crayfish *Procambarus clarkii. Journal of Experimental Biology* 203: 765-771.

Zulandt, T., Zulandt-Schneider, R.A. and Moore, P.A. 2008. Observing agonistic interactions alters subsequent fighting dynamics in the crayfish, *Orconectes rusticus*. *Animal Behaviour* 75: 13-20.

Appendices

Appendix 1.0 Dye test

1.1 Introduction

Laboratory based experiments in Chapters 4, 5, 6, and 7 investigated crayfish behavioural responses to water-borne odours. The reaction time of crayfish to these odours was one of the parameters under investigation. To facilitate discussion on this parameter, it was first necessary to determine the time for a given volume of stimulus liquid to mix uniformly throughout the aquarium.

1.2 Materials and methods

Red food dye (refer to Table 1 for volume) was squirted discretely via syringe, or adjustable pipette (for volumes under 1 mL), into the corner of an aerated aquarium (60 x 30 cm bottom, described in Section 3.1.1) containing 25 L of dechlorinated tap water, and a piece of PVC pipe for crayfish shelter (length 20 cm, diameter 7.5 cm). A digital timer was used to record the number of seconds for the dye to mix uniformly throughout the aquarium. This procedure was repeated four times, in four different aquariums, for each of the nine volumes of dye tested. Data were then analysed for differences in diffusion time using a one-way ANOVA and Scheffe's F-test to rate significant differences between treatment means.

1.3 Results

Mean time (in seconds) for uniform mixing of the dye throughout the aquarium is displayed in Table 1. Larger volumes of dye (1.25, 2.5, 5, 7.5 and 10 mL) were mixed uniformly throughout the aquarium significantly faster than volumes less than 1 mL (excluding 0.625 mL, Figure 1).

Table 1. Uniform mixing time (s) of dye in 25 L aquaria

dye volume (mL)	time (s)
0.1	63.75 ± 8.86
0.15	64 ± 8.01
0.3	61.5 ± 7.58
0.625	40.75 ± 1.11
1.25	31.75 ± 0.48
2.5	30 ± 0.71
5	28.5 ± 1.55
7.5	30 ± 1.96
10	20.5 ± 0.65

Values are mean seconds to achieve uniform mixing time ± standard error

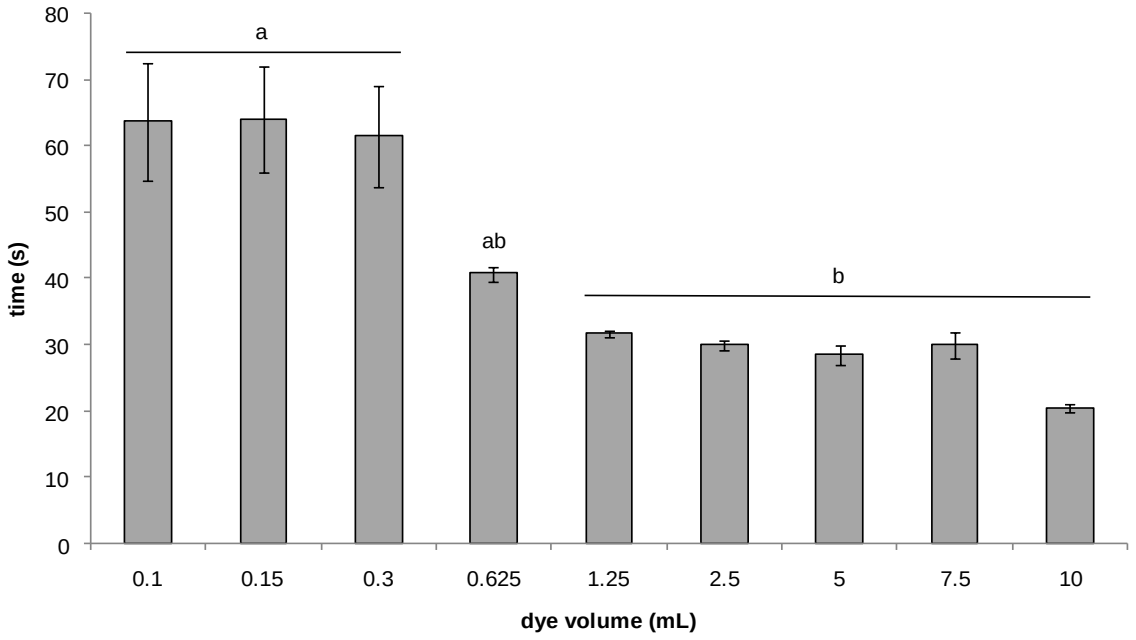

Figure 1. Mean time (s) required to achieve uniform mixing of dye in 25 L aquaria

Values are treatment means ± standard error. Different letters denote significant differences at P < 0.05.

1.4 Discussion

This experiment will aid discussion on crayfish reaction times to chemical stimuli. Mean uniform mixing times recorded in this trial can be used as a reference for the maximum length of time that would pass before crayfish receive a stimulus of a given volume (i.e. if crayfish were located closer to the point of introduction of the stimulus, then it is likely they would receive it more rapidly). This is important because crayfish reaction times often differ from the actual time they would have received the stimulus. However, it should be noted that independent variables that influence water movement, and therefore diffusion time of the stimulus within the aquarium, include: the location and orientation of the shelter; the location of the airstone and volume of air it emits; and any physical movements by resident crayfish.

Results from this trial concur with Gherardi (*et al*. 2002) and Height and Whisson (2006) who both used 10 mL stimulus volume in 25 L (60 x 30 cm bottom) aquariums.

1.5 References

Gherardi, F., Acquistapace, P., Hazlett, B.A. and Whisson, G. 2002. Behavioural responses to alarm odours in indigenous and non-indigenous crayfish species: a case study from Western Australia. *Marine & Freshwater Research* 53: 93-98.

Height, S.G. and Whisson, G.J. 2006. Behavioural responses of Australian freshwater crayfish (*Cherax cainii* and *Cherax albidus*) to exotic fish odour. *Australian Journal of Zoology* 54: 399-407.

Appendix 2.0 Supplementary water quality data

Table 1 Water quality summary data for food odour detection trial (Chapter 4)

Parameter	All treatments	
	mean ± s.e.	**range**
temperature (^{o}C)	17.96 ± 0.31	16.5 - 19
dissolved oxygen (mg/L)	8.76 ± 0.10	8.56 - 8.97
pH	7.28 ± 0.11	7.04 - 7.56
total ammonia (mg/L)	0	0
nitrite (mg/L)	0	0
nitrate (mg/L)	0	0
salinity (ppt)	0	0
conductivity (mS)	0.44 ± 0.03	0.42 - 0.51

No significant differences existed between any treatment.

Table 2 Water quality data for perch odour trial (Chapter 5)

Parameter	All treatments	
	mean ± s.e.	**range**
temperature (^{o}C)	17.91 ± 0.45	17.3 - 18.4
dissolved oxygen (mg/L)	8.59 ± 0.20	8.15 - 8.83
pH	7.11 ± 0.31	7.09 - 7.49
total ammonia (mg/L)	0	0
nitrite (mg/L)	0	0
nitrate (mg/L)	0	0
salinity (ppt)	0	0
conductivity (mS)	0.49 ± 0.07	0.41 - 0.55

No significant differences existed between any treatment.

Table 3 Water quality data for predator size trial (Chapter 6)

Parameter	All treatments	
	mean ± s.e.	**range**
temperature (^{o}C)	17.66 ± 0.56	17.1 - 18.3
dissolved oxygen (mg/L)	8.50 ± 0.27	8.15 - 8.83
pH	7.19 ± 0.25	7.03 - 7.41
total ammonia (mg/L)	0	0
nitrite (mg/L)	0	0
nitrate (mg/L)	0	0
salinity (ppt)	0	0
conductivity (mS)	0.52 ± 0.16	0.43 - 0.59

No significant differences existed between any treatment.

Table 4 Water quality data for alarm odour night trial (Chapter 7)

Parameter	All treatments	
	mean ± s.e.	**range**
temperature (^{o}C)	18.21 ± 0.31	17.9 - 18.8
dissolved oxygen (mg/L)	8.54 ± 0.27	8.23 - 8.92
pH	7.15 ± 0.19	7.02 - 7.51
total ammonia (mg/L)	0	0
nitrite (mg/L)	0	0
nitrate (mg/L)	0	0
salinity (ppt)	0	0
conductivity (mS)	0.46 ± 0.04	0.41 - 0.52

No significant differences existed between any treatment.

Table 5 Water quality data for the mesocosm trial (Chapter 8)

Parameter	All treatments	
	mean ± s.e.	**range**
temperature (^{o}C)	18.73 ± 0.04	18.5 - 18.9
dissolved oxygen (mg/L)	8.43 ± 0.12	8.10 - 8.74
pH	7.67 ± 0.14	7.28 - 7.84
total ammonia (mg/L)	0	0
nitrite (mg/L)	0	0
nitrate (mg/L)	0	0
salinity (ppt)	0	0
conductivity (mS)	0.45 ± 0.05	0.43 - 0.49

Appendix 3.0 Summary data tables – Chapters 4, 5, 6 and 7

Table 1.1 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of marron in the presence of six different concentrations of food solution (Chapter 4)

	Marron					
	Treatment	**Control**	**Test**	**Magnitude of change**	**Direction of change**	**P - value**
Reaction time	T1 (0.1ml)	127.5 (17.20)	93.89 (15.87)	65.28 (12.38)	-	0.093
	T2 (0.15ml)	131.67 (16.43)	70.00 (16.49)	65.00 (16.22)	-	0.002**
	T3 (0.30ml)	99.17 (16.23)	55.83 (12.24)	60.00 (15.34)	-	0.016*
	T4 (0.625ml)	140.83 (17.33)	59.44 (14.05)	95.28 (15.96)	-	0.004**
	T5 (1.25ml)	122.5 (17.54)	45.83 (13.64)	90.00 (19.44)	-	0.004**
	T6 (2.5ml)	130.83 (15.38)	51.67 (13.24)	80.83 (14.61)	-	0.001**
In shelter	T1 (0.1ml)	2.56 (2.56)	0 (0)	2.56 (2.56)	-	0.317
	T2 (0.15ml)	5.56 (5.56)	5.13 (5.13)	0.43 (0.43)	-	0.317
	T3 (0.30ml)	0 (0)	0.85 (0.59)	0.08 (0.59)	+	0.180
	T4 (0.625ml)	0 (0)	0 (0)	0 (0)		1.000
	T5 (1.25ml)	0 (0)	0.85 (0.85)	0.85 (0.85)	+	0.317
	T6 (2.5ml)	0 (0)	2.14 (2.14)	2.14 (2.14)	+	0.317
Locomotion	T1 (0.1ml)	6.41 (2.80)	6.41 (2.80)	10.26 (2.98)		0.905
	T2 (0.15ml)	8.55 (3.93)	15.38 (4.94)	11.97 (3.00)	+	0.119
	T3 (0.30ml)	13.25 (4.12)	17.52 (5.56)	15.38 (3.88)	+	0.479
	T4 (0.625ml)	11.11 (3.69)	11.97 (4.83)	12.82 (4.08)	+	0.859
	T5 (1.25ml)	8.12 (4.10)	15.38 (4.61)	15.81 (4.92)	+	0.181
	T6 (2.5ml)	2.14 (1.50)	10.26 (4.22)	11.54 (4.18)	+	0.137
Raised posture	T1 (0.1ml)	6.84 (5.20)	6.41 (5.54)	2.14 (1.04)	-	0.581
	T2 (0.15ml)	7.26 (5.54)	7.26 (5.61)	3.42 (1.55)		1.000
	T3 (0.30ml)	10.26 (6.46)	9.83 (4.48)	12.39 (5.39)	-	0.893
	T4 (0.625ml)	5.56 (5.56)	7.26 (5.72)	1.71 (1.71)	+	0.317
	T5 (1.25ml)	4.70 (2.79)	11.11 (5.67)	12.39 (5.57)	+	0.249
	T6 (2.5ml)	13.25 (7.64)	18.80 (8.39)	11.54 (6.26)	+	0.465

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water; empty cell = no change. Test solutions comprised: T1, 0.1 mL food solution + 2.4 mL water; T2, 0.15 mL food solution + 2.35 mL water; T3, 0.3 mL food solution + 2.2 mL water; T4, 0.625 mL food solution + 1.875 mL water; T5, 1.25 mL food solution + 1.25 mL water; T6, 2.5 mL food solution. Control water and test solutions were compared by Wilcoxon-Mann-Whitney test. *P<0.05, **P<0.01.

Table 1.2 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of marron in the presence of six different concentrations of food solution (Chapter 4)

		Marron				
	Treatment	**Control**	**Test**	**Magnitude of change**	**Direction of change**	**P - value**
Intermediate posture	T1 (0.1ml)	40.60 (10.96)	38.46 (10.42)	10.68 (4.49)	-	0.600
	T2 (0.15ml)	41.03 (10.98)	46.15 (10.50)	23.08 (7.51)	+	0.534
	T3 (0.30ml)	32.05 (10.09)	44.02 (10.02)	35.04 (8.53)	+	0.254
	T4 (0.625ml)	29.49 (10.48)	42.31 (10.92)	18.80 (7.92)	+	0.173
	T5 (1.25ml)	53.42 (10.98)	51.28 (10.22)	17.52 (7.03)	-	0.722
	T6 (2.5ml)	32.48 (10.80)	35.04 (9.70)	28.21 (8.99)	+	0.644
Lowered posture	T1 (0.1ml)	52.56 (11.44)	55.13 (10.90)	10.26 (4.40)	+	0.395
	T2 (0.15ml)	51.71 (1156)	46.58 (10.80)	19.66 (7.77)	-	0.575
	T3 (0.30ml)	52.14 (11.53)	40.60 (11.38)	29.49 (9.80)	-	0.356
	T4 (0.625ml)	64.96 (11.02)	50.43 (11.39)	18.80 (7.92)	-	0.116
	T5 (1.25ml)	41.88 (11.45)	37.61 (10.75)	8.55 (5.70)	-	0.715
	T6 (2.5ml)	54.27 (11.49)	46.15 (10.61)	22.65 (8.33)	-	0.326
Feeding	T1 (0.1ml)	10.68 (3.58)	30.77 (7.79)	21.79 (5.97)	+	0.006**
	T2 (0.15ml)	12.39 (4.31)	39.74 (7.99)	31.62 (7.25)	+	0.007**
	T3 (0.30ml)	4.70 (2.25)	38.03 (7.05)	33.33 (6.34)	+	0.001**
	T4 (0.625ml)	5.98 (3.08)	35.04 (7.26)	33.33 (6.81)	+	0.005**
	T5 (1.25ml)	8.55 (2.98)	44.02 (8.34)	5.98 (2.29)	+	0.004**
	T6 (2.5ml)	8.12 (4.84)	60.26 (7.55)	6.41 (3.53)	+	0.001**
Antennae flicking	T1 (0.1ml)	11.11 (4.14)	11.97 (4.41)	11.11 (2.93)	+	1.000
	T2 (0.15ml)	19.23 (6.96)	23.93 (6.46)	19.23 (4.41)	+	0.432
	T3 (0.30ml)	5.13 (1.76	26.07 (7.07)	21.79 (6.96)	+	0.003**
	T4 (0.625ml)	14.96 (5.44)	26.50 (8.23)	21.79 (6.53)	+	0.091
	T5 (1.25ml)	10.68 (5.32)	21.37 (6.57)	20.94 (6.49)	+	0.169
	T6 (2.5ml)	3.42 (2.26)	27.35 (5.71)	25.64 (5.56)	+	0.002**
Antennule flicking	T1 (0.1ml)	22.22 (6.92)	20.94 (5.89)	25.21 (5.76)	-	0.753
	T2 (0.15ml)	30.77 (7.67)	37.61 (5.86)	26.50 (5.57)	+	0.255
	T3 (0.30ml)	31.62 (6.37)	28.63 (5.01)	19.23 (4.66)	-	0.975
	T4 (0.625ml)	15.81 (4.05)	29.49 (7.10	22.22 (5.73)	+	0.069
	T5 (1.25ml)	17.52 (4.52)	36.75 (6.36)	21.79 (4.83)	+	0.005**
	T6 (2.5ml)	23.50 (5.92)	39.32 (6.69)	32.05 (6.01)	+	0.021*

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water. Test solutions comprised: T1, 0.1 mL food solution + 2.4 mL water; T2, 0.15 mL food solution + 2.35 mL water; T3, 0.3 mL food solution + 2.2 mL water; T4, 0.625 mL food solution + 1.875 mL water; T5, 1.25 mL food solution + 1.25 mL water; T6, 2.5 mL food solution. Control water and test solutions were compared by Wilcoxon-Mann-Whitney test. *P<0.05, **P<0.01.

Table 2.1 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of yabbies in the presence of six different concentrations of food solution (Chapter 4)

		Yabby				
	Treatment	**Control**	**Test**	**Magnitude of change**	**Direction of change**	**P - value**
Reaction time	T1 (0.1ml)	168.33 (10.82)	80.00 (16.63)	93.33 (15.98)	-	0.001**
	T2 (0.15ml)	158.06 (14.51)	71.67 (15.32)	86.39 (15.60)	-	0.001**
	T3 (0.30ml)	174.17 (10.72)	70.17 (16.38)	101.67 (16.78)	-	0.001**
	T4 (0.625ml)	180.83 (9.19)	65.00 (14.90)	115.83 (15.85)	-	0.000**
	T5 (1.25ml)	180.83 (9.19)	75.83 (16.25)	105.00 (16.40)	-	0.001**
	T6 (2.5ml)	156.67 (17.46)	27.50 (3.69)	132.50 (15.68)	-	0.000**
In shelter	T1 (0.1ml)	72.22 (10.86)	56.41 (10.61)	15.81 (6.80)	-	0.043*
	T2 (0.15ml)	77.35 (10.04)	64.53 (10.09)	12.82 (5.83)	-	0.018*
	T3 (0.30ml)	61.11 (11.82)	61.54 (11.21)	2.99 (1.88)	+	1
	T4 (0.625ml)	64.10 (11.07)	56.84 (10.72)	15.81 (6.01)	-	0.344
	T5 (1.25ml)	60.68 (11.75)	52.14 (11.15)	9.40 (5.04)	-	0.078
	T6 (2.5ml)	67.52 (11.17)	54.27 (9.64)	20.09 (6.44)	-	0.066
Locomotion	T1 (0.1ml)	0 (0)	10.26 (4.82)	10.26 (4.82)	+	0.017*
	T2 (0.15ml)	2.14 (1.21)	17.52 (6.37)	18.80 (6.23)	+	0.041*
	T3 (0.30ml)	3.42 (3.00)	14.10 (3.00)	10.68 (3.47)	+	0.008**
	T4 (0.625ml)	3.42 (2.26)	17.52 (5.27)	18.38 (4.82)	+	0.023*
	T5 (1.25ml)	0.85 (0.59)	13.25 (4.85)	13.25 (4.85)	+	0.017*
	T6 (2.5ml)	10.68 (6.50)	16.67 (5.36)	16.24 (4.89)	+	0.314
Raised posture	T1 (0.1ml)	0 (0)	1.28 (1.28)	1.28 (1.28)	+	0.317
	T2 (0.15ml)	5.56 (5.56)	11.97 (6.44)	6.41 (3.85)	+	0.109
	T3 (0.30ml)	0 (0)	1.71 (1.33)	1.71 (1.33)	+	0.180
	T4 (0.625ml)	0 (0)	1.71 (1.33)	1.71 (1.33)	+	0.180
	T5 (1.25ml)	3.85 (3.85)	0.43 (0.43)	3.42 (3.42)	-	0.317
	T6 (2.5ml)	0.43 (0.43)	1.28 (1.28)	1.71 (1.33)	+	0.655

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water. Test solutions comprised: T1, 0.1 mL food solution + 2.4 mL water; T2, 0.15 mL food solution + 2.35 mL water; T3, 0.3 mL food solution + 2.2 mL water; T4, 0.625 mL food solution + 1.875 mL water; T5, 1.25 mL food solution + 1.25 mL water; T6, 2.5 mL food solution. Control water and test solutions were compared by Wilcoxon-Mann-Whitney test. *P<0.05, **P<0.01.

Table 2.2 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of yabbies in the presence of six different concentrations of food solution (Chapter 4)

		Yabby				
	Treatment	**Control**	**Test**	**Magnitude of change**	**Direction of change**	**P - value**
Intermediate posture	T1 (0.1ml)	9.40 (6.33	35.04 (8.35)	26.50 (7.26)	+	0.006**
	T2 (0.15ml)	19.66 (8.91)	35.47 (9.39	23.50 (7.26)	+	0.074
	T3 (0.30ml)	20.51 (9.14)	22.22 (8.70)	13.68 (6.39)	+	0.310
	T4 (0.625ml)	5.56 (5.56)	47.86 (9.86)	44.87 (9.80)	+	0.004**
	T5 (1.25ml)	3.42 (2.35)	33.76 (8.95)	30.34 (8.45)	+	0.008**
	T6 (2.5ml)	29.06 (10.75)	62.82 (9.64)	36.32 (9.26)	+	0.009**
Lowered posture	T1 (0.1ml)	90.60 (6.33)	63.68 (8.68)	27.78 (7.72)	-	0.006**
	T2 (0.15ml)	74.79 (9.87)	52.56 (10.74)	23.08 (7.82)	-	0.017*
	T3 (0.30ml)	79.49 (9.14)	76.07 (9.33)	14.53 (7.09)	-	0.345
	T4 (0.625ml)	94.44 (5.56)	50.43 (10.24)	44.02 (10.14	-	0.005**
	T5 (1.25ml)	92.74 (5.72)	65.81 (9.12)	26.92 (8.40)	-	0.012*
	T6 (2.5ml)	70.51 (10.74)	35.90 (9.59)	38.03 (9.40)	-	0.009**
Feeding	T1 (0.1ml)	1.71 (1.71)	34.62 (8.07)	32.91 (8.20)	+	0.001**
	T2 (0.15ml)	2.56 (1.52)	44.87 (7.49)	42.31 (7.23)	+	0.001**
	T3 (0.30ml)	0.43 (0.43)	26.50 (6.53)	26.07 (6.50)	+	0.003**
	T4 (0.625ml)	0 (0)	50.00 (8.09)	50.00 (8.09)	+	0.001**
	T5 (1.25ml)	0 (0)	35.90 (8.16)	35.90 (8.16)	+	0.002**
	T6 (2.5ml)	1.71 (1.33)	66.24 (7.12)	64.53 (7.20)	+	0.000**
Antennae flicking	T1 (0.1ml)	0 (0)	18.80 (5.25)	18.80 (5.25)	+	0.002**
	T2 (0.15ml)	2.99 (1.41)	24.36 (6.53)	22.22 (6.22)	+	0.008**
	T3 (0.30ml)	2.99 (2.25)	10.26 (4.17)	8.12 (3.91)	+	0.017*
	T4 (0.625ml)	0.85 (0.85)	30.77 (7.20)	29.91 (7.14)	+	0.001**
	T5 (1.25ml)	0.43 (0.43)	17.09 (4.76)	17.52 (4.69)	+	0.002**
	T6 (2.5ml)	5.56 (4.12)	29.91 (6.61)	27.78 (6.64)	+	0.005**
Antennule flicking	T1 (0.1ml)	8.97 (2.58)	52.56 (7.42)	44.44 (6.14)	+	0.000**
	T2 (0.15ml)	21.37 (5.85)	47.44 (8.04)	27.78 (5.25)	+	0.001**
	T3 (0.30ml)	15.81 (5.98)	52.14 (7.37)	37.18 (7.26)	+	0.001**
	T4 (0.625ml)	14.10 (4.14)	56.84 (7.07)	46.15 (6.37)	+	0.001**
	T5 (1.25ml)	15.81 (4.71)	52.14 (8.24)	36.32 (6.31)	+	0.000**
	T6 (2.5ml)	15.81 (4.50)	59.40 (6.78)	43.59 (6.22)	+	0.000**

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water. Test solutions comprised: T1, 0.1 mL food solution + 2.4 mL water; T2, 0.15 mL food solution + 2.35 mL water; T3, 0.3 mL food solution + 2.2 mL water; T4, 0.625 mL food solution + 1.875 mL water; T5, 1.25 mL food solution + 1.25 mL water; T6, 2.5 mL food solution. Control water and test solutions were compared by Wilcoxon-Mann-Whitney test. *P<0.05, **P<0.01.

Table 3 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of small marron and yabbies in the presence of food and perch odour (Chapter 5)

		Small marron						Small yabby						
	Odour	Control	Test	Magnitude of change	Direction of change	*Z*		Odour	Control	Test	Magnitude of change	Direction of change	*Z*
Reaction time	FOOD	111.15 (13.45)	20.05 (4.15)	90.28 (13.05)	-	-2.877**	Reaction time	FOOD	150.83 (13.37)	30.83 (4.77)	120.00 (13.23)	-	-3.213**
	PERCH	118.91 (11.63)	25.67 (2.93)	94.82 (11.17)	-	-2.851**		PERCH	162.50 (10.38)	20.83 (2.75)	141.67 (10.52)	-	-3.502**
In shelter	FOOD	30.77 (10.74)	21.79 (9.50)	9.83 (5.99)	-	-1.461	In shelter	FOOD	77.35 (9.64)	52.99 (10.08)	25.21 (7.71)	-	-2.601**
	PERCH	37.18 (11.42)	34.19 (11.32)	2.99 (2.99)	-	-1.000		PERCH	61.54 (11.70)	35.04 (9.94)	29.06 (9.15)	-	-2.213*
Locomotion	FOOD	25.21 (8.32)	23.08 (6.55)	18.38 (4.58)	-	-0.561	Locomotion	FOOD	5.56 (3.22)	60.26 (7.47)	54.70 (7.17)	+	-3.624**
	PERCH	4.27 (3.06)	17.09 (5.55)	13.68 (4.47)	+	-2.549*		PERCH	14.96 (7.45)	69.23 (6.93)	61.11 (7.78)	+	-3.283**
Raised posture	FOOD	38.03 (10.78)	33.33 (11.43)	11.54 (6.13)	-	-0.730	Raised posture	FOOD	0 (0)	0 (0)	0 (0)		0.000
	PERCH	5.56 (5.56)	17.95 (7.69)	20.09 (8.18)	+	-1.219		PERCH	0 (0)	0.85 (0.59)	0.85 (0.59)	+	-1.414
Intermediate posture	FOOD	23.08 (9.05)	28.21 (10.81)	11.97 (6.10)	+	-0.674	Intermediate posture	FOOD	16.67 (8.14)	60.68 (9.12)	44.02 (9.01)	+	-3.066**
	PERCH	44.44 (12.05)	30.34 (9.48)	32.91 (9.77)	-	-0.986		PERCH	19.23 (8.46)	85.90 (5.50)	66.67 (8.82)	+	-3.42**
Lowered posture	FOOD	38.89 (11.82)	38.46 (11.70)	0.43 (0.43)	-	-1.000	Lowered posture	FOOD	83.33 (8.14)	39.32 (9.12)	44.02 (9.01)	-	-3.066**
	PERCH	50.00 (12.13)	51.71 (11.83)	12.82 (7.67)	+	-0.272		PERCH	75.21 (9.48)	13.25 (5.49)	62.82 (9.37)	-	-3.363**
Feeding movements	FOOD	5.13 (2.64)	22.22 (5.80)	17.95 (4.74)	+	-2.906**	Feeding movements	FOOD	0 (0)	35.04 (4.74)	35.04 (4.75)	+	-3.422**
	PERCH	3.42 (1.55)	24.79 (5.22)	23.08 (5.31)	+	-2.987**		PERCH	2.56 (1.87)	40.17 (5.89)	38.46 (5.77)	+	-3.645**
Antennae flicking	FOOD	24.36 (8.02)	24.36 (5.97)	20.51 (4.57)	+	-0.105	Antennae flicking	FOOD	6.41 (3.90)	70.51 (6.04)	64.10 (6.00)	+	-3.727**
	PERCH	5.56 (3.56)	21.37 (6.48)	16.67 (5.32)	+	-2.673*		PERCH	8.97 (5.39)	75.21 (5.37)	66.24 (6.84)	+	-3.698**
Antennule flicking	FOOD	35.47 (7.49)	42.74 (6.00)	20.09 (3.94)	+	-0.871	Antennule flicking	FOOD	26.5 (5.81)	79.49 (5.35)	52.99 (6.37)	+	-3.518**
	PERCH	30.34 (5.03)	44.02 (5.66)	22.22 (3.57)	+	-2.250*		PERCH	19.24 (4.41)	79.06 (3.92)	59.83 (3.66)	+	-3.726**

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water; empty cell = no change. Control water and test solutions were compared by Wilcoxon Signed ranks test. *P<0.05, **P<0.01

Table 4 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of medium marron and yabbies in the presence of food and perch odour (Chapter 5)

Medium marron							Medium yabby						
Marron	Odour	Control	Test	Magnitude of change	Direction of change	*Z*	Yabby	Odour	Control	Test	Magnitude of change	Direction of change	*Z*
Reaction time	FOOD	142.55 (9.98)	22.90 (4.55)	118.49 (9.64)	-	-3.066**	Reaction time	FOOD	125.83 (15.68)	23.33 (2.49)	105.83 (14.06)	-	-2.877**
	PERCH	148.87 (10.74)	28.18 (7.79)	121.38 (10.92)	-	-2.679**		PERCH	139.17 (14.89)	20.83 (3.67)	123.33 (14.27)	-	-3.255**
In shelter	FOOD	11.54 (7.60)	14.10 (7.42)	11.11 (6.29)	+	-0.365	In shelter	FOOD	50.43 (11.01)	17.09 (6.86)	34.19 (8.82)	-	-2.851**
	PERCH	55.56 (12.05)	54.70 (11.90)	0.85 (0.85)	-	-1.000		PERCH	50.00 (11.49)	7.69 (2.41)	45.73 (10.09)	-	-2.785**
Locomotion	FOOD	11.54 (4.54)	32.91 (6.75)	30.77 (6.93)	+	-2.535*	Locomotion	FOOD	21.37 (7.02)	82.9 (3.60)	61.54 (6.12)	+	-3.73**
	PERCH	2.14 (1.36)	12.39 (4.74)	10.26 (3.73)	+	-2.679**		PERCH	16.67 (6.32)	86.75 (2.97)	71.79 (6.25)	+	-3.673**
Raised posture	FOOD	12.39 (6.84)	11.11 (7.62)	12.39 (6.84)	-	-0.365	Raised posture	FOOD	4.70 (3.58)	14.53 (4.57)	12.39 (3.69)	+	-1.829
	PERCH	27.78 (10.86)	30.34 (10.78)	13.68 (7.83)	+	-0.272		PERCH	1.71 (1.71)	2.56 (1.24)	4.27 (1.99)	+	-0.680
Intermediate posture	FOOD	26.50 (9.39)	55.56 (10.42)	40.17 (9.11)	+	-2.054*	Intermediate posture	FOOD	48.72 (10.72)	69.66 (5.72)	37.18 (6.44)	+	-2.075*
	PERCH	38.89 (11.82)	30.77 (10.83)	8.12 (5.98)	-	-1.342		PERCH	52.14 (11.58)	89.74 (2.41)	43.59 (9.67)	+	-2.497*
Lowered posture	FOOD	61.11 (11.32)	33.33 (9.69)	27.78 (8.62)	-	-2.536*	Lowered posture	FOOD	46.58 (11.10)	15.38 (6.09)	31.12 (8.26)	-	-2.805**
	PERCH	33.33 (11.43)	38.89 (11.82)	5.56 (5.56)	+	-1.000		PERCH	46.15 (11.72)	7.69 (1.97)	40.17 (10.38)	-	-2.545*
Feeding movements	FOOD	0.43 (0.43)	26.07 (6.64)	26.50 (6.56)	+	-2.851**	Feeding movements	FOOD	0.43 (0.43)	44.44 (6.69)	44.02 (6.72)	+	-3.526**
	PERCH	2.99 (1.41)	14.96 (2.81)	12.82 (2.92)	+	-3.024**		PERCH	1.28 (0.93)	40.60 (6.18)	41.03 (5.70)	+	-3.649**
Antennae flicking	FOOD	11.54 (3.95)	42.31 (7.10)	33.33 (6.90)	+	-3.209**	Antennae flicking	FOOD	20.08 (6.47)	85.47 (3.40)	65.38 (5.36)	+	-3.734**
	PERCH	1.28 (0.93)	6.41 (4.75)	5.98 (3.91)	+	-1.473		PERCH	18.38 (6.10)	78.63 (5.41)	60.26 (7.65)	+	-3.728**
Antennule flicking	FOOD	32.48 (6.24)	59.83 (5.85)	35.04 (6.82)	+	-2.461*	Antennule flicking	FOOD	43.59 (8.06)	85.04 (2.52)	45.73 (6.91)	+	-3.289**
	PERCH	32.05 (4.58)	34.62 (5.18)	15.38 (4.17)	+	-0.308		PERCH	25.21 (6.15)	79.49 (3.73)	54.27 (6.26)	+	-3.724**

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water. Control water and test solutions were compared by Wilcoxon Signed ranks test. *P<0.05, **P<0.01

Table 5 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of large marron and yabbies in the presence of food and perch odour (Chapter 5)

Large marron							Large yabby						
Marron	Odour	Control	Test	Magnitude of change	Direction of change	*Z*	Yabby	Odour	Control	Test	Magnitude of change	Direction of change	*Z*
Reaction time	FOOD	119.67 (9.13)	25.50 (3.92)	92.27 (8.18)	-	-3.213**	Reaction time	FOOD	160.01 (11.11)	55.56 (13.09)	104.44 (14.93)	-	-2.913**
	PERCH	125.27 (13.33)	30.32 (10.40)	91.26 (11.87)	-	-2.907**		PERCH	176.67 (3.33)	37.50 (4.41)	139.17 (5.26)	-	-3.489**
In shelter	FOOD	0 (0)	2.99 (2.99)	2.99 (2.99)	+	-1.000	In shelter	FOOD	72.22 (10.86)	52.14 (10.58)	20.09 (7.72)	-	-2.207*
	PERCH	5.56 (5.56)	5.56 (5.56)	0 (0)		0.000		PERCH	77.78 (10.08)	42.73 (8.49)	37.61 (8.13)	-	-2.907**
Locomotion	FOOD	11.54 (4.79)	37.61 (5.96)	27.78 (5.46)	+	-3.097**	Locomotion	FOOD	2.14 (1.74)	41.45 (8.6)	39.32 (8.22)	+	-3.185**
	PERCH	5.13 (1.76)	41.88 (7.92)	36.75 (7.43)	+	-3.577**		PERCH	1.28 (1.28)	60.68 (7.11)	59.40 (6.98)	+	-3.627**
Raised posture	FOOD	16.67 (9.04)	23.93 (9.07)	16.67 (7.55)	+	-1.153	Raised posture	FOOD	0 (0)	2.99 (2.99)	2.99 (2.99)	+	-1.000
	PERCH	10.68 (6.41)	16.67 (6.73)	16.24 (6.15)	+	-0.845		PERCH	0 (0)	12.82 (6.28)	12.82 (6.28)	+	-1.826
Intermediate posture	FOOD	50.85 (11.55)	58.12 (10.61)	31.20 (9.38)	+	-0.561	Intermediate posture	FOOD	5.56 (3.82)	50 (9.4)	44.44 (9.30)	+	-3.192**
	PERCH	51.71 (11.19)	55.98 (8.59)	44.44 (8.17)	+	-0.259		PERCH	5.56 (5.56)	60.26 (6.56)	54.70 (6.92)	+	-3.527**
Lowered posture	FOOD	32.48 (10.75)	17.95 (8.93)	15.38 (7.87)	-	-1.761	Lowered posture	FOOD	94.44 (3.82)	47.01 (9.79)	47.44 (8.93)	-	-3.192**
	PERCH	38.46 (11.77)	27.35 (9.06)	29.91 (8.99)	-	-0.816		PERCH	88.89 (7.62)	26.07 (4.82)	65.38 (6.56)	-	-3.536**
Feeding movements	FOOD	4.27 (2.17)	64.10 (5.13)	59.83 (5.44)	+	-3.727**	Feeding movements	FOOD	2.56 (2.56)	43.16 (7.31)	40.60 (7.69)	+	-3.522**
	PERCH	2.14 (0.84)	60.68 (7.54)	58.55 (7.36)	+	-3.523**		PERCH	0 (0)	43.16 (5.81)	43.16 (5.81)	+	-3.627**
Antennae flicking	FOOD	11.97 (3.89)	47.86 (6.86)	35.90 (6.06)	+	-3.517**	Antennae flicking	FOOD	4.70 (2.93)	51.28 (6.98)	47.44 (6.79)	+	-3.427**
	PERCH	6.41 (1.79)	50.85 (7.52)	44.44 (7.73)	+	-3.622**		PERCH	1.28 (0.93)	68.37 (6.03)	67.09 (6.12)	+	-3.735**
Antennule flicking	FOOD	39.32 (4.69)	56.84 (4.94)	23.50 (3.76)	+	-2.548*	Antennule flicking	FOOD	33.76 (5.64)	85.89 (4.62)	54.70 (5.86)	+	-3.639**
	PERCH	30.34 (3.91)	63.68 (5.66)	34.19 (5.71)	+	-3.681**		PERCH	16.24 (4.21)	79.49 (2.98)	63.25 (5.37)	+	-3.725**

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water; empty cell = no change. Control water and test solutions were compared by Wilcoxon Signed ranks test. *P<0.05, **P<0.01

Table 6 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of yabbies in the presence of food and small perch odour (Chapter 6)

		Small perch				
	Odour	**Control**	**Test**	**Magnitude of change**	**Direction of change**	***Z***
Reaction time	FOOD	167.50 (52.45)	95.00 (44.10)	142.50 (45.95)	-	0.736
	PREDS	195.00 (52.96)	65.00 (32.09)	130.00 (46.42)	-	2.207*
In shelter	FOOD	33.33 (21.08)	28.57 (18.44)	4.76 (4.76)	-	1.000
	PREDS	24.60 (16.97)	0 (0)	24.60 (16.97)	-	1.342
Locomotion	FOOD	32.54 (13.82)	49.21 (14.79)	32.54 (9.15)	+	1.214
	PREDS	37.30 (16.10)	35.71 (12.52)	41.27 (12.34)	-	0.105
Raised posture	FOOD	36.51 (17.76)	15.08 (10.46)	27.78 (15.86)	-	1.069
	PREDS	12.70 (9.42)	5.56 (5.56)	11.90 (9.34)	-	0.447
Intermediate posture	FOOD	50.79 (17.89)	63.49 (16.53)	19.05 (9.36)	+	0.730
	PREDS	53.17 (15.38)	45.24 (13.45)	20.63 (6.70)	-	0.368
Lowered posture	FOOD	41.27 (19.70)	17.46 (16.53)	25.40 (16.34)	-	1.289
	PREDS	28.57 (16.54)	42.86 (16.81)	14.29 (7.38)	+	1.604
Feeding movements	FOOD	20.63 (10.48)	46.83 (12.20)	26.19 (7.75)	+	2.032*
	PREDS	0 (0)	50.79 (12.40)	50.79 (12.40)	+	2.032*
Antennae flicking	FOOD	0.79 (0.79)	1.59 (1.00)	2.38 (1.06)	+	0.577
	PREDS	7.94 (4.71)	15.87 (11.25)	19.05 (10.36)	+	0.674
Antennule flicking	FOOD	13.48 (6.06)	25.35 (9.27)	11.87 (4.04)	+	2.032*
	PREDS	1.59 (1.00)	34.03 (11.72)	32.44 (11.59)	+	2.201*

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water. Test solutions comprised: FOOD, 10 mL food odour + 10 mL water; PREDS, 10 mL food odour + 10 mL small perch odour. Control water and test solutions were compared by Wilcoxon signed ranks test. *P<0.05.

Table 7 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of yabbies in the presence of food and medium perch odour (Chapter 6)

		Medium perch				
	Odour	**Control**	**Test**	**Magnitude of change**	**Direction of change**	***Z***
Reaction time	FOOD	137.50 (56.31)	42.50 (6.02)	105.00 (52.39)	-	1.581
	PREDM	167.50 (52.45)	87.50 (46.54)	80.00 (47.38)	-	1.604
In shelter	FOOD	17.46 (16.53)	16.67 (16.67)	0.79 (0.79)	-	1.000
	PREDM	18.25 (16.42)	24.60 (16.51)	7.94 (7.03)	+	0.447
Locomotion	FOOD	44.44 (14.05)	50.00 (16.98)	35.71 (12.22)	+	0.734
	PREDM	30.95 (13.62)	22.22 (8.03)	18.25 (6.89)	-	0.730
Raised posture	FOOD	24.60 (12.56)	21.43 (6.93)	25.40 (11.51)	-	0.135
	PREDM	8.73 (8.73)	4.76 (3.89)	5.56 (4.67)	-	0.447
Intermediate posture	FOOD	13.49 (10.17)	36.51 (12.21)	27.78 (10.02)	+	1.490
	PREDM	39.68 (17.33)	84.92 (8.10)	45.24 (17.93)	+	2.207*
Lowered posture	FOOD	51.59 (21.70)	38.10 (19.56)	42.06 (19.30)	-	0.535
	PREDM	35.71 (20.45)	5.56 (3.11)	36.51 (18.83)	-	1.095
Feeding movements	FOOD	9.52 (6.02)	61.93 (6.13)	52.40 (5.35)	+	2.207*
	PREDM	0 (0)	6.35 (3.40)	6.35 (3.40)	+	1.604
Antennae flicking	FOOD	0.79 (0.79)	1.59 (1.00)	9.52 (6.02)	+	0.000
	PREDM	7.94 (4.71)	15.87 (11.25)	4.76 (3.89)	+	1.342
Antennule flicking	FOOD	9.52 (6.85)	27.72 (13.32)	18.20 (7.50)	+	2.023*
	PREDM	11.11 (5.85)	34.13 (8.19)	24.60 (6.44)	+	1.992*

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water. Test solutions comprised: FOOD, 10 mL food odour + 10 mL water; PREDM, 10 mL food odour + 10 mL medium perch odour. Control water and test solutions were compared by Wilcoxon signed ranks test. *P<0.05.

Table 8 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of yabbies in the presence of food and large perch odour (Chapter 6)

		Large perch				
	Odour	**Control**	**Test**	**Magnitude of change**	**Direction of change**	***Z***
Reaction time	FOOD	142.50 (43.26)	52.50 (11.46)	95.00 (33.02)	-	1.992*
	PREDL	120.00 (44.67)	70.00 (31.38)	75.00 (39.50)	-	0.841
In shelter	FOOD	63.49 (20.30)	65.08 (20.63)	4.76 (3.25)	+	0.447
	PREDL	16.67 (16.67)	15.08 (15.08)	1.59 (1.59)	-	1.000
Locomotion	FOOD	8.73 (3.35)	29.37 (10.39)	20.63 (9.17)	+	2.226*
	PREDL	52.38 (15.11)	44.44 (15.29)	20.63 (4.55)	-	0.841
Raised posture	FOOD	1.59 (1.59)	19.84 (12.56)	18.25 (11.69)	+	1.342
	PREDL	26.98 (16.89)	9.52 (4.43)	30.16 (14.58)	-	0.730
Intermediate posture	FOOD	47.62 (20.24)	45.24 (14.69)	35.71 (13.29)	-	0.000
	PREDL	46.03 (18.14)	44.44 (18.06)	50.79 (14.94)	-	0.105
Lowered posture	FOOD	67.41 (19.25)	31.75 (16.93)	35.67 (16.17)	-	1.826
	PREDL	30.95 (19.66)	30.16 (16.53)	30.95 (15.44)	-	0.000
Feeding movements	FOOD	11.88 (5.84)	51.46 (7.91)	39.58 (10.75)	+	2.201*
	PREDL	2.38 (2.38)	23.81 (10.14)	21.43 (9.74)	+	1.826
Antennae flicking	FOOD	1.59 (1.59)	0 (0)	1.59 (1.59)	-	1.000
	PREDL	6.35 (3.40)	11.11 (6.58)	9.52 (5.36)	+	0.535
Antennule flicking	FOOD	5.56 (3.57)	27.83 (12.73)	22.27 (12.92)	+	2.032*
	PREDL	9.52 (5.36)	34.13 (6.08)	24.60 (7.72)	+	2.023*

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water. Test solutions comprised: FOOD, 10 mL food odour + 10 mL water; PREDL, 10 mL food odour + 10 mL large perch odour. Control water and test solutions were compared by Wilcoxon signed ranks test. *P<0.05.

Table 9 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of small marron and yabbies in the presence of three test solutions (Chapter 7)

	Small marron							Small yabby						
	Odour	Control	Test	Magnitude of change	Direction of change	Z			Odour	Control	Test	Magnitude of change	Direction of change	Z
Reaction time	FOOD	124.17 (19.58)	28.33 (4.68)	95.83 (17.16)	-	3.420**		Reaction time	FOOD	125.00 (17.74)	26.67 (3.75)	98.33 (15.25)	-	3.738**
	CONS	56.67 (12.28)	22.50 (2.19)	34.17 (12.59)	-	2.692**			CONS	75.00 (13.99)	21.67 (2.49)	55.00 (14.09)	-	3.380**
	HETE	89.17 (18.57)	23.33 (2.49)	70.83 (18.26)	-	2.843**			HETE	108.33 (17.13)	21.48 (2.53)	90.00 (17.57)	-	3.452**
In shelter	FOOD	16.67 (9.04)	0.85 (0.59)	16.67 (8.78)	-	1.473		In shelter	FOOD	0 (0)	0 (0)	0 (0)		0.000
	CONS	35.90 (10.63)	30.34 (10.15)	14.96 (6.74)	-	0.674			CONS	2.14 (1.74)	10.26 (4.78)	11.54 (4.54)	+	1.612
	HETE	30.77 (9.39)	22.22 (8.52)	27.35 (8.42)	-	0.950			HETE	0 (0)	4.70 (2.57)	4.70 (2.57)	+	2.333*
Locomotion	FOOD	26.50 (7.36)	68.38 (7.03)	45.30 (6.72)	+	3.273**		Locomotion	FOOD	69.23 (4.03)	84.62 (0)	15.38 (4.03)	+	3.145**
	CONS	30.77 (5.77)	48.72 (7.62)	32.48 (5.11)	+	1.941			CONS	43.59 (7.87)	57.69 (5.29)	36.32 (5.66)	+	1.232
	HETE	41.03 (9.16)	58.12 (7.97)	38.46 (7.94)	+	1.632			HETE	46.58 (5.15)	70.94 (6.08)	34.62 (4.83)	+	2.546*
Raised posture	FOOD	20.09 (7.80)	44.44 (8.61)	39.74 (8.44)	+	2.107*		Raised posture	FOOD	38.46 (2.64)	46.15 (7.92)	28.21 (2.33)	+	1.257
	CONS	36.75 (10.55)	27.35 (6.73)	44.44 (8.12)	-	0.912			CONS	26.92 (7.87)	29.06 (5.19)	32.05 (6.96)	+	0.471
	HETE	76.07 (8.36)	70.09 (6.25)	25.64 (6.19)	-	0.903			HETE	33.76 (7.20)	26.50 (3.74)	27.78 (4.86)	-	0.803
Intermediate posture	FOOD	55.56 (8.54)	51.28 (7.62)	37.61 (8.01)	-	0.466		Intermediate posture	FOOD	58.97 (2.33)	38.46 (7.92)	35.90 (3.17)	-	2.845**
	CONS	57.26 (10.20)	70.94 (6.51)	47.01 (7.81)	+	1.080			CONS	62.82 (8.62)	64.10 (4.26)	35.47 (5.81)	+	0.142
	HETE	23.93 (8.36)	29.91 (6.25)	25.64 (6.19)	+	0.903			HETE	41.88 (6.85)	67.95 (4.98)	36.32 (5.38)	+	2.642**
Lowered posture	FOOD	24.36 (8.35)	4.27 (2.50)	24.36 (7.42)	-	2.178*		Lowered posture	FOOD	2.56 (0.88)	15.38 (0)	12.82 (0.88)	+	3.874**
	CONS	5.98 (4.33)	1.71 (1.33)	5.13 (3.88)	-	1.069			CONS	10.26 (5.77)	9.40 (3.60)	12.82 (4.65)	-	0.119
	HETE	0 (0)	0 (0)	0 (0)		0.000			HETE	24.36 (7.67)	5.56 (2.70)	18.80 (6.68)	-	2.527*
Feeding movements	FOOD	2.56 (1.39)	60.68 (6.34)	58.12 (6.29)	+	3.729**		Feeding movements	FOOD	0 (0)	79.49 (0.88)	79.49 (0.88)	+	3.874**
	CONS	14.10 (3.85)	28.63 (4.65)	18.80 (3.84)	+	2.527*			CONS	15.81 (4.84)	40.60 (5.49)	24.79 (6.17)	+	3.066**
	HETE	17.09 (4.76)	48.72 (7.28)	43.59 (6.06)	+	2.603**			HETE	11.11 (3.00)	52.99 (5.80)	44.44 (5.55)	+	3.466**
Antennae flicking	FOOD	35.47 (6.79)	69.23 (6.25)	34.62 (5.81)	+	3.533**		Antennae flicking	FOOD	69.23 (4.03)	87.18 (0.89)	17.95 (3.52)	+	3.771**
	CONS	37.18 (5.29)	49.57 (4.79)	24.36 (4.09)	+	1.762			CONS	38.46 (6.31)	63.68 (3.28)	32.05 (5.32)	+	2.871**
	HETE	36.75 (7.29)	57.69 (6.32)	35.47 (6.73)	+	1.824			HETE	47.86 (5.19)	73.50 (4.04)	25.64 (5.01)	+	3.306**
Antennule flicking	FOOD	38.46 (5.60)	35.47 (6.47)	32.91 (5.34)	-	0.655		Antennule flicking	FOOD	71.79 (3.17)	87.18 (0.90)	15.38 (2.64)	+	3.771**
	CONS	26.50 (4.79)	32.91 (4.16)	20.09 (2.72)	+	1.046			CONS	50.85 (5.06)	70.51 (3.53)	21.37 (3.71)	+	3.424**
	HETE	29.49 (4.62)	42.31 (6.16)	27.35 (4.87)	+	1.351			HETE	51.28 (4.35)	77.78 (3.83)	29.91 (3.62)	+	3.512**

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water; empty cell = no change. FOOD = food odour, CONS = conspecific odour, HETE = heterospecific odour. Control water and test solutions were compared using Wilcoxon signed ranks test (Z). *P < 0.05, **P < 0.01.

Table 10 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of medium marron and yabbies in the presence of three test solutions (Chapter 7)

	Medium marron							Medium yabby						
	Odour	Control	Test	Magnitude of change	Direction of change	Z			Odour	Control	Test	Magnitude of change	Direction of change	Z
Reaction time	FOOD	178.33 (7.56)	42.50 (6.56)	135.83 (6.80)	-	3.789**		Reaction time	FOOD	150.00 (13.83)	26.67 (2.86)	123.33 (13.15)	-	3.652**
	CONS	147.50 (16.28)	31.67 (9.99)	115.83 (16.93)	-	3.435**			CONS	128.33 (16.99)	31.67 (4.68)	96.67 (16.90)	-	3.749**
	HETE	155.00 (18.15)	68.33 (16.99)	126.67 (16.99)	-	1.874*			HETE	151.67 (17.48)	20.83 (2.75)	130.83 (17.10)	-	3.754**
In shelter	FOOD	32.05 (10.15)	23.93 (9.47)	38.03 (10.56)	-	0.821		In shelter	FOOD	38.03 (10.74)	14.96 (7.50)	23.08 (8.27)	-	2.232*
	CONS	77.78 (10.08)	58.97 (11.19)	18.80 (8.53)	-	2.000*			CONS	35.04 (10.50)	40.17 (10.17)	17.09 (5.65)	+	0.765
	HETE	84.62 (8.41)	57.26 (10.47)	35.90 (9.75)	-	2.226*			HETE	32.91 (10.68)	27.35 (9.54)	32.91 (9.83)	-	0.238
Locomotion	FOOD	5.56 (2.31)	37.18 (7.12)	33.33 (7.33)	+	3.166**		Locomotion	FOOD	8.55 (3.04)	58.55 (8.04)	50.00 (7.92)	+	3.303**
	CONS	11.97 (6.93)	42.31 (9.33)	30.34 (8.69)	+	2.958**			CONS	31.62 (8.97)	36.32 (6.92)	35.47 (7.15)	+	0.684
	HETE	11.54 (6.13)	46.58 (9.06)	42.74 (8.60)	+	2.740**			HETE	11.11 (3.79)	41.03 (6.70)	35.90 (5.70)	+	3.052**
Raised posture	FOOD	4.70 (2.57)	45.73 (6.77)	41.03 (6.49)	+	3.309**		Raised posture	FOOD	14.53 (7.69)	13.68 (4.51)	19.66 (6.62)	-	0.178
	CONS	17.09 (9.00)	9.40 (4.29)	14.53 (6.40)	-	1.219			CONS	27.78 (8.64)	23.93 (5.63)	33.76 (7.12)	-	0.411
	HETE	17.09 (9.00)	12.82 (6.46)	11.11 (5.81)	-	0.736			HETE	4.27 (2.08)	32.91 (7.61)	28.63 (6.31)	+	3.427**
Intermediate posture	FOOD	45.30 (9.31)	45.73 (5.37)	40.60 (5.73)	+	0.470		Intermediate posture	FOOD	29.91 (10.46)	70.09 (3.57)	53.85 (7.04)	+	2.861**
	CONS	44.02 (11.94)	83.33 (4.23)	46.15 (9.05)	+	2.836**			CONS	44.87 (9.70)	70.51 (5.74)	36.75 (6.80)	+	2.284*
	HETE	51.28 (10.91)	76.50 (7.58)	43.16 (9.25)	+	1.787			HETE	64.96 (9.70)	42.31 (8.35)	27.78 (6.50)	-	2.619**
Lowered posture	FOOD	50.00 (9.94)	8.55 (3.67)	48.29 (8.75)	-	2.945**		Lowered posture	FOOD	55.56 (12.05)	16.24 (3.93)	42.74 (8.91)	-	2.833**
	CONS	38.89 (11.82)	7.26 (2.67)	32.48 (9.82)	-	2.389*			CONS	27.35 (9.86)	5.56 (1.36)	25.21 (8.52)	-	2.099*
	HETE	31.62 (10.08)	10.68 (5.60)	32.91 (9.75)	-	1.588			HETE	30.77 (9.91)	24.79 (8.83)	7.69 (3.52)	-	1.577
Feeding movements	FOOD	0 (0)	53.85 (6.61)	53.85 (6.61)	+	3.640**		Feeding movements	FOOD	1.71 (1.71)	86.32 (2.02)	84.62 (2.06)	+	3.788**
	CONS	3.42 (2.35)	19.23 (5.64)	17.52 (5.69)	+	2.546*			CONS	20.51 (5.73)	24.79 (4.68)	23.08 (4.17)	+	0.743
	HETE	1.71 (1.71)	32.48 (6.21)	31.62 (6.34)	+	3.333**			HETE	3.42 (2.08)	35.04 (5.71)	31.62 (5.49)	+	3.424**
Antennae flicking	FOOD	21.79 (3.85)	40.60 (5.99)	29.91 (4.57)	+	2.206*		Antennae flicking	FOOD	18.80 (3.30)	73.08 (6.62)	54.27 (6.54)	+	3.532**
	CONS	13.25 (6.31)	43.59 (8.82)	32.05 (8.35)	+	2.833**			CONS	34.19 (8.35)	49.57 (5.77)	35.90 (6.81)	+	1.504
	HETE	12.82 (5.53)	53.85 (8.53)	48.72 (7.96)	+	2.977**			HETE	23.93 (5.20)	54.27 (6.01)	31.20 (4.67)	+	3.445**
Antennule flicking	FOOD	19.23 (4.45)	23.93 (5.59)	29.49 (4.18)	+	0.524		Antennule flicking	FOOD	47.86 (3.96)	82.05 (3.11)	39.32 (4.69)	+	3.298**
	CONS	27.35 (6.04)	54.70 (7.91)	37.61 (7.01)	+	2.471*			CONS	55.56 (5.41)	70.94 (4.38)	27.35 (4.91)	+	1.993*
	HETE	18.80 (4.87)	57.26 (8.02)	48.72 (6.25)	+	2.967**			HETE	37.18 (4.09)	65.81 (4.75)	28.63 (4.93)	+	3.299**

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water; empty cell = no change. FOOD = food odour, CONS = conspecific odour, HETE = heterospecific odour. Control water and test solutions were compared using Wilcoxon signed ranks test (Z). *P < 0.05, **P < 0.01.

Table 11 Mean values (± s.e.) of the reaction time (s), behaviours and postures (% time) of large marron and yabbies in the presence of three test solutions (Chapter 7)

	Large marron							Large yabby						
	Odour	Control	Test	Magnitude of change	Direction of change	Z			Odour	Control	Test	Magnitude of change	Direction of change	Z
Reaction time	FOOD	172.50 (8.85)	45.00 (7.07)	127.50 (10.80)	-	3.740**		Reaction time	FOOD	100.83 (18.42)	18.33 (1.51)	82.50 (18.28)	-	3.652**
	CONS	119.17 (19.04)	27.50 (9.94)	91.67 (19.05)	-	3.327**			CONS	145.00 (17.24)	23.33 (2.49)	121.67 (16.75)	-	3.749**
	HETE	92.50 (19.11)	25.83 (3.60)	71.67 (17.85)	-	2.748**			HETE	155.00 (14.09)	23.33 (2.18)	131.67 (14.73)	-	3.754**
In shelter	FOOD	39.74 (11.69)	31.62 (8.73)	32.05 (8.53)	-	0.767		In shelter	FOOD	66.67 (11.43)	21.37 (7.55)	45.30 (10.20)	-	2.873**
	CONS	32.05 (9.94)	29.91 (8.59)	6.41 (1.67)	-	1.141			CONS	20.51 (9.43)	15.38 (6.78)	11.11 (5.74)	-	0.674
	HETE	33.76 (10.48)	22.22 (8.73)	18.38 (6.41)	-	1.309			HETE	33.33 (11.43)	14.96 (7.58)	20.94 (8.94)	-	1.761
Locomotion	FOOD	16.24 (5.86)	36.32 (7.71)	28.63 (6.06)	+	2.263*		Locomotion	FOOD	17.52 (6.49)	69.23 (7.74)	51.71 (8.83)	+	3.543**
	CONS	38.46 (8.68)	46.15 (6.31)	23.93 (6.28)	+	0.514			CONS	31.62 (4.69)	45.30 (5.63)	29.06 (3.76)	+	1.571
	HETE	24.36 (7.70)	58.12 (7.26)	40.60 (7.81)	+	2.705**			HETE	12.82 (4.13)	45.30 (7.30)	39.32 (6.22)	+	2.910**
Raised posture	FOOD	10.68 (7.01)	26.50 (7.28)	16.67 (4.23)	+	2.985**		Raised posture	FOOD	3.85 (2.09)	34.62 (3.90)	30.77 (4.61)	+	3.570**
	CONS	12.39 (4.94)	17.95 (6.12)	13.25 (3.40)	+	1.210			CONS	22.65 (6.45)	35.47 (6.64)	31.62 (5.35)	+	1.471
	HETE	17.52 (7.86)	18.38 (6.03)	14.53 (4.61)	+	0.237			HETE	6.84 (2.05)	14.96 (4.00)	13.25 (3.45)	+	1.936
Intermediate posture	FOOD	56.84 (10.35)	46.58 (7.02)	24.79 (4.06)	-	1.603		Intermediate posture	FOOD	36.75 (9.44)	55.13 (4.79)	28.63 (3.97)	+	2.310*
	CONS	58.12 (7.55)	64.96 (6.56)	41.88 (5.74)	+	0.589			CONS	60.68 (8.06)	51.71 (7.11)	28.63 (5.27)	-	0.924
	HETE	35.47 (9.45)	58.97 (6.90)	43.16 (6.64)	+	2.038*			HETE	46.15 (9.41)	43.16 (6.44)	38.89 (6.42)	-	0.032
Lowered posture	FOOD	41.03 (10.18)	24.36 (6.71)	25.21 (6.27)	-	1.926		Lowered posture	FOOD	58.97 (9.87)	10.26 (2.78)	48.72 (7.46)	-	3.370**
	CONS	29.49 (8.35)	17.09 (4.84)	30.34 (6.54)	-	1.025			CONS	16.67 (7.60)	12.82 (5.49)	14.10 (5.18)	-	0.763
	HETE	47.01 (11.07)	22.65 (7.34)	34.62 (8.19)	-	2.167*			HETE	47.01 (9.95)	41.88 (8.18)	41.03 (7.36)	-	0.398
Feeding movements	FOOD	0.85 (0.59)	64.10 (5.28)	63.25 (5.62)	+	3.730**		Feeding movements	FOOD	0 (0)	88.03 (1.42)	88.03 (1.42)	+	3.831**
	CONS	2.14 (0.84)	15.38 (4.61)	15.81 (4.10)	+	2.419*			CONS	9.40 (3.66)	28.21 (3.57)	23.08 (3.46)	+	2.911**
	HETE	2.56 (1.52)	39.74 (8.07)	37.18 (7.52)	+	3.298**			HETE	4.27 (2.65)	30.77 (3.41)	27.35 (3.74)	+	3.448**
Antennae flicking	FOOD	21.37 (3.32)	48.29 (7.06)	33.76 (5.74)	+	2.639**		Antennae flicking	FOOD	35.47 (5.74)	79.49 (3.93)	44.02 (3.67)	+	3.764**
	CONS	41.45 (7.94)	47.01 (5.63)	26.92 (5.18)	+	0.364			CONS	33.76 (4.70)	50.85 (4.18)	28.21 (4.48)	+	1.963*
	HETE	29.91 (8.06)	67.95 (6.73)	46.58 (6.91)	+	2.962**			HETE	17.95 (3.78)	50.43 (4.49)	35.90 (3.57)	+	3.561**
Antennule flicking	FOOD	50.43 (4.04)	45.30 (7.49)	25.64 (2.98)	-	0.722		Antennule flicking	FOOD	44.02 (7.59)	83.76 (3.46)	39.74 (5.21)	+	3.441**
	CONS	40.60 (5.04)	45.73 (5.75)	24.79 (3.76)	+	0.856			CONS	47.44 (6.20)	68.38 (4.57)	22.65 (3.49)	+	3.397**
	HETE	34.19 (5.06)	52.99 (6.78)	25.64 (4.74)	+	2.378*			HETE	45.30 (3.46)	62.82 (4.95)	27.78 (4.36)	+	2.074*

Magnitude of change (± s.e.) is the mean difference in absolute values between control and test. Direction of change: (-) lower or (+) higher value test solution than control water; empty cell = no change. FOOD = food odour, CONS = conspecific odour, HETE = heterospecific odour. Control water and test solutions were compared using Wilcoxon signed ranks test (Z). *P < 0.05, **P < 0.01.

Appendix 4.0 The influence of gender, size, life-stage and prior residence on shelter acquisition by marron and yabbies

---

This publication can be found in *Freshwater Crayfish 15*:

Height, S.G., Marsh, B. and Whisson, G.J. 2006. The influence of gender, size, life-stage and prior residence on shelter acquisition by marron (*Cherax tenuimanus*) and yabbies (*Cherax albidus*). *Freshwater Crayfish 15*: 79-86.

1.0 Introduction

Species invasions can result in substantial loss of biodiversity due to competitive interactions, predation and associated introductions of diseases and parasites (Diamond and Case 1986, Horwitz 1990, Vitousek et al. 1996). In aquatic ecosystems, invasive species have been identified as one of the greatest threats to freshwater biodiversity and ecosystem function (Lodge et al. 2000). Freshwater crayfish are well documented as an invasive species, having been translocated by humans frequently over the recent past, mostly for aquaculture ventures (Gherardi and Holdich 1999, Lodge *et al* 2000). Crayfish have escaped from farm ponds, invaded natural water bodies, and in several instances, given rise to breeding populations that have replaced populations of native species (Holdich 1988, Gherardi et al. 2002a). The invasive success of non-indigenous crayfish species depends on a number of biological characteristics including the ability to tolerate environmental extremes (Huner and Lindqvist 1995), polytrophism (Gherardi et al. 2002a), rapid growth (Paglianti et al. 2001), high fecundity (Huner 2001), disease resistance (Evans and Edgerton 2001), and the level of competitive ability and plasticity (Gherardi et al. 2000, Hazlett et al. 2003).

Marron (*Cherax tenuimanus* Smith 1912) are native to the permanent rivers and streams in the south-west of WA. Yabbies (*Cherax albidus* Clark 1936) were first introduced to farm dams in Western Australia in 1932 (Morrissy and Cassells 1992) from the eastern states of Australia. Although the present distribution of yabbies in this

region is uncertain, a number of breeding populations are known to exist as a result of escape from manmade impoundments, placing pressure on native marron populations as both species compete for limited resources. These competitive interactions between marron and yabbies are not well understood, particularly in the case of shelter acquisition. Shelter is an important, limited resource for crayfish (Bovbjerg 1970) providing protection against predation and cannibalism (Lodge and Hill 1994, Figler et al. 1999). Inferiority in competition for shelter may lead to increased predation risk, and contribute to species displacement (DiDonato and Lodge 1993, Blank and Figler 1996, Guiasu and Dunham 1999).

While there is evidence that invasive freshwater crayfish can be detrimental to native species (Gherardi and Holdich 1999, Gherardi et al. 2002b), it appears that in some water bodies in Western Australia, where displacement had previously been documented (Whisson 2003), marron populations are expanding in the presence of well-established yabby populations (Campbell and Whisson 2002). This is an unexpected occurrence, and very important to the current aquatic translocation debate in Western Australia. It suggests that the mechanisms underlying displacement and competitive exclusion in mixed crayfish populations may be more complex than originally thought. This study investigates the influence of gender, relative size, life-stage, and prior residence on shelter acquisition by marron and yabbies in an attempt to better understand the nature of those factors shaping these inter-specific interactions.

2.0 Materials and methods

2.1 Experimental animals

Marron and yabbies were sourced from a crayfish farm in Parkerville, Western Australia (32^{o}S, 116^{o}E) and held in tanks at the Aquatic Science Research Laboratory at Curtin University of Technology. Size and weight classes of marron and yabbies used in the trials are displayed in Table 1. Crayfish were not fed for the duration of the trials.

Table 1. Crayfish size grades used in the shelter acquisition trial

Crayfish	Number	Weight (g)	Carapace (mm)
large male marron	12	95.6 ± 1.9	70.0 ± 1.8
large female marron	12	87.1 ± 3.9	70.0 ± 1.2
small male marron	9	44.0 ± 1.2	52.4 ± 1.0
small female marron	12	46.9 ± 2.7	54.1 ± 1.4
male yabbies	15	51.4 ± 2.5	46.3 ± 0.8
female yabbies	15	41.2 ± 2.0	43.5 ± 0.9
berried yabbies	12	52.4 ± 1.2	47.1 ± 0.4

2.2 Experimental design

Experiments were conducted in the Aquatic Science Research Laboratory in October 2003. Each experiment ran for 24 h. Artificial lighting was provided at 12:12 light:dark. An individual marron or yabby (the prior resident) was placed into an aquarium (30 x 60 cm) containing 40 L of conditioned tap water and a single piece of polyvinyl chloride tube (length 20 cm, diameter 9 cm) for shelter. Each aquarium was covered in black plastic for visual isolation except for one end, which was left uncovered for observation of the test animals. After a 24 h acclimation period, tanks containing a marron then received a yabby; tanks containing a yabby received a marron (Plate 1). The shelter occupant was recorded after 10 minutes, and then every 4 h for 24 h. Shelter occupancy was recorded as one of the following: resident in shelter; intruder in shelter; both crayfish in shelter; neither crayfish in shelter. For a crayfish to be deemed ‘in shelter’, it was required to have at least two appendages inside the shelter.

Intruding crayfish included same or opposite sex heterospecifics that were the same size or larger than the resident. All permutations of the different classes of marron and yabbies were tested as residents/intruders; each combination was replicated three times in a Latin square design. Total ammonia, nitrite, nitrate, salinity, pH, dissolved oxygen and temperature were monitored in aquariums at the beginning and end of each experiment.

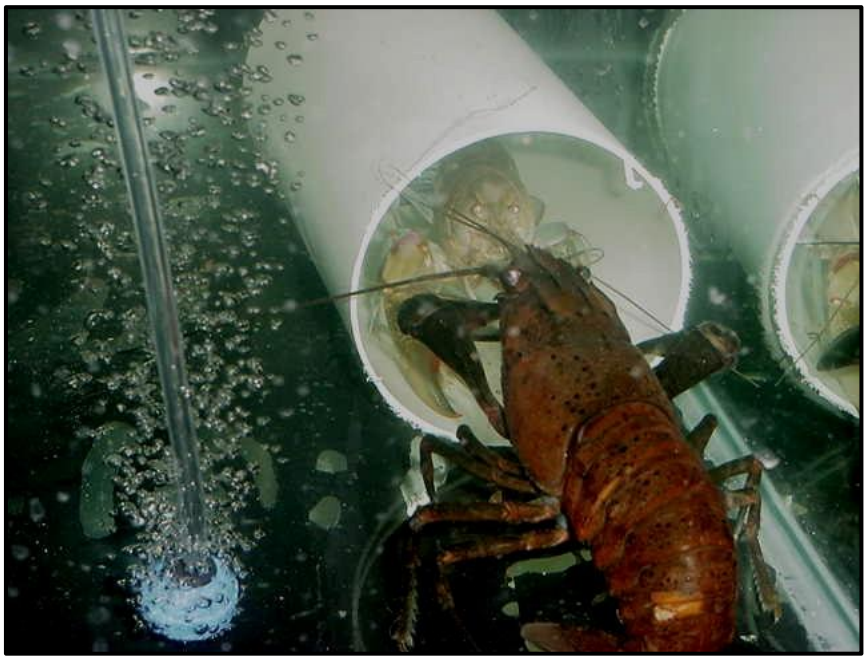

Plate 1 Shelter competition between a resident yabby and a marron intruder

2.3 Statistical analysis

Comparisons between and within treatments were made using the Mann-Whitney *U*-test. Comparisons between treatments for pooled data used Chi-square analysis (χ^2). Water quality data were analysed using a one-way ANOVA. All statistical analyses used SPSS statistical software package.

3.0 Results

Size and gender did not affect shelter use by marron or yabbies in any of the treatments that marron were prior residents (P > 0.1). The presence of an intruding yabby did not affect the shelter use of marron (treatments that contained both marron and yabbies were compared to treatments that contained marron only; P > 0.1) with the exception of large female marron, which used the shelter more frequently when male yabbies were intruders (z =-2.121, P = 0.034). Large female marron also occupied the shelter more frequently with male yabby intruders than female yabby intruders (z = -1.650, P = 0.099). When data were pooled across treatments, marron (as prior residents) occupied the shelter more frequently than did intruding yabbies ($\chi^2(1) = 4.455$, P = 0.035; Fig. 1), and the larger marron used the shelter more often than smaller marron ($\chi^2(1) = 9.966$, P = 0.002).

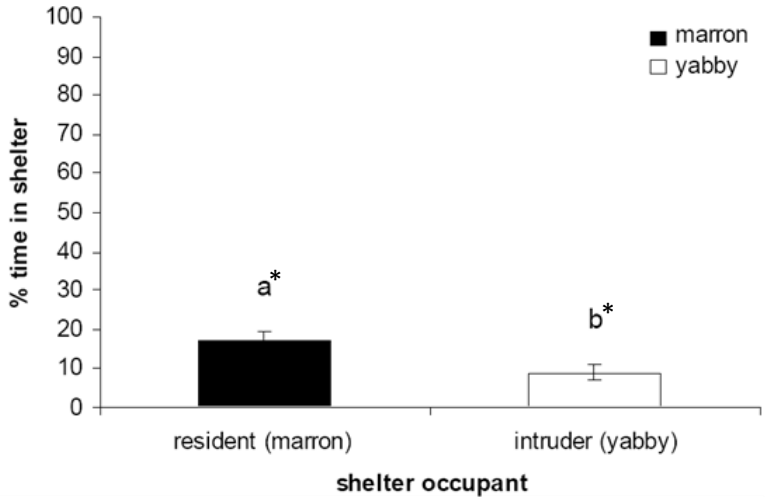

Figure 1. Time spent in shelter by resident marron and intruding yabbies

Values represent pooled treatment means ± standard error. Different letters between groups indicate significantly different means at *P < 0.05.

When berried yabbies were tested as intruders on marron, both large male marron and small female marron occupied the shelter more frequently than did the berried yabbies (z = -1.650, P = 0.099; and z = -2.121, P = 0.034 respectively; Figure 2a, Figure 2b), however the presence of marron did not affect shelter use by the berried yabbies (P > 0.1).

When yabbies were prior residents, intruding large female marron occupied the shelter more frequently than resident male yabbies (z = -2.023, P = 0.043; Figure 2c), and were more successful in excluding male yabbies than female yabbies from shelter (z = -1.798, P = 0.072). Prior residence affected shelter usage by female yabbies, which were more successful in acquiring shelter as a resident than as an intruder in the presence of large female marron (z = -2.087, P = 0.037). When data were pooled, marron occupied the shelter more frequently as prior residents than yabbies as prior residents ($\chi^2(1) = 4.457$, P = 0.035).

Water quality data remained within optimum ranges for crayfish used in the experiments (Morrissy, N.M. 1992, Mosig 1998). No significant differences existed (P > 0.05) between tanks for any given parameter (total ammonia, nitrite, nitrate, salinity, pH, dissolved oxygen and temperature).

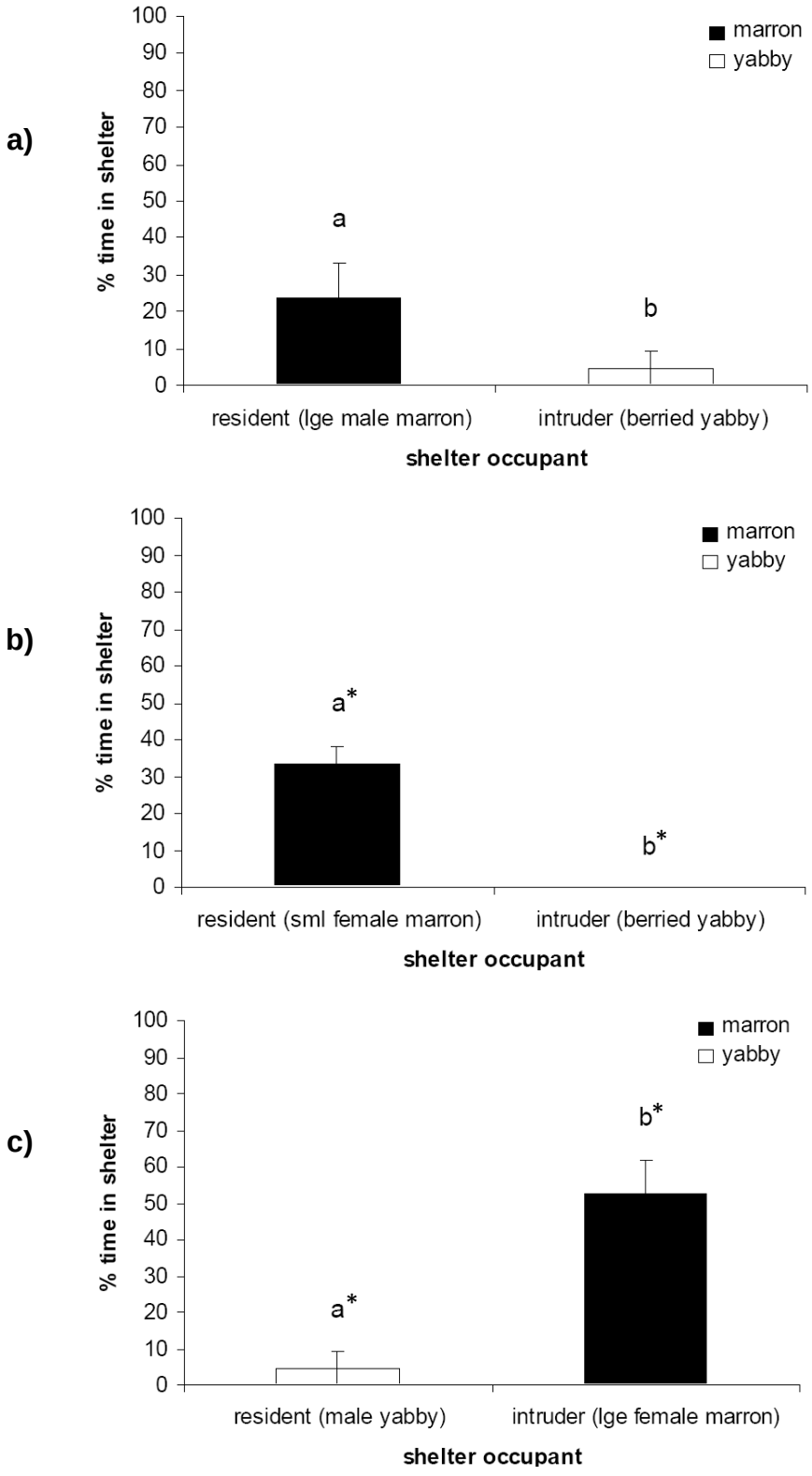

Figure 2. Shelter utilisation by prior resident crayfish: a) large male marron; b) small female marron; c) male yabbies

Values are treatment means ± standard error. Different letters between groups indicate significantly different means at $P < 0.1$, *$P < 0.05$.

4.0 Discussion

Previous studies have documented the relative importance of prior residence, size and gender on interactions in decapod crustaceans (Evans and Shehadi-Moacdieh 1988, Ranta and Lindström 1993, Peeke et al. 1995, 1998, Figler et al. 1999, Takahashi et al. 2001). The prior residence effect, where the initial resident in a geographic area typically has an advantage over intruders (Peeke *et al* 1998), may be compromised when the intruder has a body-size advantage over the resident (Wazlavek and Figler 1989, Nakata and Goshima 2003). In the present study, given the advantage of prior residence, neither species was inherently dominant in shelter occupation. However, when data were pooled across treatments, the results suggested that both prior residence and crayfish size assisted marron in shelter occupation.

The advantage of crayfish size in both intra- and interspecific interactions has been documented by a number of researchers (Söderbäck 1995, Figler *et al* 1999, Vorburger and Ribi 1999, Nakata and Goshima 2003). In Western Australia, native marron possess a size advantage over non-indigenous yabbies. Söderbäck (1995) reported that the size advantage of *Pacifastacus leniusculus* resulted in increased success in aggressive interactions with native *Astacus astacus* and decreased vulnerability to gape-size limited predators. Similar mechanisms may be preventing yabby populations from expanding in some waterbodies in Western Australia due to decreased success in interspecific interactions with marron (where marron possess a size advantage over yabbies), and size selective predation by finfish, especially where shelter is a limited resource.

Vorburger and Ribi (1999) examined aggression and competition for shelter between native *Austropotamobius torrentium* and introduced *P. leniusculus*. Similar to the present study, neither species was inherently dominant in aggressive interactions, but dominance was strongly size-dependent, favouring the larger species, *P. leniusculus*. Nakata and Goshima (2003) reported that a body-size advantage strongly influenced the outcome of both intra- and interspecific contests for shelter between *Cambaroides japonicus* and *P. leniusculus*, overcoming the prior residence effect. A similar situation

was observed in the present study; intruding large female marron excluded resident male yabbies from shelter. This is a significant finding; if female marron are capable of acquiring shelter over smaller heterospecific crayfish, then the chances of reproductive success increase, whilst heterospecific crayfish will be excluded to less-favourable habitat, possibly facing increased pressure from predators.

Berried yabbies were used as a resident/intruder to test the assertion that this life-stage is the most successful in acquiring shelter. Mason (1977) discovered that female *P. leniusculus* were most aggressive around hatching time. Figler *et al* (1995, 1997) reported maternal aggression in *Procambarus clarkii*- resident maternal females that were ovigerous or brooding hatched progeny showed a significantly stronger shelter competition advantage over intruding crayfish than did non-maternal residents. Considering these results from previous researchers, it was surprising to find that berried yabbies did not display any shelter competition advantage as a resident or intruder. Furthermore, both large male marron and small female marron occupied the shelter more frequently than intruding berried yabbies. It is recommended that these results be confirmed with higher replication of specific size combinations of male and female marron and yabbies.

The results of this research indicate that body size is a key factor influencing shelter competition between marron and yabbies. This finding is consistent with previous authors (Vorburger and Ribi 1999, Nakata and Goshima 2003) who identified the advantage of body size on competitive interactions between other pairs of native and invasive crayfish (*A. torrentium* and *P. leniusculus*; *C. japonicus* and *P. leniusculus* respectively). While many influencing factors have been identified (burrowing, habitat type and complexity, presence of macrophytes, water depth and quality), in Western Australia, the smaller body size of yabbies compared to marron may be an important factor limiting the expansion of yabby populations in the presence of marron, especially in waterbodies where shelter is a limited resource.

References

Blank, G.S. and Figler, M.H. 1996. Interspecific shelter competition between the sympatric crayfish species *Procambarus clarkii* (Girard) and *Procambarus zonangulus* (Hobbs and Hobbs). *Journal of Crustacean Biology* 16:300-309.

Bovbjerg, R.V. 1970. Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). *Ecology* 51:225-236.

Campbell, L. and Whisson, G. 2002. The changing composition of crayfish at Lake Moyanup, Western Australia: the influence of an invasive congener and three predaceous finfish species (Abstract). 14th symposium of The International Association of Astacology, Queretaro,Mexico, 4 -10 August 2002.

Diamond, J. and Case, T.J. 1986. Overview: introductions, extinctions, exterminations, and invasions. In: *Community Ecology* (Diamond, J. and Case, T.J. eds), pp. 65–79. Harper & Row Publishers, New York.

DiDonato, G. T. and Lodge, D.M. 1993. Species replacements among *Orconectes* crayfishes in Wisconsin lakes: the role of predation by fish. Canadian Journal of *Fisheries and Aquatic Sciences* 50:1484–1488.

Evans, D.L. and Shehadi-Moacdieh, M. 1988. Body size and prior residency in staged encounters between female prawns, *Palaemon elegans* Rathke (Decapoda: Palaemonidae). *Animal Behaviour* 36:452–455.

Evans, L.H. and Edgerton, B.F. 2001. Pathogens, parasites and commensals. In: *Biology of Freshwater Crayfish* (Holdich, D.M. ed), pp. 377-438. Blackwell Science, Oxford.

Figler, M.H., Blank, G.S. and Peeke, H.V.S. 1997. Maternal aggression and post-hatch care in red swamp crayfish, *Procambarus clarkii* (Girard): the influences of presence of offspring, fostering, and maternal molting. *Marine and Freshwater Behaviour and Physiology* 30:173–194.

Figler, M.H., Cheverton, H.M. and Blank, G.S. 1999. Shelter competition in juvenile red swamp crayfish (*Procambarus clarkii*): the influences of sex differences, relative size, and prior residence. *Aquaculture* 178:63-75.

Figler, M.H., Twum, M., Finkelstein, J.E. and Peeke, H.V.S. 1995. Maternal aggression in red swamp crayfish (*Procambarus clarkii*, Girard): the relation between reproductive status and outcome of aggressive encounters with male and female conspecifics. *Behaviour* 132:107–125.

Gherardi, F., Acquistapace, P., Hazlett, B.A. and Whisson, G. 2002a. Behavioural responses to alarm odours in indigenous and non-indigenous crayfish species: a case study from Western Australia. *Marine and Freshwater Research* 53:93-98.

Gherardi, F. and Holdich, D.M. 1999. Crayfish in Europe as Alien Species: how to make the best of a bad situation? (eds. Gherardi, F., & Holdich, D.M.), A.A. Balkema, Rotterdam. 299p.

Gherardi, F., Raddi, A., Barbaresi, S. and Salvi, G. 2000. Life history patterns of the red swamp crayfish, *Procambarus clarkii*, in an irrigation ditch in Tuscany, Italy. *Crustacean Issues* 12:99–108.

Gherardi, F., Smietana, P. and Laurent, P. 2002b. Interactions between non-indigenous and indigenous crayfish species. Roundtable Session 2B. Bulletin. Fr. Piscic 367:899-907.

Guiasu, R.C. and Dunham, D.W. 1999. Aggressive interactions between the crayfishes *Cambarus bartonii bartonii* and *C. robustus* (Decapoda: Cambaridae): interspecific and intraspecific contests. *Journal of Crustacean Biology* 19:131-146.

Hazlett, B.A., Burba, A., Gherardi, F. and Acquistapace, P. 2003. Invasive species use a broader range of predation-risk cues than native species. *Biological Invasions* 5:223–228.

Holdich, D.M. 1988. The dangers of introducing alien animals with particular reference to crayfish. *Freshwater Crayfish* 7:15-30.

Horwitz, P. 1990. The translocation of freshwater crayfish in Australia: Potential impact, the need for control and global relevance. Biological Conservation 54:291-305.

Huner, J.V. and Lindqvist, O.V. 1995. Physiological adaptations of freshwater crayfish that permit successful aquacultural enterprises. American Zoologist 35:12-18.

Huner, J.V. 2001. *Procambarus* In: *Biology of Freshwater Crayfish* (Holdich, D.M. ed), pp. 541-584. Blackwell Science, Oxford.
Hyatt, G.W. 1983. Qualitative and quantitative dimensions of crustacean aggression. In: *Studies of Adaptation: The Behaviour of Higher Crustacea* (Rebach, S. and Dunham, D.W. eds), pp. 113-139. Wiley-Interscience, New York.

Lodge, D.M. and Hill, A.M. 1994. Factors governing species composition, population size and productivity of cool water crayfishes. *Nordic Journal of Freshwater Research* 69:111-136.

Lodge, D.M., Taylor, C.A., Holdich, D.M. and Skurdal, J. 2000. Non-indigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. Fisheries 25:7–20.

Mason, J.C. 1977. Copulatory behaviour of the crayfish, *Pacifastacus trowbridgii* (Stimpson). *Canadian Journal of Zoology*, 48:969-976.

Morrissy, N.M. 1992. An Introduction to Marron and Freshwater Crayfish Farming in Western Australia. Fisheries Department ofWestern Australia, 36 pp.

Morrissy, N. M., and Cassells, G. 1992. Spread of the introduced yabby *Cherax albidus*, Clark, 1936 in Western Australia. Fisheries Department ofWestern Australia, Research Report No. 92:1-27.

Mosig, J. 1998. *The Australian Yabby Farmer*. Landlinks Press, Victoria. 214p.

Nakata, K. and Goshima, S. 2003. Competition for shelter of preffered sizes between the native crayfish species *Cambaroides japonicus* and the alien crayfish species *Pacifastacus leniusculus* in Japan in relation to prior residence, sex difference and body size. *Journal of Crustacean Biology* 23:897-907.

Paglianti, A., Gualtieri, M. and Gherardi, F. 2001. Growth rate in YOY crayfish: combined effects of temperature and diet. Knowledge-based Management of European Native Crayfishes: Dialogues between researchers and managers, Potiers, September 13-15, p.35.

Peeke, H.V.S., Figler, M.H. and Chang, E.S. 1998. Sex differences and prior residence effects in shelter competition in juvenile lobsters, *Homarus americanus* Milne-Edwards. *Journal of Experimental Marine Biology and Ecology* 229:149–156.

Peeke, H.V.S., Sipple, J. and Figler, M.H. 1995. Prior residence effects in shelter defense in adult signal crayfish (*Pacifastacus leniusculus* (Dana)): results in same- and mixed-sex dyads. *Crustaceana* 68:873–881.

Ranta, E. and Lindström, K. 1993. Body size and shelter possession in mature signal crayfish, *Pacifastacus leniusculus*. *Annales Zoologici Fennici* 30:125-132.

Söderbäck, B. 1995. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms. *Freshwater Biology* 33: 291-304.

Takahashi, M., Suzuki, N. and Koga, T. 2001. Burrow defense behaviors in a sand-bubbler crab, *Scopimera globosa*, in relation to body size and prior residence. *Journal of Ethology* 19:93–96.

Vitousek, P.M., D'Antonio, C.M., Zloope, L.L. and Westbrooks, R. 1996. Biological invasions as global environmental change. *American Scientist* 84:468-478.

Vorburger, C. and Ribi, G. 1999. Aggression and competition for shelter between a native and an introduced crayfish in Europe. Freshwater Biology 42: 111-119.

Whisson, G. 2003. Human impacts on marron populations in the South-West. Natural Heritage Trust, Project Final Report. Project No. 983284. 24p.

Wazlavek, B.E. and Figler, M.H. 1989. Territorial prior residence, size asymmetry, and escalation of aggression in convict cichlids *Cichlasoma nigrofasciatum* Gunther. *Aggressive Behaviour* 15:235–244.

Appendix 5.0 Diet formulations for crayfish and silver perch

Table 1 Composition of marron pellet used for experiments in Chapters 4, 5, 6 and 8

Protein	23.0%
Fat	6.0%
Crude fibre	8.3%
Calcium	2.9%
Phosphorus	1.0%
Salt	0.3%
Metabolisable energy	9.9 MJ/kg
Biotin	125 mg/kg
Calcium pantothenate	21 mg/kg
Choline	170 mg/kg
Copper	0.8 mg/kg
Folic acid	0.8 mg/kg
Iodine	0.1 mg/kg
Iron	2 mg/kg
Manganese	10 mg/kg
Nicotinic acid	33 mg/kg
Riboflavin	3 mg/kg
Thiamine	2.5 mg/kg
Vitamin A	1700 IU/kg
Vitamin D	250 IU/kg
Vitamin E	10 mg/kg
Vitamin K	0.3 mg/kg
Zinc	4 mg/kg

Pellet supplied by Glen Forrest Stockfeeders™ Pty Ltd

Table 2 Formulation and biochemical composition of silver perch pellet used for experiments in Chapters 6 and 7

Ingredients	**%**
Fish meal	27.0
Soybean meal	20.0
Blood meal	2.0
Corn gluten meal	4.0
Wheat	28.4
Sorghum	11.0
Millrun	2.0
Cod liver oil	1.0
Di-calcium phosphate	2.0
Vitamin/mineral premix	2.5
L-methionine	0.15

Proximate composition	
Crude protein	
Crude fat	
Linoleic series fatty acids	
Fibre	
Carbohydrate	

	g/kg
Total methionine	7.4
Total lysine	22.6

Pellet supplied by Glen Forrest Stockfeeders™ Pty Ltd