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The role of shelter in Australian freshwater crayfish (*Cherax spp.*) polysystems

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Executive summary

Research into the polyculture of finfish and crayfish has been conducted in Western Australia for over a decade now. This research was instigated out of a need to increase revenues from freshwater crayfish farmers wishing to diversify their income base with a view to increasing profitability and reducing risk. It has become clear that several key variables dictate how the polyculture system (i.e. polysystem) will perform. These include biological factors like: size of participating species, relative densities, gender, planktonic turbidity, natural feeds; and abiotic factors like: light intensity, clay turbidity, floating cages for segregation, water quality, and habitat/shelter complexity. Many of these factors can be controlled/adjusted by the manager of the polysystem to maximise performance, production and profitability.

While much of the research to date has focussed on the marron (*Cherax tenuimanus*) industry, it is also important to realise that an understanding of these factors can also assist other crayfish polysystems, like integrated agri-aquaculture systems containing yabbies (*Cherax albidus*). Some of the factors that influence how the system will perform may become more prevalent, like suspended clay turbidity and the associated role of light intensity in species interactions, or shelter complexity and the resulting choice of shelter material. But overall, they are the same basic variables and we must understand how they affect the particular multi-species system that we are dealing with.

There is a lot to be learned from the literature on how these variables affect multi-species aquatic environments in the wild. Perhaps aquaculturists have not considered this enough in the past. Some farmers seem to believe that these variables are different JUST because it is a culture system. This is not true. The variables will take on different levels in a culture system (i.e. a manager will stimulate turbidity, provide artificial feeds, stock different sizes, and supply particular types of shelter) BUT the actual variables themselves (e.g. food, density, light, shelter) are basic to ALL aquatic ecosystems.

Other researchers have looked at important factors like density, gender, and light intensity/turbidity in crayfish polysystems - but the issue of habitat complexity and the role of shelter has not been adequately addressed. This thesis will investigate some

basic questions about shelter and endeavour to apply them to crayfish polysystems, with the emphasis on marron (*C. tenuimanus*) and yabbies (*C. albidus*) because these are the two most commercially important species of crayfish in Western Australia. Importantly, it should be noted that due to the invasive nature of yabbies, and their apparent ability to displace native marron in the wild, findings will be related to yabby-marron competition/displacement where relevant.

We need to know many things about shelter: what type is best in a multi-species system? Should the shelter size match the crayfish size? Do marron have different requirements for shelter than yabbies? Does it matter who gets first use of a shelter (i.e. prior residence effect)? Can we learn about crayfish shelter requirements by examining the behaviour/plasticity of crayfish species? If crayfish are stocked with finfish and they retreat into shelter as a predator-avoidance measure, is the complexity important given that their densities will be higher? If densities of crayfish inside shelters are higher in polysystems, will cannibalism be a concern, particularly when conditions are right for moulting? Does visual recognition and/or chemo-detection of a predator affect the shelter usage by marron or yabbies? Does temperature affect shelter usage behaviour for a burrowing species like yabbies?

Shelter is an important factor in the life history of a freshwater crayfish and an understanding of its influence on different species is important for maximising system performance. Crayfish are categorised depending on their ability to construct shelters (i.e. burrows). Yabbies have evolved in systems with fluctuating water quality and many predators and, as such, have learned to burrow (to escape drought and also to escape predators). Marron, on the other hand, are a non-burrowing native crayfish species that have existed with relatively few predators in the South-West. As a result, marron are less capable of modifying their behaviour when confronted with a predator (i.e. low behavioural plasticity). Species with high plasticity, like yabbies, are more capable of adapting to new environments, because they can change their behaviour to increase their chance of survival. Therefore we can expect yabbies and marron to utilize habitats differently and we should compare these behaviours as a basis to developing management strategies. This type of knowledge may also assist with managing the translocation and spread of yabbies in the wild and their displacement of native marron.

Within multi-species systems, the physical structure of shelter plays an important role in protecting crayfish and the perfect shelter would not only provide safety from co-stocked finfish, but also from conspecific cannibalism. Given the different life histories and behaviours, it is probable that both species of crayfish will have different refuge requirements.

Over the course of this four-year investigation, trials were conducted in four culture systems (72L aquariums, 300L circular tanks, 80t mesocosm tank, and 720m^2 earthen ponds) using marron and yabbies as the species of interest. Silver perch and Murray cod were chosen as the finfish species of interest as they appear to have the highest aquaculture potential for native freshwater finfish in Australia at the present time. Further, both of these fish have been documented as potential predators of crayfish, resulting in a challenge to understand the role of shelter in minimising the negative effects of fish-crayfish interactions within a polysystem.

This study has confirmed that shelter plays a critical role in multi-species system dynamics. In the case of polysystems, it will affect both interspecific and intra-specific interactions, ultimately governing production and profitability, along with the other, previously defined factors. This means that the manager of a polysystem can influence productivity by understanding: a) the behavioural characteristics and biology of the crayfish; b) the feeding biology of the finfish; and c) the system variables (both biotic and abiotic) that will affect the overall well being of the fish and crayfish. In the case of shelter, the manager should understand the available shelter types, the appropriate densities, the importance of matching complexity to the crayfish size, and the prior residence effect when choosing a timing strategy for stocking and harvesting.

Prior residence increased resource holding potential for both marron and yabbies in the short term. In fact, prior residence was a stronger determinant of successful sheltering than crayfish gender or species. However, in longer-term trials the physical size of the crayfish (larger animals evicted smaller animals) and reproductive status (berried females were successful at evicting all other crayfish) were more important factors in determining successful shelter acquisition, although the temporal variations (i.e. growth and release of young) complicate the issue.

When stocking crayfish of different sizes, and in polysystems, the correct size of shelter becomes critical, as smaller individuals will be forced to leave over-sized shelter and locate a shelter commensurate with their own body size to avoid predators. This is relevant to crayfish nurseries where complex habitat is paramount for juvenile cohorts that display variation in sizes and gender.

The expansion of crayfish polyculture holds considerable promise; however, further investigations are required into shelter complexity within floating fish cages, shelter types and arrangement of shelters within ponds (for increased production and ease-of-harvesting), potential of yabbies in polyculture (comparison of monosex and hybrid strains), and the impact of shelter on escape behaviour of marron in a polysystem.

This thesis is dedicated to my parents and grandparents

and my best friends… Joey and Risa Wangpen

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form of another degree or diploma at any university or other institution of tertiary education. Information derived from published or unpublished sources has been acknowledged in the text and a list of references is given.

Prayadt Wangpen   **29 June 2007**

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Prayadt Wangpen   **29 June 2007**

Acknowledgements

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1. Wangpen, P., Whisson, G. (2005), Effect of size, gender and physical factors on behavioural response and shelter acquisition in freshwater crayfish under threat from a finfish predator. Biothon Event, Department of Environmental Biology, Muresk Institute, Curtin University of Technology p25 (Abstract and poster presentation).

2. Wangpen, P., Whisson, G. (2005, in press), Effect of size, gender and physical factors on behavioural response and shelter acquisition in freshwater crayfish under threat from a finfish predator. World Aquaculture Society, Bali, Indonesia. (Abstract and oral presentation).

3. Wangpen, P. (2005), When a predator becomes a prey, Short communication, Crayfish News Vol. 27 Issue 3 p3.

4. Whisson, G., Tomlinson, C., Hoschke, A., and Wangpen, P. (2005) Comparing catch composition of two designs of samplers deployed on the Ningaloo Reef, Western Australia, with an emphasis on crustaceans. Australian Marine Sciences Association annual conference 2005, Darwin, Northern Territory (poster presentation).

5. Wangpen, P., Whisson, G., and Height, S. (2006, in press), Combined effect of temperature and shelter availability on survival of famished juvenile yabbies (*Cherax albidus* Clark*), Freshwater Crayfish 16* (Abstract and oral presentation).

6. Wangpen, P. (2007), The role of shelter in Australian freshwater crayfish (*Cherax spp.*) polysystems*, Crayfish News* Vol.28 Issue (under prep.).

Chapter one

Introduction

The aim of this introductory chapter is to provide an overview of the state of world fisheries (including aquaculture production) and outline Australia's status within a global context. There are many reasons why aquaculture should prosper in Australia; however, the key to the future of this type of farming is a sound understanding and application of sustainable development principles, within a framework of biodiversity protection. In this thesis, polyculture is presented as a multi-species approach that can be relevant to both natural systems and farming systems. An understanding of the importance of shelter as a key factor governing interactions and productivity within these systems is therefore justified.

1.1 Global fisheries overview

1.1.1 Global fisheries production

Global capture fisheries production reached a maximum yield of 100 million tonnes in the early 1990s (FAO 2006). The production has stabilised with annual growth of 1.2 percent since 1970. Natural phenomena and anthropogenic pressures are major causes of fluctuations in production (FAO 2006). Overfishing and habitat destruction are the major constraints to the recovery of fish stock (Pafit 1995, Kurkansky 1997, Moore 1999). Illegal fishing combined with greater technology have contributed to the collapse of capture fisheries in many countries (Silvani 1999, Valdemarsen 2001): Canada (Pafit 1995), the Americas (Kurkansky 1997) and Australia (Wainwright & Kirkness 1997). In contrast, the demand for seafood as a source of prime quality protein and lipids has increased at an alarming rate, while capture fisheries encounter high operating costs and deplete the world's fisheries resources (Love & Langenkamp 2003). At the same time, the world population is expanding far in excess of the supply of fishery products (UN 2004); the consumption of seafood per capita has therefore slightly declined (FAO 2004). Thus the price of seafood is rising and becoming less affordable.

The fisheries industry is facing increasing operating costs, especially fuel and labour (Hannesson 2003); and restricted areas for fishing due to conservation or fisheries rehabilitation programmes (Tidwell & Allan 2001). Fishers are forced to operate in more remote fishing grounds, incurring even higher travelling costs whilst returns are dwindling due to yield. This situation highlights the need for alternative sources of seafood.

1.1.2 A case for aquaculture

Aquaculture, defined simply as farming animals and plants in water, is playing an increasingly important role in securing world food supplies and therefore supporting the world economies (FAO 1997, 1999, 2004). Aquaculture is a means of providing raw materials and employment for seafood industries (Yearsley *et al.* 1999). Historically, aquaculture has provided alternative high-quality staple foods for centuries (Naylor *et al.* 2000; Tidwell & Allan 2001). In recent years, aquaculture has made significant technical advances, and is a rapidly expanding and profitable sector in many countries. This

technical progress provides greater quality control and makes aquaculture products more affordable for all people (Losordo 1998, Halachmi *et al.* 2005). By the year 2010, approximately half of the world seafood available for human consumption is expected to be provided by aquaculture (FAO 2004). Recently, Subasinghe (2006) suggested that the emergence of this sector as a significant source of food would contribute to the alleviation of poverty; Aguilar-Manjarrez *et al.* (2006) endorse this view and added that aquaculture, being renewable and thereby sustainable in nature, can also reconcile conflict over resources.

The FAO (1999) stated that aquaculture has expanded rapidly (Fast & Leung 2003) and can now be found in almost every country in the world (Medley *et al.* 1994, FAO 2004). China remains the major finfish aquaculture producer, contributing up to 67 % of world production (FAO 2006, Subasinghe 2006). Not surprisingly, most of world finfish aquaculture production comes from polyculture ponds (FAO 1997, Milstein 1997, Liang *et al.* 1999, Sharma *et al.*1999, Wurts 2000).

1.2 World aquaculture

1.2.1 Present status of world aquaculture

World seafood supply is shifting towards aquaculture, which generated a total of $US63.4 billion worth of product in 2004 for the world market (FAO 1997, 2004). It is estimated that 36 million people worldwide are employed directly through fishing and aquaculture (Naylor *et al.* 2000) and that as many as 200 million people receive direct and indirect revenue from the seafood industry (Garcia & Newton 1994, Naylor *et al.* 2000). In addition, advances in aquaculture have made a major contribution to the expansion of seafood industries, trade, and the diversification of seafood worldwide. In terms of species and products, it is currently estimated that more than 240 aquaculture species are traded globally in approximately 800 different forms, varieties and preparations (Love & Langenkamp 2003, FAO 2004). As the trading of seafood products changes according to market demand, aquaculture production techniques and the husbandry of new species need to be investigated.

1.2.2 Future trends in aquaculture

As aquaculture production becomes more complex (Hutchins *et al.* 1998, Lee 2003, Li *et al.* 2003, Zhang *et al.* 2004a, Zhang *et al.* 2004b, Westenberg *et al.* 2005, Jerry *et al.* 2005, Jerry *et al.* 2006), husbandry becomes more intensive (Burford *et al.* 2003, Paez-Osuna *et al.* 2003, Biao *et al.* 2004, Tlusty *et al.* 2005 and Avnimelech 2006). The high return on investment coupled with other favourable economic factors present aquaculture as a highly attractive option over other primary industries (Cordingley 2005). At the same time, the current climatic problems of global warming and degradation of natural environments (Vitousek *et al.* 1997, Flannery 2006) put global ecosystems under threat (Forrest & Blakemore 2006). These threats include the scarcity of water resources and diminishing global biodiversity (Stevens *et al.* 2000, Hartley 2002 and Rice 2005). In this regard, aquaculture is being hypothesized as a contributor to the severe depletion of wild fish stocks (Naylor *et al.* 2000).

The current concerns are not limited to the availability of land and water; competition for use of resources is also greater (Paez-Osuna *et al.* 2003, Herrera-Silveira *et al.* 2004, Giap *et al.* 2005, Boyd & Polioundakis 2006). Poorly managed and irresponsible aquaculture is seen as a contributor to land degradation, resource depletion, water pollution, and other issues including inefficient use of fisheries products (Li *et al.* 2004) that could be utilised for human consumption (Edwards 1992).

As with other primary producers, aquaculture must develop and grow in a sustainable fashion (Subasinghe 2006). The flagship code of practice in aquaculture (Rogers 2000) fosters environmentally friendly and sustainable aquaculture (FAO 1999, 2004). Many strategies have been investigated (Crawford *et al.* 2001, Blum 2003, Dowd 2005), from integrated agri-aquaculture (Edwards *et al.* 1997, FAO 2004, Neori *et al.* 2004), to cleaner production.

Little (1987), Milstein *et al.* (1991), Edwards (1992), Milstein (1992, 1997), Milstein *et al.*(1995) and Whisson (2000) promote the potential of polyculture, namely diversifying pond production in aquaculture through the concurrent culture of multiple compatible species, a feature intrinsic to natural aquatic ecosystems. Valdimarsson *et al.* (1997), Stanley (2003), Pruder (2004), Murray & Peeler (2005) are groups of scientists and

aquaculturists investigating how best to maximise the full potential of available species without compromising the environment (Whisson 2000, Boyd 2003, O'Bryen & Lee 2003). The aquaculture footprint is measurably reducing (Boyd 2003, Yokoyama 2003, and Martinez-Cordero & Leung 2004). These research directions will play an important part of the sustainable development of aquaculture.

1.3 Australian aquaculture

1.3.1 Introduction

Over the past two decades, Australian aquaculture production has expanded due to stronger demand from overseas for high value seafood, tougher import policies and strong exchange rates (Cordingley 2005). This has resulted in higher prices for domestic seafood compared to imports (O'Sullivan & Dobson 2002). The aquaculture sector has attracted remarkable attention from government, industry, and educational institutions, which contribute to the body of research on production, and train and develop skilled personnel for the industry's expansion (ABARE 2003).

Love & Langenkamp (2003) reported that aquaculture is a rapidly growing sector of Australian industry. Statistics show that Australian aquaculture has grown 10% per annum in the last decade, which has influenced federal government development policies (ABARE 2003). The gross value of production almost trebled between 1991/92 and 2001/02 and accounted for 30 percent of the total gross value ($733 million) of Australian fishery production in 2001/02. It is one of the most diverse industries (ABARE 2003), with operations spread throughout all states and territories. The National Aquaculture Development Committee (NADC) indicated that the value of the industry would reach $2.5 billion by 2009/10 (ABARE 2003, Love & Langenkamp 2003).

However, problems were encountered with expansion of this sector due to resource conflict, and in 2001/2002 growth of aquaculture fell to 5.1%. It was thereafter predicted that the industry was unlikely to achieve the national goal (O'Sullivan & Dobson 2002, O'Sullivan & Dobson 2003, Cordingley 2005). With the current conflicts of land and water use and other employment opportunities (ABARE 1999), aquaculture industries are searching for new species as well as new venture possibilities. Coastal aquaculture and mariculture have attracted more interest (Le Moullac *et al.* 2003,

Gardner *&* Lobkov 2005, Gifford *et al.* 2005, Guyondet *et al.* 2005, Macey & Coyne 2005, and Troell *et al.* 2006). These changes have effectively reduced most research funding and other inputs. According to Gibbs (2004), farming in the sea is one of the few alternative ways of meeting the demand for seafood products (Neori *et al.* 2004) without exacerbating conflict for use of land and freshwater.

1.3.2 Mariculture and coastal aquaculture

The competitive advantages of aquaculture in Australia revolve around the perceived 'clean green environment' (FWA 2003, UNEP 2006), low farm density (Jackson *et al.* 2003b), and the opportunity to learn from the experience in other countries (Tacon *et al.* 1995). The major concern in expanding coastal aquaculture is that initial establishment costs and labour rates are higher in comparison to other countries in the region (ABARE 1999). Legislation poses other constraints on expanding business (Crawford *et al.* 2001). Marine and coastal aquaculturists need to assess opportunities with respect to available technology and potential to utilise high value or ornamental species (Calado *et al. 2003,* Watson & Hill 2006), especially for overseas markets.

Five marine species (Southern blue fin tuna, pearls, Atlantic salmon, prawns and oysters) comprise the majority of Australian aquaculture. The value of products from these species contributed 91% of the 2001/02 total annual Australian aquaculture production (Love & Langenkamp 2003). Much of the research on production has focused on molluscs, oysters and abalone (Gifford *et al.* 2005, Jones & Iwama 1991, Mayfield & Branch 2000). Production of lobsters, prawns, and crabs has shown promise as luxury food items and thus also attracted research (Phillips & Evans 1997, James & Tong 1998, Jones & Morgan 2002, Jackson *et al.* 2003a, Phillips *et al.* 2003). In addition, aquatic plants are also being farmed in Australia for food (Cordingley 2005). However, inland aquaculture continues to face various issues limiting expansion of production (Lymbery *et al.* 2006; Prangnell & Fotedar 2006, Tantulo & Fotedar 2006). More research into sustainable resolution of resource conflict is required to facilitate further development in this area.

1.3.3 Freshwater aquaculture

Whilst aquaculture of marine species has achieved some success (Allen *et al.* 2006, Evans *et al.* 2004, Phillips *et al.* 2003, and Troell *et al.* 2006), freshwater aquaculture in

Australia has faced major limitations (ABARE 1999). The obstacles to entrepreneurship in freshwater aquaculture include difficulty of access to location, approach to venture capital, and strict legislation (O'Sullivan & Dobson 2002). Although Australia is the driest continent, there is water available in many farm dams. Whisson (1996) stated that when the price of farm produce fluctuates, farmers seek to diversify their farm production and their activities (O'Sullivan 1997, Gooley 2000). Aquaculture is one alternative solution for maximising production from water bodies dependant upon available resources and inputs (Little & Muir 1987, Alsogoff *et al* 1992, O'Sullivan 1997, Whisson 2000). There are many researchers who have been working on diversifying farm production (Pillay 1993, Whisson 1996, Milstein 1997, Whisson 2000, 2004 and others), with the intention of maximising the benefits within the water bodies. Examples include guided tours through integration with agri-eco-tourism-based services (O'Sullivan 1997); generation of valuable by-products like bait for recreational fisheries (Scott *et al.* 1988) or stocking farm dams with crayfish such as freshwater crayfish or with more than one species (Whisson 2000, Storer 2006).

1.3.3.1 Freshwater finfish aquaculture

There are some 180-190 freshwater fish species living in Australia and about 127 species are endemic (Lawrence & Morrissy 2000b). Most of these fish species are small in size, under utilised, with slow growth rates or are generally less attractive for farming (Whisson 2000). Rowland (1994a, 1994b) suggested that some of the indigenous freshwater species that have potential for aquaculture include silver perch (Rowland 1996, Rowland & Kearney 1992, Rowland *et al.* 1995), Murray cod (Francis *et al.* 2006), and golden perch (Culver & Geddes 1993, Arumugam & Geddes 1996, and Collins & Anderson 1999). Although, some species such as trout and salmon were translocated into many water bodies and performed very well in aquaculture systems (Love & Langenkamp 2003, Arthington & McKenzie 1997). There are a few species in Western Australia, such as black bream (Partridge *et al.* 2004) and cobbler which are being investigated for aquaculture potential (Fisheries Western Australia 1997). Whisson (2000) stated that while these research studies deal with single species finfish, other research has investigated the potential of combining them with other aquatic species namely freshwater crayfish.

1.3.3.2 Freshwater crayfish aquaculture

There are a number of research studies involving freshwater crayfish, a semi-aquatic animal produced within Australia and throughout the world (Morrissy 1983, Morrissy *et al.* 1990, Boulay *et al.* 1993, Medley *et al.* 1994, Lawrence *et al.* 1995, Henryon *et al.* 1999, Rouse & Kartamulia 1992, Barki & Karplus 2003). There are about 27 *Cherax* sp (Family Parastacidae) and about 120 crayfish species found throughout Australia (Riek 1969); in fact the world's largest freshwater crayfish, *Astacopsis gouldi,* is found in Australia (Horwitz 1990). These organisms have developed along an evolutionary line from marine lobsters more than 100 million years ago (Hasiotis 1999, Lawrence & Morrissy 2000). Their life history and biology have been described by many biologists including Riek (1969), Howitz & Knott (1995), Austin & Knot (1996). Lawrence and Jones (2002) described the aquaculture attributes of three major species: marron, redclaw and yabbies.

Marron (*Cherax tenuimanus*) has been well investigated (Morrissy 1974, 1988, Morrissy 2000, Whisson 2000); redclaw (*Cherax quadricarinatus*) has been described by Jones (1990), Brummett & Alon (1994), Karplus *et al*. (1995), Karplus *et al*. (1998); and the yabby *(Cherax destructor, albidus)* has been studied by Austin (1987), Morrissy & Cassells (1992), Austin *et al*. (1997), Austin (1998), Lawrence & Morrissy (2000a, b) Lawrence *et al*. (2001). These unique animals have attracted interest from researchers and aquaculturists locally and throughout the world (Huner 1994, Lawrence & Morrissy 2000a, Lawrence *et al.* 2004). The potential for polyculture of these species with other species are being investigated (Whisson 1997, 2000, Storer 2006).

1.3.3.3 Polyculture

Polyculture is defined as the stocking of several species with different feeding habits in the same water body. Basically, polyculture aims to utilise the different trophic levels in the water column (Hepher & Pruginin 1981, Pillay 1993). O'Sullivan (1997) suggested that polyculture (of ornamental finfish in aquariums for example) where several species were stocked together, gained the benefit from trophic levels within the ecosystem (Ghosh *et al.* 1994; Hendrickx *et al.* 1996). There are many polyculture examples from other countries such as pond-based aquaculture in China or tank-based in Israel (Schultz 1984, Zachritz 1985, Milstein 1992, Riise & Roos 1997). On a large scale,

determination of the right combination of species, space and temporal preferences presents a more complex challenge (Inko & Mckenzie 1976, Eldani & Primavera 1981, Ra'anan & Cohen 1983, Cange *et al.* 1986, Tian *et al.* 1997, Jones *et al.* 2001). Although polyculture has been practised for thousands of years (Brown & Gratzek 1980, Zhong & Power 1997), dynamic demands from various markets and new species mean that preliminary research from an ecological as well as commercial perspective is imperative in order to develop polyculture systems (Hulata *et al.* 1990, Prinsloo & Schoonbee 1992, Karplus *et al.* 1995, Whisson 2000, Vromant & Chau 2005).

1.3.3.4 Candidate species for polyculture

Due to a lack of native species, researchers are seeking potential polyculture species throughout Western Australia. Recently, legislation has been introduced which prohibits translocation of some aquatic species beyond their natural habitat. There are native species (endemic to the South-West, Western Australia) considered suitable for aquaculture, for example the freshwater cobbler (*Tandanus tandanus*) (Fisheries Western Australia 1997). It is, however, considered low value and dangerously poisonous (Yearsley *et al.* 1999); in addition catfish generally have highly predaceous habits (De Graaf *et al.* 1996). The other species considered suitable is the redfin perch (*Perca fluviara)* which is prohibited due to its prolific reproduction which affects the biodiversity of natural ecosystems. Some species however such as silver perch (*Bidyanus bidyanus)* are not able to spawn outside their original habitat (O'Brian pers comm.) This together with its herbivorous feeding habits, makes the silver perch an extremely good candidate for polyculture (Rowland & Kearney 1992; the characteristic for farming has been described by Rowland 1994, 1996, and Whisson 2000).

1.3.3.5 Crayfish and finfish polyculture

In the case of Western Australia, Lawrence & Morrissy (2000a) stated that commercial polyculture exists mainly as part of diversified farming activities. Gooley (2000) suggested that diversification into farming of crayfish in existing on-farm water bodies would provide extra production (FWA 1997). Crayfish and finfish polyculture systems sound promising in theory, but research is needed to identify the risks associated with production. Although considerable polyculture activity has involved introduced species (Whisson 2000, FWA 1997), this mostly occurred before the existing regulations were implemented (Horwitz 1990, Horwitz & Knott 1995). Many species have been able to

establish themselves in the new environments (Horwitz & Knott 1995). Rowland *et al.* (1995) and Whisson (2004) indicated that the introduced species silver perch is an omnivore, foraging mainly within the surface of substrate, as opposed to crayfish which are benthic foragers. This suggests that silver perch may be a perfect candidate for polyculture with crayfish; however, Whisson (1996, 2000) observed that silver perch predate on crayfish or show opportunistic predatory behaviour. These led to the study of the role of shelter in polysystems and their impacts on polyculture and multi-species systems.

1.4 Thesis rationale

Whisson (2000) recommended silver perch (*Bidyanus bidyanus*) and marron (*Cherax tenuimanus*) as excellent candidates for polyculture. These could provide alternative strategies to diversify farm production. After many years of research into polyculture systems, Whisson (2000) and Storer (2006) identified which factors governed silver perch and marron polyculture production. Both species can interact in a synergetic relationship. However, the survival rate of marron in the system was very low. The authors recommended that this could be improved by providing appropriate shelter.

Shelter availability is an important factor governing mortality in crayfish aquaculture. In polyculture, shelter reduces interaction between co-stocked species, enhances carrying capacity of the system, and reduces aggressive behaviour. However, it is obvious that providing shelters will incur extra investment therefore the actual role of shelter needs to be confirmed. This study will provide a better understanding of shelter competition between crayfish and their response to finfish predators. The effect on crayfish of prior residence, and shelter size and type will be investigated. The effect of temperature, and spatial and temporal change on shelter use will also be discussed. The role of shelter on crayfish and finfish production and implications for polysystems will also be provided.

The study of the role of shelter in a multi-species system presented in this thesis should assist the crayfish industry by providing guidelines for husbandry and polyculture management strategies. Those who aim to use shelter in crayfish polyculture would benefit from this work.

1.5 Aim and objectives

This study aims to improve productivity of the polyculture systems and contribute to shelter manipulation strategies in crayfish polysystems. The major objectives are:

1 To determine the behavioural responses and shelter competition of yabbies under threat from silver perch.

2. To determine the effect of prior residence between marron and yabbies under threat from silver perch, in relation to prior resident effect, and conspecific and congeneric competition for shelter.

3. To determine the shelter preference and shelter selection of yabbies under threat from silver perch.

4. To investigate the effect of shelter availability and famished yabbies at different temperatures on yabbies survival.

5. To determine the effect of temporal and spatial change on shelter selection in marron

6. To investigate the effect of shelter on predator and cannibalistic avoidance of yabbies and marron in polysystems.

7. To investigate how shelter availability effects survival, growth and production of crayfish and finfish in polyculture.

These objectives were addressed through the following experiments

1) Chapter 4: shelter competition between yabbies, Chapter 5: shelter competition between marron and yabbies

2) Chapter 4: competition between yabbies, Chapter 5: shelter competition between marron and yabbies

3) Chapter 6:shelter selection and shelter preference in yabbies

4) Chapter 7: the effect of shelter availability and temperature on yabby survival

5) Chapter 8: the effect of spatial and temporal changes on shelter selection in marron

6) Chapter 9: the impact of prior residence on dominance cannibalism

7) Chapter 9: the impact of shelter complexity on cannibalism and predation in polysystems

Chapter two

Literature review

This chapter provides a global overview of multi-species approaches to aquaculture and then reviews polyculture systems that have been investigated in Australia to date. Further, the system-specific variables underpinning polyculture production are introduced, before turning the discussion to the role of shelter within crayfish polysystems. Other related work is compared, and the importance of shelter in natural aquatic systems is highlighted as a basis for the research conducted in this thesis.

2.1 Interactions occurring in crayfish polysystems

Researchers at Curtin University of Technology have developed a crayfish-finfish polyculture system that includes farmed marron (*Cherax tenuimanus*) and silver perch (*Bidyanus bidyanus* Mitchell) (Whisson 2000). Fisheries Western Australia (1999) and Whisson (2000) indicated that this crayfish-finfish polyculture technique has been adopted by Australian farmers, and crayfish-finfish polyculture also has been intensively studied by overseas researchers (Rouse 1987, Wahab *et al.* 1995, Karplus *et al.* 1995, Liang *et al.* 1999, Barki 2001). The technique has been extended to field trials in order to utilise water resources in abandoned mine lakes found throughout Western Australia (Whisson & Storer 2003).

These multi-species aquatic system applications showed that participating species can co-exist in a synergetic relationship (Whisson 2000, Barki 2001, Whisson & Storer 2003). In a case study in Western Australia, Whisson (1997, 2006) found that freshwater crayfish (marron) in polyculture systems grew significantly larger than those in monoculture ponds. The author also identified economic benefits of culturing marron together with silver perch in this system. However, low survival of crayfish remains a major disadvantage. Crayfish polyculture systems could achieve high productivity and higher economical returns if the major factors causing stock loss are resolved.

Interactions between finfish predators and their prey are complex (Beukema 1968). The response of crayfish to fish predators appears to be related to its vulnerability (Blake & Hart 1995). Crayfish tend to reduce their overall activity in the presence of fish predators (Milstein 1995). Barki *et al.* (2001) studied the interaction between red claw crayfish (*Cherax quadricarinatus*) and red tilapia (*Oreochromis niloticus*), and found no effect on growth and survival of red claw in a small-scale intensive system. He noted that the actual behavioural responses (both intra and inter specific) need to be investigated in order to explore the undisclosed nature of ecological niches and species relationships.

2.2 Interspecific interactions in polysystems

2.2.1 Competition

The ultimate goal of polyculture is to maximise carrying capacity and improve net yield by exploiting ecological niches (Mills & Mclound 1983, Terhune & Chwedler 1997, Nunes *et al. 2003*). These man-made systems aim to stock species with compatible aquaculture characteristics (Rouse *et al.* 1987, Hulata *et al.* 1990, Siddiqui *et al.* 1996, and Whisson 2004). Sometimes participating species compete for resources and display antagonistic behaviour (interference competition) (Vahl 2006); in other cases there is an overlap of trophic position, resulting in competition for limited resources (Schultz 1984, Miller *et al.* 1992, Barki *et al.* 2001). Food, habitat and interaction between species have a strong influence on spatial distribution (Barki *et al.* 2001, Nakata & Goshima 2003), abundance and system production (Hendrickx *et al.* 1996, Jordan *et al.* 1996, Ronnback *et al.* 2002 and Malavasi *et al.* 2005). In several studies into redclaw polyculture in Australia and overseas (Barki 1997, Lawrence & Jones 2002, Karplus *et al.* 2003), growth and survival were shown to be affected by interspecific interactions including predation.

2.2.2 Predation

Predation is another factor that governs the population structure in multi-species systems (Mason 1994, Coulas *et al.* 1998, Baeta *et al.* 2005). Predators can be either carnivorous or omnivorous in behaviour (Hobbs 1993, Blake & Hart 1995, Kestemont *et al.* 2003). Hogger (1988) quoted that even though there are many potential crayfish predators, not many of them have been shown to have a significant effect on crayfish abundance. Lima (1998), however, suggested that while predator presence may not affect prey abundance, it could affect anti-predator response, ultimately affecting prey foraging behaviour and growth performance.

However, there is little evidence in the literature of crayfish predation on finfish. Noble crayfish (*Astacus astacus*) have been recorded preying on trout fry (*Salmo trutta*) (Rubino *et al.* 1990), while Minckley & Craddock (1961) graphically describe how crayfish (*Cambarus bartoni* and *Orconectes nais*) were observed attacking a variety of partially incapacitated but swimming fish in Kentucky streams. According to Huner

(1986), crayfish are likely predators of fish eggs on spawning mats, and compete with fish for natural and artificial food.

2.2.3 Predator recognition

Many crustaceans in aquatic environments utilise and adopt olfactory nerves and rely heavily on chemical sensory communications throughout their life span (Grasso & Basil 2002). Behaviour in crayfish such as courtship, food searching, and predator detection are chemically activated (Derby 2000). Detection occurs through odour recognition by the neutron, which is then carried to the olfactory lobes (Mitamura *et al.* 2005). Processing and distinguishing of information occurs in the brain with the use of two pairs of lobes (olfactory and accessory lobes) and nerve cell clusters found during an amphibian study by Flowers & Graves (1997). Frog larvae were able to detect and avoid chemical cues from their respective predators namely the garter snake and salamander. Diaz *et al.* (2001) reported that crabs appear to have two sets of behavioural responses: avoiding response and antagonistic behaviour response. They exhibited an escape response in offshore water, and a combination of escape and alarm responses in the presence of chemical cues.

2.2.4 Predator avoidance

Crayfish display many ways of avoiding unfavourable interactions by reducing their movement and sheltering while predators are present (Stein & Magnuson 1976, Appelberg & Oldelström 1993, Blake & Hart 1993), or seeking a more secure refuge (Sih 1987, Lima & Dill 1990). This increased time in shelter does, however, result in reduced movement and foraging activity (Rahel & Stein 1988, Brown & Godin 1999, Bouwma & Hazlett 2001). Red swamp crayfish, native to north eastern Mexico, burrow to avoid predators in the case of low water, dry conditions and reproductive periods (Huner & Burras 1995). The prairie crayfish inhabiting eastern and central Mississippi and western Alabama have been observed digging a deep burrow up to 4.5m to avoid terrestrial predators (Hobbs 1942). The signal crayfish, which did not burrow in its native land, has been shown to construct burrows after its introduction into Britain (Holdich 2002). The avoidance measures include antagonistic responses (such as raising chelae to a strike position and moving towards the predator), described by Hazlett (1994).

Avoidance and anti-predator behaviour in crayfish have been studied by many ecologists including Sih (1987), Hazlett (1994), Blake and Hart (1993), Hazlett & Schoolmaster (1998), Kats and Dill (1998), Lima (1998), Hazlett *et al.* (2002) and Woodley & Peterson (2003). Strategies used by crayfish in order to avoid potential predator and conspecific cannibalism depend on the species and their ability to learn (Hazlett 1971, 1994). There are also many factors that affect the ability of crayfish to detect and recognise predators.

2.2.5 Predator and prey interaction

Predator-prey relationships have been studied by many biologists (Alexander 1987, Dulvy & Polunin 2003, Elvira *et al.* 1996, Fisher & Grant 1994). Kornijow (1997) viewed predation as an important process from three dimensions: community structure, prey distribution and shelter complexity. Prey alter their behaviour in the presence of a predator, and predators are selective in the size, sex and condition of the prey they hunt (Warner & Hall 1974, Stein 1977). The size of fish predators is also important. For example, reef fish predating on western rock lobster showed a clear preference for small, newly settled juveniles (Howard 1988). Momot (1967) reported that trout only fed on 0+ crayfish (less than one year old) in a marl lake, and Svensson (1993) suggested that moulting behaviour is more synchronized amongst young crayfish, to reduce the risk from predators. In another example, minnows and other small ornamental species had little impact on the red swamp crayfish (*Procambarus clarkii*) in the United States; however, large catfish almost eliminated *P. clarkii* from established culture ponds (Huner 1986). Faragher (1983) found that as the size of rainbow and brown trout increased, so did the size of their crayfish prey (*C. destructor*) in Lake Eucumbene, Eastern Australia. Predation of decapod crustaceans is usually size selective, where predation intensity is highest on smaller-sized prey (Warner & Hall 1974, Stein 1977, Howard 1988, Persson & Greenberg 1990, Wahle 1992, Blake & Hart 1995).

One example of the effect of predation on prey is the potential to restrict distribution or reduce abundance (Hendrickx *et al.* 1996). This simple model established in 1925-26, "the Lotka-Volterra equations" (also known as the *predator-prey equations*) (Campbell 1994), describes the dynamics of biological systems in which two species interact, one a predator and one its prey.

The usual forms of the equations are:

$$\frac{dx}{dt} = x(\alpha - \beta y)$$

$$\frac{dy}{dt} = -y(\gamma - \delta x)$$

Where:

Y is the number of some predator (for example, silver perch, Murray cod)

X is the number of its prey (for example, yabbies, marron)

T represents the growth of the two populations against time, and

α, β, γ and δ are parameters representing the interaction of the two species.

This predator-prey cycle has been analysed by Charles Elton and interpreted as an example of a natural predator–prey fluctuation (Elton & Nicholson 1942); however, this assumption is apparently not correct. Keith (1983) has shown that food shortage during the winter initiates the decline in prey numbers, and predators play a secondary role in prolonging the decline. Although predator populations depend on prey, the prey numbers fluctuated because of interactions with their food plants.

2.3 Intraspecific interactions in polysystems

2.3.1 Competition

Direct intraspecific or within species competition has contributed to the growth performance in subordinate animals (Segal & Roe 1975), resulting in reduced food consumption, increased species interaction and decreased food conversion efficiency (Karplus *et al.* 1992). Campbell (1994) reported that the Logistic growth models explained the effects of competition between species, social interaction, and population growth. In crayfish there are many interactions involving competition within species. Studies include symmetric competition (Rutherford *et al.* 1995, Pavey & Fielder 1996, Barki *et al.* 1997); moult stage (Tamm & Cobb 1978); gender (Sinclair 1977); prior resident (Peeke *et al.* 1995, Huntingford & deLeaniz 1997); and agonistic interaction (Rubenstein & Hazlett 1974, Gössmann *et al.* 2000). Thomas *et al.* (2003) reported that several factors affecting species interaction led to cannibalism.

2.3.2 Cannibalism

Laboratory observations where the stomach content of crustaceans have been investigated suggest that cannibalism frequently occurs in many natural crayfish populations (Goddard 1988). However, Holdich (2002) suggested that very few field studies relating the frequency of cannibalism to food availability and temperature have been completed (Crear *et al.* 2000).

Cannibalism occurs when foods are scarce and animals are hungry (Thomas *et al.* 2003). Evidence shows that changes in food level, size and hunger are factors affecting the frequency of cannibalism in natural populations (Dong & Polis 1992, Guan 1998). Among these factors, low availability of food is the most important ecological factor favouring cannibalism (Edgar & Crespi 1992). Cannibalism has both direct and indirect effects on wild crayfish populations. MacNeil *et al.* (2003) showed that parasitised amphipods contributed to infection of other animals in the system. This suggests that cannibalism not only reduces survival rate but also increases the risk of disease outbreak (Matthews & Reynolds 1992, Evans & Edgerton 2002).

2.3.3 Density effects

Verhoef & Austin (1999), Morrissy (2000), and Lawrence & Jones (2002) reported that growth and survival of Australian freshwater crayfish were inhibited by stocking densities. In redclaw crayfish (*C. quadricarinatus*), juveniles displayed agonistic behaviour over food in high stocking densities (Karplus *et al.* 2003). Similar findings have shown slow growth in yabbies (Verhoef & Austin 1999). Barki & Karplus (2004) demonstrated that density has a strong effect on growth in redclaw crayfish. Density affected growth and uniformity of size even though food was provided at satiation level. In redclaw held at low density presumably with fewer social interactions, a high proportion of juveniles attained a larger size and growth was relatively uniform; whereas at high density, growth was reduced and size variation increased.

2.3.4 Dominance hierarchies

Dominance hierarchies also govern the distribution of crayfish and resources (Hobbs 1988, 1993, Statzner & Peltret 2006). The strongest organisms will dominate habitats and the resources needed to live and reproduce offspring. This phenomenon and the

associated regulation of populations is therefore dictated by the prevailing ecological limits (Mills & McCloud 1983, Terhune & Schwedler 1997), carrying capacity, and social structure (Turvey & Merrick 1997, Moutou *et al.* 1998). Dominance hierarchies are a function of food availability and crayfish density (Kitaya *et al.* 2005).

2.3.5 Visual, chemical and physical cues in crayfish

Height (2002) recorded that exotic yabbies exhibit greater behavioural plasticity than native marron in response to chemical cues. The study also observed they were more alert to visual and chemical stimuli (Jenkinson 2004, Storer 2006). Sih & Moore (1993), and Storfer & Sih (1998) reported that predator avoidance responses in terrestrial animals are inherent from many generations. However, this ability seems not to be innate in all types of crayfish (Hazlett 1971) and sometimes requires learning (Mathis & Smith 1993, Chivers & Smith 1998, Dicke & Grostal 2001). There are also different levels of response to stimuli depending on the crayfish's ability to utilise the information. Hazlett (1994) reported that *Oncornectes virilis* displays predator recognition while *O. propinquus* does not respond to the same odour. This could be another area whether species selection for polyculture would benefit from knowledge of behavioural responses.

Further studies on predator detection are reported by Nyström (2002) suggesting that this ability is useful in poor visibility conditions. Predator recognition also assists fishers avoid using predatory bait for crayfish capture. Taugbøl *et al.* (1997) observed the use of predatory pike (*Esox lucius* L.) and perch (*Perca fluviatilis* L.) as bait and found that they were less attracted to juvenile noble crayfish. Recent studies in crayfish demonstrate learned behaviours of crayfish and the existence of long-term memory (Gherardi *et al.* 2002). Gherardi *et al.* (2002) confirmed that invasive crayfish learn faster and have longer memory retention than native crayfish. This will assist the researchers investigating crayfish behavioural responses to chemical stimuli, which will, in turn, provide more knowledge on the conspecific and congenic interaction in the polysystems.

2.4 Interactions affecting production in crayfish polysystems

A number of studies have been done on multi-species ecology, investigating the relationship between fish and crayfish in polysytems (Moav *et al.* 1977, Rouse 1987, Milstein 1992, Karplus 1995, Milstein 1997, Whisson 1997, Barki 2001). Whisson (2000) stated that marron utilise the benthic environment and are simply scavengers of organic matter on the substrate; however, co-stocking finfish and crayfish involved complex interactions (Stein 1977, Karplus *et al.* 1995). These complex interactions depend on community structures, predator-prey relationships, and cannibalism. Karplus *et al.* (1995) suggested that low survival of crayfish in their studies were due to predation from various sources such as freshwater crabs, *Potamon potamobios* (Gherardi 2002). In polysystems, silver perch play a major role in utilising the lower trophic level; nevertheless, sometimes they can be opportunistic predators (Rowland & Barlow 1991). Moav *et al.* (1977) suggested that to reduce the interaction between crayfish and finfish, it would be best to use finfish which were lower categorized consumers in the food chain (herbivores and omnivores).

2.4.1 Biotic factors

Habitats in polysystems consist of many types of structures (Whisson 2000), both biotic and abiotic (Campbell 1994). Biotic structures include plants and vegetation (Whisson 2000); abiotic refers to non-living materials found in all areas of the culture system (Fellows 1995, Whisson 1997, Storer 2006).

2.4.1.1 Food

Food availability is the single most important factor in crayfish ecology; it can trigger agonistic behaviour in juvenile crayfish. Supplementary feed improves crayfish growth rate but, in turn, affects cannibalism rate during the moulting period: when they moult they are highly palatable and therefore more at risk of cannibalism and predation. Most crayfish are considered omnivorous, consuming a wide range of food items: detritus, algae, macrophytes, invertebrates, fish and fish eggs (Capelli 1980, Westman *et al.* 1986, Guan & Wiles 1998). Since crayfish often forage nocturnally, this increases their exposure to predators.

2.4.1.2 Density-related factors

In polysystems, in addition to selecting the right combination of species, it is also important to have the right stocking densities (Shepherd & Bromage 1992). High densities increase species interaction and effect growth, survival and yield (Morrissy 1979). Several studies have reported the effect of stocking density on the economic feasibility of crayfish such as marron (*C. tenuimanus*) (Morrissy 1992), redclaw (*C. quadricarinatus*) (Pinto & Rouse 1996, Jones & Ruscoe 2000), red swamp crayfish (*Procambarus clarkii*) (Lutz & Wolters 1986, Vilagran 1993) and signal crayfish (*Pacifastacus leniusculus*) (Savlainen *et al.* 2004).

2.4.1.3 Planktonic turbidity

Turbidity in water can impair the hunting ability of predators because most aquatic animals rely on vision in their search for food (Guthrie 1986). Crayfish, however, rely on chemo-sensitivity. This allows them to eliminate the impact of water turbidity thereby reducing the threat of cannibalism and predation (Whisson 2000, Storer 2006). High planktonic levels could benefit crayfish growth as it provides a natural food especially for juvenile crayfish (Nyström 2002). However, high planktonic concentrations in ponds can result in depleted Dissolved Oxygen (DO) levels in water (Boyd 2002).

Predators have been shown to be more active in light rather than dark conditions. The effect of plankton turbidity reduces light penetration, thereby reducing the intensity of predation (Miner & Stein 1996, Utne 1997). However, Chesney (1989) reported that turbidity has been shown to increase predation rates in some systems: more light intensity together with increased turbulence increases the contrast of prey against water, resulting in higher rates of predation (Hinshaw 1985, Mackenzie *et al.* 1994).

2.4.1.4 Relative size variations

The relative size of each species has been shown to affect the intensity of cannibalism and predation (Warner & Hall 1974, Stein 1977, Blake & Hart 1993). Issues related to size differentials have been studied by Blaxter and Stains (1970), while Ware (1973) considered the role of prey size, and Preston *et al.* (1999) studied population density, with other studies on encounter rates by Utne-Palm (1999). The stocking regime (size

and density) also impacts on survival and predation rates within the ecological structure (Pillay 1993). Generally predator size is positively correlated with prey size, as larger predators require larger amounts of food, therefore larger prey are preferred as a greater source of energy (Stein 1976).

2.4.2 Abiotic factors

2.4.2.1 True turbidity (clay-based)

Many studies have investigated turbidity effects on crayfish populations (Hartnoll 1982, Benfield & Minello 1996, Chivers *et al.* 1996, 2001, Clark *et al.* 2002, Reynolds 2002). Turbidity influences crayfish visibility, and reduces interaction with others. Yellow clay soil in the Western Australian wheatbelt region provides low light penetration into yabby dams (Lawrence & Morrissy 2002). The suspended solids in the water provide detritus and organic matter for crayfish. However high turbidity could interfere with respiratory systems (Boyd 1998).

2.4.2.2 Segregation of participating species

Segregation of participating species can lead to more interactions with conspecifics. In both natural and artificial systems, crayfish tend to concentrate around macrophytes or artificially-provided structures. Some studies have found that providing substrate is positively correlated with survival (Clarke *et al.* 2002, Gazdewich & Chivers 2002). In crayfish polyculture ponds, provision of substrate has been shown to have some benefit on crayfish survival (Whisson 2004, Storer 2006); insufficient or unsuitable substrate has been shown to have a negative effect on both growth and survival (Jones & Ruscoe 2000). There have been some reports suggesting that fish cages in themselves could provide extra substrate for crayfish, especially when they are vulnerable to other predators, like birds or rats (Ivlev 1961, Mason 1975, Stein 1977). Whisson (2000) found that under free-range culture conditions marron were hiding under floating silver perch cages where the water temperatures conditions were more favourable.

2.4.2.3 Shelter and burrows

Crear *et al.* (2000) and Thomas *et al.* (2003) revealed that shelter plays an important role in reducing cannibalism in crustaceans. This, in turn, impacts on the design of

aquaculture systems. In crayfish ponds there are mechanisms to keep populations from exceeding the carrying capacity including escaping, burrowing or cannibalism (Adamowicz & Purvis 2004).

2.5 Role of physical structures in multi-species systems

Hill (1994) indicated that abiotic structures such as rocks, sticks, logs and crevices can serve as a refuge for marron. Marron selected the appropriate habitat location and then looked for shelter according to their need. Stein (1977) reported that predator odour is retained in material such as plants (Hutchings 1988, Merrick & Lambert 1991). Stein (1977) also observed that predatory animals alter their search behaviour and increase their turning frequency in order to maintain close proximity to prey aggregations. Physical structures provide a safer place for prey seeking shelter (Ivlev 1961, Momot 1967). This type of predation pressure emphasises the need for a greater complexity of refuges (Jones & Ruscoe 2000). The study in silver perch and marron polyculture shows that shelter availability and complexity improve the survival of crayfish in multi-species systems (Whisson 2000). However, further study is warranted.

2.6 Role of shelter in crayfish-finfish polysystems

Many researchers have investigated the importance of shelter in aquaculture (Teichert-Coddington 1996, Eklov 1997, Carss *et al.* 1999, Ratchford & Eggleston 2000, Taylor & Elwood 2003, Tews *et al.* 2004). Shelter availability and complexity in crayfish aquaculture has also been investigated (Fellows 1995, Whisson 1997, Jones & Ruscoe 2000). However, the role of shelter in management strategies to maintain and maximise productivity is still unclear. There has been little study on the impact of shelter on increasing crayfish survival or growth performance (Meager *et al.* 2005).

The difficulty is, in the case of crayfish and finfish polyculture, the role of shelter is regarded as relatively inconsequential in comparison to enhancing carrying capacity by improving water quality (Boyd 1998) or quality of supplemental feed (Tacon *et al.* 1983, 1995). This is likely due to the fact that approximately 70% of the operating cost for aquaculture is based on feed; improving those qualities could therefore significantly reduce production costs (Fotedar 1994, New & Tacon 1993). Furthermore, effort has been directed towards the extension of modern technology, genetical manipulation or

hybridizations to create a "super-animal" which requires suboptimal habitat but can perform extremely well with minimum space. In comparison, there has been relatively little interest in improving basic habitat requirements (Cobb 1969). The habitat is home to all kind of organisms (Vannini & Cannici 1995), some of which provide extremely important architectural structures (Keshavanath *et al.* 2004) in the ecosystems. This area of research has been neglected in terms of securing resources, perhaps sceptically due to the lack of economic benefits. Further studies in this area will provide a better understanding of the role of habitat, shelter and refuge and their complexity, allowing ecologists and aquaculturists to improve animal quality and well-being in culture ecology.

Stevens & Swiney (2005) found that the role of shelter varies between the type of organism and their life stage. For example, one to three-year-old red king crabs (*Paralithodes camtschaticus*) selected their shelter according to the presence of other predators in the system and whether or not they were at the pre-moult stage. Lamouroux *et al.* (1999), Booth (2001) and Atkinson *et al.* (2004) demonstrated that habitat preference impacts highly on species abundance. The key to success in species survival is selecting a suitable habitat (Gilinsky 1984) or shelter as refuge, which virtually becomes a home for the animals (Vannini & Cannici 1995). For instance red swamp crayfish do not require additional shelter (Huner 2002); however, their production in rice paddies is significantly dependent on remnant stubble (Huner 1994, McClain *et al.* 1998).

2.6.1 Importance of shelter for freshwater crayfish

Abundance of crayfish populations increases with shelter availability (Skurdal *et al.*1988, Englund & Krupa 2000). This suggests that suitable shelter provides a basic substrate for crayfish communities. The importance of shelter for crayfish has been studied by many researchers (Krause *et al.* 1998, Hojesjo *et al.* 2003, Johnston & Robson 2005, Meager *et al.* 2005, Hellstedt & Henttonen 2006). Impacts of shelter on crayfish ecosystems have been investigated (Horwitz 1990, Jernakoff 1990). Researchers have endeavoured to gather more knowledge on the interactions in communal culture (Blake & Hart 1993). These studies have agreed that shelter is a limiting factor governing survival of crayfish (Blake & Hart 1993, Dennis *et al.* 1997, Bremset & Berg 1999, Englund & Krupa 2000, Decaestecker *et al.* 2002, Brown 2003,

Dennis & Sparksa 2006). Further study will enhance opportunities of rehabilitation and promote other technological advances such as system design and engineering, information technology, biotechnology for ecological sustainability and crayfish aquaculture.

Whisson (1996), Lawrence & Morrissy (2000a) and Whisson (2000; 2004) have shown that pond production of freshwater crayfish, considered in terms of kg/area, is very low; however, crayfish prices are based on size, which is an important variable (Brummett & Alon 1994). The reduction of stock loss will contribute to increased productivity and reduced input, which means less capital investment but higher profit margins (Whisson 1996). O'Sullivan & Dobson (2002) state that input can be reduced in polyculture and that crayfish polyculture systems could be productive and economically viable if the major factors causing loss of stocks are controlled (Whisson 2000).

2.6.2 Shelter attributes affecting crayfish preferences

Crayfish display preferences when selecting certain habitats over others (Stevens 2003). This has also been documented in other decapods (Westin & Gydemo 1988, Lamouroux *et al.* 1999; Booth 2001, Atkinson *et al.* 2004). Booth (2001) and Atkinson *et al.* (2004) investigated the settlement and substratum preference and survival of red king crab (*Paralithodes camtschaticus*). It is clear that the selection of habitat is an important key to a species survival (Gilinsky 1984), particularly as a refuge from predators.

In field studies, shelter size is a crucial factor for lobster shelter preference. Cobb (1969) observed that lobsters generally occupy shelters where the height was less than the width, and that there was a correlation between lobster size and shelter size. In the laboratory situation lobster prefer low profile shelters when given a choice of flat (height = 1/2 width) versus square (height = width) profile openings. Vannini & Cannici (1995) reported that while other shelter is important, burrows are preferred as the primary refuge for some crayfish. Cases of relocation of food resources and mates have also been reported (Holdich 2002). Some decapods occupy a single shelter, while others tend to visit on a more or less regular basis. In polyculture experiments, manipulating ecosystems through shelter provision allows animals to perform to their full potential. However, measurement of shelter preference in crayfish is affected by many factors such as prominent predators (Gherardi *et al.* 2002, Whisson 2004). Although much

research has concentrated on particular species, fields and locations, many species have not been studied and the response toward shelter remains inconclusive (Andersen *et al.* 2006). To date no scientist has studied the types of shelter preferred by freshwater crayfish under threat from predators.

2.6.3 Shelter's role in social interactions

Natural forms of shelter/refuge include aquatic plants, sticks, submerged logs, rocks, ledges, and other debris (Mills *et al.* 1994). Vegetation and organic matter increase turbidity, which reduces the hunting efficiency of predators and cannibalism (Dehli 1981). Substrates not only provide refuge; they also influence the population dynamics of prey, providing a substrate for periphyton, which comprise the major source of crayfish diet (Azim *et al.* 2003, 2004, Keshavanath *et al.* 2004). Using structures for shelter is particularly important when crayfish are in their vulnerable stages (Gherardi *et al.* 2000). Bovbjerg (1970) and Westin & Gydemo (1988) observed that some crayfish leave their shelter when they moult. This could be a strategy to avoid conspecific interaction, however it increases the risk of predation.

Recent interest has focused on the impact of non-indigenous species as invasive species (Morrissy & Cassells 1992, Söderbäck 1995, Elvira *et al.* 1996, Lawrence & Morrissy 2000b). This concern is evidenced by the increased research into their behavioural plasticity and competition with native species (Gherardi *et al.* 2002, Height 2002, Petrovskii *et al.* 2002, Hazlett 2003a, Height *et al.* 2004, Paavola *et al.* 2005). This issue has stimulated research into crayfish ecology and shelter competition. Some aquaculture studies have suggested that, under most conditions, the use of shelter is unnecessary for the intensive indoor rearing of juvenile yabbies (Verhoef & Austin 1999). On the other hand, Figler *et al.* (1999) highlighted the necessity of shelter in culture facilities especially for non-burrowing crayfish such as marron

2.6.4 Predator avoidance in crayfish

The most important predators of freshwater crayfish are predatory fish (Nyström 2002), which can influence crayfish abundance, growth and behaviour. Englund & Krupa (2000) found the distribution of small crayfish was independent of depth in pools without fish, whereas large crayfish occupied deep water in the presence of fish

predators. The intensity of fish predation is not simply a function of food abundance, but rather a complex result of many interrelated environmental, biological and chemical factors. Stein (1977) explained that these factors relate directly to the feeding biology of the predator. Whisson (2004) stated that the vulnerability of prey in free-range polyculture varies with turbidity, prey size, life stage of predator and prey, and prevailing habitat complexity (artificial or natural). The author suggested that additional food sources (artificial or natural), all contribute to the intensity of predation. According to Blake & Hart (1995) smaller crayfish (*Pacifastacus leniusculus*) avoid confrontation with predators (*Perca fluviatilis* and *Esox lucuis*) by spending more time in macrophytes; this is also true for lobster (Spanier *et al.* 1998). Spanier *et al.* (1998) found that under predation risk, sub-dominant lobsters spent more time in shelter during the day and built a higher percentage of alternative shelters than either single or dominant lobsters. The importance of habitat has been studied by many researchers (Cobb 1969, Jernakoff 1990, Blake & Hart 1993, Dennis *et al.* 1997, Bremset & Berg 1999). These studies have made an important contribution to the study of multi-species polysystems.

2.7 Shelter selection and utilisation in crayfish polysystems

Freshwater crayfish occuring in various habitats have evolved physiological and morphological as well as behavioral adaptations (Hazlett 2003b). Hasiotis (2002) and Fletcher & Miller (2006) reported that suitable shelter is necessary to reproduce offspring and survive in these habitats. Gilinsky (1984) stated that although crayfish are a low level benthic consumer, they do however play a major role in the trophic ecology of aquatic ecosystems. Crayfish are generally considered to be polytrophic omnivores (Goddard 1988, Huner 1994). Freshwater crayfish can adapt to a wide range of temperatures, availability of food, water quality and various types of predator (Morrissy *et al.* 1990b). However, many young crayfish are predated by higher level consumers such as finfish (Acosta & Butler 1997, Stein 1977). Predation is common in many food webs including crayfish and finfish, and may reduce crayfish numbers (Lodge & Hill 1994) especially in juveniles (Mather & Stein 1993). Mather & Stein (1993) reported that smallmouth bass (*Micropterus dolomieu*) did not discriminate among prey, but ate similar numbers of equal-sized *O. rusticus* and *O. sanborni*; but when size mimicked

those in the field (i.e., *Orconectus. rusticus* 4 mm> *Orconectes saborni*), fewer *O. rusticus* were eaten.

Figler *et al.* (1999b) reported that small crayfish spent more time in shelter during the presence of predators. Thus the availability of shelters is an important factor in predator avoidance for crayfish. The effect of shelter on predator avoidance is influenced by several factors such as habitat complexity (Whisson 2000), and/or habitat heterogeneity (Eklov 1995). Furthermore, young crayfish moult more frequently than older crayfish (Merrick & Lambert 1991, Mills *et al.* 1994, Timmermans *et al.* 1995, Wheatly 1995), therefore making them highly prone to predation (Svensson 1993). Young crayfish use stored energy quicker and are less tolerant to starvation than larger crayfish (Svensson 1993); they also have higher level of foraging and are therefore unable to spend as much time in shelter, increasing susceptibility to predatory interactions (Whisson 2004).

2.7.1 Shelter competition between native and non-native crayfish

Most of the review articles concentrate on mechanisms which result in species displacement and the competition for habitat, which occurred when a non-indigenous crayfish was introduced. However historically, only a tenth of introduced species become invader species (Elton 1958). Shelter is one of the most important limited resources which invasive crayfish will compete for often displaying antagonistic behaviour towards native crayfish; this competition can be directed towards both conspecific and heterospecific crayfish (Capelli & Munjal, 1982). Lodge & Hill (1994) confirmed that crayfish that are inferior competitors are excluded from shelter and are therefore more vulnerable to predation by fishes. These inferior competition skills and competition for shelter may ultimately lead to higher susceptibility to predation and eventually result in species displacement (Divine 1998, Usio *et al.* 2001, Gherardi 2002). Vorburger & Ribi (1999), Gherardi (2002) and Height *et al.* (2004) investigated aggressive interactions between a native and introduced crayfish for shelter concluding that successful shelter acquisition was strongly related to size, favouring the faster growing and larger invading species. They did note however, that on occasions the smaller native species were able to defend the shelter against the larger invasive species, suggesting that aggression played a role in the outcome of shelter competition. Similarly, Ranta & Lindströem (1993) and Height *et al.* (2004) identified the effect of

body size on the outcome of shelter ownership. The other undesirable behaviour needed to be addressed in crayfish conspecific relationships.

2.7.2 Prior residence in crayfish polysystems

Original residence or prior residence refers to organisms that physically rule the shelter where new comers are classified as intruders (Braddock 1949). Prior residence is affected by various factors resulting in highly aggressive behaviour in larger crayfish (Figler *et al.* 1999, Height *et al.* 2004), which seems to indicate that the most important factor is gender. Mariappan & Belasundaram (2002) indicated that males generally are dominant over intruders of either sex in the waterbody except when females are berried. Findings with berried female yabbies indicate that they gain a privilege over shelter, although the ulterior objective is for the survival of species (Height *et al.* 2004). Further, the changing dynamics of prior residency in the presence of predators is not well understood.

2.7.3 Spatial and temporal shelter issues

Previous studies have found a positive correlation between habitat heterogeneity (homogenous) and animal species diversity (Tews *et al.* 2004). However this relationship may vary considerably between species groups depending on whether structural attributes in ecosystems are perceived as heterogeneous or fragmented. Crayfish abundance is positively related to shelter availability (Lodge & Hill 1994). It governs daily activities and patterns of behavioural responses toward other conspecifics. In the more specific ecological systems, numerous studies have been conducted to identify the role of shelter on spatial and temporal changes in ecosystems (Nakata *et al.* 2001, Fellows 1990, Jordan *et al.* 1996 and Lawrence *et al.* 2001). Englund & Krupa (2000) observed spatial utilisation in pools without fish: the distribution of small crayfish was independent of depth, whereas large crayfish occupied deep water. However, in the situation where predation has become a significant pressure (Barki *et al.* 2001), shelter availability is of key importance as a limiting resource (Jonsson 1992, Blake & Hart 1993, Kozak *et al.* 2002). There is little documentation relating to predation of Australian freshwater crayfish by the finfish predator, silver perch. While no work has been done between the spatial and temporal variations for yabbies and marron, Ratchford & Eggleston (2000) reported that juvenile spiny lobster (*Panulirus*

argus) aggregation is controlled by the location and concentration of predator chemical cues.

2.7.4 Cannibalism in yabbies and the impact of shelter availability

Food availability and crayfish density directly influence cannibalistic activity (Kitaya *et al.* 2005). Evidence shows that changes in food level (Thomas *et al.* 2003), starvation and size differentiation are factors affecting the frequency of cannibalism in natural populations (Hazlett 2003, Kestemont *et al. 2003,* Stenroth & Nyström 2003). Among these factors, the scarcity of food is the most important ecological factor driving cannibalism (Elgar & Crespi 1992, Thomas *et al.* 2003). It is well documented that cannibalism occurs in both natural populations and crayfish aquaculture (Goddard 1988), this some extent is to maintain populations at carrying capacity. However, Crear *et al.* (2000) revealed that shelter plays an important role in reducing cannibalism leading to transformation in the design of aquaculture systems. Holdich (2002) suggests that very few studies have been conducted relating the frequency of cannibalism to shelter availability and other factors affecting cannibalistic behaviour. The crayfish growth strategy and moults is a transitional stage, which attracts predators and intra-specific cannibalism. Stevens & Swiney (2005) believe that providing shelter (Figler *et al.* 1999) results in reduced cannibalism. Another hypothesis is that shelter provides a bio-film that contains epiphyton and periphyton - an alternative source of food for crayfish (Lodge *et al.* 1994, Azim *et al.* 2003*,* Kraufvelin *et al.* 2006)

2.7.5 The role of substrate in freshwater crayfish systems

Shelter type for freshwater crayfish has been investigated in both natural ecosystems (Partridge 1978, Gherardi & Barbaresi 2000) and aquaculture (Mattila 1991; Whisson 2000, Lawrence *et al.* 2004, Tews *et al.* 2004). Vorburger & Ribi (1999) indicated that use of shelter in different forms, and natural vegetation, did increase the survival of both *Asellu* and *Corophium*. Their survival increased by providing habitat complexity (Mattila 1991). An autotrophic organism such as periphyton also benefits from the addition of artificial substrate (Lodge *et al.* 1994, Azim *et al.* 2004, Keshavanath *et al.* 2004). Nenke (2000) described the experience of using plant products in extensive yabby farm systems. It has been shown in Western Australia that regularly providing hay as feedlots (substrate for bacteria) in yabby dams improves yabby production.

Different substrate types were also investigated by Mason (1994), who found that particular types of benthic substrate were preferred by different groups of crayfish depending on their behaviour (e.g. burrowing or non-burrowing). Mather & Stein (1993) reported that types of shelter also impact on light intensity and turbidity, therefore affecting survival and growth (Minagawa 1994, Whisson 2000). This will impact on the design and application of shelter for crayfish polyculture.

Types of available shelter will influence marron population dynamics. Whisson (2000) observed that the ribbon-leaved *Vallisneria sp.* played an important nutritional role, in addition to a complex natural habitat, for juvenile marron. However, as crayfish grow larger they require a stronger structure for shelter, such as crevices between rocks or a safer place such as under the roots of trees. This finding was supported by Peterson *et al.* (1996) who found that *Cambarellus shufeldtii* occurred in sites with lower relative abundance of emergent vegetation, while the smaller *C. diminutus* preferred submerged vegetation. Yabbies on the other hand did not require additional shelter in high density rearing (Verhoef & Austin 1999). Yabby farmers use hides mainly to prevent avian predation (Mosig 1996, Lawrence & Jones 2002). Proper placed car tyres (not steel belt), in the reef type arrangement as shelter in yabby dams, can result in greater production (McCormack 1996). Redclaw (*C. quadricarinatus*) is also vulnerable to predation (Boulay *et al.* 1993) and minimal provision of shelter in redclaw intensive culture can result in high mortality (40-95%). On the other hand, Queensland farmers using car tyres in redclaw farming achieved production levels four times higher than those reported for yabbies (Mosig 1996). The importance of shelter was also reported by Whisson (1996, 2000) who noted its critical role during the moulting period when marron could not protect themselves from predators. The use of roof tiles, plant pots, or aquatic macrophytes was shown to increase survival rates in adult marron.

2.7.6 Human intervention on habitat sustainability of freshwater crayfish

Human activities have many negative impacts on freshwater crayfish habitats. In the past, the improvement of natural habitats was purely for the benefit of capture fisheries. However, recently available technologies (Tang *et al.* 2003, Karthik *et al.* 2005), combined with more knowledge on species interaction, has resulted in more positive effects on ecosystem management (Lamouroux & Souchon 2002, Rosenfeld *et al.* 2005). Some research has identified the role of artificial shelter for rehabilitation

(Molony *et al.* 2005). Since the collapse of fishing stocks, habitat rehabilitation and the role of shelter in different scenarios have been investigated (Fellows 1990, Figler *et al.* 1999, Nakata & Goshima 2003, Westin & Gydemo 1988). However further studies should continue in order to find better solutions, and to solve many potential environmental crises especially when new technologies became available.

Some research is being conducted to help turn threats into benefits such as inland salinity (McIntosh & Fitzsimmons 2003, Lymbery *et al.* 2006) or acidic mine lakes (Viadero & Tierney 2003). Whisson & Storer (2003) show that acidified mine voids could be used to benefit the public. Other research concentrates on using wastewater treatments (Qin *et al.* 2005) for aquaculture. These strategies are helping to transform problems into opportunities. Considering the example of production gained from using carnivorous native species to control tilapia (Fisher & Grant 1994), results are promising. Inko & Mckenzie (1976), Lin & Diana (1995), Tian *et al.* (1997), Rouse & Kahn (1998), Wang *et al.* (1998), and Yi *et al.* (2001) have reported the benefits of predatory polyculture.

Another approach would be to use indigenous species to control introduced species. Species that can control introduced species (Elvira *et al.* 1996) were studied at Ruidera Lake (central Spain). They chiefly ate the recently introduced crayfish *Procambarus clarkii* which was the dominant prey in occurrence, number and biomass for every size class and season. That type of information is important to resource managers and is relevant to crayfish biology and conservation (Hansen & Richardson 2002). The use of information from museums has helped identify three endangered Tasmanian crayfish: *Astacopsis franklinii, A. tricornis*, and the world's largest freshwater crayfish, *A. gouldi*. A research project funded by the National Heritage Trust in Western Australia showed that native marron (*Cherax tenuimanus*) populations were increasing in the presence of exotic yabbies (Campbell & Whisson 2002). The result was probably due to the large amount of benthic macrophytes in the system and a large array of shelter types, sizes and complexities.

2.8 Project rationale

It is clear that several key variables dictate how polysystems function, and therefore how profitable they are. These include biological factors (e.g. relative size and density,

gender, availability of food, presence of predators, etc.) and abiotic factors (e.g. light intensity, turbidity, water quality, habitat complexity). Shelter/habitat has not been studied in as much depth as the other factors and requires investigation. In carrying out such a study it is pertinent to examine processes occurring in nature, and apply these principles to aquaculture where possible. This is particularly relevant for a shelter study because aquaculture managers can easily manipulate the type and abundance of shelter in a culture environment. The result can be a profound impact on interspecific and intraspecific interactions, system performance, production and ultimately profitability.

Chapter three

Experimental systems, sites and materials

3.1 Experimental systems and sites

3.1.1 Aquarium-based trials

All aquarium-based experiments were conducted at the Curtin Aquatic Research Laboratories (CARL), Curtin University of Technology, Perth, Western Australia. Perth is considered to be the most isolated city in the world and lies 31°59'S and 115° 53'E. Average maximum air temperature ranges from mean daily minima of 7.8°C in August to mean daily maxima of 30.8°C in February. Inside CARL, temperatures range from 15 to 25°C all year round.

Prior to 2004, aquarium-based experiments were conducted in an open space area within CARL (Plate 3.1). Following a significant upgrade to CARL in 2004, all aquarium-based trials were conducted in Wet Lab 123 (room size 9.7 x 4.4 x 2.5m), a purpose-built room for behavioural research with no windows to enable researchers to fully control light conditions, air flow and temperature. Fifty-six aquaria (60cm length x 30cm width x 40cm depth) were used in the trials. The experimental system consisted of three parallel stands, with each stand holding 9 aquaria on each of its three shelves. Each aquarium had a capacity of 72 L of water and was provided with constant aeration using single 1.5-inch diameter air stones (Plate 3.2).

Plate 3.1 Aquarium system in CARL open space for all experiments pre-2004

The research room contained twelve 40w fluorescent lights fixed to the ceiling to provide adequate visibility for working and recording crayfish behaviour. Additional artificial lighting of (100w bulbs) was controlled using timers, providing total control of desirable light/dark. An LCD torch was used during observations to avoid disturbance to animals.

Plate 3.2. Individual aquarium showing PVC pipe shelter and air-stone

Only one side of each aquarium was accessible for observations. The top shelves were situated at a height that allowed researchers to stand and observe, while the middle shelves required a chair to be comfortably observed. The bottom shelves were observed with researchers sitting on the floor. Generally a piece of pipe shelter (various sizes according to experimental design) was placed in the aquarium for animal refuge. The sides and tops of all aquaria were visually isolated to minimize disturbance and reduce the possibility of crayfish interaction between aquaria. The tank used untreated ground water available from taps marked "freshwater" for all experiments. The chemical analysis of water is shown in Table 3.1.

Table 3.1 Chemical composition of water supply at CARL

Major Ions	Freshwater (Curtin bore)
Salinity (ppt)	0
Conductivity (mS/m)	40
TDS	270
Na^+ (mg/L)	51
K^{2+} (mg/L)	3.2
Ca^{2+} (mg/L)	4.4
Mg^{2+} (mg/L)	11
Cl^- (mg/L)	1-210
SO^{2-}_4 (mg/ L)	25
Hardness ($CaCO_3$)	56
Al- Unfiltered ICP	0.014
Fe-Unfiltered	0.045
Mn-Unfiltered	0.006
Total Nitrogen (mg/L)	3.4

Source: Curtin: SGS Environment Services, Queens Park, WA.

3.1.2 Recirculating tank-based trials

All recirculating system-based experiments were conducted at the Curtin Aquatic Research Laboratory (CARL). The tanks were housed in the same area as the aquaria (refer to 3.1.1). Nine blue plastic circular tanks were used for experiments reported in Chapters 7 and 9 and for holding animals (Plate 3.3). The recirculation system consisted of three parts: rearing tanks, water supply and treatment, and aeration (Plate 3.4). The water supply and treatment system consisted of 32 mm centrifuge pumps moving water from a sump, through a 2 μm cartridge filter into two coupled biofilters, then via spray bars into the experimental tanks, and finally through independent central drains with 40 mm stand pipes (covered by 10 mm mesh) by gravity back into the sump. The refill system on the sump would activate according to a controlling float valve. Each rearing tank had a top diameter of 100 cm, a bottom diameter of 88 cm, and a height of 50 cm. When filled, the tank contained 300L of water. The exchange rate into each tank was 125L/h. Each tank was aerated from the central CARL blower.

Plate 3.3 Nine x 300L tanks in recirculation system
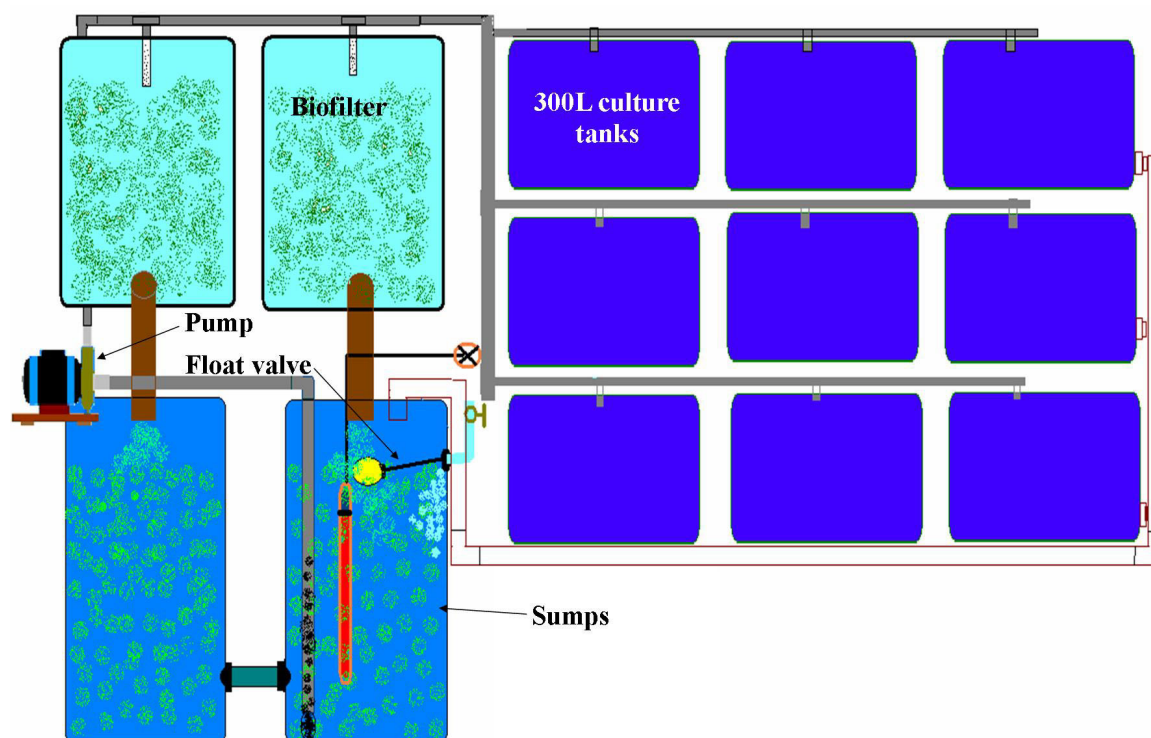
Plate 3.4 Schematic representation of recirculation system (not to scale)

3.1.3 Pond-based experiments

The experiment that investigates the impact of shelter on marron and silver perch polyculture (Chapter 9, Section 9.3) was conducted at the Collie Aquafarm, Western Australia (34.5S, 116E) where a series of experimental ponds were constructed for the Centre for Sustainable Mine Lakes (CSML). This field research site was created to demonstrate how crayfish polyculture could be used as a beneficial end use of final mining voids once mining has ceased (Evans *et al*. 2000, Storer & Evans 2003). Water is pumped from a 'mine lake' into a water treatment facility (i.e. anoxic limestone drain) and then into a settlement pond from where it is gravity fed to the culture ponds (Plate 3.5).
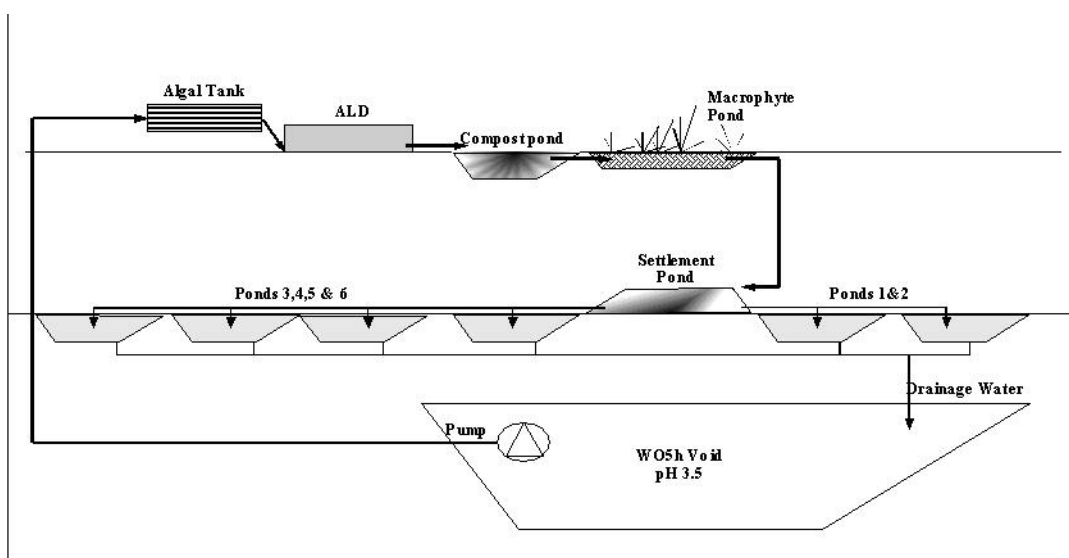
Plate 3.5 Collie Aquafarm, Western Australia, photograph and schematic representation of the water treatment system. ALD = anoxic limestone drain

Six rectangular, purpose-built earthen ponds, surrounded with an electric fence to prevent terrestrial predators, were used for polyculture trials. The dimensions of the earthen ponds are described in Table 3.2. Details of water quality in ponds are shown in Table 3.3.

Table 3.2 Pond specifications at the Collie site (from Storer 2006)

Dimensions	Measurement (m)
Length	40
Width	18
Depth range	1.25-1.75
Average depth	1.8
Effective surface area	0.072 ha
Side batter	3 : 1

Table 3.3 Chemical composition of water supply at Collie Aquafarm

Major Ions	Aquafarm (December to March 2004)
Salinity (ppt)	0.81
Conductivity (mS/m)	1.60
TDS	200
Total Nitrogen (mg/L)	0.00

Aeration was supplied by paddlewheel aerators that started automatically at 9pm and turned off at 8am daily. Three ponds were provided with shelter consisting of synthetic loose mesh marron hides (Custom Network Pty Ltd) shown in Plate 3.6.

Plate 3.6 Loose mesh crayfish shelters and paddlewheel aerator at Collie Aquafarm

3.1.4 Mesocosm-based experiment

The mesocosm situated at CARL is an excellent research facility for observational research. The oval shaped tank is 8m long, 4m wide and 2.45m deep. It is filled with 80 tonnes of freshwater and houses various finfish and crayfish. There are three observation windows along the front side (Plate 3.7). Video recording tools were used to observe the interaction between crayfish and finfish predators with regards to shelter.

Plate 3.7 Mesocosm tank where the underwater video camera was installed and crayfish behaviour toward finfish predators was investigated

Bunches of synthetic loose mesh marron shelters and bundles (32 pieces) of 90mm PVC pipes were constructed and placed randomly in the mesocosm according to the experimental design. The underwater cameras were installed at two different sites and linked to a data recorder system. Data were recorded continuously at 5-min. intervals. This study followed an unusual finding at Lake Moyanup in Western Australia (Campbell and Whisson 2004) where a population of native marron showed clear signs of resurgence following partial displacement from an expanding yabby population. Campbell and Whisson (2004) hypothesized that the type, size and complexity of prevailing habitat was the major factor contributing to the unexpected finding.

3.2 Test Species

3.2.1 Yabbies (*Cherax albidus*)

Yabbies (Plate 3.8) used in this study were sourced from a commercial yabby farm Cambinata^{TM}, Kukarin, and a farm dam in Western Australia's Wheatbelt region (32°S, 116°E). The yabby, originally from Victoria, was introduced to Western Australia in 1932 (Morrissy & Cassells 1992). Farming yabbies is now widespread throughout regional WA (Lawrence and Jones 2002); although according to FWA (1997), stocking yabbies is only allowed on the east of Albany Highway between Perth and Albany. Yabbies mature at an early age and are highly fecund, and are therefore a threat to slower-growing native crayfish (Morrissy 1990).
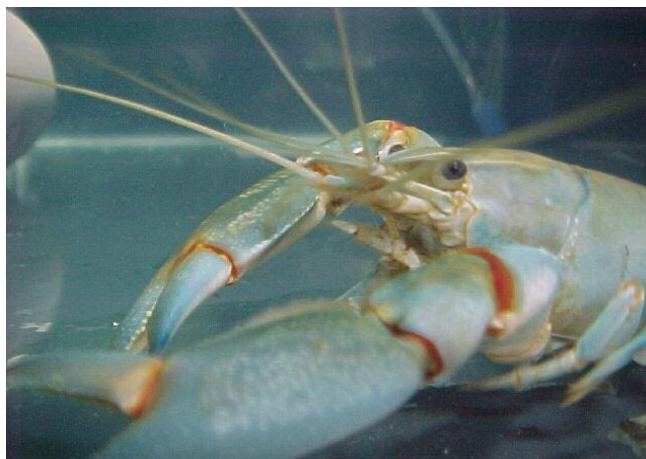
Plate 3.8 Yabby (*Cherax albidus*)

3.2.2 Marron (*Cherax tenuimanus*)

Marron (Plate 3.9), one of the world's largest freshwater crayfish, is native to the south-west drainage systems of Western Australia. Marron production is relatively low with the crayfish requiring at least two years to reach marketable size (Whisson 2000).

Plate 3.9 Marron (*Cherax tenuimanus*)

3.2.3 Silver perch (*Bidyanus bidyanus*)

Silver perch (Plate 3.10) is native to the Murray-Darling Basin of eastern Australia (Lake 1971). They have been translocated and stocked in farm dams in WA for many years. A hardy species, silver perch can tolerate a wide range of conditions, and being an omnivorous scavenger, silver perch are a candidate for polyculture with marron (Whisson 2000). However, silver perch evolved with yabbies in the Eastern States and may prefer the warmer waters of yabby dams; as such, yabby-silver perch polyculture is worthy of further consideration.

Plate 3.10 Silver perch (*Bidyanus bidyanus*)

3.2.4 Murray Cod *(Maccullochella peelii peelii)* (Mitchell)

Murray cod (Plate 3.11) is the biggest freshwater cod in Australia. It is native to the Murray Darling region. Due to its large size and aquaculture attributes they have been translocated to Western Australia (FWA 2003). Murray cod can tolerate harsh aquatic environments, are ambush predators and prey on freshwater crayfish (Chivers *et al.* 1996, Allen *et al.* 2002).

Plate 3.11 A Murray cod (*Maccullochella peelii peelii*) in the CARL mesocosm

Chapter four

Shelter competition and behavioural responses of yabbies exposed to silver perch odour

*This chapter explores the effect of size, gender and reproductive status
of yabbies on the process of shelter acquisition while exposed
to silver perch odour in laboratory conditions. The experiment
explores the yabby's degree of behavioural plasticity and adaptation
to the environment. Results support the notion of a competitive advantage
in shelter acquisition and thus the potential for species dominance
in culture and in nature.*

4.1 Introduction

Shelter is essential to increase production of crayfish monoculture (Booth 2001) and polyculture (Whisson 2000). Shelter in the correct abundance and complexity will increase crayfish survival (Karplus 1995). It affects the foraging strategies of predators (Warner & Hall 1974, Stein 1977) and is therefore a key predator avoidance mechanism (Blake & Hart 1993, Jordan *et al.* 1996). Further, in culture systems the shelter complexity can be manipulated, for example, natural versus artificial (Whisson 2000).

Yabbies (*Cherax albidus*) are an introduced species to Western Australia (Morrissy & Cassells 1992), being native to New South Wales, Victoria and South Australia. They were stocked into farm dams in WA in 1932 and can now be found in some rivers and irrigation dams of the South West. They are thought to out-compete marron and the general belief is that as an invader they will, in time, displace the native species. Height (2004) studied behavioural modifications of yabbies to chemical stimuli and showed that yabbies display greater behavioural modifications when presented with chemical stimuli than marron.

The aim of the present study was to continue the work of Height (2004) but focus on shelter competition between yabbies of different sizes and genders when presented with silver perch odour.

4.2 Materials and methods

4.2.1 Location and system design

This experiment was conducted at the Curtin Aquatic Research Laboratories (CARL) Curtin University of Technology, Western Australia. Fifty-four aquaria (60cm length x 30cm width x 40cm depth) were used in the trial. The system consisted of 3 parallel experimental stands, with each stand housing 6 aquaria on each of its three shelves (Plate 4.1). Each aquarium contained 40L of water and was provided with constant aeration using single 1.5 inch air stones connected to the low pressure air supply in the laboratory (as described in Chapter 3).

Plate 4.1 Experimental system consisting of 54 aquaria on 3 stands

Ten 40w fluorescent bulbs were used to provide artificial light with the photoperiod set for 12 hours light and 12 hours dark. In order to mimic changes in light intensity during sunrise and sunset, an additional 100w globe was turned on at 0600 and off at 1800 daily; in addition the fluorescent ceiling lights turned on at 0800 and off at 1600. No artificial lights operated at night except during the recording period when an LED headlight (Energizer[Tm]) was used to observe the positions and behaviour of animals. Each aquarium was provided with shelter in the form of a single, straight piece of grey, unplasticised polyvinyl chloride (UPVC) pipe of 20cm length and 90mm diameter. All aquaria were visually isolated from one another by black plastic sheets covering three sides and the top to minimize disturbance of experimental animals and eliminate the possibility of visual interaction between aquaria; only the ends remained uncovered to allow for observation of the crayfish.

4.2.2 Experimental design

The experiment comprised three treatment groups; these are summarized in Table 4.1. Each treatment was replicated eighteen times using a Latin square block design. Details of treatment units and allocations are given in Appendix 2.

Table 4.1 Treatment description and tested solution sequences of the experiment

Treatment	Description	Time & Test solutions			
		1st day 5 min	1st day 24 hr	3rd day 5 min	3rd day 24 hr
T1	SB *vs* LM	Control	na	Predator	na
		Predator	na	Control	na
T2	LB *vs* LM	Control	na	Predator	na
		Predator	na	Control	na
T3	SB *vs* LB	Control	na	Predator	na
		Predator	na	Control	na

Note: SB=small berried female, LM=large male, LB=large berried female, na=no additional

4.2.3 Experimental animals

4.2.3.1 Yabby (*Cherax albidus*)

All yabbies used in this trial were selected from a farm dam in Beverley in the Western Australian Wheatbelt (32ºS, 116ºE). The selected crayfish were purged together in one of three 120L tanks provided with shelter and aeration for three weeks prior to the trial. They were fed commercial crayfish pellets (Glen Forrest Stockfeeds) three times weekly, and 50% of the holding tank water was exchanged every alternate day. Prior to the commencement of the trial, yabbies were sorted and separated into experimental groups by size and gender. Groups were then held for 3 days in 120L static tanks provided with plenty of shelter made of UPVC pipe. From these original stocks, crayfish were visually graded into similar average size groups according to gender and reproductive state (i.e. berried and non-berried). Yabbies were weighed and their carapace length was measured using callipers and each was identified using white marking liquid; all were then placed into final holding tanks, as follows:

    Small berried-females (n=18)
        carapace length   42.3 ± 2.2mm
        average weight   62±4.7g
    Large berried-females (n=18)
        carapace length   47.3 ± 1.0mm
        average weight   80±3.8g
    Large males (n=18)
        carapace length   50.3 ± 3.5mm
        average weight: 85± 2.5g.

4.2.3.2 Silver perch (*Bidyanus bidyanus*)

Silver perch of various sizes ranging from 50-1,500g were sourced from a commercial farm in Parkerville, Western Australia (31.5ºS, 116 ºE). Silver perch were housed in a 5,000L recirculation system for 2 weeks prior to the start of the experiment during which time they were fed daily to satiation. Fish were transferred to 200L holding tanks 24 hours before the experiment began at a density of 2.5 kg/100L. Predator odour solution was prepared according to Hazlett (1994) and adapted according to Height (2002).

4.2.4 Experimental procedure

Yabbies were sorted, weighed, measured and marked before being placed into 120L acclimation tanks 24 hours prior to the experiment. Prior resident yabbies were allocated to each aquarium according to the experiment design and left for 24 hours to establish residency following the protocol described by Peeke *et al.* (1995). Intruder crayfish were retained in the holding tanks ready for introduction into the experimental aquarium the following day.

Silver perch predators were held in 200L covered blue plastic drums containing 150L of freshwater with two air stones providing aeration. At the onset of the experiment, intruders were gently released into each aquarium. This was designated as Time Zero (T_0) of the first recording period. Immediately following, 10mL of either the control or the predator odour solution was added via syringe in a corner of the aquaria away from the experimental crayfish. It was designated that half of each treatment (nine tanks) received the labelled control solution (as described in Chapter 3) and the other half received the labelled predator odour solution. The treatment and control syringes were strictly separated and handled with care to avoid any opportunity for cross-contamination. Tank number and treatment allocation are shown in Appendix 2.

Observations of shelter acquisition and behavioural responses were carried out on four occasions. The first observations were taken immediately after intruders were placed into the aquaria; initial observations were recorded every 30 seconds for a period of 5 minutes. The recording technique followed a protocol established by Height (2002) and included recording of shelter use, movement, posture, cleaning, climbing, flicking of antennae/antennules, fighting, and appendage losses, detailed in Table 4.2. Following

the conclusion of the first recording period, subsequent recordings were carried out every four hours until the end of the 24 hour period. At this point, 80% of aquarium and holding water was exchanged gently to minimize disturbance of either yabbies or silver perch. Tanks were then replenished with freshwater to the designated levels.

Table 4.2 Data collection protocol and observation period

Behavioural response		Observation period			
		1st day 5 min period	1st day 24 hours	3rd day 5 min period	3rd day 24hours
Shelter occupation		Yes	Yes	Yes	Yes
Movement		Yes	No	Yes	No
Cleaning		Yes	No	Yes	No
Climbing		Yes	No	Yes	No
Fighting		Yes	No	Yes	No
Ante/Antu	Antennae flicking	Yes	No	Yes	No
	Antennule flicking	Yes	No	Yes	No
	Flicking both	Yes	No	Yes	No
posture	Lowered posture	Yes	No	Yes	No
	Intermediate posture	Yes	No	Yes	No
	Raised posture	Yes	No	Yes	No
Appendage loss		Yes	No	Yes	No

Note: Ante=Antennae, Antu=Antenules

Both resident and intruder yabbies shared the aquaria for another 24 hours before the next observation period began. On the third day, the next recording period began. This time, test solutions were reversed such that aquaria that received predator solution in the first recording period now received the control solution. When the test solutions were added, the second cycle of recording began, repeating observation of shelter acquisition and behavioural responses for every 30 seconds for 5 minutes, then 4-hourly to 24 hours as per the first recording procedure. Following introduction of test solutions, the silver perch used to generate the predator odour were returned to the rearing tanks. When the 24 hours' recording of shelter occupation was complete, all yabbies were transferred back to the rearing tanks.

4.2.5 Data collection

Crayfish behaviour in this experiment was classified according to the protocol employed by Gherardi *et al. (*2002). This required a three person team to record behaviour for the 5 minute observation period. Each person was assigned to a treatment and test solution according to the experimental design. Observations recorded included the amount of time spent engaged in or exhibiting each behavioural response. The record sheets were compiled into a spreadsheet (Excel™, Microsoft Corporation). A sample of the data sheet is provided in Appendix 2.

4.2.6 Recording protocol

The recording protocol used for this experiment was adapted from Height (2002) and has also been used in a number of crayfish behavioural studies (Hazlett and Schoolmaster 1998, Hazlett 1999, Hazlett 2000, Gherardi *et al.* 2002, Hazlett *et al.* 2002). The reaction time (in seconds) and the percentage of time (for the 5 minute test period) spent exhibiting each behaviour and posture were recorded as per Appendix 2.

4.2.6.1 Reaction time

Reaction time was recorded as the interval (in seconds) from injection of the freshwater control or predator odour test solution until a change in behaviour or posture occurred relative to the behaviour/posture recorded immediately before the injection occurred.

4.2.6.2 Behaviour and posture

Crayfish were observed for the 5-minute test period, during which behaviours and posture were recorded every 30 seconds. Below is a list of behaviours and postures that were recorded, including an explanation where necessary.

Behaviours:
- use of shelter (i.e. inside or outside shelter)
- locomotion (movement of ambulatory legs)
- cleaning
- flicking movements of antennae, antennules or both
- climbing.

Postures:

- Lowered posture: body is in contact with the substratum, the chelipeds are drawn in towards the body, and the tail fan curled under the abdomen;

- Intermediate posture: body is held just off the substratum, the tips of the chelae lightly touch the substratum, and the tail fan is nearly perpendicular to the substratum;

- Raised posture: the body is elevated off the substratum, the chelipeds are held off the substratum and parallel to it or higher, and the abdomen or tail fan is extended.

4.2.6.3 Aggressive interactions

Observation and recording of interactions considered aggressive included fighting, appendage losses or mortality.

4.2.7 Water quality monitoring

Total ammonia, nitrite, nitrate, and pH were monitored using commercial test kits (Aquarium Pharmaceutical Inc™) and temperature was monitored using standard aquarium thermometers. Water was tested from each treatment and also the silver perch tank prior to the beginning of the experiment. If the levels of any water quality parameter exceeded the optimum limit according to Height (2002), then water exchange was carried out. Tests were repeated at the start and end of the experiment.

4.2.8 Statistical analysis

Treatment comparisons between prior residents and intruders in response to the test solutions, through display of shelter use and other behavioural responses, were analysed using the non-parametric Mann-Whitney U-Test. When more observations were available in the pooled comparisons, one-way analysis of variance (ANOVA) and Post hoc tests were performed.

4.3 Results

4.3.1 Prior resident vs. intruder - competition and shelter acquisition

4.3.1.1 First day (five-minute contest observation)

Prior resident yabbies were established in all treatments for a 24-hour acclimation period before intruders were released into aquaria. During the first 5 minute observation period, on average, prior residents regardless of size or gender occupied the shelter significantly longer than intruders (P< 0.001). In the pairing of small berried females as prior residents vs. large male intruders, the prior resident spent significantly more time in the shelter than intruders did (z=-3.571 P=0.000), as shown in Figure 4.1. Likewise, shelter occupation of large berried resident females paired against large males was significantly longer (z=-3.528 P=0.000), as was small berried resident females against intruding large berried female yabbies (z=-3.902 P=0.000). The latter two comparisons are shown in Figures 4.2 and 4.3, respectively.

There were no significant differences in shelter use by all resident/intruder pairs between those exposed to control or predator test solutions (P>0.05).

4.3.1.2 First day (twenty-four hour observation at four-hour intervals)

Results of 4-hourly observations during the first 24-hour observation period are summarised in Appendix 2. In contrast to findings of the first 5 minute observation period, over the following 24 hours large male intruders spent significantly more time (43.33±5.77% of time vs. 21.11±6.33%) in the provided shelter than small berried female prior resident yabbies (z=-2.146 P=0.032) when exposed to the freshwater control solution. However, when exposed to the predator solution, no significant difference was found (z=0.316 P=0.725) between the time that large male intruders occupied the shelter compared to the small berried female prior residents (38.89±4.23% for LM intruders vs. 33.33±7.26% for SB residents). In contrast, no significant difference (P>0.05) in shelter occupation was observed between either of the other two contest pairs exposed to either predator or control solution.
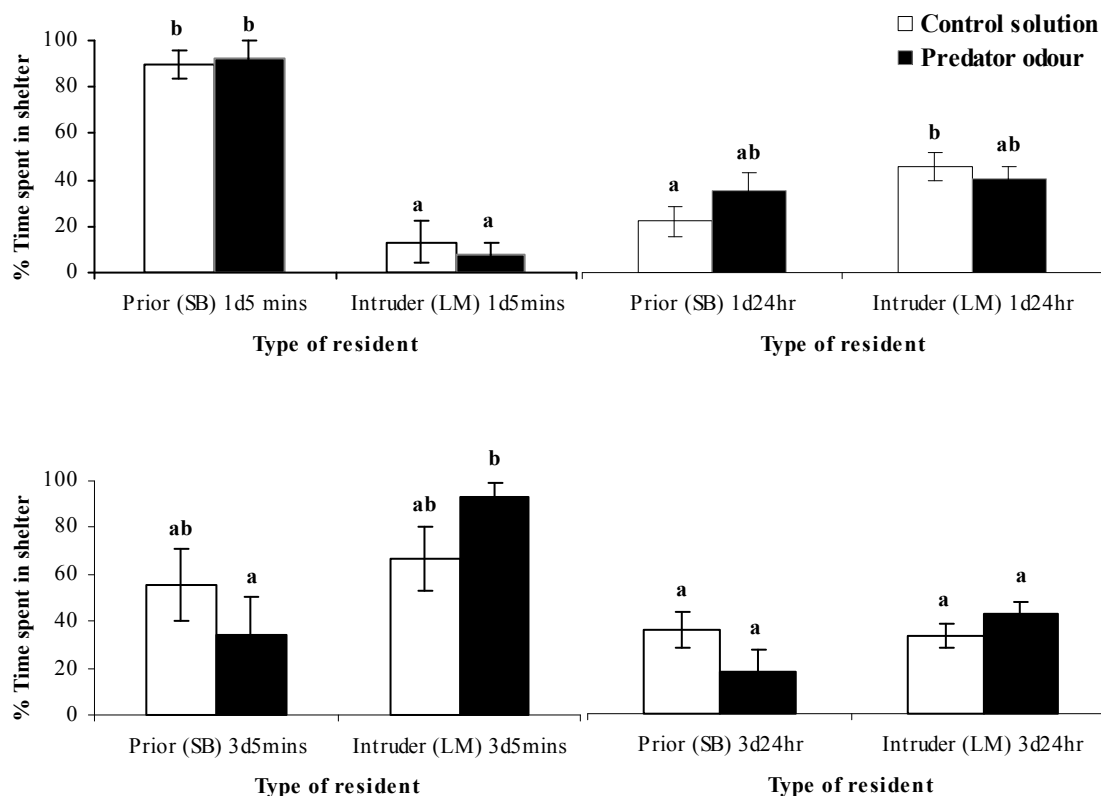
Figure 4.1 Time spent in shelter by resident small-berried female (SB) and intruding large male yabbies (LM) at different times with both control solution and predator odour
Values represent mean (± se). Note: Different letters between groups indicate significant different mean (α =0.05).

4.3.1.3 Third day (five-minute contest observation)

Results of the first 5-minute observation period of the third day are summarised in Appendix 2. The only contested pair to show significantly different (z=-2.675 P=0.007) shelter occupation times during the first 5 minutes of the third day was the small berried female prior resident yabbies (34.44±15.73% of time) *vs* the large male intruders (93.33±5.53%) when exposed to predator odour. There was no significant difference in shelter use between any other pair of prior resident vs. intruder exposed to either the predator or the control solution. However, it is interesting to note that for all but one of the prior resident/intruder pairs, the sum of average percent of time spent occupying shelter exceeded 100%. For example, results of observations of LB *vs* LM exposed to control solution, the prior resident LB spent 77.78±14.70% of time in the shelter, while the LM intruder also spent 63.33±15.90% of time. While these results are not

significantly different (z=-0.511, P=0.609), it is clear that both resident and intruder yabbies spent periods of time occupying the shelter jointly. This is the first time this phenomenon was observed in this experiment.

4.3.1.4 Third day (twenty-four hour observation at four-hourly intervals)

Summary results of 4-hourly observations during the 24-hour study period of the third day are shown in Appendix 2. There were no significant differences in shelter occupation in either the control group or the group subjected to predator odour between any of the contest pairs. However, intruders tended to spend more time occupying shelters in the response to predator odour in Treatment 1 (SB *vs* LM) and Treatment 3 (SB *vs* LB); while these observations were not significantly different (P=0.057), they are interesting.
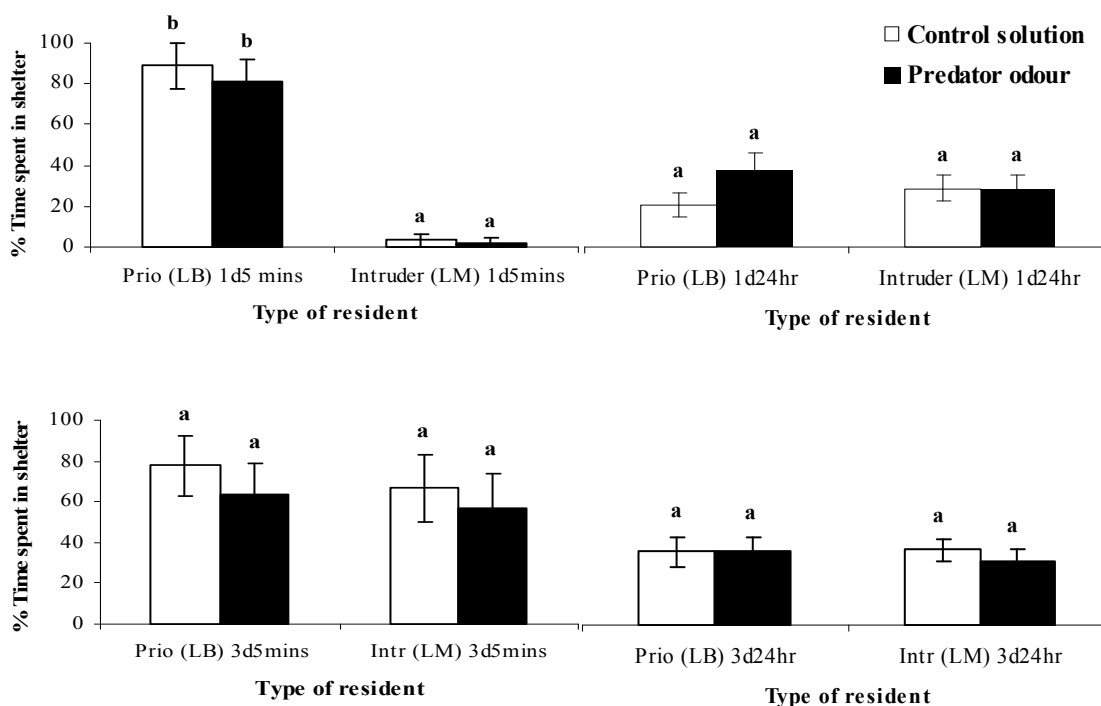
Figure 4.2 Time spent in shelter by resident large berried female and intruding large male yabbies
Value reported are means ±S.E. Columns having different lettering are significantly different (P<0.05).
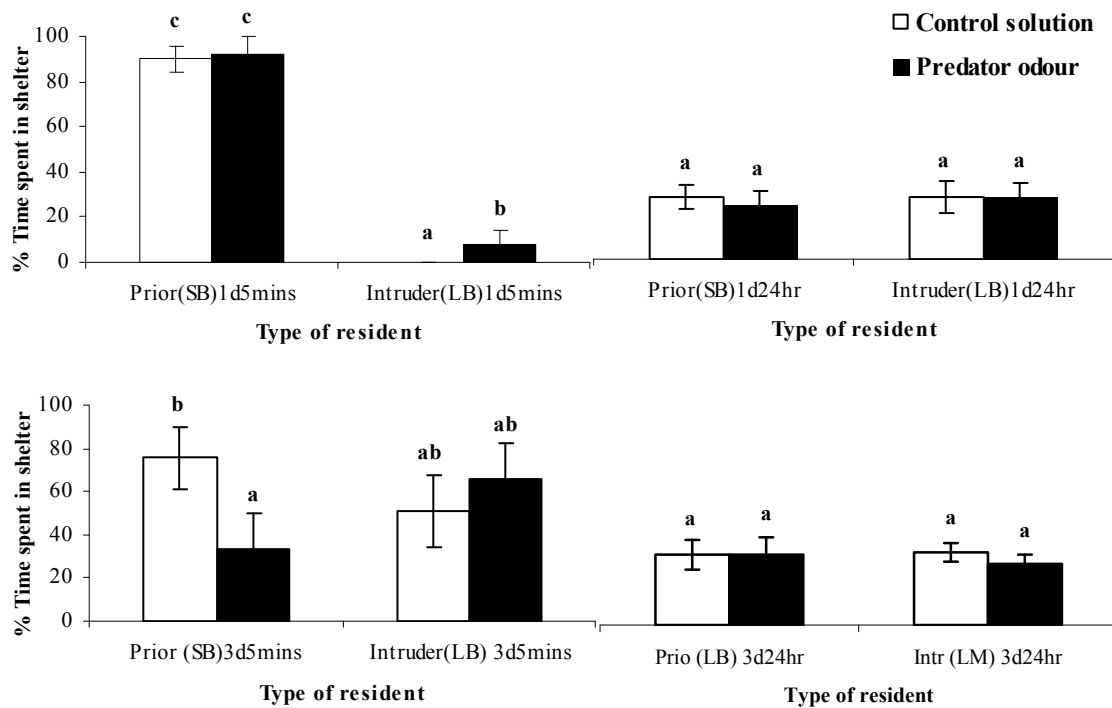
Figure 4.3 Percent time spent in shelter by resident large berried and intruding large male yabbies.
Values represent mean (± se) Note: Different letters between groups indicate significant different mean (α =0.05).

4.3.2 Time spent exhibiting movement

Table 4.3 summarises time spent displaying movement following exposure to either predator odour or control solution. Results are also depicted in Figure 4.4. Across all three treatments, in the first 5 minutes following exposure to predator odour and control solution, intruder crayfish spent significantly more time (P = 0.001 to 0.016) engaged in movement than all prior residents. However, by Day 3 in each treatment, there was no significant difference in movement of either intruders or prior residents to either predator odour or control solution.

Table 4.3 Mean values (± S.E.) of percentage of time (s) spent in movement with two different test solutions for all treatments on both recording days

Treatment		Test type	Prior resident	Intruder	Z (P)
T1	**(day1, 5mins)**	Control solution	31.11±10.60	70.00±10.00	-2.398 (0.016)
		Predator odour	14.44±9.73	64.44±7.84	-2.965 (0.003)**
	(day3, 5mins)	Control solution	36.67±10.41	27.78±8.46	-0.588 (0.556)
		Predator odour	31.11 ±7.90	17.78±7.78	-1.522 (0.128)
T2	**(day1, 5mins)**	Control solution	16.67±10.80	58.89±8.46	-2.552 (0.011)
		Predator odour	8.89±3.510	62.22±9.96	-3.433 (0.001)**
	(day3, 5mins)	Control solution	55.56± 4.44	10.00±7.64	-1.470 (0.142)
		Predator odour	18.89±7.72	14.44±7.84	-0.340 (0.734)
T3	**(day1, 5mins)**	Control solution	12.22±5.98	77.78±12.24	-2.957 (0.003)**
		Predator odour	23.33±9.57	75.56±5.50	-2.945 (0.003)**
	(day3, 5mins)	Control solution	27.78±7.95	23.33±7.45	-0.448 (0.654)
		Predator odour	23.33±7.07	11.11±.6.55	-1.512 (0.131)

** denotes results that are highly significant (P < 0.01).
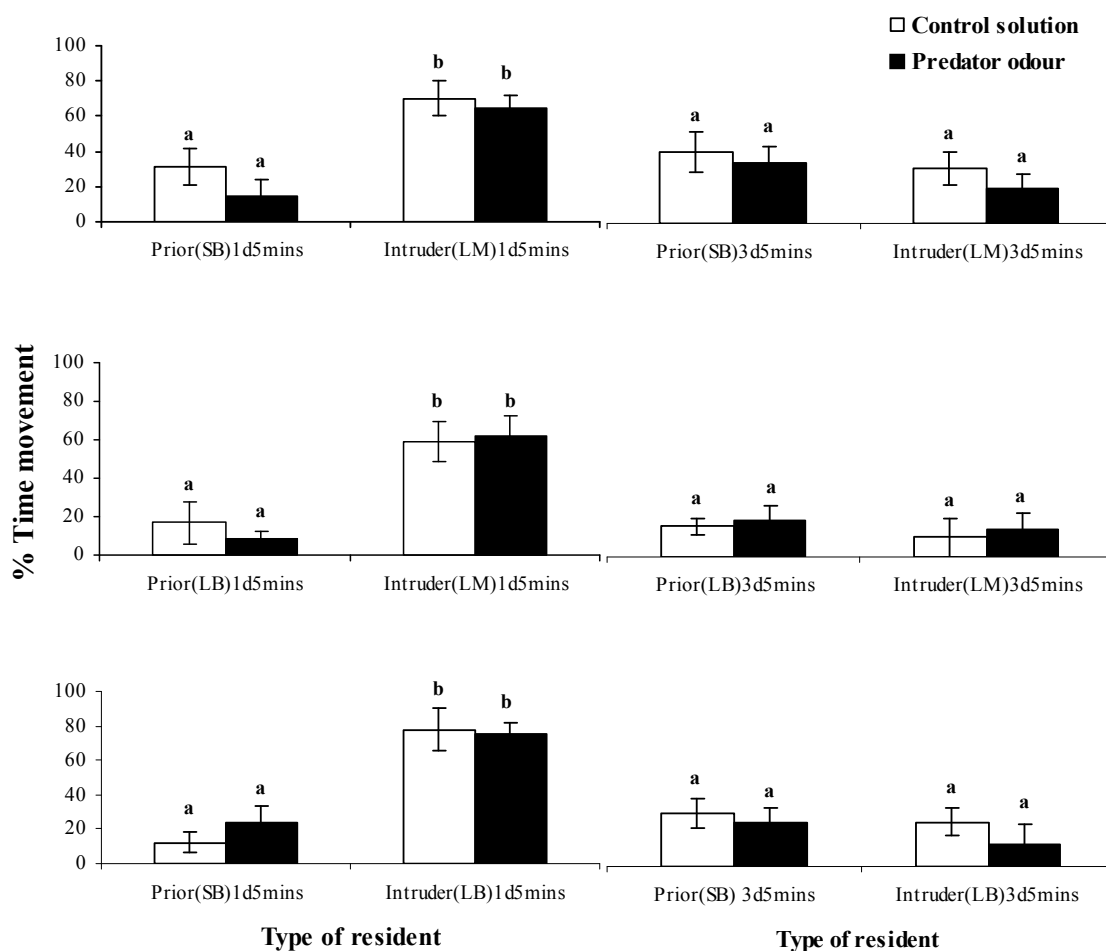
Figure 4.4 Percentage time spent in movement by resident and intruding yabbies.
Values represent means (± se) Note: Different letters within and between groups indicate significantly different means (α =0.05).

4.3.3 Behavioural modification

Behavioural modification is reflected in the response of yabbies to stimuli by displaying different behaviours, for example, changes in postures, cleaning, climbing, and flicking of antennae/antennules or both. Aggressive and avoidance behaviour in the form of fighting and appendage loss were recorded. It is worthwhile to consider the observed responses of yabbies from the perspective of behavioural modification and adaptation to circumstance.

4.3.3.1 Postures of yabbies in response to control and predator odours

4.3.3.1.1 First day (five-minute contest observation)

Comparison of the postural response of small berried female prior residents with large male intruders (Treatment 1) shows that prior residents spent significantly more time at low posture than intruders in response to both the control solution and predator odour solution ($z=-2.241$ $P=0.025$, $z=-2.184$ $P=0.029$). With respect to intermediate posture, it was noted that intruders spent a significantly larger amount of time displaying this posture than prior residents ($z=-2.179$ $P=0.029$). Both prior residents and intruders showed no significant differences in display of the raised posture ($P>0.05$). These results are depicted in Figure 4.5 and summarised in Appendix 2.
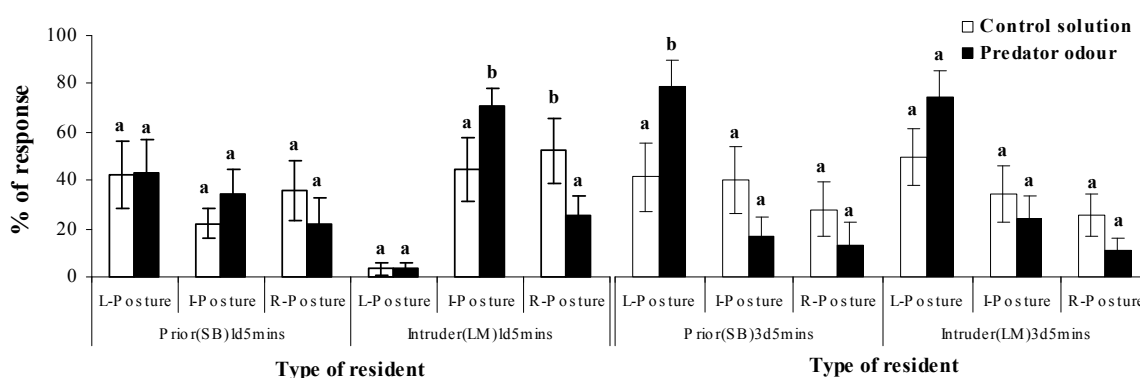
Figure 4.5 Time spent in different postures by resident SB and intruding LM in the first 5 minutes of day 1 and day 3
Values represent mean (± S.E.), Different letters between groups indicate significant different mean (α =0.05). Note: L=lowered, I=Intermediate and R=raised.

Similar results were observed in Treatment 2 where large berried female prior residents spent significantly more time in lower posture than the large male intruders (z=-3.357 P=.001) when exposed to the control solution. However, no significant difference in display of lower posture was found when the predator odour solution was tested (P>0.05). Results also showed that intruders spent more time in the intermediate posture than residents (z=-2.167 P=0.030) when exposed to predator odour, but not the control solution. There were no significant differences in time spent exhibiting intermediate or raised posture in response to either tested solution (P>0.05). These results are depicted in Figure 4.6 and summarised in Appendix 2.
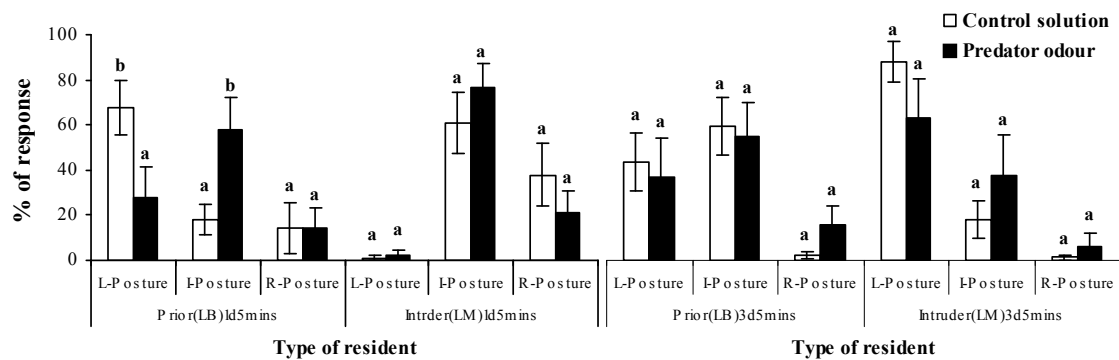
Figure 4.6 Time spent in different postures by resident LB and intruding LM yabbies in the first 5 minutes of day 1 and day 3
Values represent mean (± S.E.). Different letters between groups for each posture indicate significant different means (α =0.05). Note: L=lowered, I=Intermediate and R=raised.

Resident small berried females spent significantly more time in the lower posture than the large berried intruders (Treatment 3); this difference was consistent when exposed to both the control and predator odour solution (z=-2.791 P=0.005 and z=-2.140 P=0.032). Other postures displayed were not significantly different (P>0.05) between solutions tested or between residence status. Results are depicted in Figure 4.7 and summarised in Appendix 2.
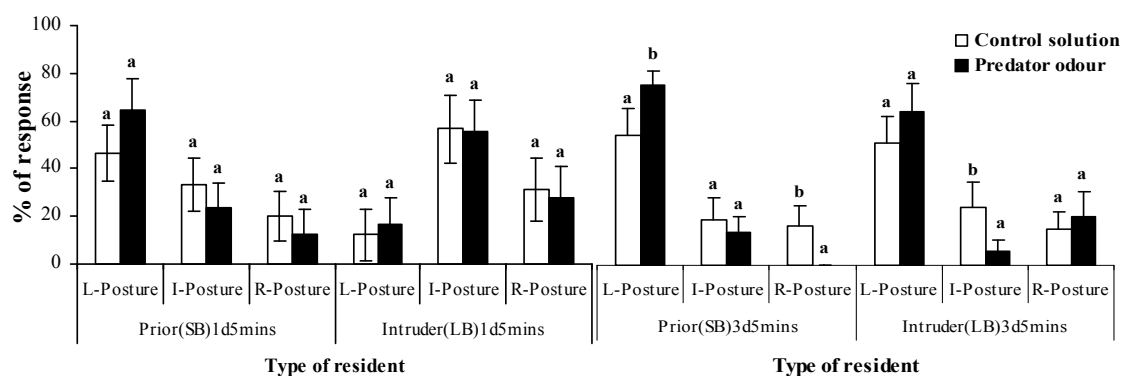
Figure 4.7 Time spent in different postures by resident SB and intruding LB yabbies in 5 minutes of day 1 and day 3

Values represent mean (± S.E.). Different letters between groups for each posture indicate significant different means (α =0.05). Note: L=lowered, I=Intermediate and R=raised.

4.3.3.1.2 Different postures displayed by prior residents and intruders on the third day (5-min recording period)

By this stage, neither the small berried female prior residents nor the large male intruders showed differences in posture (P>0.05). The only differences were observed when exposed to the control solution between intruding large males and prior resident large berried females. In this case, the large male intruders spent significantly more time at the lowered posture compared to the prior resident (z=-2.559 P=0.010). In contrast, in the same experiment, prior residents spent significantly more time displaying the intermediate posture than intruders (z=-2.380 P=0.017). There were no other significant differences in response to the control or predator odour solutions. Results are depicted in Figures 4.5, 4.6 and 4.7, and are summarised in Appendix 2.

4.3.3.2 Flicking of antennae and antennule of yabbies in response to predator odour and control solution

4.3.3.2.1 Flicking of antennae and antennules in response to test solutions on the first day (five-minute contest observation)

In the first five minutes of the experiment, prior residents utilised antennules alone significantly more often than all of the intruders used antennules alone (P<0.05). In contrast, intruders used both antennae and antennules together significantly more than all prior residents across all treatments (P<0.05). There were no significant differences in use of antennae alone, and there were no significant differences in use of antennae

alone, antennules alone, or both together in response to the tested solutions (P>0.05). Results are depicted in Figure 4.8 and are summarised in Appendix 2.
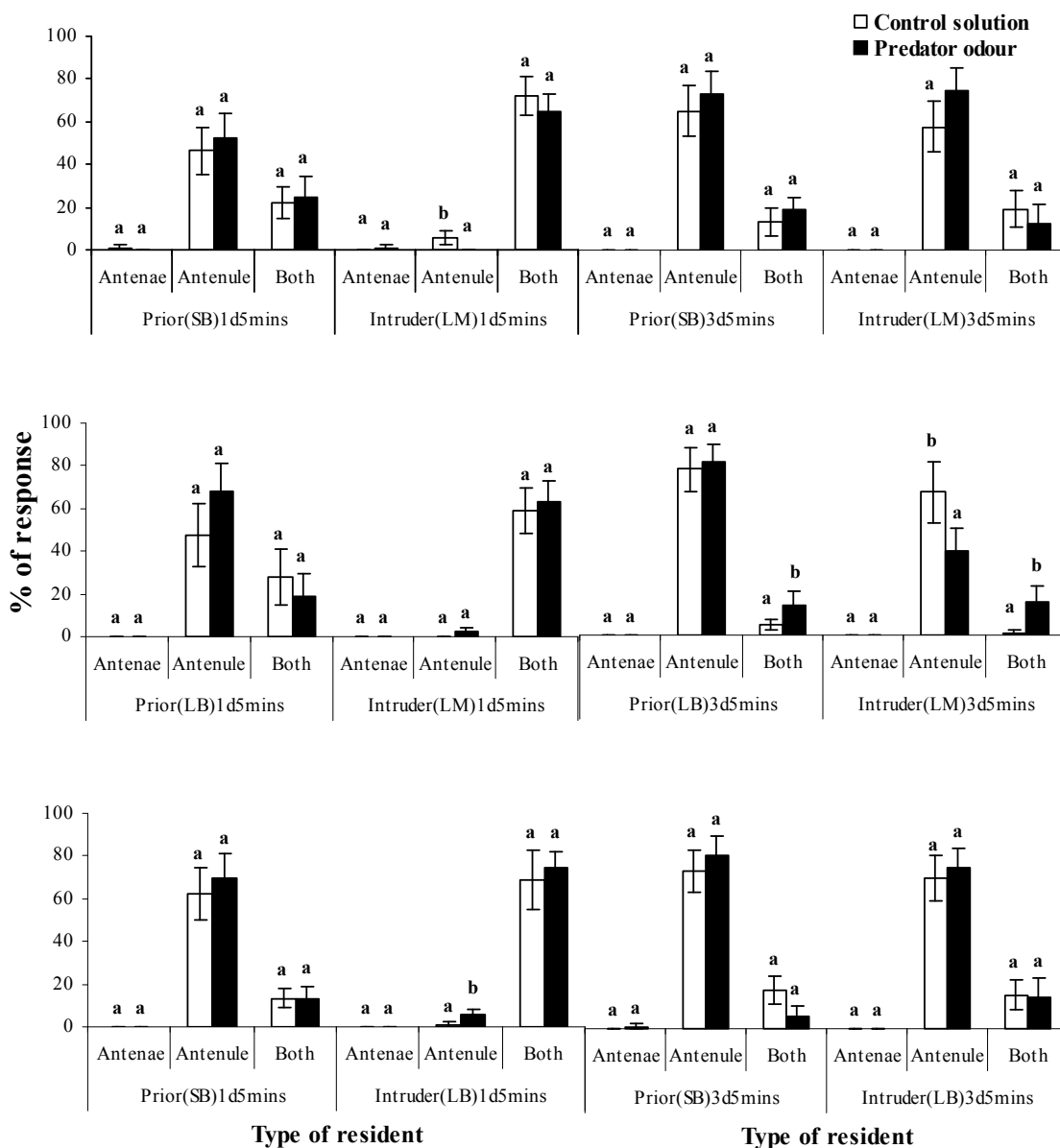
Figure 4.8 Comparisons of the flicking of antennae/antennule between all resident and intruders with control and predator odour in 5-minute contest observation

Values represent mean (± S.E.). Different letters between groups for each antennae/antennule flicking indicate significant different means (α =0.05). Note: Ante=Antennae, Antu=Antennules.

4.3.3.2.2 Flicking of antennae and antennules in response to test solutions on the third day (five-minute contest observation)

Neither residence status nor test solution had any effect on the proportion of time spent flicking antennae and/or antennules by the test crayfish in the first 5 minutes of the third day (P>0.05). During this time, both prior residents and intruders spent 40 - 80% of time flicking antennules, less than 20% of time flicking both their antennae and antennules together, and no time flicking antennae alone. Results are depicted in Figure 4.8 and are summarised in Appendix 2.

4.3.3.3 Time spent by yabbies cleaning in response to control and predator odour

There was no significant difference in the proportion of time spent on cleaning between any combination of prior residents and intruders or when exposed to predator odour or control solution (P>0.05). Results are shown in Figure 4.9.
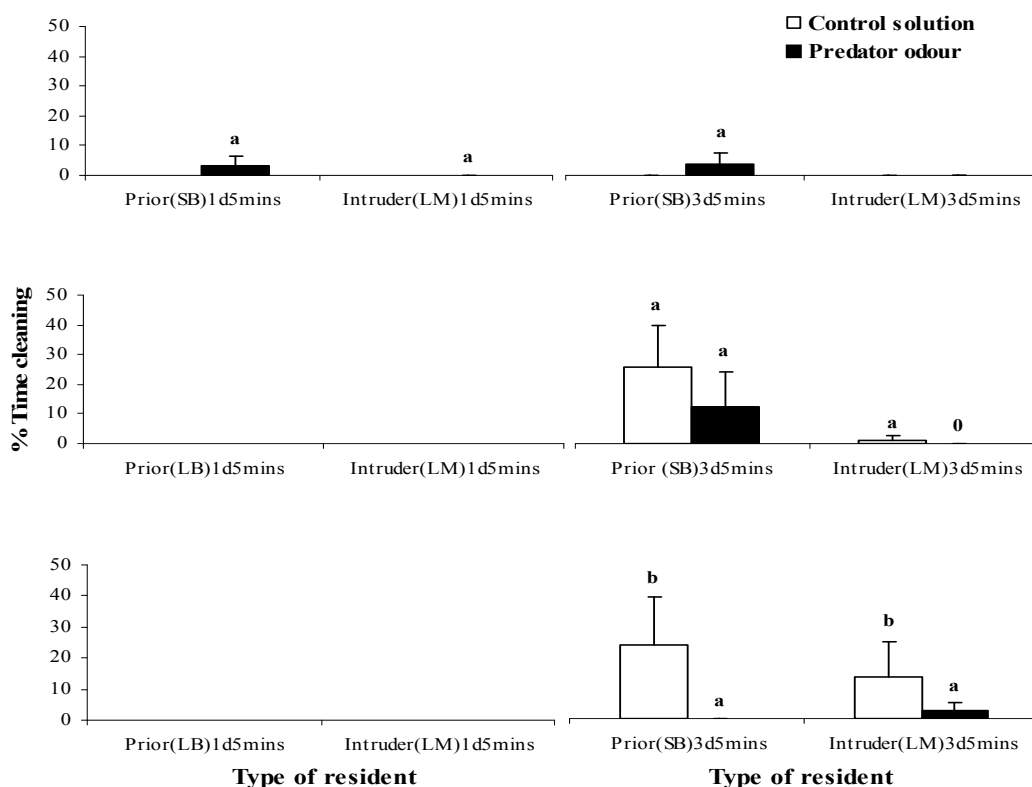
Figure 4.9 Time spent cleaning between yabbies with control and predator odour on day 3 Values represent mean (± S.E.). Different letters between groups for each behavioural response indicate significant different means (α =0.05.

4.3.4 Aggressive behaviour of yabbies in response to control and predator solutions

In this experiment, aggressive behaviour was measured by observation of climbing, fighting, and appendage losses during or after fighting. Results of comparisons between prior residents and intruders showed that only large male intruders during the first 5 minutes of the first day spent significantly more time engaged in climbing (P<0.05), whether exposed to predator odour or control.

There were no significant differences in time spent fighting between any treatment pairs with either tested solution (P>0.05). No appendage loss or mortality was recorded in this experiment. Results are depicted in Figure 4.10 and summarised in Appendix 2.
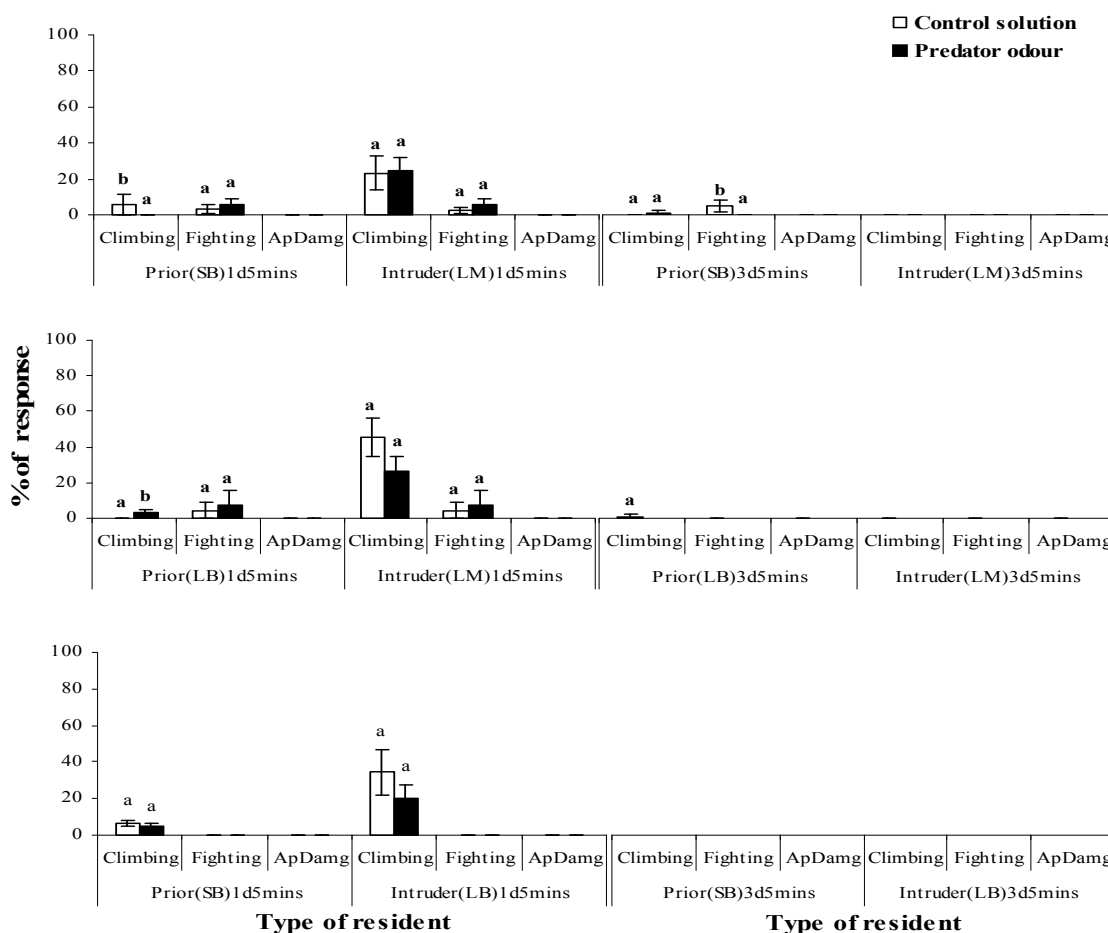
Figure 4.10 Comparisons of aggressive behaviour of resident and intruder yabbies with control and predator odour in first 5 minutes of day 1 and day 3 observations
Values represent mean (± S.E.). Different letters between groups for each behavioural response indicate significant different means (α =0.05

4.4 Discussion

4.4.1 Effect of prior resident status on shelter acquisition

A prior resident effect was clearly demonstrated in the first 5 minutes of observations from the first day of this experiment. When intruders were placed into the aquaria, followed by addition of control or predator odour solution, both types of intruders (large male and large berried female yabbies) did not gain possession of the shelter immediately. Figler (1999) also demonstrated similar results when observing the behaviour of the red swamp crayfish (*Procambarus clarkii*). These results suggest that the behavioural responses observed were based on visual cues from the prior resident rather than on chemical cues from the test solution. This conclusion is supported by Jenkinson (2004) and Storer (2006) who found that the reaction time of yabbies to both chemical and visual cues was significantly shorter than visual cues alone. Dingle (1983) showed that crayfish evaluate their Resource Holding Potential (RHP) and their circumstances before taking further action; visual cues are clearly critical to determining relevant competitor characteristics. Finally, Taylor & Elwood (2003) found that when shelters were already occupied by other crayfish, intruders reacted by avoiding confrontation and open competition for shelter. Results of this experiment agree with the notion of a prior resident effect on response of intruder crayfish when competing for shelter.

4.4.2 Effect of gender and physical condition

It has been hypothesized that physical and reproductive condition (e.g. berried females) will affect the outcome of competition for shelter (Whisson 2000). Berried female crayfish have been shown to take control of shelter and out-compete smaller or similar-sized males for shelter; this makes sense in terms of survival of the species (Krebs 1985). The results of the present study showed that prior resident berried yabbies (*Cherax albidus*) showed a heightened agonistic response toward conspecific intruders when competing for shelter. Behaviours observed included increased fighting and climbing. Under natural conditions, yabbies will establish their home by burrowing to avoid threat from either predators, other yabbies competing for shelter, or from intra-specific cannibalism (Lawrence *et al.* 2001). In this trial, as no substrate was provided and shelter was the limited resource, intruders tended to spend more time searching for

alternative shelter. This resulted in increased time spent engaged in locomotion and climbing. According to Figler *et al.* (1999), the provision of a single shelter forces intruders into the open where they may be vulnerable to potential predators. Peeke *et al.* (1995), Ranta and Linström (1993), and Takahashi *et al.* (2001) reported that sometimes aggressive responses from the prior resident could lead to dominance cannibalism. Clearly, prior residents tend to have a considerable advantage over shelter when competition for shelter exists (Boulay *et al* 1993). However, on the arrival of an intruder with greater RHP (Peeke *et al.,* 1998), prior residents tended to lose residency status (Nakata and Goshima, 2003). This was observed in the present study.

By the end of the first 24 hour observation period, large male intruders had spent significantly more time in shelter than small berried female prior residents. This shows clearly that the larger crayfish was eventually able to dominate the shelter, which agrees with the findings of Nakata and Goshima (2003). In contrast, large berried female prior residents were observed sharing the shelter with large male intruders and did not display significant levels of agonistic behaviour during the course of observations. Figler *et al.* (1999) revealed that crayfish that were relatively larger always won the shelter regardless of sex or reproductive condition. Marsh (2004 unpublished) also confirmed this with similar findings when physically larger marron dominated shelter over prior resident yabbies under experimental conditions. The results of the present experiment did not show consistent dominance of shelter by the larger competitor. This could be due to several factors. The difference in size of crayfish used in this experiment was comparatively less than that reported by other authors. Crayfish used in this experiment were purged together prior to the start of the experiment among crayfish of more widely disparate size; this familiarity may have diminished competitive behaviour even though duration of acclimatisation of prior residents has previously been demonstrated to be sufficient to establish and sustain a prior resident advantage (Height *et al.* 2004).

4.4.3 Behavioural modification

The results of this trial reflect the diversity and complexity of behavioural responses and patterns of yabbies. It is controversial whether yabby behaviour is instinctive or adaptive (Height 2005). Observations of behavioural changes in the present study were similarly difficult to interpret. The range of behavioural modifications described by

Hazlett (1999) has been assumed to cover the majority of yabby behaviours. During the present experiment, the duration of observation periods was sufficient to allow for observation of a range of different reactions similar to those described by Hazlett (1999). However, when expression of a given behaviour occurred for a significant proportion of the observation period, the duration of observation time may have then limited observation of the full range of other possible responses and behaviours; this may also have artificially added significance to the relative proportion of time spent engaged in a given behaviour. For example, if crayfish were observed to spend more time climbing, this activity would reduce the remaining proportion of observation time engaged in other behaviours such as shelter occupation or cleaning. Nonetheless, yabbies in the present study were observed to spend some time cleaning, an activity which usually disappears when animals are under threat. This observation implies that crayfish were displaying a normal complement of behaviour throughout the experimental period. This supports the idea that observation times used in the present study were appropriate and sufficient to observe a full range of behaviour.

In general, yabbies have been noted to spend the majority of time in low posture (Height 2002). Low posture is considered indicative of retreat, or rest, according to circumstance (Hazlett 1999). In the present study, prior residents tended to display low posture whereas intruders tended to display intermediate posture which is interpreted by Hazlett (1999) to indicate responsiveness. Findings of the present study agree with these general behaviour interpretations. Furthermore, raised posture is considered to be a defensive or dominant pose, and was not commonly observed in the present study. This finding supports conclusions drawn regarding agonistic behaviour (see Section 4.4.6).

4.4.4 Predator recognition and shelter occupation

Predator odour was not shown in this experiment to influence shelter competition. Gilinsky (1984) reported that the presence of fish predators greatly reduced the number of benthic species and the density of macroinvertebrates. Yabbies are considered to be highly adaptive to a range of ecosystems however; adult yabbies defend to survive in the presence of a variety of natural predators such as eels and tortoises (Lawrence & Morrissy 2000). Height (2002) found that when food was mixed with predator odour, yabbies spent a higher proportion of time in shelter than the control solution

(freshwater). However, in the present experiment, the response to predator odour and control groups in terms of shelter acquisition did not result in a clear pattern. Prior resident yabbies were shown by Jenkinson (2004 unpublished) to display an immediate response to visual cues from predators, which adds a dimension to the findings of Height (2002) with respect to the influence of predators on crayfish behaviour. Collectively, these results could imply that yabbies are able to utilise information to distinguish immediate threats (i.e. visual) from potential threat of predators (i.e. chemical cues from fish odour only). This could explain the lack of observed effect of predator odour on behaviour over the course of the present experiment.

4.4.5 Chemosensory predator detection and predator avoidance

The presence of a predator will influence the behaviour and abundance of crayfish (Gilinsky 1984). Crayfish have been shown to rely on both visual and chemical cues to locate food and predators (Blake & Hart 1993). Appelberg *et al.* (1993) found that *Astacus astacus* increased time in shelter when exposed to predator odour. Results of this experiment did not support the effect of predator odour on shelter occupation. However, intruding crayfish spent most of the observation periods in motion and displaying avoidance behaviour by climbing on the aquarium walls. It is difficult to determine whether this avoidance behaviour constituted a response to predator threat, or to the prior residents. This experiment was structured to provide two different types of stimuli simultaneously; conspecific visual cues and silver perch chemosensory cues. It is possible that exposure to a single stimulus may have resulted in a different and perhaps more uniform pattern of response. Peeke *et al.* (1998) and Hazlett (2000) showed that crayfish in possession of shelter distinguished or protected their territory through release of an alarm odour and they observed retreat of newcomers in response. These authors concluded that the non-specific alarm appeared to successfully repel some conspecific invaders. In the present experiment, the simultaneous introduction of conspecific intruders and predator odour may have resulted in confusion, thereby affecting the response pattern in both prior residents and intruders when visual cues did not match chemosensory stimuli. This may also explain the apparent lack of response to chemosensory predator cues.

Wild yabbies and silver perch coexisted in the Murray-Darling River systems before being translocated to Western Australia. Yabbies play a part in the perch diet in these river ecosystems. Generally, crayfish respond to information from their chemoreceptive organs (Tierney 1985). Prior resident crayfish in the present study were confronted by both conspecific intruders and predator odour. While responses to intruders were observed, there was no clear pattern of response to fish odour by either prior residents or intruders. Some disagreement exists about degree of concentration of fish odour appropriate for such experiments (Hazlett 2003). Although predator odour solution was prepared according to Height (2002), this solution may have been insufficiently concentrated for the conditions of the present experiment.

4.4.6 Agonistic behaviour in yabbies

Yabbies are similar to other crayfish with respect to social hierarchy, the display of pronounced agonistic behaviour, and cannibalism, especially among larger adults (Morrissy *et al.* 1990, Holdich 2002). Other studies of freshwater crayfish found that aggressive behaviour occurs more commonly among juveniles than adults (Gherardi & Daniels 2004, Holdich 2002). In the present experiment using only adult crayfish, few instances of fighting were observed and there were no significant differences in time spent fighting between any treatment pairs in the presence of either tested solution. Storer (2006) reported that smaller, subdominant yabbies tend to retreat from larger yabbies, and interpreted this as 'flight without taking fight'. This could explain the low incidence of fighting as prior residents were clearly smaller than intruders in two of three treatment pairs in this experiment. While it is clear from existing research regarding yabby behaviour that the species does indeed tended to be aggressive, these findings and the conclusions of Storer (2006) suggest that yabbies 'pick their fights' by displaying agonistic behaviour only when they sense an advantage. Furthermore, yabbies in the present experiment appeared to distinguish between 'fight for survival' (e.g. avoidance of aggression by both competitors when one contestant was a berried female), and 'fight to win' (e.g. cannibalism). This implies more sophisticated and adaptable behaviour than previously recognised and invites further study. This conclusion could explain the varied results of other authors quoted here, where circumstance and condition may have influenced the expression and observation of their results.

Lynas (2002) found that adult yabbies display agonistic behaviour towards other crayfish species of similar size, and concluded that this tendency posed a major advantage because it was presumed to increase mortality and reduce productivity in competitor species such as marron. While the present experiment investigating conspecific competition for shelter did not record high levels of aggression, it was concluded that aggressive behaviour appears to be selectively displayed depending on circumstance (e.g. competition for limited resources) and condition (e.g. relative size and reproductive state). This finding supports the notion that yabbies possess a competitive advantage as invaders in nature.

Early reproduction of offspring can lead to species dominance in aquatic ecosystems (Morrissy 1983), and overpopulation in aquaculture units (Lawrence & Morrissy 2002). Yabbies generally grow fast and use *r*-selected strategies with early maturation (Chapman 1958, Campbell 1993). They may also brood up to 5 times a year (Morrissy *et al.* 1990). Yabbies can utilise organic matter, detritus and periphyton on benthic substrates (Duffy 2006). These characteristics together with adaptive behaviour patterns pose challenges for farming of yabbies in multispecies dam systems, and also raise concerns regarding escape of yabbies and displacement of native species. The Department of Fisheries, Western Australia, recently investigated the use of all-male seed to address these concerns using the male hybrid (*C. albidus* x *C. rotundus*) to stock farm water bodies (Lawrence & Morrissy 2000). This creates opportunity for a new type of aquaculture and polyculture with finfish to increase productivity of farm dams. However, while the present study contributes to understanding behaviour of yabbies in general, knowledge of behaviour with respect to life stage, gender and physical condition of yabbies when reared with commercial finfish and other species remains poorly understood. Behaviour of single-sex hybrid populations in polyculture invites further study; such systems show considerable promise.

Yabbies have been shown to be more reactive to chemical stimuli from finfish predators than native marron, *Cherax tenuimanus/canii* (Gherardi *et al.* 2002, Height & Whisson 2004). Yabbies in monoculture are hardy and can thrive under various climatic conditions including extremes of temperature and drought by manipulating their environment to survive, for example, by burrowing (Lawrence *et al.* 2001). However, commercial production of yabbies is considered to be very low and inefficient

(Lawrence & Morrissy 2002). Polyculture of yabbies has not been successful due to their aggressive behaviour towards finfish, resulting in poor fish growth and injury (Whisson 2003, pers. comm.). Whisson suggested that silver perch could be profitably reared in cages in polyculture with yabbies, or yabbies in cage *vs* free-range silver perch. However, the performance of yabbies under such conditions is not known. Although single-sex rearing of yabbies holds promise together with the prospect of polyculture with cages, commercial investment is unlikely without further research. Finally, Whisson (2003 pers. comm.) suggested that provision of shelter could hold the key to boosting crayfish productivity and the success of polyculture of finfish. Results of the present study of shelter acquisition behaviour and response to fish odour support this notion.

4.4.7 Providing shelter in crayfish polysytems

Several authors describe the importance of shelter for crayfish survival (Huolila *et al.* 1997, Figler *et al.* 1999, Lawrence *et al.* 2001, Nakata *et al.* 2001). These authors agree that providing shelter is particularly important for juveniles as they are more vulnerable to predation. Verhoef and Austin (1999) successfully reared juveniles in high density without shelter by grading according to size. However, this useful finding is only relevant to tank-based monoculture. Boulay *et al.* (1993) found that minimal provision of shelter in intensive culture of redclaws (*C. quadricarinatus*) resulted in high mortality ranging from 40-95%. Similar results have been observed in polyculture of adult marron with silver perch (Whisson 1996), especially during moulting periods when crayfish are vulnerable to predators. Clearly, provision of shelter is important to improve the survival and enhance the productivity in both monoculture and polyculture of crayfish under a range of circumstances. This is supported by results of the present experiment. Further study of shelter-related behaviour is warranted to fully understand the influence of size, gender, and physical condition of crayfish on survival and production efficiency, including predation and cannibalism in polyculture.

4.5 Key findings

1. Prior resident status was established in all size of yabbies; this affected the behavioural response of intruders competing for shelter.

2. The prior resident effect was short-lived (less than 24 hr) and had no significant impact on behaviour of yabbies thereafter.

3. In this trial, predator odour did not show any effect on prior resident *vs* intruder competition.

4. At times, prior residents and intruders of similar size were observed to share shelter jointly.

5. Shelter occupation in this experiment may have been influenced by shelter preference of yabbies. Further research into shelter preference of crayfish is recommended.

6. Results of this experiment support the high degree of plasticity of yabby behaviour and adaptiveness to circumstance, and support the notion that this constitutes a competitive advantage in nature with potential for species dominance in culture and in nature.

7. Further work is required to examine interspecific shelter acquisition behaviour of crayfish, particularly marron in competition with yabbies under threat from silver perch (i.e. can yabbies and marron productively coexist in polysystems?).

Chapter five

Shelter competition between marron and yabbies under threat from silver perch

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*This chapter examines the impacts of prior residence and shelter competition between native marron (*Cherax tenuimanus*) and invasive yabbies (*Cherax albidus*). The impacts are examined in term of behavioural modification and the responses to silver perch (*Bidyanus bidyanus*) odours. It is known that intraspecific and interspecific conflicts can leave crayfish vulnerable to opportunistic predators such as silver perch in polysystems. Shelters in this study act as the limiting resource, where marron and yabbies are in competition for the resource as a consequence of exposure to silver perch odour. This study follows on from previous chapters, where the combination of prior resident yabbies and intruder were investigated according to shelter-related behaviour responses and modifications. In this Chapter the combination of prior residents and intruders, either native or invasive, were assessed by shelter competition under pressure from silver perch. The results of this study provide useful behavioural information about shelter competition and manipulation in aquaculture and natural water systems.*

5.1 Introduction

In Western Australia, yabbies (*Cherax albidus*) have been extensively cultured since being introduced from the eastern states in the 1930s (Morrissy & Cassells 1992). Considered a low cost investment, yabby production has gained popularity for increasing income of Wheatbelt communities (Lawrence & Morrissy 2002). Morrissy & Cassells (1992) reported that its biological attributes of fast growth, easy breeding, ability to tolerate a wide-range of temperature and low requirement for protein have contributed extensive stocking in rural farm dams. Yabbies, have now spread throughout the Western Australia region (Horwitz 1990, Horwitz & Knott (1995). After nearly a century of farming, yabbies have become domesticated and sometimes misnamed as the native species "koonac" (*Cherax preissii* Erichson) (Morrissy & Cassells 1992). Yabby production from both trapping and purpose-built farms reached 300 tonnes in 1997 (Lawrence & Jones 2002). Strong demand from overseas markets, coupled with business support, have enabled them to strengthen their position in the rural economy.

Marron, is the third largest freshwater crayfish in the world. Native to Western Australia, it has been recognised for its delicate flavour and is highly regarded by chefs in preference to yabbies and redclaw (Lawrence & Jones 2002). In the past, production of marron was based on wild capture; however, numbers have been in decline since the mid-1970s (Fisheries Western Australia 2004). Since Smith (1912) first recognised the potential of marron aquaculture, there has been a major development in husbandry and aquaculture techniques (Morrissy 2000). However, the production of farmed marron was not significant economically until 1979 (Lawrence & Jones 2002). Compared to yabbies, marron are slower to grow, take longer to mature, and require higher dissolved oxygen and a cooler, more stable climate (Morrissy 2000). In addition a weaker response to predator odour, together with less aggressive behaviour has reduced their competitiveness in the wild compared to invasive species 'yabbies' (Gherardi *et al.* 2002 and Height 2002).

While aquaculture production of marron is increasing, the wild population is being threatened. Many natural factors affect wild marron. Both low rainfall and the changing distribution and pattern of precipitation have been identified as major influences on marron populations (Morrissy 1978, Morrissy *et al.* 1984 and Morrissy & Fellows

1990). Low water levels severely limit nursing grounds, and increase level of intraspecific and interspecific interaction resulting in inhospitability. Habitat degradation such as loss of vegetation on the riparian zone has contributed to a reduction in suitable habitat for marron (Jackson 1997). Finally, the recent significant threat to rural communities the rise of inland salinity –also threatens marron, which can tolerate up to 15 ppt but cease to grow at salinity in excess of 6 ppt (Lawrence 1998). If the level of inland salinity increases at this rate, the major rivers in southwest Western Australia and marron populations will be in crisis (Fotedar 2004 pers comm., Molony & Bird 2002, Lymberry 2006).

Human activities either legal or illegal are major causes of fishing mortality (FWA 2004). Even though no commercial fishing is allowed, recreational fishing has greatly affected marronits population (Molony and Bird 2002). The increase of human population has contributed to watercourses being dammed and diverted for irrigation, resulting in less water and increased nutrient run-off into the rivers (Lane & McComb 1988). Whilst intensive agriculture has contributed to the increased concentration of toxic substances, the other major concern for marron populations is the introduction of non-indigenous species which become a silent threat, competing for habitat. Yabbies, with their greater adaptation capacities (Height 2002), have been introduced from the eastern states of Australia and are now widely spread into throughout marron habitats (Horwitz 1990, Morrissy & Cassells 1992 and Campbell & Whisson 2000).

Resource competition between native and non-native species has been a major concern in Western Australia (Fisheries Western Australia 2004) and around the world (Gherradi 2000, Hazlett 2000, Holdich 2002, and Taylor 2002). Yabbies pose a threat to marron by competing for habitat and increasing water turbidity. Yabbies display higher behavioural plasticity. This, coupled with their prolific reproductive ability poses a high risk to the sustainability of aquatic habitat and native marron in Western Australia. Lynas (2002) indicated that yabbies are more aggressive and able to successfully utilised shelter more effectively than marron of a similar size thus making marron population highly vulnerable.

Shelter is the most important visible limiting resource, under the polysystems there is no information is shelter competition. It is therefore important to investigate the

behavioural interaction, between the two species modified by the threat from a finfish predator, and how these responses affect the crayfish polysystems.

The aim of this experiment was to gather information on shelter-related responses of marron and yabbies according to the effect of prior residence size, life stage, and physical condition under laboratory conditions.

5.2 Materials and methods

5.2.1 Location and system design

Fifty-four aquariums (60cm length x 30cm width x 40cm depth) were used in this experiment. The experimental system was located at Curtin Aquatic Sciences Research Laboratory (CARL). The configuration of aquaria and tanks used was the same as that described in Chapter 4. The water provided and methods of aeration used were also described in Chapter 4.

5.2.2 Experimental animals

Marron (*Cherax tenuimanus*) was sourced from a commercial farm in Parkerville, Western Australia (32.9ºS, 116ºE). Marron were sorted by size and placed in holding tanks for one week prior to commencement of this experiment. The marron were fed three times weekly using a commercial crayfish pellet as described in Chapter 4, and were purged by 50% exchange with new water every 24 hours during this period. Continuous, vigorous aeration was also used throughout this period.

The average weight of large male marron (n = 12) was 95.57±1.92g with an average carapace length of 70.00±1.76mm. The large female marron (n = 12) average weight was 87.15±3.94g with an average carapace length of 69.96±1.19mm. Small male marron (n = 9) averaged 44.00±1.17g in weight with an average carapace length of 53.21±1.01mm. The average weight of small female marron (n=12) was45.94±2.74 g with average carapace lengths of 53.93 ±1.25. These animals were used in the other experiment with yabbies however; none of these animals have experience with silver perch.

All yabbies (*Cherax albidus*) used in this trial were selected from a stocked farm dam in Beverley, Western Australia (32ºS, 116ºE). Yabbies were selected according to size

and physical condition (male, berried and non-berried). They were placed in one of three 120L aerated tanks provided with shelter for two weeks prior to the trial. They were fed three times weekly as described for marron, and were purged on every alternate day in the same manner described for marron. Prior to commencement of the trial, 20 yabbies were sorted, weighted and carapace length was measured. Yabbies were then marked and individually placed into an aquarium as the prior resident for 24 hours as described for marron. Remaining yabbies were reserved for use as intruders.

The average weight of male yabbies (n = 15) was 53.57±2.92g with an average carapace length of 47.12±1.76mm. The female yabbies (n = 12) average weight was 51.54±3.94g with an average carapace length of 46.27±1.19mm. Berried female yabbies (n = 12) averaged 52.40±1.50g in weight with an average carapace length of 47.21±2.01mm. These animals were used in the other experiment exposed to marron before however; none of these animals have experience with silver perch

Silver perch (*Bidyanus bidyanus*) were sourced from the same commercial farm at Parkerville that provided marron. In this experiment, various sizes of silver perch (range: 250g to 1,500g with a total biomass of 5,000g) were placed in a 200L holding tank for approximately one hour before the trial.

5.2.3 Experimental procedure

Experiments were conducted on two consecutive days beginning at approximately 11.00AM with the last replicate completed by 4.00PM each day. On the first day, each treatment allocation received either control or predator solution. On the second day, this was reversed. Behavioural responses of crayfish are considered homogenous during daylight with no known effect of biorhythm reported (Holdich, 2002). Observations were therefore expected to be consistent throughout the experimental period each day. The Latin Square design was employed for six trials, each containing three treatments (contest pairs). Each experiment was replicated three times. Seven crayfish types were compared as follows: large male marron (LMM), large female marron (LFM), small male marron (SMM), small female marron (SFM), male yabby (MY), berried yabbies (BY) and female yabby (FY). A summary of treatment allocation is shown in Table 5.1.

Crayfish were observed continuously during each 6 minute experiment, and observations were recorded every 30 seconds. Many studies of individual crayfish

behaviour employ 15-second recording intervals, including Height (2002), Gherardi *et al.* (2000), and others. A 30-second recording interval was set for this study to allow for full observation and recording of both crayfish each pair of prior residents and intruders studied.

Table 5.1 Treatment allocation summary

Trial	Treatment	Resident type		Record type	
		Prior resident	**Intruder**	**1st day**	**2nd day**
1	1	Male yabby (MY)	No intruder	Control	Predator
	2		Female yabby (FY)		
	3		Berried yabby (BY)		
2	1	Large male marron (LMM)	No intruder	Control	Predator
	2		Large female marron (LFM)		
	3		Small female marron (SFM)		
3	1	Berried yabby (BY)	No intruder	Control	Predator
	2		Female yabby (FY)		
	3		Male yabby (MY)		
4	1	Large female marron (LFM)	No intruder	Control	Predator
	2		Large male marron (LMM)		
	3		Small male marron (SMM)		
5	1	Female yabby (FY)	No intruder	Control	Predator
	2		Male yabby (MY)		
	3		Berried yabby (BY)		
6	1	Small male marron (SMM)	No intruder	Control	Predator
	2		Small female marron (SFM)		
	3		Berried yabby (BY)		

Resident crayfish were placed individually in each aquarium 24 hours prior to the study to establish prior residence status (Peeke *et al.* 1995) and to acclimate (Height & Whisson 2002). At the onset of each experiment, intruders were gently released into

each aquarium in the corner opposite to prior residents. This was immediately followed by addition of the test solutions. This was recorded as 'Time zero', after which both prior resident and intruder behaviour was recorded every 30 seconds for a total of 6 minutes. Thereafter, intruders were returned to separate holding tanks. Intruders were allowed to acclimatize and were then randomly reused the next day. All equipment and water used were maintained strictly separate according to individual tank and species.

When observations were complete on the first day, 80% of water in each aquarium was replaced. At the end of the experiment, water quality was tested and all crayfish and finfish were returned to the rearing tanks.

The predator solution was prepared as described by Hazlett and Schoolmaster (1998). The water volume in the predator tank was calculated according to tank dimensions and was then reduced to 100L to concentrate fish odour. However, according to Hazlett (1999), if total predator tank ammonia levels exceed 0.5ppm, predator species begin to experience stress which alters the secretion of fish odour. In addition, this level of ammonia in predator test solution will overwhelm the perception of predator odour by crayfish. Total ammonia in the predator tank water was therefore tested prior to collection of the experimental solution. Following initial testing of predator tank water, the protocol was modified to include hourly replacement of a proportion of water in the predator tanks prior to collection of test solutions such that predator tank ammonia levels never exceeded 0.5ppm.

Pre-trial testing with dye showed that added solutions dispersed uniformly throughout experimental aquaria within 30 seconds. This is similar to previous work by Storer (2006), Height (2002) and Marsh (2004) which employed the same protocol.

5.2.4 Recording protocol

The recording protocol used in this experiment was derived from Height (2002) and has been used in numerous crayfish behavioural studies (Hazlett and Schoolmaster 1998, Hazlett 1999, Hazlett 2000, Gherardi *et al.* 2002, Hazlett *et al.* 2002, Height and Marsh 2004). The reaction time in seconds and the percentage of time within each 30 second interval spent exhibiting various behaviours and postures were recorded as described in Chapter 4.

5.2.5 Data collection

Data was collected using logbooks. Data collected included behavioural observations for each trial replicate. In addition to this, initial water quality was tested for all aquaria, holding and rearing tanks for all species for use as baseline data. Data was recorded by any one of three observers. However, the same observer recorded all observations within a given experimental unit. After completion of recording of each experiment, data was transferred to a spreadsheet (Microsoft Excel®) and tallied. Copies of each data set were secured as archives.

5.2.6 Water quality monitoring

Total ammonia, nitrite, nitrate, pH, and temperature were recorded for each treatment and also for the silver perch holding tank prior to the beginning of each experiment. If any water quality parameter exceeded the optimal level (Boyd 2000), water exchange was carried out in accordance with Hazlett (1999). Water quality tests were repeated at the end of the experiment to confirm that results remained within the recommended range.

5.2.7 Statistical analysis

Data were transformed to percentages before performed statistical analysis. Comparisons between prior resident and intruder behaviour and response to test solutions were analysed using the non-parametric Mann-Whitney U-Test (Ott 1998, Keller and Warrack 2000). When within-species observations were pooled for inter-species comparison, a chi-square analysis was performed. For comparison between treatment, and for comparison between test solution and resident type, one-way and two-way analysis of variance (ANOVA) was used, respectively (Selvanathan *et al.* 2000). Post hoc comparison was performed when ANOVA showed a significant different at $P < 0.05$.

5.3 RESULTS

The observations of each experiment were compared for differences between species, within and between resident types, and responses to test solutions. The 30-second recording interval was adequate to record all observations of both prior resident and intruders. There was no significant difference within the group of experimental animals (P>0.05).

5.3.1 Reaction time

Prior residents reacted within 30-60 seconds of introduction of intruders and administration of test solution. In this experiment, the reaction time ranged between 49.09±4.31s and 41.09±4.34s for marron. There was no significant difference in reaction time for either marron and yabby prior residents or intruders (P>0.05) There was also no significant difference in reaction time in response to control (47.80±4.98s) or predator odour solutions (50.10±5.54s), P>0.05.

5.3.2 Behaviour response comparison between marron and yabby

Comparison of the two species revealed no significant differences in most behavioural responses including movement, climbing, fighting, cleaning and flicking antennae/antennules (P>0.05). However, analysis of variance showed significant difference in shelter use between crayfish species. Yabbies spent significantly more time in shelters than marron (df=178 F=3.955 P=0.04). Yabbies prefered intermediate posture while marron spent more time in raised postures (df=178 F=7.134 P=0.008). A comparison of behaviour responses between the species is shown in Figure 5.1. No loss of appendage or mortality was observed in this experiment; these categories are therefore combined in Figure 5.1.
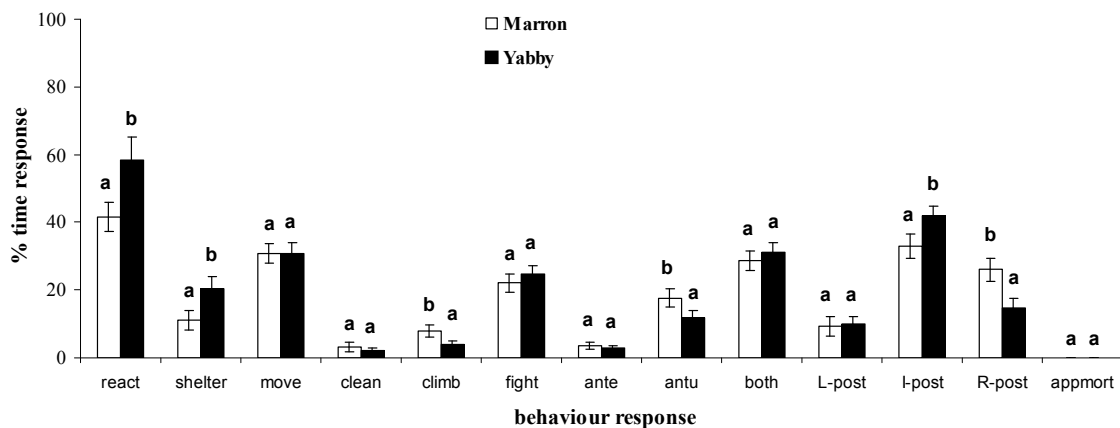
Figure 5.1 Comparison of behaviours between marron and yabbies
Bars represent percentage of means (±SE) for each comparison except reaction time (seconds). Different letters between groups indicate significantly different percentages (α=0.05 Tukey HSD).

5.3.3 Behavioural modification of marron and yabbies

The purpose of comparison in this section was to distinguish any differences in response between marron and yabbies of different type, size and condition. All behavioural responses were included in the analysis regardless of their resident status, condition and any differences in response to test solutions.

Analysis of variance showed no significant difference in shelter use, movement, cleaning, Fighting, antennae flicking, or flicking both antennae/antennules and lowered posture (P>0.05). Appendage lost or mortality was not observed. However, there were significant differences in climbing behaviour (df=6 F=4.988 P=0.000), antennule flicking (df=6 F=2.421 P=0.028), intermediate posture (df=6 F=2.439 P=0.027) and raised posture (df=6 F=2.309 P=0.036).

5.3.3.1 Climbing behaviour

Size of marron had the greatest influence on time spent climbing. Small female marron spent 22.92±6.66% of time climbing, which was similar to small male marron (11.46±4.45% of time). As depicted in Figure 5.2, this was significantly longer than berried yabby, large female marron, male yabby, female yabby and large male marron (P<0.05).
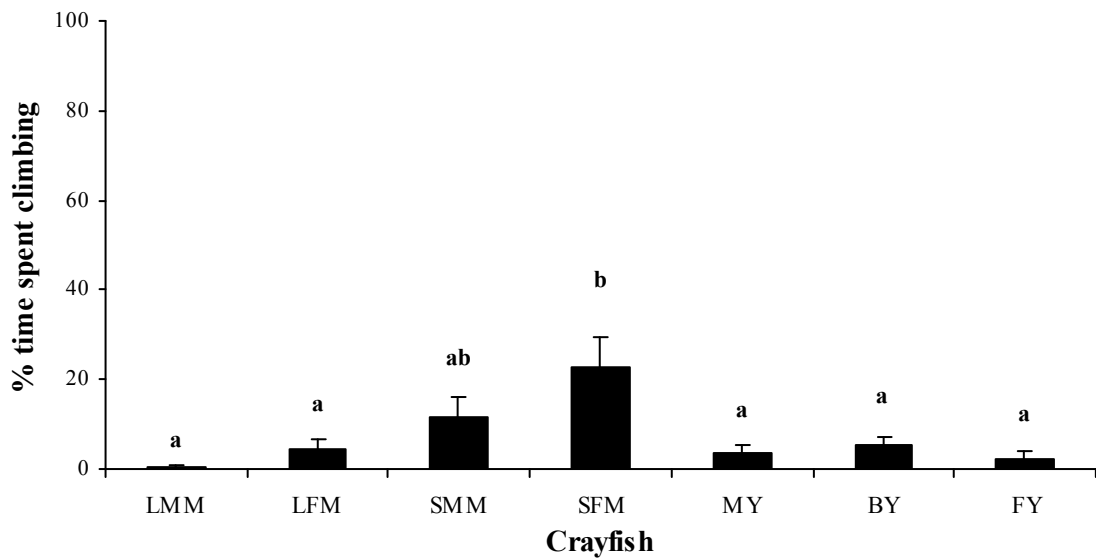
Figure 5.2 Climbing response comparison between different types of crayfish
Values represent means (±SE) for each comparison (Tukey HSD). Different letters between groups indicate significantly different means (α=0.05).

5.3.3.2 Antennule flicking

Antennule flicking behaviour was analysed by one-way ANOVA, and shows significant differences in this behaviour between types of crayfish (*df* =6 F=2.421 P=0.028). Post hoc and multiple comparisons were performed which also confirmed significant differences between the groups (α = 0.05) as shown in Figure 5.3.

There was no significant difference in antennule flicking between large female marron and all classes of yabbies (P>0.05). However, in comparison to this group, large male and small male marron displayed a significantly higher proportion of antennule flicking, and small female marron displayed significantly less antennule flicking (P<0.028).
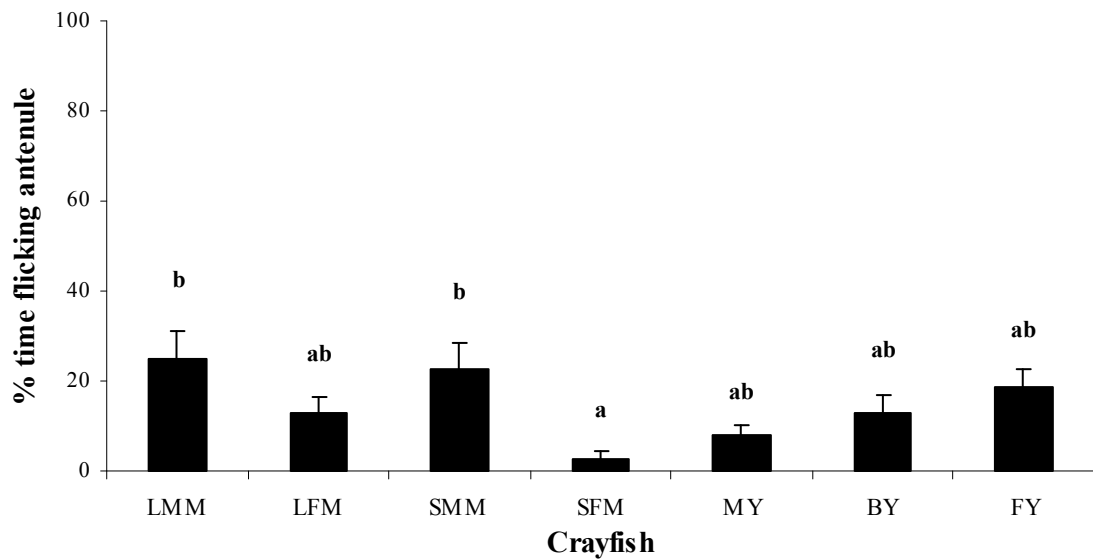
Figure 5.3 Antennule flicking comparison between different types of crayfish
Bars represent means (± SE) for each comparison (Tukey HSD). Different letters between groups indicate significantly different means (α= 0.05).

5.3.3.3 Intermediate posture

Three different postures were compared. Overall, marron and yabbies spent a similar proportion of time exhibiting lowered posture. However, while engaged in resident-intruder competition, the two species spent significantly different proportions of time displaying intermediate posture (P=0.027) as shown in Figure 5.4. Comparison of individuals using the Mann-Whitney U Test showed that large male marron spent significantly less time (27.08±6.35%) than male yabbies (50.00±4.94%), z = -2.747 P=0.006 in this posture. However, post hoc comparisons did not reveal significant differences between groups due to high variability in responses within groups.
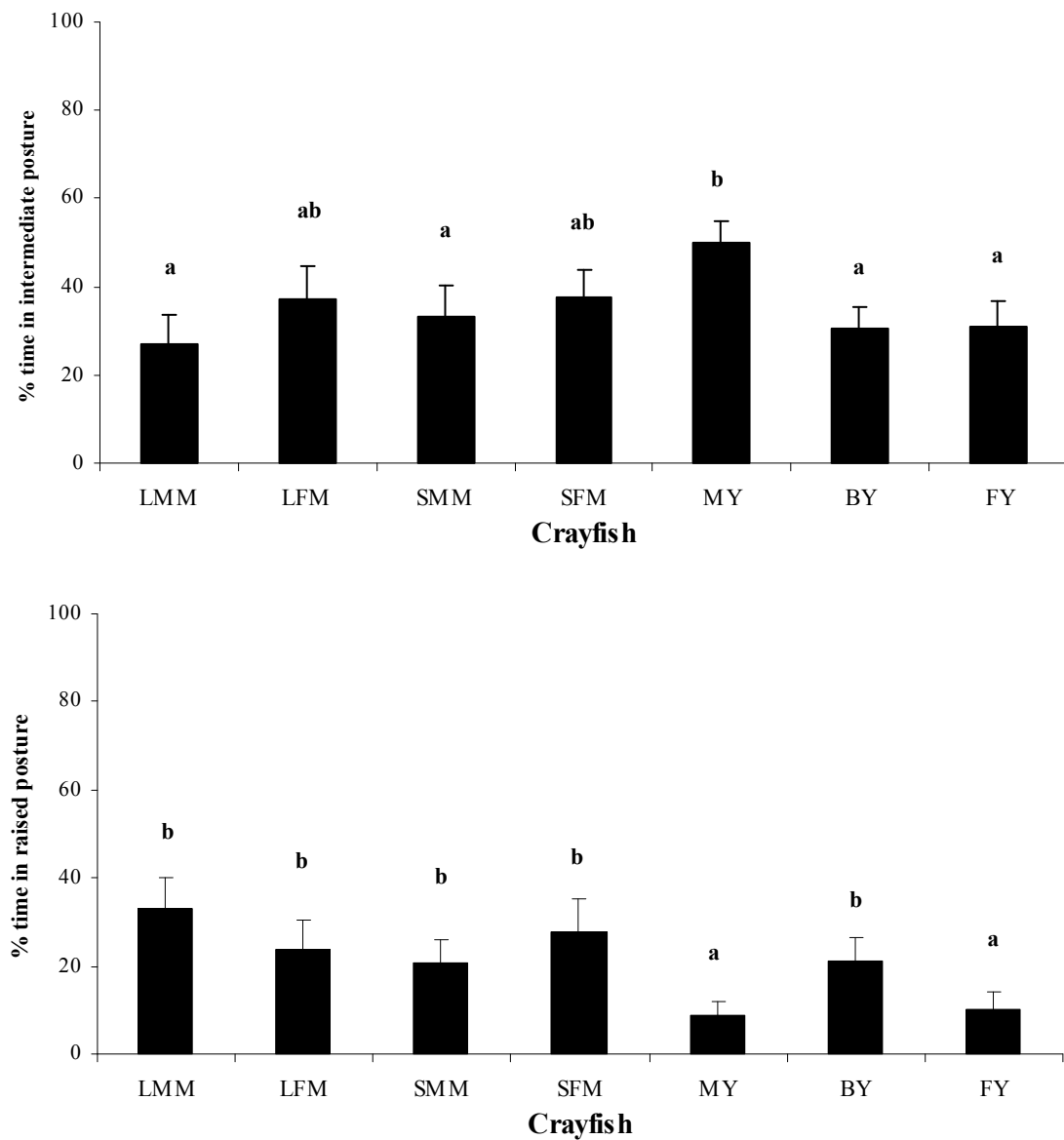
Figure 5.4 Time spent in intermediate and raised posture between different types of crayfish Values represent means (±SE) for each comparison (Tukey HSD). Different letters between groups indicate significantly different means (α=0.05).

5.3.3.4 Raised posture

As shown in Figure 5.4, results for display of the raised posture were similar to that of intermediate posture where the mean of time spent exhibiting raised posture was significantly different between individuals (Mann-Whitney U Test). However, post hoc comparison likewise revealed no significant difference within groups due to high variability in incidence of this posture.

5.3.4 Behavioural responses of different combinations of residents using marron and yabbies

5.3.4.1 Marron as prior resident

Behavioural responses of different combinations of marron as prior resident with and without predator odour were compared and are shown in Figure 5.5. There were no significant differences in response to predator odour in shelter use, climbing, movement, proportion of time flicking antennae, and time spent displaying lowered posture (P>0.05). Prior-resident marron spent significantly more time displaying cleaning, flicking antennules, flicking both antennae/antennules and intermediate posture (z = -2.057, P=0.04; z = -2.077, P=0.038; z = -3.728, P=0.001) in response to predator odour. In contrast, prior-resident marron exposed to predator odour spent significantly less time in the raised posture than prior-resident marron exposed to control solution (z = 2.694, P=0.007).
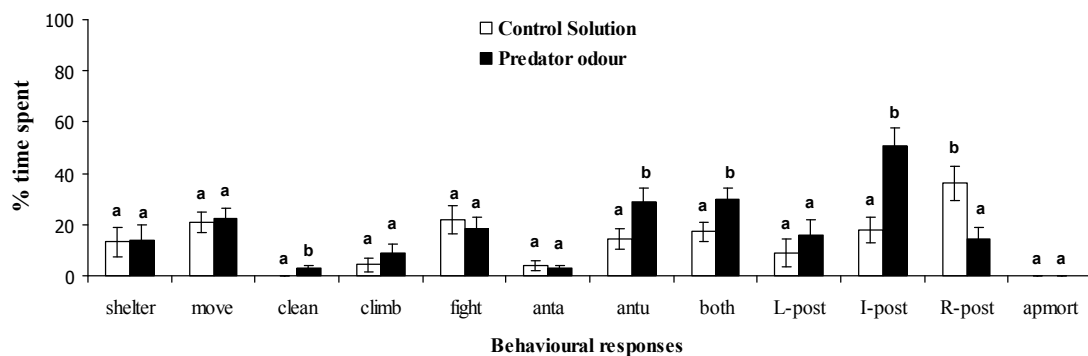
Figure 5.5 Behavioural responses of prior-resident marron

Values represent means (±S.E.) for each comparison (Tukey HSD). Different letters between groups indicate significant different mean (α=0.05).

5.3.4.1.1 Shelter occupation of large male marron as prior residents

The results of observations of shelter occupation by large male marron solo and in combination with various intruders are displayed in Table 5.3. No significant differences (P>0.05) were observed in shelter occupation time between different combinations or intruder types, with either control or test solution.

Table 5.3 Shelter occupation of large male marron as prior resident

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	LMMc : LMMp	33.33±33.33	0.00±0.00	0.000	1.000
	LMMc: LMM*v*LFMc	33.33±33.33	8.33±4.81	-1.000	0.317
	LMMc: LMM*v*LFMp	33.33±33.33	19.44±19.44	-0.258	0.796
	LMMc :LFM*v*SFMc	33.33±33.33	27.78±20.03	-0.258	0.796
	LMMc : LFM*v*SFMp	33.33±33.33	36.11±32.04	-0.471	0.637
	LMMp : LMM*v*LFMc	33.33±33.33	0.00±0.00	-1.000	0.317
	LMMp : LMM*v*LFMp	33.33±33.33	16.67±16.67	-0.258	0.796
	LMMp : LMM*v*SFMc	33.33±33.33	36.11±32.04	-0.258	0.796
	LMMp : LMM*v*SFMp	33.33±33.33	19.44±19.44	-0.471	0.637
	LMM*v*LFMc: LMM*v*LFMp	0.00±0.00	16.67±16.67	-1.000	0.317
	LMM*v*LFMc: LMM*v*SFMc	0.00±0.00	19.44±19.44	-1.000	0.317
	LMM*v*LFMc: LMM*v*SFMp	0.00±0.00	36.11±32.04	-1.549	0.121
	LMM*v*LFMp: LMM*v*SFMc	16.67±16.67	19.44±19.44	-0.258	0.796
	LMM*v*LFMp: LMM*v*SFMp	16.67±16.67	36.11±32.04	-0.696	0.487
	LMM*v*SFMc: LMM*v*SFMp	19.44±19.44	36.11±32.04	-0.696	0.487

Note: Values represent mean (±SE) for each comparison (Mann-Whitney U Test)
c=control, p=predator and *v*=versus intruder

5.3.4.1.2 Shelter occupation of large female marron as prior residents

Comparison between large female marron as prior resident solo and with various types of intruders is shown in Table 5.4. There were no significant differences (P>0.05) in shelter occupation with either test solution. However, large female marron spent considerably less time in shelter during the study period. This finding indicates that the behavioural responses of large female marron to intruders differs significantly from that of large male marron.

Table 5.4 Shelter occupation of large female marron as prior residents

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	LFMc : LFMp	0.00±0.00	2.78±2.78	-1.000	0.317
	LFMc : LFMvLMMc	0.00±0.00	5.56±5.56	-1.000	0.317
	LFMc : LFMvLMMp	0.00±0.00	0.00±0.00	0.000	1.000
	LFMc : LFMvSMMc	0.00±0.00	22.22±22.22	-1.000	0.317
	LFMc : LFMvSMMp	0.00±0.00	2.78±2.78	-1.000	0.317
	LFMp : LFMvLMMc	2.78±2.78	5.56±5.56	-0.258	0.796
	LFMp : LFMvLMMp	2.78±2.78	0.00±0.00	-1.000	0.317
	LFMp : LFMvSMMc	2.78±2.78	22.22±22.22	-0.258	0.796
	LFMp LFMvSMMp	2.78±2.78	2.78±2.78	-1.000	1.000
	LFMvLMMc: LFMvLMMp	5.56±5.56	0.00±0.00	-1.000	0.317
	LFMvLMMc: LFMvSMMc	5.56±5.56	22.22±22.22	-0.258	0.796
	LFMvLMMc: LFMvSMMp	5.56±5.56	2.78±2.78	-1.000	0.317
	LFMvLMMp: LFMvSMMc	0.00±0.00	22.22±22.22	-1.000	0.317
	LFMvLMMp: LFMvSMMp	0.00±0.00	2.78±2.78	-1.000	0.317
	LFMvSMMc: LFMvSMMp	22.22±22.22	2.78±2.78	-0.258	0.796

Values represent means (±SE) for each comparison (Mann-Whitney U Test). Note: Values represent mean (±SE) for each comparison (Mann-Whitney U Test); c=control, p=predator and *v*=versus intruder

5.3.4.1.3 Shelter occupation of small male marron as prior residents

Results of observations of shelter occupation by small male marron in combination with various intruders are shown in Table 5.5. Similar to observations of large male marron, there were no significant differences (P>0.05) in shelter occupation between any combination of intruder and test solution.

Table 5.5 Shelter occupation of small male marron as prior residents

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	SMMc : SMMp	33.33±33.33	33.33±33.33	-0.000	1.000
	SMMc : SMM*v*SFMc	33.33±33.33	2.78±2.78	-0.258	0.796
	SMMc : SMM*v*SFMp	33.33±33.33	0.00±0.00	-1.000	0.317
	SMMc : SMM*v*BYc	33.33±33.33	2.78±2.78	-0.258	0.796
	SMMc : SMM*v*BYp	33.33±33.33	0.00±0.00	-1.000	0.317
	SMMp : SMM*v*SFMc	33.33±33.33	2.78±2.78	-0.258	0.796
	SMMp : SMM*v*SFMp	33.33±33.33	0.00±0.00	-1.000	0.317
	SMMp : SMM*v*BYc	33.33±33.33	2.78±2.78	-0.258	0.796
	SMMp : SMM*v*BYp	33.33±33.33	0.00±0.00	-1.000	0.317
	SMM*v*SFMc: SMM*v*SFMp	2.78±2.78	0.00±0.00	-1.000	0.317
	SMM*v*SFMc: SMM*v*BYc	2.78±2.78	2.78±2.78	0.000	1.000
	SMM*v*SFMc: SMM*v*BYp	2.78±2.78	0.00±0.00	-1.000	0.317
	SMM*v*SFMp: SMM*v*BYc	0.00±0.00	2.78±2.78	-1.000	0.317
	SMM*v*SFMp: SMM*v*BYp	0.00±0.00	0.00±0.00	0.000	1.000
	SMM*v*BYc : SMM*v*BYp	2.78±2.78	0.00±0.00	-1.000	0.317

Note: Values represent mean (±SE) for each comparison (Mann-Whitney U Test)
c=control, p=predator and *v*=versus intruder

5.3.4.2 Yabbies as prior residents

All three types of yabbies as prior residents spent similar proportions of time in the shelters regardless of exposure to either test or control solution. There were no significant differences (P>0.05) between yabbies as prior residents in movement, cleaning, climbing, fighting, antennae flicking or both antennae/antennule flicking, or displaying lowered or intermediate posture (P>0.05). However, when prior-resident yabbies were confronted with predator odour they spent significantly more time flicking antennules and significantly less exhibiting raised posture compared to resident yabbies exposed to control solution (z = -2.094 P=0.036). Results are shown in Figure 5.6.
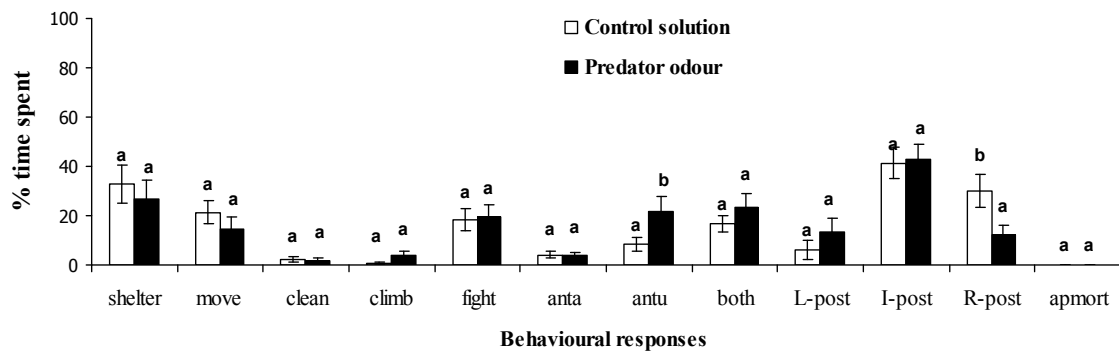
Figure 5.6 Behaviour of yabbies as prior residents
Bars represent means (±SE) for each comparison (Mann-Whitney U Test). Different letters between groups indicate significantly different means (α=0.05).

5.3.4.2.1 Shelter occupation of male yabbies as prior residents

Time spent in shelters by prior resident male yabbies was used to compare the response to intruders and to test solutions. No significant differences (P>0.05) were found between any combination of male yabbies with various types of intruders, with and without predator odour. Results are shown in Table 5.6. Other behavioural responses observed will be considered in the discussion.

Table 5.6 Shelter occupation of male yabbies as prior residents

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	MYc : MYp	33.33±33.33	0.00±0.00	-1.000	0.317
	MYc: MY*v*FYc	33.33±33.33	8.33±4.81	-0.232	0.817
	MYc: MY*v*FYp	33.33±33.33	19.44±19.44	-0.258	0.796
	MYc : MY*v*BYc	33.33±33.33	27.78±20.03	-0.232	0.817
	MYc : MY*v*BYp	33.33±33.33	11.11±7.35	-0.232	0.817
	MYp : MY*v*FYc	0.00±0.00	8.33±4.81	-1.549	0.121
	MYp : MY*v*FYp	0.00±0.00	19.44±19.44	-1.000	0.317
	MYp : MY*v*BYc	0.00±0.00	27.78±20.03	-1.549	0.121
	MYp : MY*v*BYp	0.00±0.00	11.11±7.35	-1.549	0.121
	MY*v*FYc : MY*v*FYp	8.33±4.81	19.44±19.44	-0.232	0.817
	MY*v*FYc : MY*v*BYc	8.33±4.81	27.78±20.03	-0.674	0.500
	MY*v*FYc : MY*v*BYp	8.33±4.81	11.11±7.35	-0.225	0.822
	MY*v*BYp : MY*v*BYc	19.44±19.44	27.78±20.03	-0.696	0.487
	MY*v*BYp : MY*v*BYp	19.44±19.44	11.11±7.35	-0.232	0.817
	MY*v*BYc : MY*v*BYp	19.44±19.44	11.11±7.35	-0.443	0.658

Note: Values represent mean (±SE) for each comparison (Mann-Whitney U Test)
c=control, p=predator and *v*=versus intruder

5.3.4.2.2 Shelter occupation of berried yabbies as prior residents

Comparison of shelter occupation of berried yabbies in combination with various intruders and in response to test and control solutions is summarised in Table 5.7. Similar to observations of male yabbies, there were no significant differences found between any combination of berried yabbies with various intruder types, with and without predator odour (P>0.05).

Table 5.7 Shelter occupation of berried yabbies as prior residents

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	BYc : BYp	27.78±27.78	44.44±29.40	-0.696	0.487
	BYc : BY*v*FYc	27.78±27.78	11.11±11.11	-0.258	0.796
	BYc : BY*v*FYp	27.78±27.78	0.00±0.00	-1.000	0.317
	BYc : BY*v*MYc	27.78±27.78	58.33±30.05	-0.696	0.487
	BYc : BY*v*MYp	27.78±27.78	36.11±32.04	-0.696	0.487
	BYp : BY*v*FYc	44.44±29.40	11.11±11.11	-0.943	0.346
	BYp : BY*v*FYp	44.44±29.40	0.00±0.00	-1.549	0.121
	BYp : BY*v*MYc	44.44±29.40	58.33±30.05	-0.225	0.822
	BYp : BY*v*MYp	44.44±29.40	36.11±32.04	-0.225	0.822
	BY*v*FYc : BY*v*FYp	11.11±11.11	0.00±0.00	-1.000	0.317
	BY*v*FYc : BY*v*MYc	11.11±11.11	58.33±30.05	-1.159	0.246
	BY*v*FYc : BY*v*MYp	11.11±11.11	36.11±32.04	-0.696	0.487
	BY*v*FYp : BY*v*MYc	0.00±0.00	58.33±30.05	-1.549	0.121
	BY*v*FYp : BY*v*MYp	0.00±0.00	36.11±32.04	-1.549	0.121
	BY*v*MYc :BY*v*MYp	58.33±30.05	36.11±32.04	-0.225	0.822

Note: Values represent mean (±SE) for each comparison (Mann-Whitney U Test)
c=control, p=predator and *v*=versus intruder

5.3.4.2.3 Shelter occupation of female yabbies as prior residents

Shelter occupation by prior-resident female yabbies in combination with various intruders and in response to test and control solutions was slightly higher than the previous comparisons of both male and berried yabbies as prior residents. Results are summarised in Table 5.8. When female yabbies were alone and exposed to predator odour, they spent up to 97.22±2.78% of time in shelters. The only significant differences found occurred when prior resident female yabbies were exposed to predator odour with male yabby intruders (27.78±27.78%, z = -2.023, P=0.043) and with berried female intruders (2.78±2.78%, z = -2.023, P=0.043). In both instances, the prior resident female responded by spending significantly less time in the shelter.

Table 5.8 Shelter occupation of female yabbies as prior residents

Behavioural response	Paired comparison a: b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	FYc : FYp	30.56±30.56	97.22±2.78	-1.826	0.068
	FYc : FY*v*MYc	30.56±30.56	58.33±30.05	-0.696	0.487
	FYc : FY*v*MYp	30.56±30.56	27.78±27.78	-0.258	0.796
	FYc : FY*v*BYc	30.56±30.56	38.89±30.05	-0.696	0.487
	FYc : FY*v*BYp	30.56±30.56	2.78±2.78	-0.258	0.796
	FYp : FY*v*MYc	97.22±2.78	58.33±30.05	-1.159	0.246
	FYp : FY*v*MYp	97.22±2.78	27.78±27.78	-2.023	0.043*
	FYp : FY*v*BYc	97.22±2.78	38.89±30.93	-1.159	0.246
	FYp : FY*v*BYp	97.22±2.78	2.78±2.78	-2.023	0.043*
	FY*v*MYc : FY*v*MYp	58.33±30.05	27.78±27.78	-0.696	0.487
	FY*v*MYc : FY*v*BYc	58.33±30.05	27.78±27.78	-0.225	0.822
	FY*v*MYc : FY*v*BYp	58.33±30.05	38.89±30.93	-1.159	0.246
	FY*v*MYp : FY*v*BYc	27.78±27.78	38.89±30.93	-0.696	0.487
	FY*v*MYp : FY*v*BYp	27.78±27.78	2.78±2.78	-0.258	0.796
	FY*v*BYc : FY*v*BYp	38.89±30.93	2.78±2.78	-1.159	0.246

Note: Values represent mean (±SE) for each comparison (Mann-Whitney U Test)
c=control, p=predator and *v*=versus intruder

5.3.5 Behavioural responses of different combinations of intruders using marron and yabbies

5.3.5.1 Marron as intruders

No significant differences were found between most behavioural responses of marron when introduced as intruders (Figure 5.7). The only observed differences were that marron spent significantly more time at lowered and intermediate postures when in the presence of predator odour than marron exposed to the control solution (z = -2.107 P=0.035 and z-2.805 P=0.005), and that those exposed to the control treatment spent more time flicking antennae than those exposed to predator odour (z = -2.396 P=0.017).
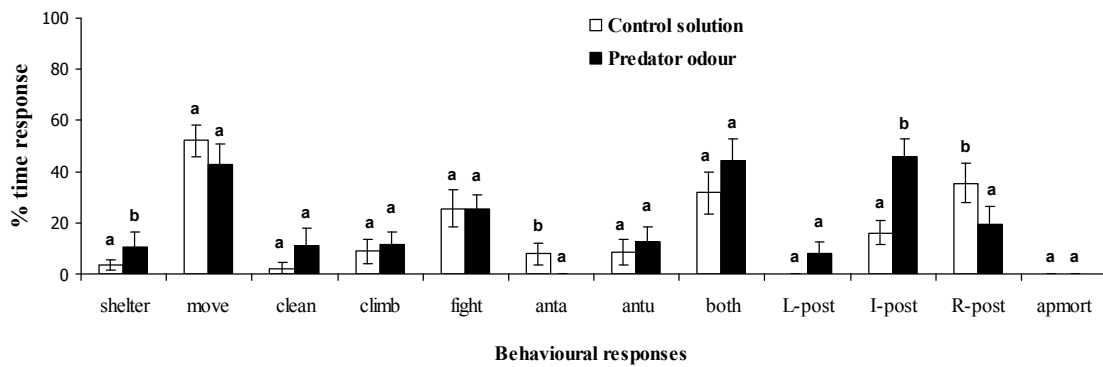
Figure 5.7 Behaviour of marron as intruders
Values represent means (±SE) for each comparison (Mann-Whitney U Test). Different letters between groups indicate significantly different means ($\alpha = 0.05$).

5.3.5.1.1 Large female marron as intruders

Shelter occupation behaviour of large female marron intruding on various types of prior residents is displayed in Table 5.9. There were no significant differences between behaviours observed ($p>0.05$).

Table 5.9 Shelter occupation of large female marron as intruders

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E B	Z	P
% Time in shelter	LMM*pri* : LFM*int*c	0.00±0.00	0.00±0.00	0.000	1.000
	LMM*pri* : LFM*int*p	16.67±13.67	19.44±19.44	-0.258	0.796
	LFM*int*c : LFM*int*p	0.00±0.00	19.44±19.44	-1.000	0.317
	LFM*int*c : SFM*int*c	0.00±0.00	13.89±7.35	-1.549	0.121
	LFM*int*c : SFM*int*p	0.00±0.00	33.33±17.35	1.549	0.121
	LFM*int*p : SFM*int*c	19.44±19.44	13.89±7.35	-0.232	0.817
	LFM*int*p : SFM*int*p	19.44±19.44	33.33±17.35	-0.696	0.487

Note: *pri*=prior resident, *int* =intruder, c=control and p=predator
Values represent means (±SE) for each comparison (Mann-Whitney U Test).

5.3.5.1.2 Shelter occupation of small male marron as intruders

Observations of small male marron intruding on small male marron and large female marron as prior residents exposed to test solution are shown in Table 5.10. None of the combinations tested showed any differences in shelter occupation time.

Table 5.10 Shelter occupation of small male marron as intruders

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	LFM*pri* : SMM*int*c	22.22±22.22	0.00±0.00	-1.000	0.317
	LFM*pri* : SMM*int*p	2.78±2.78	0.00±0.00	-1.000	0.317
	SMM*int*c : SMM*int*p	0.00±0.00	0.00±0.00	0.000	1.000

Note: *pri*=prior resident, *int* =intruder, c=control and p=predator
Values represent means (±SE) for each comparison (Mann-Whitney U Test).

5.3.5.1.3 Small female marron as intruders

A number of paired comparisons with small female marron as intruders were tested, and results are shown in Table 5.11. There were no significant differences in shelter occupation between any of the pairs tested.

Table 5.11 Shelter occupation of small female marron as intruders

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
	LMM*pri* : SFM*int*c	19.44±19.44	13.89±7.35	0.232	0.817
	LMM*pri* : SFM*int*p	36.11±32.04	33.33±17.35	0.000	1.000
% Time in shelter	SFM*int*c : SFM*int*p	13.89±7.35	33.33±17.35	-0.886	0.376
	SMM*pri* : SFM*int*c	2.78±2.78	2.78±2.78	0.000	1.000
	SMM*pri* : SFM*int*p	0.00±0.00	2.78±2.78	-1.000	0.317
	SFM*int*c : SFM*int*p	2.78±2.78	2.78±2.78	0.000	1.000

Note: *pri*=prior resident, *int* =intruder, c=control and p=predator
Values represent means (±SE) for each comparison (Mann-Whitney U Test).

5.3.5.2 Yabbies as intruders

Yabbies as intruders generally did not exhibit different behaviour when exposed to either test solution. The only exceptions to this were that after predator odour was introduced, yabbies reduced movement, and spent significantly more time at lowered posture than yabbies exposed to control solution (z = -3.451; P=0.001). A summary of results is shown in Figure 5.8.
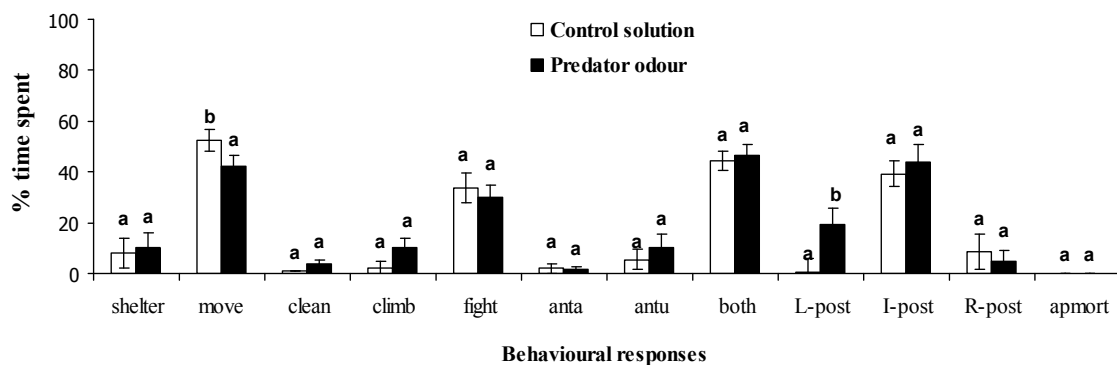
Figure 5.8 Behavioural of yabbies as intruders
Values represent means (±SE) for each comparison (Mann-Whitney U Test). Different letters between groups indicate significantly different means (α = 0.05).

5.3.5.2.1 Female yabbies as intruders

Observations of shelter occupation time of female yabbies as intruders are shown Table 5.12. None of the combinations tested showed any differences in shelter occupation time in response to either predator odour or the control solution.

Table 5.12 Shelter occupation of female yabbies as intruders

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	MY*pri* : FY*int*c	8.33±4.81	0.00±0.00	-1.549	0.121
	MY*pri* : FY*int*p	19.44±19.44	16.67±12.78	-1.000	0.317
	BY*pri* : FY*int*c1	11.11±11.11	2.76±2.78	-0.258	0.796
	BY*pri* : FY*int*p1	0.00±0.00	33.33±33.33	-1.000	0.317
	FY*int*c1 : FY*int*p1	2.78±2.78	33.33±33.33	-0.258	0.796
	FY*int*c : FY*int*p	0.00±0.00	16.67±12.78	-0.696	0.487
	FY*int*c : BY*int*c	0.00±0.00	30.56±30.56	-0.258	0.796
	FY*int*c : BY*int*p	0.00±0.00	11.11±11.11	-1.549	0.121
	FY*int*p : BY*int*c	16.67±12.78	30.56±30.56	-0.232	0.817
	FY*int*p : BY*int*p	16.67±12.78	11.11±11.11	-0.232	0.817
	BY*int*c : BY*int*p	30.56±30.56	11.11±11.11	-0.232	0.817

Note: *pri*=prior resident, *int* =intruder, c=control and p=predator
Values represent means (±SE) for each comparison (Mann-Whitney U Test).

5.3.5.2.2 Berried yabbies as intruders

Shelter occupation by berried female yabbies as intruders was tested in combination with various types of prior residents. Results are shown Table 5.13. None of the combinations tested showed any significant differences in shelter occupation time in response to either predator odour or the control solution.

Table 5.13 Shelter occupation of berried female yabbies as intruders

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	MY*pri* : BY*int*c	27.78±20.03	30.56±30.56	-0.232	0.817
	MY*pri* : BY*int*p	11.11±7.35	11.11±11.11	-0.232	0.817
	BY*int*c : BY*int*p	30.56±30.56	11.11±11.11	-0.258	0.796
	FY*v*BYc : BY*int*c	39.89±30.93	0.00±0.00	-1.549	0.121
	FY*v*BYc : BY*int*p	2.78±2.78	5.55±2.78	0.745	0.456
	BY*int*c : BY*int*p	0.00±0.00	5.55±2.78	-1.581	0.114
	SMM*pri* : BY*int*c	2.78±2.78	2.78±2.78	0.000	1.000
	SMM*pri* : BY*int*p	0.00±0.00	2.78±2.78	-1.000	0.317
	BY*int*c : BY*int*p	2.78±2.78	2.78±2.78	0.000	1.000

Note: *pri*=prior resident, *int* =intruder, c=control and p=predator
Values represent means (±SE) for each comparison (Mann-Whitney U Test). (Table 5.14 included)

5.3.5.2.3 Male yabbies as intruders

No significant differences were found between any behavioural responses of male yabbies when introduced as intruders. A comparison of results is shown in Table 5.14.

Table 5.14 Shelter occupation of male marron as intruders

Behavioural response	Paired comparison a : b	Mean ± S.E a	Mean± S.E b	Z	P
% Time in shelter	BY*pri* : MY*int*c	58.33±30.05	19.45±12.11	-0.886	0.376
	BY*pri* : MY*int*p	36.11±32.04	0.00±0.00	-1.549	0.121
	FY*pir* : MY*int*c	58.33±30.05	0.00±0.00	-1.549	0.121
	FY*pri* : MY*int*p	28.78±27.78	0.00±0.00	-1.000	0.317
	MY*int*c : MY*int*p	0.00±0.00	0.00±0.00	0.000	1.000

5.3.6 Water quality

The water quality was monitored before and after each experiment. Test results consistently fell within the recommended optimum range for crayfish (Langdon 1988) across all experimental aquaria and also for the predator holding tank. Results of water quality parameters tested are therefore summarised and simply expressed as a range of results, and are shown in Table 5.15.

Table 5.15 Summary of results of water quality testing

Source	Parameter	Optimum (Boyd, 2000)	Observed range
Aquaria	Temperature (ºC)	24-28	22-24
	pH	6.5-8.5	7.8-8.1
	Total ammonia (mg/L)	<0.5	<0.25
	Nitrite (mg/L)	<0.5	<0.25
	Nitrate (mg/L)	<1.0	<0.50
Predator tank	Temperature (ºC)	24-28	22-24
	pH	6.5-8.5	7.8-8.1
	Total ammonia (mg/L)	<0.5	<0.25
	Nitrite (mg/L)	<0.5	<0.25
	Nitrate (mg/L)	<1.0	<0.50

5.4 Discussion

5.4.1 Reaction time to intruders

When crayfish were placed in the vacant experimental aquaria, 24 hours was allotted for crayfish to settle, acclimate and to establish residence as described in Chapter 4. During this period, crayfish would be expected to display normal behaviour such as shelter occupation or various movements. However, it was noted that when crayfish visually detected movement by observers, they often showed avoidance reactions such as backward movement. This agrees with the conclusions of Storer & Jenkinson (2004) that crayfish are highly sensitive to visual stimuli. For this reason, special care was taken by observers in these experiments to avoid any sudden movement and thereby to reduce external visual stimuli as a source of experimental error. This approach has not been previously reported, and likely accounts for some of the differences in response times observed in the present study. Furthermore, no mortality or appendage loss was observed in the present study.

Studies of reaction time have been conducted by Height (2002) and Gherardi *et al.* (2000) - both authors recorded in fifteen-second intervals. Height (2002) found that the reaction time of yabbies and marron to freshwater control solution was 161±19.99 and 214±20.3 seconds, respectively. In contrast, Height (2002) reported that the response to cobbler and redfin perch odour was considerably faster, namely 80.83±24.70s and 80.83±24.70s for marron, and 28.75±3.43s and 37.5±5.38s for yabbies in response to each of the two predators, respectively. Gherardi *et al.* (2000) also reported reduced reaction times when marron and yabbies were exposed to heterospecific alarm odour compared to conspecific alarm odour.

The present experiment found that both marron and yabbies reacted immediately after the introduction of all types of intruders. This agrees with the findings of Storer & Jenkinson (2004) who observed that the reaction times of marron and yabbies to visual cues were shorter than to chemical cues, and that the combination of both visual and chemical cues proved to stimulate faster reaction times of both crayfish than to either visual or chemical cues alone. In the present experiment however, addition of silver perch odour was not observed to reduce reaction time to intruders alone for either crayfish species.

In this experiment, behavioural responses were observed at 30s intervals to allow detailed recording for both resident and intruder crayfish. However, this longer recording interval limited the precision of recording some observations. This may account for the high standard deviation of recorded behaviours. An example of this limitation in the present study is where the recorded reaction times differed greatly from Height (2002), Gherardi *et al.* (2002) and Jenkinson (2004). Some of these observed differences are likely a product of the recording interval employed in this study.

5.4.2 Shelter competition

Shelter plays an important role in crayfish distribution in the wild by providing a permanent habitat (Nyström *et al.* 1999, Partridge 1978) and protection from predation (Stein & Magnuson 1976, Whisson 2000), and cannibalism (Dong & Polis 1992). In general, crayfish with resource holding potential (RHP) will dominate the limited resource (in this case, shelter) or successfully fight to retain it (Dingle 1983). RHP is influenced by body size; this is the key factor affecting shelter acquisition (Figler *et*

*al.*1999). In this regard, results of the present study are consistent with the findings of Figler *et al.* (1999), Nakata & Goshima (2003), and Vorburger & Ribi (1999).

5.4.2.1 Interspecific and intraspecific behaviour differences in shelter competition

A summary of all behavioural responses of marron and yabbies are shown in Figure 5.1. Behavioural responses were dominated by fighting which constituted up to 30% of experimental observation time. In contrast, time in shelters accounted for only 15-25% of total activities. In this study it was thus found that crayfish spend more time displaying agonistic behaviour, and that there were no significant differences between marron and yabbies in this regard. This agrees with the findings of Storer & Jenkinson (2004).

There were several significant behavioural differences between marron and yabbies noted in this study. The first of these was the greater frequency of antennule flicking displayed by marron, which contradicts the findings of Height (2002). It should first be noted that antennule flicking behaviour was increased in both species of crayfish when exposed to predator odour, but marron nonetheless exhibited significantly more of this behaviour overall.

This observation could be the result of artefact; Height (2002) investigated unpaired behaviour of individuals whereas in this study, as also described in Chapter 4, paired yabbies often shared shelter such that any antennule movement would not have been observed. Another possible explanation for differences in antennule flicking behaviour could be that body size of marron in this study was generally larger than that of yabbies. Therefore, when yabbies were placed as intruders with comparatively larger marron, the marron appeared to respond to the smaller intruder as a source of prey by displaying behaviour consistent with perception of food, characterised by antennule flicking (Vogt 2002). This fits with the description of marron by Holdich (2002) as an opportunistic cannibal. Such behaviour has also been observed by other researchers in crayfish, particularly when starved (Capelli & Munjal 1982, and Hazlett 2003). Finally, when smaller yabbies were placed as intruders with larger marron displaying opportunistic predatory behaviour, investigative antennule flicking behaviour by yabbies was replaced by avoidance behaviour, namely, shelter seeking. This could also explain the

significantly greater shelter occupation time by yabbies compared to marron shown in Figure 5.1.

Yabbies appeared to display intermediate posture more frequently than marron in the present study. This disagrees with Height (2002) and also the findings of the previous study reported in Chapter 4 which showed the lowered posture to be more common than the intermediate posture. Intermediate posture is generally interpreted as indicative of alertness and preparation for movement whereas lowered posture is considered a display of relaxation (Hazlett 1999). It is therefore not surprising to find an increased incidence of intermediate posture by yabbies in the present study investigating shelter acquisition and competition. Finally, differences in body size between marron and yabbies used in the present study could account for interspecies differences in display of the intermediate posture.

Marron were observed climbing and displaying the raised posture more than yabbies. The raised posture is considered to be an agonistic behaviour (Hazlett 1999), which fits the interpretation of a predatory response of larger marron to smaller intruder yabbies. In contrast, climbing is considered to be escape behaviour (Hazlett 1999). In the present study, climbing was most commonly displayed by small marron and is interpreted as escape behaviour. In contrast, while yabbies displayed less climbing than marron, they spent more time seeking and occupying shelters. This is also interpreted as escape behaviour by yabbies. This observed difference in avoidance or escape tactics between marron and yabbies could reflect a basic species behaviour difference in this regard.

Hazlett (1990) and Gherardi *et al.* (2002) concluded that yabbies tended to respond to intruders as to a disturbance or an alarming cue by movement of antennae. This was also noted by Vogt (2002) who reported the use of antennae by yabbies to detect the movement of other animals. This was not observed in the present study, possibly due to insufficient recording time.

5.4.2.2 Comparison of shelter occupation between prior residents and intruders

The advantage of prior residence in terms of longer shelter occupation time by various sizes of prior residents was shown in Chapter 4 and is supported by the findings of Capelli & Munjal (1982). In the present experiment however, prior residence appeared to have less influence on shelter occupation when residents were faced with an intruder

of another species. While interspecies competition could explain this difference, these findings could also be an artefact of experimental design, namely insufficient replication. This conclusion is supported by the high standard error of observations in the present study.

Marsh (2004 unpublished) investigated interspecies shelter occupation behaviour between marron and yabbies, and in accordance with the conspecific findings in Chapter 4, reported that prior residents displayed longer shelter occupation times. However, Marsh forced prior residents into shelters before intruders were introduced which may have interfered with subsequent behaviour. Nakata and Goshima (2003) also reported longer shelter use by prior residents, but the species investigated were *Cambaroides japonicus* and *Pacifastacus leniusculus*. It is not known how comparable the responses of these crayfish species are with those used in the present study.

The introduction of intruders into resident territory is considered to pose a threat and spark an aggressive/defensive response by prior resident crayfish resulting in mobilising or fighting behaviour (Rubenstein & Hazlett 1974). As a result, time spent in shelters is minimised. Huber *et al.* (1997) discovered that serotonin governs aggressive behaviour and that the level of this neuro-secretic pheromone is influenced by the physical condition of crayfish. In the present study, prior residents were undisturbed and in good condition, and were free to move around the territory surrounding shelters. The conditions of the present study and the crayfish used in the investigation were conducive to normal levels of serotonin and associated behavioural responses.

It is possible that reduced shelter occupation times reported here could be an artefact of the recording interval used such that observations were recorded while crayfish were still engaged in fighting behaviour. Finally, the influence on shelter occupation of other factors such as response to predators, diurnal rhythms, and shelter type are poorly characterised and may also have affected the observed outcome.

5.4.2.3 Effect of gender on shelter competition

In this experiment, no effect of gender was detected on shelter occupation by either prior residents or intruders of either species. This differs from results of Chapter 4 where it was found that female yabbies (both berried and non-berried) generally spent more time in shelter than males. Similarly, Marriapan and Balasundaram (2003) found

that ovigerous female freshwater prawns (*Macrobrachium noblii*) spent more time in the shelter than males, but males spent more time in shelters than non-ovigerous females.

Peeke *et al.* (1998) also reported that resident male American lobster (*Homarus americanus)* spent more time in shelters than female residents when exposed to juvenile intruders, but did not investigate gender differences with respect to female reproductive state. This disagrees with those of Marsh (2004), who argued that female marron were highly successful in gaining privilege over shelters. This was also supported by Ranta and Linström (1993) who found that intruder female *Pacifastacus leniusculus* could successfully evict smaller conspecific residents when only 1.5 times larger than residents, whereas intruding males needed to have twice the body size of residents in order to successfully evict them. In contrast, Little (1974) found that males and non-ovigerous females did not compete with conspecific ovigerous females for shelter, leaving ovigerous females free to dominate available shelter. In studies conducted by Bovbjerg (1956), and Figler *et al.* (1995), ovigerous females or females with larvae exhibited aggressive responses to other crayfish and retained more time in shelter.

Mason (1970) found that the behavioural responses of conspecific males and females engaged in competition for shelter gave way to pair matching games, a finding later confirmed by Barki *et al.* (2003). Villanelli & Gherardi (1998) found that when two males competed for shelter, the result was highly aggressive fighting behaviour.

Clearly, gender, reproductive state and relative size of females can influence the success of shelter competition in crayfish. This was not demonstrated in the present experiment, even though they were conducted during the peak of the reproductive season when a gender effect is likeliest to be observed, if present. It should be noted that female yabbies tended to have a higher proportion of shelter use overall (Table 5.8), but the difference was not statistically significant. There may be species differences in the expression of this behaviour, which could explain the lack of observed gender effect in this case. Exposure to predator odour in this study may have interfered with the expression of gender effects; however, there was no significant difference in behaviour by gender when exposed to either treatment or control solution. Finally, it is possible that the number of replicates was insufficient to detect the expression of gender effects in this study.

5.4.2.4 Effect of size difference on shelter competition

Larger crayfish possess greater resource holding potential and are therefore highly aggressive according to Dingle (1983). Certainly crayfish with larger claws or spines are better equipped to defend shelter. Gender differences in claw and spine size in crayfish add to differences in carapace length and weight. These body size advantages increase success in shelter competition (Söderbäck 1995; Figler *et al.* 1999; Vorburger & Ribi 1999; Nakata & Goshimal 2003, Height *et al.* 2004) such that larger males dominate shelter competition. These results agree with Ranta and Linström (1993) who found that larger crayfish used shelter more than smaller conspecifics. The prior resident effect does confer a slight advantage in retention of shelter (Figler *et al.* 1999) even when the prior resident is of smaller size (Edman & Jonsson 1996). This was also demonstrated in Chapter 4 for at least the initial period during observation. When shelter is a limited resource, prior residents are motivated to fight even when relative size leaves them at a disadvantage. When competition for the resource persists, size will eventually predominate.

In the present experiment, size did not appear to influence the proportion of time spent in shelter in either conspecific or interspecific comparisons even though research clearly shows that size can play a significant role in shelter competition between crayfish. As discussed in reference to the gender effect in this study, it is possible that species differences may influence the expression of size effects. Finally, it is possible that the number of replicates and/or the recording interval was insufficient to detect the expression of size effects in this study.

5.4.2.5 Effect of life stage and physical condition on shelter competition

Ameyaw-Akumfi (1976) found that the frequency of agonistic behaviour was significantly higher in ovigerous female crayfish (*Orconectes virilis* and *Procambarus clarkia)*. This was not confirmed in the present study. Berried female yabbies were studied as both prior residents and intruders but there was no evidence that the ovigerous state resulted in either increased display of agonistic behaviour or increased shelter occupation. As noted in Section 5.4.2.3, shelter occupation tended to be higher for female yabbies but not berried females. A possible explanation for this was put forward by Figler *et al.* (1995 and 1997) who argued that maternal aggression appears

to drive berried female crayfish out of shelter to defend territory. This is considered to form a plausible explanation of otherwise seemingly contradictory findings, and points to the complexity of interaction between agonistic and shelter competition behaviour in female crayfish.

5.4.3 Behavioural modification in response to predatory threat

Yabbies appear to modify behaviour in response to visual cues from intruders. Figler (1999) noted that yabbies did not display agonistic responses immediately but rather first appeared to observe and retreat when confronted by an intruder with greater resource potential. This is supported by Height (2002) who observed display of antennae flicking typical of investigative behaviour, and lowered posture typical of avoidance (Lawrence & Morrissy 2002, and Hobbs 1942). In this study, such behaviours were also observed. However, except for delayed response to chemical and visual cues, these other behavioural observations were not significantly different, likely because of study design with respect to range of behaviours recorded, observation time and number of replicates.

5.4.4 Differences between behavioural responses of invasive and native species

The issue of the invasion of non-indigenous species (NIS) and displacement of native species has been put forward as the most important factor driving bio-diversity disasters (Elton 1958; Hill *et al.* 1999; Lodge 1988; Söderbäck 1994). These authors all recommend further investigation as a matter of urgent priority. Lynas (2002) pointed out that yabbies, an introduced species from eastern Australia, are more aggressive than marron native to the west. Lynas argued that the naturally more aggressive behaviour of yabbies served as a warning to authorities worthy of action to protect native species immediately. While Elvey *et al.* (1997) pointed out that *Cherax destructor* did not out-compete the native *Astocopsis franklinii* in Tasmania, these authors concluded that this was likely due to native superiority in shelter occupation in Tasmanian water bodies. On the other hand, Usio *et al.* (2001) reported that shelter use of the native *Cambaroides japonicus* was reduced in the presence of the invasive *Pacifastacus leniusculus* compared to when no intruder was present. Marsh & Height (2004) agreed with Lynas, and argued that while shelter competition strategy plays an important part in the success or failure of species invasion, there are many contributing behavioural factors which

also influence the outcome of invasion. Further research into the interaction of native and non-native species was recommended.

The mechanism of species displacement through invasion is not only a function of successful shelter competition but also the degree of agonistic behaviour, ability to adapt and response to other stimuli. No difference in agonistic behaviour between marron and yabbies was observed in this study. Marron possess an advantage in size, and also show superior reaction time in response to visual and chemical stimuli compared to yabbies. However, results of the present study lend support to the notion of superior adaptive ability of yabbies (Section 5.4.3) and demonstrate their superior shelter occupation. With regard to potential for successful invasion by yabbies and displacement of native marron, the most significant finding of the present study is the difference in response to predator odour between yabbies and marron while engaged in shelter competition. In this regard, the present study showed that while marron responded faster to stimuli overall, their response to predator odour was significantly diminished while engaged in either conspecific or interspecific shelter competition. This increases vulnerability of marron to predation by finfish and may confer a significant competitive advantage to invasive yabbies.

5.4.5 Interspecific agonistic behaviour

The effect of species, size, life stage, gender and prior resident status on the expression of dominating behaviour and shelter occupation has been documented (Evans & Shehadi-Moachieh 1988). Evidence exists to suggest that different types of crayfish can nonetheless co-exist in ecosystems if there is no conflict of resources and territory (Noble 1939). However, most ecosystems will experience periodic conflict or competition for resources, in which case the Darwinian theory of evolution will apply. In the present study, several factors affecting the expression of agonistic behaviour were investigated. These are categorised and discussed in the following sections.

5.4.5.1 Size variation

Results of this study show that when two crayfish confront one another, they approach with outspread chelae, come into contact, and wrestle until one of the pair submits, usually the smaller will retreat from the individual with greater RHP (Bovbjerg 1953 and Söderbäck 1995). Other ecologists agree that body size is generally considered to be

the most important sign of resource holding potential (Bovbjerg 1953; Lowe 1956; Momot & Leering 1986; Ranta & Lindström 1993; Edman & Jonsson 1996; Pavey & Fielder 1996: Dingle 1983, and Holdich 2002). The key contributor to RHP is the bulk and morphology of crayfish chelipeds. In most crustaceans, these appendages have transformed into different shapes and sizes depending on feeding habit, as described by Dingle (1983), and are key contributors to the success of each species. Larger chelae are usually advantageous in intraspecific and interspecific agonistic encounters (Gheradi *et al.* 1999). In general, male crayfish have larger chelae than females and therefore tend to dominate smaller crayfish or females. In this study; however, no effect of size on agonistic behaviour was demonstrated. Furthermore, there were no instances of mortality or appendage loss observed, which suggests that the degree of agonistic behaviour displayed by subjects in the present study may have been less than that observed in other studies.

5.4.5.2 Gender variation

Agonistic behaviour is expressed by all combinations of gender and size classes (Huber *et al.,* 1997). In the present experiment, it was found that in general, prior resident crayfish became more aggressive toward intruders of the other gender. Guiaşu & Dunham (1997) reported that during the reproductive season, male *C. robustus* differentiated into reproductive and non-reproductive forms; reproductive males were significantly more successful in battle. This finding supports that of the present study in that gender effects influence levels of aggression in contest. For example, small male marron as prior residents exhibited more aggressive behaviour when intruders were berried female yabbies as opposed to non-berried females. In this instance, aggression and Fighting persisted for the entire observation period. Some postulate that gender-related aggression depends on level of hormones and perception of pheromone (Tierney & Dunham 1982 and 1984), which also explains increased aggressiveness of females when carrying eggs or larvae. Others have suggested that the motivation for ovigerous females or those with larvae to show an aggressive response to other crayfish is to ensure survival of offspring (Bovbjerg 1956; Figler *et al.* 1995). This motivation could itself be hormone or pheromone-driven. In contrast, small male marron have only territory to protect. Increased aggression of small male marron in combination with a berried female intruder could simply reflect a heightened response of the resident to the

high level of aggression of the berried female. However, this is the only interspecies/gender combination in which this phenomenon was observed in the present study.

5.4.5.3 Physical factors

Little (1974) found that maternal pheromones were produced by ovigerous *O. virilis* and *P. Clarkii* as soon as they released eggs and that these neurocrine stimulants persisted for up to 3 months. During this period, females exhibit aggressive behaviour under the influence of the pheromones (Fingerman 1997). Maternal aggressive/protective behaviour declines after larvae leave the female. Instances of cannibalism by the females have been detected as pheromone levels decline. For this reason, and perhaps in response to perception of an absence of pheromones, juveniles of *Procambarous spp.* have been noted to avoid male and non-berried females (Little 1976).

It was noted by Guiaşu & Dunham (1997) that during the *Cambaridae* reproductive season, the breeding form predominates in inter-form contests between size-matched pairs. Males in general are more involved in fighting and tend have a higher rate of appendage loss (Gherardi *et al.* 1999 and 2000). Single chelae or loss of chelae will impair Fighting ability and reduce ability to defend themselves against predators. Edsman & Jonsson (1996) also found that loss of antennae resulted in lowered competitive ability.

5.4.5.4 Species variation in general behavioural responses

Tierney & Dunham (1982 and 1984) found that *Orconectes spp.* use pheromones to distinguish themselves from other crayfish speices. The same authors noted that such pheromones are species-specific and can be discriminated between species. However, not all crayfish species use pheromones in this way. No study of pheromone in marron or yabbies has been reported. Height (2002) showed that yabbies are more receptive to predator odour or alarm cues than marron. Similar results were reported by Jenkinson (2004) and Storer (2006). These findings are supported by results of the empirical approach employed in the present experiment.

5.4.6 Strategic advantage in species displacement in freshwater crayfish

Results of the present study serve to enhance understanding of some crayfish behavioural responses which influence success in shelter acquisition and aggressive contestsi and are relevant to the threat of displacement of native species by invaders. This study investigated various aspects of shelter-related behaviour of marron native to Western Australia, and invasive yabbies can not draw a solid conclusion of species displacement. Due to there was no baseline support on behaviour response Vorburger & Ribi (1999), from both native and invasive crayfish species which similar finding with this experiment.

There is a number of general visually observation factors from literatures that plays an important role in the successful of some species. to date that issue on species displacement in crayfish as raised by Usio *et al.(2001)* derive from several tactics including combination of individual body size as describe by Nakata & Goshima (2003), prior resident effect; Ranta & Lindström, 1993, Blank & Figler (1996) and (Figler *et al. 1999),* Physical condition such as berried female Marriapan and Balasundaram (2003)

In addition to exploring the advantage of physical appearances the aggressiveness behaviour also dictated the winner Lynas 2002, Capelli&Munjal, 1982, Lodge & Hill (1994), Vorburger & Ribi (1999). However when the shelter resource are scarce the size of animal are the most advantages factor in the competiton.

The other factor that organism use to dominate the ecosystem is reproductive mechanism, yabbies shows an *r-select* strategy (Campbell 1990, Gonzales-Oreja 1999, Krebs 1985) provides significantly high fecundity, fast growth and hardiness than marron in the variation of ecosystems. Other important part of life history of yabby is an ability to withstand when an adverse of environment poor water quality, marron will suffer or leave the habitat to find a better location (normally found the carcases in the surrounding water). Yabbies will show their ability to survive in the harsh environment by burrowing, Hobbs, (1942), Lawrence &Morrissy (2002) when the water temperature drop during winter or the water table fall.

(Height, (2002) and Height & Whisson, (2004) discovered that invasive yabbies are more acquiescent to alarm odour from finfish predator than native and spend more time in shelter than the marron when confront with predator. Similar to Westin and Gydiemo (1988) drawn conclusion after observed that *O. virilise, P. leniusculus and A. astacus* move out the crevices or shelters to moult in the open area, to avoid conspecific cannibalism this may trap in to other threat. These species with limited of adaptive will not survive in this situation, in this point the conflict occurs between two congeners make an opening opportunity for predator. Yabbies in this sense have inherent of recognise the predator threat where similar finding supported by Hartman and O'Neill (1999) who found that juvenile *P. clarkia*, if solitary, moulted either out of or in the shelter (but always in the shelter when a fish predator was present)

This result conclude that yabbies may not a large aggressive creatures but inheritably adapt all strategies in species survival and from be able to use alert with alarm cues, ability to reproduce and escaping from the impact from the climatic changes and harsh environment. Surprisingly, study by Campbell and Whisson 2002 in Lake Moyanup South-West, Western Australia that the marron number of recaptured has been increase from previous surveyed, but the reasons of this finding still awaiting to clarify.

Key Findings

1. High variability and apparent adaptability of behaviour responses of yabbies precluded drawing specific conclusions about patterns of behaviour and contributed to a high standard deviation of recorded observations in this experiment. The number of replicates should be increased to eliminate this problem.

2. In general, as prior residents, yabbies successfully defended and retained shelter more often than marron. As intruders however, there was no difference in success of shelter acquisition between yabbies and marron. This difference in shelter competition may confer an advantage to yabbies over native marron in the wild.

3. Interspecies fighting was greatest when both crayfish were of similar size regardless of other differences such as gender and physical condition. This aggression contributed to reduced shelter occupation.

4. No mortality or appendage loss was observed in this experiment. This is attributed to body size and condition (intermoult) of crayfish used and the relatively short (6 minutes) observation period employed in the study.

5. 30s interval is not sufficient to accurately determined the reaction time

6. Marron tend to climb as an escape tactic in response to threat while yabbies tend to remain in or seek shelter when confronted by competitors or predators. This difference in response to threat may confer an advantage to yabbies over native marron in the wild.

7. Low percentage of time spent in shelter in both species may be shelter were not suitable?

Chapter six

Shelter selection and shelter preference in yabbies

This aquarium-based trial examined the influence of predator and conspecific threats on small, medium and large yabbies of each gender. Yabbies were randomly distributed among 56 aquaria and provided with a bundle of shelters comprised of PVC pipes measuring 25, 50 and 90mm in diameter. The yabbies were randomly allocated in three different combinations of small, medium and large sized males or females, and with predator odour (silver perch). Observations were conducted at hourly intervals for 24 hours. The results of shelter preference under the influence of predator odour provide insight into behavioural plasticity in yabbies.

6.1 Introduction

In crayfish polysystems, the presence of predators is a major reason why small crayfish spend more time in shelter (Stein 1977). Thus, the availability of shelter is an important factor for crayfish survival (Figler 1999, Nakata & Goshima 2003). Furthermore, young crayfish moult more frequently than older crayfish (Merrick & Lambert 1991, Mills *et al.* 1994, Timmermans *et al.* 1995, Wheatly 1995), therefore making them highly prone to predation (Svensson 1993). The effectiveness of shelter on predator avoidance is influenced by several factors such as its complexity (Whisson 2000), interaction with conspecifics, and crayfish behavioural plasticity and modification. Studies from previous chapters in this thesis have shown yabbies to be highly capable in using information and adapting to new environments and threats accordingly.

Vannini & Cannici (1995) reported that although shelter is important for crayfish, some species prefer burrows as their shelter. Gherardi (2002) reported that burrows can be divided into three categories depending on the origin and type of pressure from predators. Noble crayfish (*Pacifastacus leniusculus*) did not burrow in its native North America; however, it became a burrower when translocated to a new environment (Holdich 2002). Substrate preference was also found to be different between species. Suitable substrate can provide better protection and increase food availability (Gilinsky 1984). In the absence of substrate crayfish rely solely on the shelters provided such as in aquaculture systems.

Many crayfish have been translocated to various habitats around the world (Hobbs *et al.* 1989). They have to adapt their physiology and morphology, as well as behaviour, to a new environment (Hazlett 2003b). Yabbies are no exception; they have to use available materials and substrates as shelter. Suitable shelter is necessary for reproduction and survival in these habitats (Hasiotis 2002, Fletcher & Miller 2006).

6.2 Materials and methods

6.2.1 Site and experimental system

This experiment was conducted using the laboratory aquarium systems, as described in Chapter 3, located at the Curtin Aquatic Research Laboratories (CARL). Fifty-six aquaria measuring 30 x 60 x 25cm (40L capacity) were used for this experiment. Three

different sizes of PVC pipe were bundled to provide shelter and were arranged in the same pattern of 25, 90 and 50mm from the left to right (Figure 6.1). These were placed in the centre of the aquaria to enhance crayfish visibility during observation. For the same reason, all airstones were moved behind the shelters. In addition to the interior of the PVC pipes, there were gaps of 2.5cm^2 (cross-sectional area) between the 25 and 90mm pipes, and a gap of 4.6 cm^2 between the 50 and 90mm pipes, which could also serve as shelter. The room provided artificial light (12h dark - 12h light).
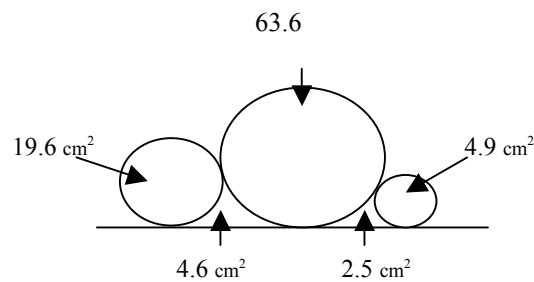
Figure 6.1 Shelter arrangement in an aquarium and cross-sectional areas of pipes

Yabbies of three different sizes were weighed and measured (carapace length) and then allocated to each aquarium according to the experimental design summarised in Table 6.1. Yabbies were acclimatised for one hour prior to commencement of the experiment.

Table 6.1 Experimental design and treatment allocation with application of test solutions

Treatment	Labels	Yabbies	Test solutions			
			Block 1st day		Block 2nd day	
			A	B	A	B
T1A	T1	Small males	control	pred odour	pred odour	control
T1B	T2	Medium males	control	pred odour	pred odour	control
T1C	T3	Large males	control	pred odour	pred odour	control
T2A	T4	Small+Medium males	control	pred odour	pred odour	control
T2B	T5	Small+Large males	control	pred odour	pred odour	control
T2C	T6	Medium+Large males	control	pred odour	pred odour	control
T3	T7	Small+Med+Large males	control	pred odour	pred odour	control
T4A	T8	Small females	control	pred odour	pred odour	control
T4B	T9	Medium females	control	pred odour	pred odour	control
T4C	T10	Large females	control	pred odour	pred odour	control
T5A	T11	Small+Medium females	control	pred odour	pred odour	control
T5B	T12	Small+Large females	control	pred odour	pred odour	control
T5C	T13	Medium+Large females	control	pred odour	pred odour	control
T6	T14	Small+Med+Large females	control	pred odour	pred odour	control

6.2.2 Experimental animals

Yabbies (*Cherax albidus*) were sourced from a commercial yabby farm, "Cambinata Yabbies", Western Australia, and allowed to breed for two years at a commercial marron farm in Parkerville (32.9^{o}S, 116°E). Yabbies were sorted according to size and then placed in holding tanks at CARL for 2 weeks prior to commencement of this experiment. The yabbies were purged during this period with water exchanges every 24 hours. Average weight of large yabbies (n=64) was 58.42±1.92g, with an average carapace length of 53±1.76mm. Medium-sized yabbies (n=64) averaged 27.15±3.94g with an average carapace length of 44.45±1.19mm. Small yabbies (n=64) averaged 3.3±1.5g with a carapace length of 31.21±2.01mm.

Silver perch (*Bidyanus bidyanus*) were provided by CARL. They had been relocated from a commercial farm at Parkerville, Western Australia, and reared in a 5000L recirculating system at CARL for approximately one year. Fish were selected, weighed, and then transferred into a 300L blue plastic circular tank near the experimental aquarium system. Two 1.5 inch airstones were placed to provide constant aeration from a main air compressor as described in Chapter 3. A black plastic sheet was used to cover the tank containing the fish. Water exchanges were conducted every 2 hours during the 24-hour period prior to the trial. Water quality parameters were checked every two hours during this time.

6.2.3 Experimental procedure

Each treatment replicate of yabbies was placed into an aquarium containing shelter (randomly within blocks) according to the experimental design (Table 6.1). Within each block, experimental units were variously positioned to reduce any bias of aquarium shelf location. On the first day of recording, A-block received the control solution, and the B-block received predator odour (Table 6.1). On the following day of recording, this was reversed. Test solution was applied by squirting 10mL of solution into aquaria as described in Chapters 4 and 5. All aquaria received either control or predator odour solution approximately 2 to 5 minutes prior to the start of each hourly observation. To prevent cross-contamination, the use of syringes was strictly separated between control and predator odour treatments. The experiment commenced at 3pm each day, and

observations were recorded every hour for 24 hours. The total recording time of each observation cycle was completed within 7 to 8 minutes.

A red LED spotlight affixed to the forehead of the observer (*Energizer™*) provided sufficient visibility to observe yabby behaviour. With this type of light, crayfish seemed calmer than with a normal bright light torch. The red spotlight was used in every observation during recording to eliminate lighting as a source of variation.

6.2.4 Data collection

Shelter selection by each yabby or combination of yabbies (i.e. size and gender) was observed. The size of shelter occupied and the location of yabbies within the aquaria were recorded using recording sheets. When recording was complete, data was tallied and transferred to a spreadsheet. Arcsine transformations were applied to all percentage data prior to statistical analysis.

Water quality of the predator (silver perch) tanks was tested prior to the start of the experiment. The water volume in the tanks was calculated and reduced to 100L for concentration of odour as described by Hazlett (1999) and Hazlett & Schoolmaster (1998).

6.2.5 Water quality monitoring

Total ammonia, nitrite, nitrate, pH, and temperature were recorded from each treatment and from the silver perch tank prior to the beginning of recording. The tests were repeated at the beginning and at the end of the experiment. As described in Chapter 4, if any water quality parameter exceeded the optimal limit according to Boyd (1998), water was exchanged until the desired parameter(s) were restored.

The total ammonia level in the predator tank was specially monitored as, according to Height (pers. comm. 2002), if ammonia exceeds 0.5ppm this will overwhelm the perception of predator odour by crayfish. When required, water exchange was therefore performed to eliminate any influence of ammonia on crayfish response (Hazlett 1999).

6.2.6 Statistical analysis

The first observations of the recording period were excluded from the statistical analysis as described in Chapter 3. Total time spent in shelter and out of shelter for different test solutions was compared using Student's *t*-test. Yabby size, gender and combination of different sizes of yabbies with respect to shelter selection and occupation were compared every hour during the 24 hours and were analysed using one-way analysis of variance (ANOVA). Differences in time spent in shelter in response to the test solutions were analysed by the non-parametric Mann-Whitney U-Test (Ott 1998). For comparison between treatments and to test significance of differences between tested solutions, one-way and two-way ANOVA were used (Selvanathan *et al.* 2000). Post hoc analysis (Sheffe & Tukey HSD) was performed when results showed a significant difference at $P<0.05$.

6.3 Results

6.3.1 Overall activity of yabbies

6.3.1.1 Total active time spent by yabbies

Total active time spent by all sizes, genders and combinations of yabbies outside the shelters during the 24 hours is presented in Figure 6.2. There were significantly different amounts of active time between yabbies exposed to predator odour compared to those exposed to the control solution; t (23) = 4.06226 P< 0.01 (Appendix 2). This reduction in active time was consistent throughout the 24-hour observation period. There was a pronounced diurnal pattern in activity observed for all sizes, genders and combinations of yabbies outside the shelters exposed to both test solutions.
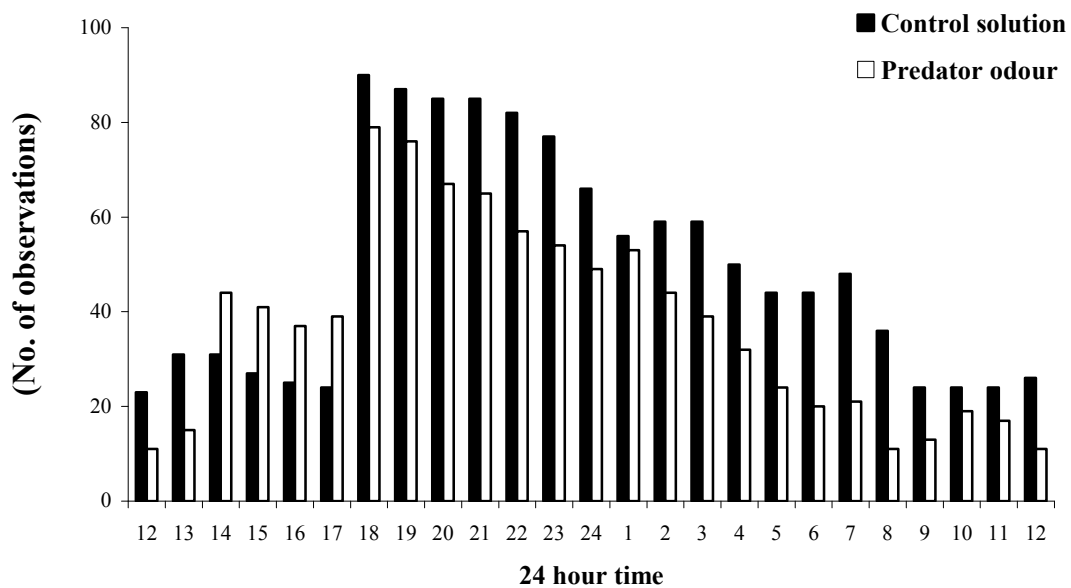
Figure 6.2 Total active time spent by all yabbies comparing response to test solutions

6.3.1.2 Comparison between active times of different size of yabbies

Comparisons between the active times of different sized yabbies in response to test solutions are presented in Table 6.2. With the control solution, medium and large yabbies were significantly more active (20.50±1.31%, 20.50±128%) than small yabbies (10.75±1.89%, z=-2.139 P=0.000). In contrast, predator odour appeared to have a strong

influence on yabby activity indicated by a reduction in active time of medium yabbies, which fell to 13.79±1.31% of observation time when exposed to predator odour (z=-3.338 P=0.001). Similar results were observed in large yabbies that spent 20.50±1.28% of time outside shelter following exposure to control solution, which is significantly higher than that of yabbies confronted with predator odour (13.79±1.31%, z=-3.388 P=0.001). While small yabbies also tended to reduce their active time when exposed to predator odour compared to activity following control solution, this was not significantly different (P>0.05).

Table 6.2 Comparison of active time spent (mean percentage ± S.E.) by different sizes of yabbies during the experiment with control and predator odour solutions

Active time	Paired comparison (a : b)	Mean ± S.E (a)	Mean± S.E (b)	Z	P
Total active time spent by yabbies	SYc : SYp	10.750±1.89	7.625±1.42	-1.282	0.200
	SYc : MYc	10.750±1.89	20.50±1.31	-3.375	0.000**
	SYc: MYp	10.750±1.89	13.79±1.31	-2.139	0.032*
	SYc : LYc	10.750±1.89	20.50±1.28	-3.758	0.000**
	SYc: LYp	10.750±1.89	13.79±1.31	-2.139	0.032*
	SYp : MYc	7.625±1.42	20.50±1.31	-4.748	0.000**
	SYp : MYp	7.625±1.42	13.79±1.31	-3.025	0.002**
	SYp : LYc	7.625±1.42	20.50±1.28	-4.748	0.000**
	SYp : LYp	7.625±1.42	13.79±1.31	-3.025	0.002**
	MYc : MYp	20.50±1.31	13.79±1.31	-3.338	0.001**
	MYc : LYc	20.50±1.31	20.50±1.28	0.000	1.000
	MYc :LYp	20.50±1.31	13.79±1.31	-3.388	0.001**
	MYp : LYc	13.79±1.31	20.50±1.28	-3.388	0.001**
	MYp : LYp	13.79±1.31	13.79±1.31	-0.000	1.000
	LYc : LYp	20.50±1.28	13.79±1.31	-3.388	0.001**

Note: SY=small yabbies, MY=medium yabbies, LY=large yabbies, c=control and p=predator odour (*α=0.05, **α= 0.01)

6.3.1.3 Comparison of active time of yabbies of different gender and size

Figure 6.3 shows the total time spent outside shelter (active time) by small male and small female yabbies with different test solutions during the 24-hour observation period. There were no significant differences in active time between small male and small female yabbies exposed to the control solution, t (23) =0.202, P=0.842, or predator odour t (23) =1.701, P=0.102. However, when compared by gender, both small male and small female yabbies show a significant reduction in active time in response to predator odour, i.e. SM Cont *vs* SM Pred*, t* (23)=2.077, P=0.049 and SF Cont *vs* SF Pred, t (23)=2.891, P=0.008 (see Appendix 2).
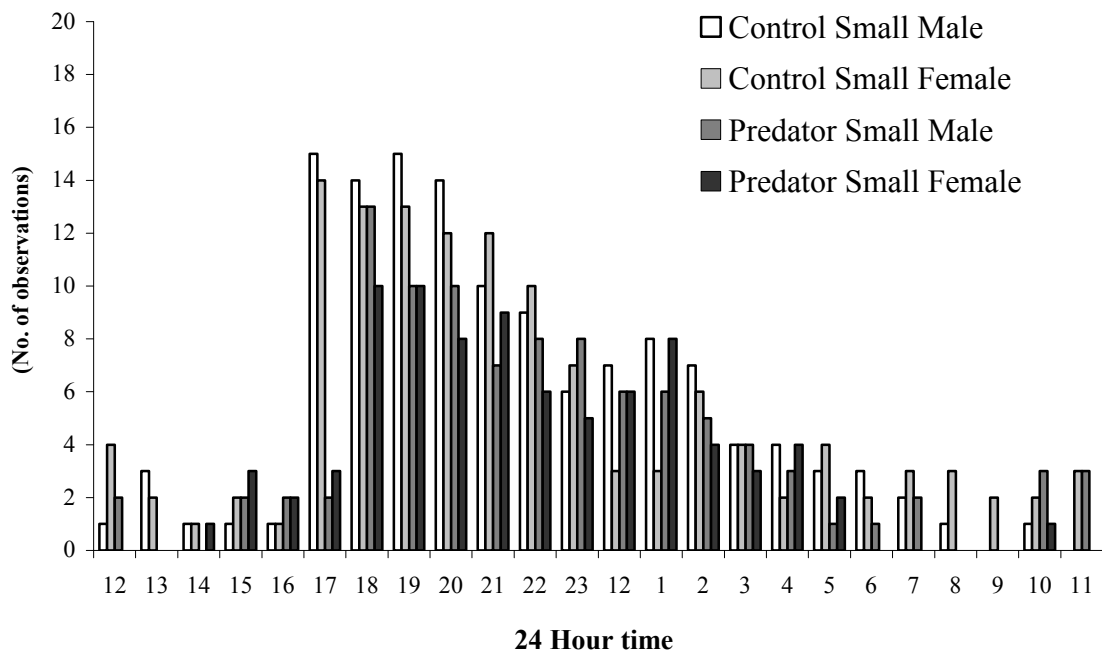
Figure 6.3 Total active time of small male and small female yabbies exposed to control and predator odour

In Figure 6.4, the total active time of medium sized yabbies with test solutions is shown. Male and female yabbies of medium size spent significantly more active time in response to the control solution than to predator odour (*t* (23) =3.194, P=0.004). Both males and females spent similar amounts of active time when exposed to predator odour (*t* (23) =0.363, P=0.720). However, when the medium size was compared within gender and with control solution and predator odour, both genders showed a significant reduction of active time when exposed to predator odour, i.e. MM Cont *vs* MM Pred*, t*(23)=7.114, P=0.000 and MF Cont *vs* MF Pred, *t* (23)=6.179, P=0.0001.
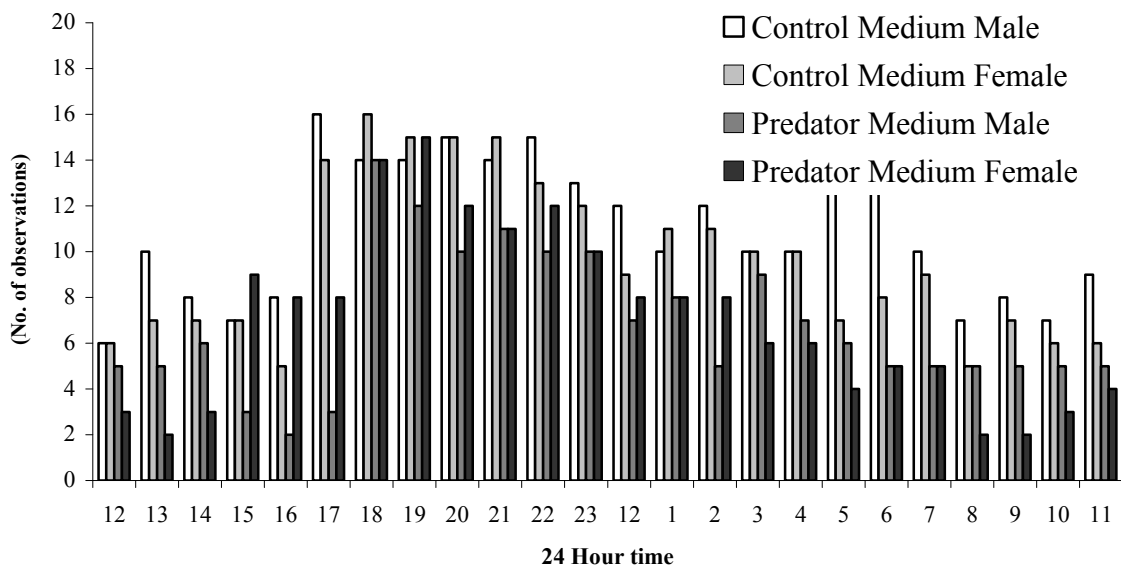
Figure 6.4 Total active time of medium male and medium female yabbies exposed to control and predator odour

The total active time spent by large male and female yabbies in response to either control or predator odour is shown in Figure 6.5. The active response of large yabbies to control solution was different from that to predator odour for large males but not large females. Large females were significantly more active than large male yabbies (*t* (23) = -2.654, P=0.014 and *t* (23) =-3.542, P=0.001) in response to both control solution and predator odour. In addition, large female yabbies did not reduce activity when confronted with predator odour (*t* (23)=1.316, P=0.201), while large male yabbies reduced their active time significantly when exposed to predator odour (*t* (23) = 2.618, P=0.015).
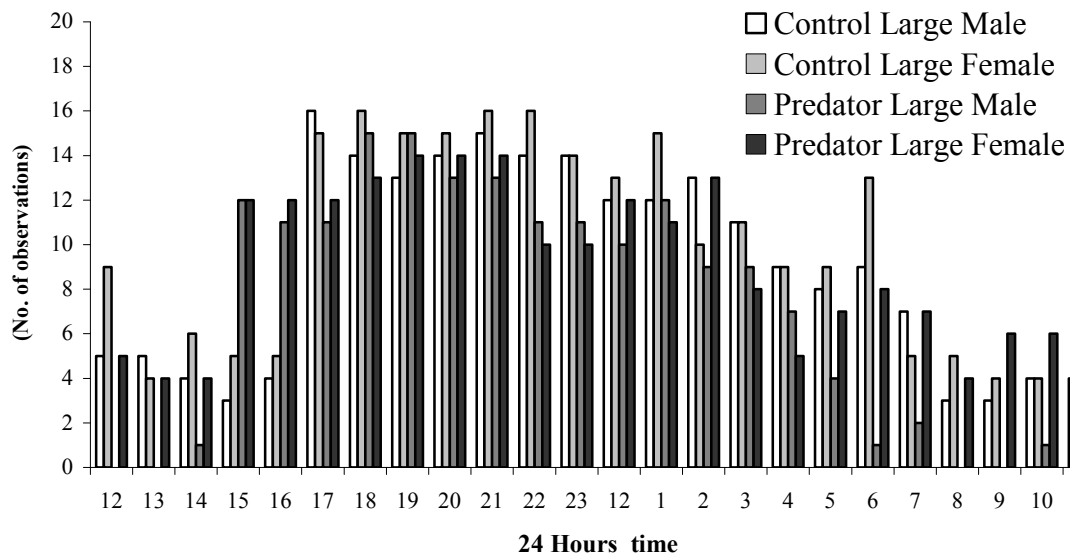
Figure 6.5 Total active time of large male and large female yabbies exposed to control and predator odour

6.3.2 Shelter selection pattern of yabbies

6.3.2.1 Overall comparison of time in shelter and active time of yabbies

The combination of active time outside with total time spent in shelter was analysed, and the results are graphically presented in Figure 6.6. The total amount of time that small, medium and large yabbies spent in shelter or being active was compared during day and night, and in response to control solution and predator odour. The bars represent the total active time observed spent in each activity. There was a clear difference between active time and time spent in shelter across all sizes of yabbies when exposed to control solution compared to predator odour, and also during day vs. night. Statistical analysis is summarised and shown in Table 6.2.
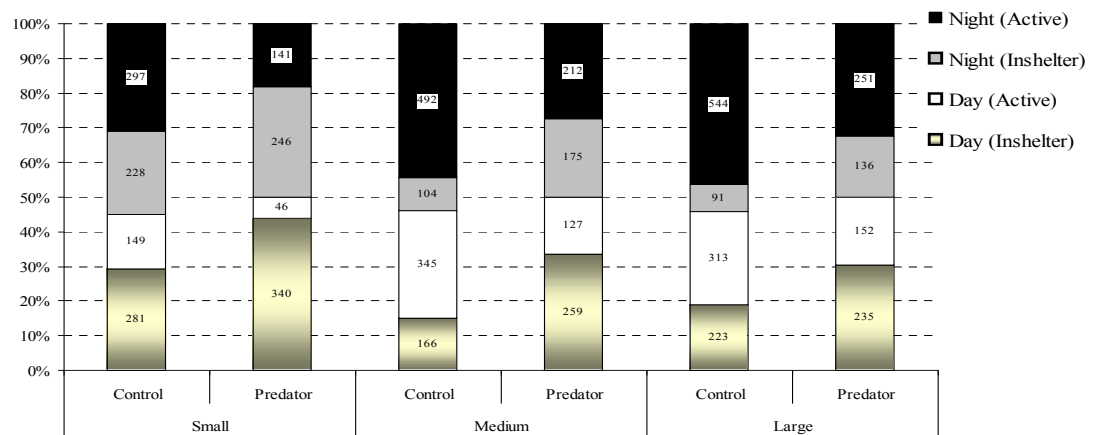
Figure 6.6 Percent of time that small, medium and large yabbies were observed in shelter and being active during day and night with control solution and predator odour
Number of each type of observation is shown within the respective bar.

6.3.2.2 Shelter preference of different sizes of yabbies

Small, medium and large yabbies spent time in each selected shelter in various combinations of day, night and test solution, and results are presented in Table 6.3. In this table, diurnal comparison highlights changes in shelter occupation with differences between control solution and predator odour.

Table 6.3 Mean of percentage of time spent in each shelter ± S.E by different sizes of yabbies during day and night in response to test solutions

Yab.	% of Time	Tr	Shelter size (mm)					Active
			25mm	50mm	90mm	25&90	50&90	
Small	Day	Cont	$_1$13.13±2.87^b	$_{12}$0.88±0.23^a	$_1$1.25±0.45^a	$_1$2.50±1.44^a	$_{12}$17.38±3.94^b	$_{12}$12.88±1.77^b
		Pred	$_1$12.88±2.32^b	$_1$0.25±0.25^a	$_1$0.38±0.26^a	$_1$1.25±0.49^a	$_2$27.75±2.27^c	$_1$5.38±0.92^a
	Night	Cont	$_1$11.88±3.93^b	$_{12}$1.25±0.37^a	$_1$1.13±0.35^a	$_1$1.00±0.38^a	$_1$13.25±3.28^b	$_2$19.80±3.36^b
		Pred	$_1$6.88±1.86^a	$_2$1.50±0.38^a	$_1$1.25±0.31^a	$_1$0.75±0.41^a	$_{12}$20.38±3.49^b	$_2$17.25±2.94^b
Medium	Day	Cont	$_{12}$14.13±3.8^b	$_1$1.25±0.49^a	$_1$5.25±1.31^{ab}	na	$_1$0.13±0.13^a	$_2$27.25±3.46^c
		Pred	$_2$22.13±1.90^b	$_1$0.75±0.25^a	$_1$5.25±2.31^a	na	$_1$4.13±1.80^a	$_1$15.50±1.66^c
	Night	Cont	$_1$8.50±3.18^a	$_1$1.88±0.23^a	$_{12}$2.25±1.00^a	na	$_1$0.38±0.13^a	$_2$35.00±3.18^b
		Pred	$_{12}$16.25±1.7^b	$_1$1.63±0.73^a	$_{12}$2.25±0.70^a	na	$_1$1.13±1.12^a	$_2$26.13±1.09^c
Large	Day	Cont	na	$_2$19.38±2.32^b	$_1$8.25±1.53^a	na	na	$_1$20.13±2.12^b
		Pred	na	$_2$19.00±1.20^b	$_1$10.30±1.55^a	na	na	$_1$18.63±1.99^b
	Night	Cont	na	$_1$4.25±1.41^a	$_1$7.00±1.24^a	na	na	$_2$36.63±1.60^b
		Pred	na	$_1$7.38±1.22^a	$_1$9.38±1.39^a	na	na	$_2$31.00±1.93^b

Note: Yab=yabbies, Cont=Control solution, Pred=Predator odour. Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences within results for each size of yabby (α=0.05)

6.3.2.2.1 Small yabbies

With control solution, small yabbies preferred the 25mm and the 50&90mm shelters significantly more than other shelter (P<0.05). However, there was no significant difference in time spent in the 25mm and the 50&90mm shelters (P>0.05). After predator odour was introduced, small yabbies increased time in the 50&90mm shelter significantly both day and night (P<0.05).

There were significant differences in temporal shift in use of the 50mm and 50&90mm shelters, and active time. The difference in shelter selection between day and night was greater than the difference in shelter selection in response to control solution and predator odour (P<0.05). When predator odour was introduced, small yabbies spent significantly more time in the 25mm and the 50&90mm shelters during the day than during the night. This was opposite to the pattern shown with active time.

Active time was greater during the night when small yabbies were exposed to predator odour. Active time was also greater during the night with control solution but the difference observed was not statistically significant (P>0.05).

6.3.2.2.2 Medium yabbies

Medium-sized yabbies strongly preferred the 25mm shelter overall other shelter types (P<0.05). The 25&90mm shelter was not used. When exposed to predator odour, medium-sized yabbies increased time spent in the 25mm shelter, and also significantly reduced active time. They were more active than small yabbies and spent significantly more time outside shelter (P<0.05). Medium yabbies spent significantly more time outside shelter when exposed to control solution than when exposed to predator odour. However, they also significantly reduced active time during the day when exposed to predator odour (P>0.05).

6.3.2.2.3 Large yabbies

Large yabbies did not show significantly different responses to predator odour (P>0.05). They spent more time in shelter during the day and were highly active at night (P<0.05). Large yabbies did not use the 25mm, 25&90mm or 50&90mm shelters. During the day, the 50mm shelter was preferred over the 90mm shelter (P<0.05). There were no significant differences in the use of these shelters during the night.

Shelter preference of yabbies of different size is influenced by shelter size, time of day (i.e. day vs night), and by the presence of control or predator odour.

6.3.2.3 Comparison of shelter use by different sizes and genders of yabbies

Shelter occupation by different sizes and genders of yabbies with regard to time spent in each size of shelter, shelter selection comparison between day and night, and differences in response to control solution and predator odour are compared and summarised in the following sections. The active time of yabbies were excluded from these comparisons.

6.3.2.3.1 Small male and small female yabbies

There were significant differences in shelter size use between small male and female yabbies: *df* (9), F=16.69, P=0.000. These are summarised in Table 6.4 and depicted in Figure 6.7. In the control treatment, small male yabbies spent significantly more time utilising the 25mm shelters (16.15±2.99 %) during the day than females. With predator odour, males reduced time spent in 25mm shelter from $16.15 \pm 1.31\%$ during the day to $3.13 \pm 1.80\%$ at night (*df* (3) F=5.99 P=0.01).
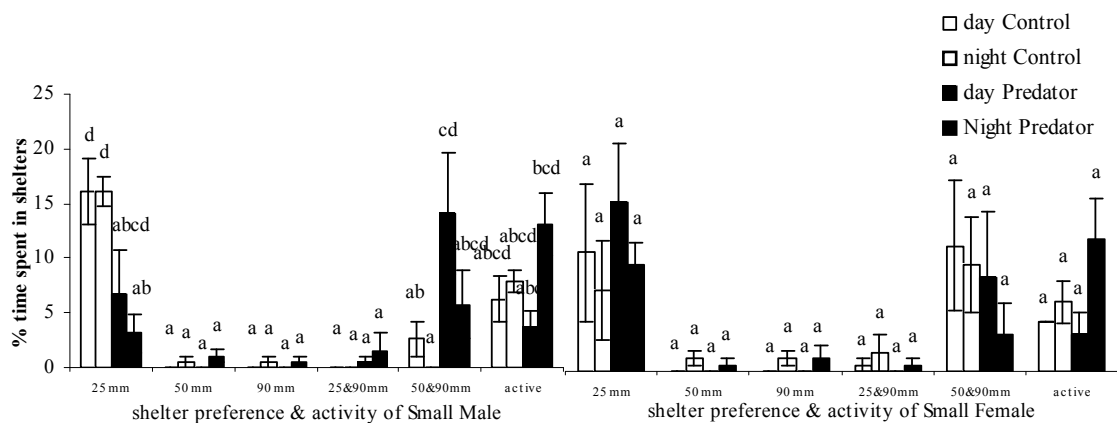
Figure 6.7 Shelter preference of small male and small female yabbies with both control and predator odour
Note: different letters indicate significant differences (α =0.05) between shelter sizes

Table 6.4 Comparison of shelter preferences of small yabbies

Yabbies	Times &	Shelter size				
	Test solutions	25mm	50mm	90mm	25&90mm	50&90mm
Small male	Day control	$_2 16.15 \pm 2.99^{b}$	na	na	na	$_1 2.60 \pm 1.56^{a}$
	Night control	$_2 16.15 \pm 1.31^{b}$	$_1 0.52 \pm 0.52^{a}$	$_1 0.52 \pm 0.52^{a}$	na	na
	Day predator	$_{12} 6.77 \pm 3.93^{a}$	na	na	$_1 0.52 \pm 0.52^{a}$	$_1 14.06 \pm 5.54^{a}$
	Night predator	$_1 3.13 \pm 1.80^{a}$	$_1 1.04 \pm 0.60^{a}$	$_1 0.52 \pm 0.52^{a}$	$_1 1.56 \pm 1.56^{a}$	$_1 5.73 \pm 3.11^{a}$
Small female	Day control	$_1 9.90 \pm 5.73^{a}$	na	na	$_1 0.52 \pm 0.52^{a}$	$_1 10.42 \pm 5.45^{a}$
	Night control	$_1 6.77 \pm 4.11^{a}$	$_1 1.04 \pm 0.60^{a}$	$_1 1.04 \pm 0.60^{a}$	$_1 1.56 \pm 1.56^{a}$	$_1 8.85 \pm 3.93^{a}$
	Day predator	$_1 14.06 \pm 4.91^{a}$	na	na	na	$_1 7.81 \pm 5.40^{a}$
	Night predator	$_1 8.85 \pm 1.78^{b}$	$_1 0.52 \pm 0.52^{a}$	$_1 1.04 \pm 1.04^{a}$	$_1 0.52 \pm 0.52^{a}$	$_1 3.13 \pm 2.48^{a}$

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences within each shelter type for each gender of yabby (P<0.05)

Use of the 25mm shelter by males was significantly higher than all other shelters (P<0.05). The control group did not use the 25&90mm shelter, however, the time spent in that shelter by male yabbies exposed to predator odour was increased. There was no significant difference in use of that shelter when day and night was compared (P>0.05).

Small female yabbies showed a similar pattern of shelter use compared to males. However, when comparing all shelter types used during day or night by small female yabbies, no significant preference was distinguished (P>0.05) with respect to treatment group. However, when exposed to predator odour at night, small female yabbies spent more time in the 25mm shelter (8.85±1.78%) than any other shelter (P<0.05). In contrast to males, shelter use of small female yabbies was remarkably variable.

6.3.2.3.2 Medium male and medium female yabbies

Patterns of shelter use by medium-sized yabbies were not clear compared to small yabbies. Medium males did not show a significant preference for any shelter, day or night with either test solution (Table 6.5). They did not use the 25&90mm shelter.

Table 6.5 Comparison of shelter preferences of medium yabbies

Yabbies	Times & Test solutions	Shelter size				
		25mm	50mm	90mm	25&90mm	50&90mm
Medium male	Day control	$_1$8.85±5.27^a	$_1$1.04±0.60^a	$_1$6.25±4.42^a	na	na
	Night control	$_1$5.21±3.12^a	$_1$0.52±0.52^a	na	na	0.52±0.52^a
	Day Predator	$_1$14.58±5.03^a	na	$_1$6.77±4.91^a	na	na
	Night	$_1$10.42±5.31^a	$_1$1.04±0.60^a	$_1$1.56±1.00^a	na	na
Medium female	Day control	$_1$17.71±2.42^b	na	$_1$0.52±0.52^a	na	na
	Night control	$_1$10.94±4.28^b	$_1$0.52±0.52^a	$_1$0.52±0.52^a	na	0.52±0.52^a
	Day Predator	$_1$8.33±4.28^a	$_1$0.52±0.52^a	$_1$9.37±4.29^a	na	na
	Night	$_1$6.77±4.11^a	$_1$1.56±1.00^a	$_1$1.56±1.00^a	na	na

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences within each shelter type for each gender of yabby ($P<0.05$)

Medium females strongly preferred the 25mm shelter as did small male and female yabbies. When exposed to predator odour, medium females significantly reduced occupation of the 25mm shelter both day and night ($P>0.05$). These results are depicted in Figure 6.8. Medium females also did not use the 25&90mm shelter.
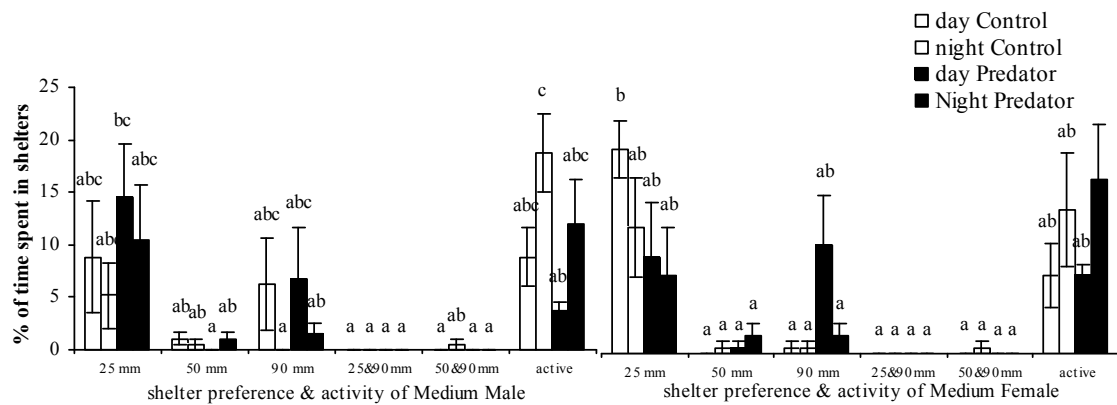
Figure 6.8 Time spent in shelters by medium yabbies with control and predator odour
Values represent mean (± se). Note: Different letters between groups indicate significantly different
means (α<0.05)

6.3.2.3.3 Large male and large female yabbies

Large male yabbies used only the 50mm and 90mm shelters. During day time with control solution, large males spent 15.63±2.17% of time in the 50mm shelter, significantly longer than in the 90mm shelter (3.12±1.99%, P<0.05). There were no other significant differences in shelter occupation between day and night, or between use of the 50mm and 90mm shelter occupation when exposed to predator odour (P>0.05).

Shelter occupation by large females followed a similar pattern to that of large males, but none of the differences across shelter type were statistically significant. Results by day and night time, and with control or predator odour, are summarised in Table 6.6. Large yabbies did not use the 25mm, 25&90mm or 50&90mm shelters.

Table 6.6 Comparison of shelter preferences of large yabbies

Yabbies	Times &	Shelter size				
	Test solutions	25mm	50mm	90mm	25&90mm	50&90mm
Large male	Day control	na	$_1$15.63±2.71^b	$_1$3.12±1.99 ^{a}	na	na
	Night control	na	$_1$4.17±1.70^a	$_1$4.17±0.85 ^{a}	na	na
	Day Predator	na	$_1$9.90±5.13 ^{a}	$_1$11.46±3.94 ^{a}	na	na
	Night predator	na	$_1$4.17±3.51 ^{a}	$_1$8.33±2.08 ^{a}	na	na
Large female	Day control	na	$_1$5.21±5.21 ^{a}	$_1$6.25±4.25 ^{a}	na	na
	Night control	na	$_1$1.56±1.00 ^{a}	$_1$1.04±0.60 ^{a}	na	na
	Day Predator	na	$_1$7.81±3.44 ^{a}	$_1$7.29±4.29 ^{a}	na	na
	Night predator	na	$_1$4.69±4.02 ^{a}	$_1$7.81±0.00 ^{a}	na	na

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences within shelter type for each gender of yabby (P<0.05)

6.3.2.4 Comparison between active time and shelter use by pairs of yabbies of different size

In this section, active time and shelter use of different sized pairs (trios) of yabbies are compared with shelter use of individual yabbies. Results were compared based on the hypothesis that shelter selection by individuals within groups of yabbies will be similar to that of individuals of the same size and gender on their own. Exposure to control and predator odour was also compared.

6.3.2.4.1. Small male yabbies paired in various combinations

This section compares shelter selection of small males on their own (SM) with other small males sharing with medium males (SM+MM) or large males (SM+LM), and when sharing with a medium and large male together (SM+MM+LM). Shelter preference with control and with concurrent conspecific and predator odour were compared during day and night. Results are summarised in Table 6.7.

There were significant differences in shelter selection of SM in groups compared to solo male yabbies. SM with control solution spent more time in the 25mm shelter (16.15±3.99% day and 16.15±1.31% night, P<0.05) than SM in any combination. The other comparisons show no significant differences, however, in the SM+LM, small male yabbies spent significant more time in the 50&90mm shelter (P<0.05).

Table 6.7 Comparison of shelter selection of small male yabbies individually and in various combinations with other males.

Yabbies	Shelter size					
Times & Test solutions	25mm	50mm	90mm	25&90mm	50&90mm	Active
SM Day Control	$_2$16.15±3.99^b	na	na	na	$_1$2.60±1.56^a	$_{12}$6.25±2.08^a
Night Control	$_2$16.15±1.31^c	$_1$0.52±0.52^a	$_1$0.52±0.52^a	na	na	$_{12}$7.81±1.00^b
Day Predator	$_{12}$16.77±3.93^{ab}	na	na	$_1$0.52±0.52^a	$_1$14.06±5.54^b	$_1$3.65±1.56^{ab}
Night Predator	$_1$3.13±1.80^a	$_1$1.04±0.60^a	$_1$0.52±0.52^a	$_1$1.56±1.56^a	$_1$5.73±3$^{.ab}$	$_2$13.02±2.87^b
SM+MM Day Control	$_1$3.65±3.65^{ab}	$_1$0.52±0.52^a	$_1$0.52±0.52^a	na	$_1$15.10±5.06^b	$_{12}$5.21±0.60^{ab}
Night Control	$_1$4.69±4.96^{ab}	$_1$1.04±1.04^a	na	na	$_1$3.12±1.99^{ab}	$_2$16.15±4.99^b
Day Predator	$_1$5.73±3.69^a	na	na	na	$_1$16.67±3.99^b	$_{12}$2.60±0.52^a
Night Predator	$_1$2.08±2.08^{ab}	$_1$0.52±0.52^a	$_1$1.04±0.60^{ab}	na	$_1$9.37±5.00^{ab}	$_{12}$11.98±3.33^b
SM+LM Day Control	$_1$8.33±4.74	$_1$0.52±0.52	$_1$1.04±0.60	$_1$5.21±5.21	$_1$4.17±2.55^a	$_{12}$5.73±1.00
Night Control	$_1$1.04±0.60	$_1$0.52±0.52	$_1$0.52±0.52	na	$_{12}$11.46±3.85^b	$_2$11.46±3.56^b
Day Predator	$_1$3.65±3.56^a	na	na	$_1$2.08±2.08^a	$_2$18.23±3.74^b	$_1$1.04±0 .60^a
Night Predator	$_1$1.04±1.04^a	$_1$0.52±0.52^a	$_1$1.04±0.60^a	$_1$1.04±1.04^a	$_2$19.79±1.34^b	$_1$1.56±1.00^a
SM+MM+LM Day Control	na	$_1$0.52±0.52^a	$_1$0.52±0.52^a	$_1$0.52±0.52^a	$_1$19.79±0.60^c	$_1$3.65±1.02^b
Night Control	$_1$2.08±2.08^a	na	na	$_1$1.04±1.04^a	$_1$10.42±3.29^{ab}	$_1$11.46±3.85^c
Day Predator	$_1$5.21±3.94^{ab}	na	na	$_1$1.04±1.04^a	$_1$13.54±5.67^b	$_1$5.21±1.80^{ab}
Night Predator	$_1$1.04±0.60^a	$_1$1.56±1.00^{ab}	$_1$1.04±0.60^a	na	$_1$9.90±4.69^{ab}	$_1$11.46±3.45^b

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences within results for each gender of yabby (P<0.05)

6.3.2.4.2 Small female yabbies paired in various combinations

Solo small female yabbies did not show a clear pattern of shelter use. However, when paired with other yabbies the results were notable. During day and night with control

solution, small females paired with medium females (SF+MF) showed no significant shelter preference. However, when faced with predator odour, SF+MF spent significantly more time in the 50&90mm shelter (P<0.05). There were no significant differences in any variable when SF shared with LF (P>0.05). When results of the combination of SF+MF+LF were compared, SF preferred the 50&90mm shelter except during daytime with control solution. Results are summarised in Table 6.8.

Table 6.8 Comparison of shelter preference and shelter selection of small female yabbies in various combinations with other females

Yabbies		Shelter size					
Times & Test solutions		25mm	50mm	90mm	25&90mm	50&90mm	Active
SF	Day control	$_1$9.90±5.73^a	na	na	$_1$0.52±0.52^a	$_1$10.42±5.45^a	$_1$4.17±0.00^a
	Night control	$_1$6.77±4.11^a	$_1$1.04±0.60^a	$_1$1.04±0.60^a	$_1$1.56±1.56^a	$_1$8.85±3.93^a	$_1$5.73±1.78^a
	Day Predator	$_1$14.06±4.91^b	na	na	na	$_1$7.81±5.40^{ab}	$_1$3.13±1.80^a
	Night predator	$_1$8.85±1.78^{ab}	$_1$0.52±0.52^a	$_1$1.04±1.04^a	$_1$0.52±0.52^a	$_1$3.13±2.48^{ab}	$_1$10.94±3.44^b
SF+MF	Day control	$_4$4.17±4.17^a	1.04±1.04^a	$_1$1.56±1.00^a	$_1$0.00±0.00^a	$_{12}$7.81±2.99^a	$_1$10.42±3.29^a
	Night control	$_1$7.81±4.37^a	na	$_1$0.52±0.52^a	$_1$0.52±0.52^a	$_2$2.60±1.00^{ab}	$_1$13.54±4.62^b
	Day Predator	$_1$3.65±2.15^a	na	na	na	$_1$17.71±1.80^b	$_1$3.65±1.56^a
	Night predator	$_1$3.13±2.48^a	na	na	na	$_{12}$10.42±3.71^{ab}	$_1$11.46±3.56^b
SF+LF	Day control	$_1$10.42±5.45^a	$_1$0.52±0.52^a	na	na	6.25±5.58^a	$_1$7.81±4.37^a
	Night control	$_1$4.17±3.51^{ab}	$_1$1.56±1.56^a	0.52±0.52^a	$_1$0.52±0.52^a	$_1$3.65±2.99^{ab}	$_1$14.58±3.51^b
	Day Predator	$_1$8.85±5.54^a	$_1$1.04±1.04^a	na	$_1$0.52±0.52^a	$_1$12.50±4.47^a	$_1$2.08±1.47^a
	Night predator	$_1$6.25±4.89^a	$_1$0.52±0.52^a	na	na	$_1$10.94±6.44^a	$_1$7.29±5.28^a
SF+MF+LF	Day control	$_1$5.73±5.06^a	$_1$0.52±0.52^a	$_1$1.56±1.00^a	$_1$4.17±4.17^a	$_1$9.37±4.38^a	$_1$3.65±0.52^a
	Night control	$_1$3.12±1.99^{ab}	$_1$0.52±0.52^a	$_1$1.56±0.52^{ab}	$_1$0.52±0.52^a	$_1$11.98±1.00^c	$_1$7.29±1.80^{bc}
	Day Predator	$_1$5.73±4.45^{ab}	na	$_1$1.04±1.04^a	$_1$1.04±1.04^a	$_1$15.63±4.85^b	$_1$1.56±1.00^a
	Night predator	$_1$3.65±2.31^{ab}	$_1$1.56±1.00^a	$_1$1.04±0.60^a	na	$_1$15.10±5.47^b	$_1$3.65±1.78^{ab}

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences within results for each gender of yabby (P<0.05)

6.3.2.4.3 Medium male yabbies paired in various combinations

Results of comparison of medium male yabbies in combination with others are shown in Table 6.9. Medium male yabbies (MM) displayed a similar pattern when on their own and when sharing with LM and in combination with SM and LM. When sharing with small males (MM*v*SM) however, MM preferred the 25mm shelter during the day with control solution (16.15±4.69%) compared to MM during the day with control solution when on their own (8.85±5.27%). There were no other significantly different comparisons (P>0.05).

Table 6.9 Comparison of shelter selection of medium male yabbies individually and in various combinations with other males

Yabbies		Shelter size					
Times & Test solutions		25mm	50mm	90mm	25&90mm	50&90mm	Active
MM	Day control	$_1$8.85±5.27 [a]	$_1$1.04±0.60 [a]	$_1$6.25±4.42 [a]	na	na	$_{12}$8.85±2.74 [a]
	Night control	$_1$5.21±3.12 [a]	$_1$0.52±0.52 [a]	na	na	0.52±0.52 [a]	$_2$18.75±3.71 [b]
	Day Predator	$_1$14.58±5.03 [b]	na	$_1$6.77±4.91 [ab]	na	na	$_1$3.65±1.00 [a]
	Night predator	$_1$10.42±5.31 [a]	$_1$1.04±0.60 [a]	$_1$1.56±1.00 [a]	na	na	$_{12}$11.98±4.20 [a]
MM+SM	Day control	$_1$16.15±4.69 [b]	$_1$1.04±0.60 [a]	$_1$0.00±0.00 [a]	na	na	$_1$7.81±4.37 [ab]
	Night control	$_1$5.73±3.55 [a b]	$_1$1.04±1.04 [a]	$_1$4.17±1.90 [a b]	na	na	$_1$14.06±3.74 [b]
	Day Predator	$_1$10.42±4.96 [a]	na	$_1$1.56±1.56 [a]	0.52±0.52 [a]	$_1$4.17±4.17 [a]	$_1$8.33±4.42 [a]
	Night predator	$_1$5.73±3.55 [a]	$_1$3.65±2.46 [a]	$_1$2.08±1.47 [a]	na	$_1$1.56±1.56 [a]	$_1$11.98±5.13 [a]
MM+LM	Day control	na	na	2.08±2.08 [a]	na	na	$_1$22.92±2.08 [b]
	Night control	$_1$0.52±0.52 [a]	$_1$1.04±1.04 [a]	na	na	na	$_1$23.44±1.00 [b]
	Day Predator	$_1$7.29±5.98 [a]	$_1$0.52±0.52 [a]	$_1$1.56±1.00 [a]	na	$_1$6.25±5.58 [a]	$_1$9.37±4.85 [a]
	Night predator	$_1$9.90±5.40 [a]	$_1$0.52±0.52 [a]	$_1$0.52±0.52 [a]	na	$_1$1.56±1.00 [a]	$_1$12.50±5.58 [a]
MM+SM+LM	Day control	$_1$2.08±2.08 [a]	na	$_1$4.17±3.51 [a]	na	na	$_1$18.75±3.71 [b]
	Night control	na	1.04±0.60 [a]	$_1$0.52±0.52 [a]	na	na	$_1$23.44±1.00 [b]
	Day Predator	$_1$10.94±6.44 [a]	na	$_1$1.56±1.56 [a]	na	$_1$1.56±1.56 [a]	$_1$10.94±6.44 [a]
	Night predator	$_1$3.65±3.65 [a]	na	na	na	$_1$4.69±4.69 [ab]	$_1$16.67±4.89 [b]

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences within results for each gender of yabby (P<0.05)

6.3.2.4.4 Medium female yabbies paired in various combinations

Similarly to medium male yabbies, MF tended to show a preference for the 25mm shelter across all combinations (Table 6.10). This preference was highly significant when on their own with the control solution where MF spent 17.71±2.48% during the day (P<0.05). However, this was not significantly different from use of this shelter during the day when exposed to predator odour. Notably though, when MF was paired in any other combination, the 25mm shelter attracted highly significant use during the day when exposed to predator odour. There were no other significant differences in shelter use in any other combinations (P>0.05).

Table 6.10 Comparison of shelter selection of medium female yabbies individually and in various combinations with other females

Yabbies		Shelter size					
Times & Test solutions		25mm	50mm	90mm	25&90mm	50&90mm	Active
MF	Day control	$_1$17.71±2.48 ^{b}	na	$_1$0.52±0.52 ^{a}	na	na	$_1$6.77±2.74 ^{a}
	Night control	$_1$10.94±4.28 ^{ab}	$_1$0.52±0.52 ^{a}	$_1$0.52±0.52 ^{a}	na	0.52±0.52 ^{a}	$_1$12.50±4.89 ^{b}
	Day Predator	$_1$8.33±4.81^a	$_1$0.52±0.52 ^{a}	$_1$9.37±4.29 ^{a}	na	na	$_1$6.77±1.00 ^{a}
	Night predator	$_1$6.77±4.11 ^{ab}	$_1$1.56±1.00 ^{a}	$_1$1.56±1.00 ^{a}	na	na	$_1$15.10±4.84 ^{b}
MF+SF	Day control	$_1$6.77±3.44 ^{ab}	$_1$2.08±2.08 ^{ab}	$_1$4.17±1.90 ^{ab}	na	$_1$0.52±0.52 ^{a}	$_{12}$11.46±3.24 ^{b}
	Night control	$_1$0.52±0.52 ^{a}	$_1$1.56±1.56 ^{a}	$_1$2.60±1.31 ^{a}	na	na	$_2$20.31±1.00 ^{b}
	Day Predator	$_1$13.54±5.56 ^{b}	na	$_1$1.04±1.04 ^{a}	na	$_1$4.17±3.51 ^{ab}	$_1$6.25±2.41 ^{ab}
	Night predator	$_1$10.42±4.89 ^{ab}	$_1$0.52±0.52 ^{a}	na	na	$_1$0.52±0.52 ^{a}	$_1$13.54±5.00 ^{b}
MF+ LF	Day control	$_1$4.17±2.55 ^{ab}	$_1$0.52±0.52 ^{ab}	$_1$4.17±2.95 ^{a}	na	na	$_1$16.15±3.65 ^{b}
	Night control	$_1$2.60±1.97 ^{a}	$_1$1.04±1.04 ^{a}	$_1$0.52±0.52 ^{a}	na	0.52±0.52 ^{a}	$_1$20.31±2.15 ^{b}
	Day Predator	$_1$13.54±5.21^b	$_1$1.04±1.04 ^{a}	na	na	na	$_1$10.42±4.34 ^{ab}
	Night predator	$_1$10.94±4.28 ^{a}	na	$_1$1.04±0.60 ^{a}	0.52±0.52 ^{a}	na	$_1$12.50±4.25 ^{b}
MF+ SF+LF	Day control	$_1$7.81±4.99 ^{ab}	$_1$0.52±0.52 ^{a}	$_1$1.04±1.04 ^{a}	na	na	$_1$15.63±5.00 ^{b}
	Night control	$_1$5.21±3.12 ^{a}	$_1$1.04±1.04 ^{a}	$_1$0.52±0.52 ^{a}	na	na	$_1$18.23±4.45 ^{b}
	Day Predator	$_1$14.06±3.55 ^{c}	$_1$0.52±0.52 ^{a}	$_1$1.04±0.60 ^{ab}	na	na	$_1$9.37±3.45**c**
	Night predator	$_1$9.37±3.85 ^{ab}	na	$_1$1.56±1.00 ^{a}	na	na	$_1$14.06±3.93 ^{b}

Note: Different letters between rows indicate significant differences within rows, and different number between columns indicate significant differences within results for each gender of yabby (P<0.05)

6.3.2.4.5 Large male and female yabbies paired in various combinations

As shown in Tables 6.11 and 6.12, neither LM nor LF used the 25mm, 25&90mm or the 50&90mm shelters when alone or in any combination with others. The only significant preference shown by LF was for the 50mm shelter when in combination with MF during the day when exposed to predator odour; no other significant differences were shown by LF alone or in any combination. LM alone and LM+MM strongly preferred the 50mm shelter during the day when receiving the control solution. LM+MM also exhibited this preference in response to predator odour during the day, as did LM+SM+MM. In contrast, LM+SM preferred to use the 90mm shelter at night with either test solution.

Table 6.11 Comparison of shelter selection of large male yabbies individually and in various combinations with other males

Yabbies		Shelter size					
Times & Test solutions		25mm	50mm	90mm	25&90mm	50&90mm	Active
LM	**Day control**	na	$_1$15.63±2.17 ^{b}	$_1$3.12±1.99 ^{a}	na	na	$_1$6.25±1.90 ^{a}
	Night control	na	$_1$4.17±1.70 ^{a}	$_1$4.17±0.85 ^{a}	na	na	$_2$16.67±1.47 ^{b}
	Day Predator	na	$_1$9.90±5.13 ^{a}	$_1$11.46±3.94 ^{a}	na	na	$_1$3.65±1.56 ^{a}
	Night predator	na	$_1$4.17±3.51 ^{a}	$_1$8.33±2.08 ^{ab}	na	na	$_{12}$12.50±3.26^b
LM+SM	**Day control**	na	$_1$14.58±4.96 ^{a}	$_1$2.08±1.20 ^{a}	na	na	$_{12}$8.33±4017 ^{ab}
	Night control	na	$_1$0.52±0.52 ^{a}	$_1$6.25±1.20 ^{b}	na	na	$_2$18.23±1.56 ^{c}
	Day Predator	na	$_1$10.94±4.76 ^{a}	$_1$8.85±4.53 ^{a}	na	na	$_1$5.21±1.80 ^{a}
	Night predator	na	$_1$2.08±1.20 ^{a}	$_1$5.73±0.52 ^{b}	na	na	$_2$17.19±1.56 ^{c}
LM+ MM	**Day control**	na	$_1$11.46±3.24 ^{b}	$_1$4.69±2.15 ^{a}	na	na	$_1$8.85±2.74 ^{b}
	Night control	na	$_1$1.04±0.60 ^{a}	$_1$2.08±0.85 ^{a}	na	na	$_2$21.88±0.60 ^{b}
	Day Predator	na	$_1$9.37±3.45 ^{b}	$_1$6.25±3.29 ^{a}	na	na	$_1$9.37±0.60 ^{b}
	Night predator	na	$_1$3.65±2.46 ^{a}	$_1$7.29±2.76 ^{ab}	na	na	$_{12}$14.06±4.20^b
LM+ SM+MM	**Day control**	na	$_1$8.85±4.84 ^{a}	$_1$2.08±2.08 ^{a}	na	na	$_1$14.06±5.73 ^{a}
	Night control	na	$_1$0.52±0.52 ^{a}	$_1$6.25±5.58 ^{ab}	na	na	$_1$18.23±5.40 ^{b}
	Day Predator	na	$_1$11.46±3.45 ^{b}	$_1$6.25±3.61 ^{a}	na	na	$_1$7.29±0.60 ^{ab}
	Night predator	na	$_1$2.60±0.52 ^{a}	$_1$3.65±2.99 ^{a}	na	na	$_1$18.75±3.51^b

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences within results for each gender of yabby (P<0.05)

Table 6.12 Comparison of shelter selection of large female yabbies individually and in various combinations with other females

Yabbies		Shelter size					
Times & Test solutions		25mm	50mm	90mm	25&90mm	50&90mm	Active
LF	**Day control**	na	$_1 5.21 \pm 5.2^a$	$_1 6.25 \pm 4.25^a$	na	na	$_1 13.54 \pm 4.85^a$
	Night control	na	$_1 1.56 \pm 1.00^a$	$_1 1.04 \pm 0.60^a$	na	na	$_1 22.40 \pm 1.56^b$
	Day Predator	na	$_1 7.81 \pm 3.44^a$	$_1 7.29 \pm 4.29^a$	na	na	$_1 9.90 \pm 3.93^a$
	Night predator	na	$_1 4.69 \pm 1.00^a$	$_1 7.81 \pm 4.02^a$	na	na	$_1 12.50 \pm 4.58^b$
LFνSF	**Day control**	na	$_1 6.77 \pm 4.11^{ab}$	$_1 5.73 \pm 2.31^a$	na	na	$_1 12.50 \pm 4.42^b$
	Night control	na	$_1 2.08 \pm 1.47^a$	$_1 2.08 \pm 0.00^a$	na	na	$_1 20.83 \pm 1.47^b$
	Day Predator	na	$_1 8.33 \pm 4.81^{ab}$	$_1 2.08 \pm 0.00^a$	na	na	$_1 14.58 \pm 3.99^b$
	Night predator	na	$_1 5.73 \pm 4.45^a$	$_1 5.73 \pm 3.11^{ab}$	na	na	$_1 13.54 \pm 4.21^b$
LFν MF	**Day control**	na	$_1 11.98 \pm 5.13^a$	$_1 2.08 \pm 1.47^a$	na	na	$_1 10.42 \pm 3.99^a$
	Night control	na	$_1 3.12 \pm 3.12^a$	$_1 4.17 \pm 1.90^a$	na	na	$_1 17.71 \pm 3.01^b$
	Day Predator	na	$_1 8.85 \pm 1.78^b$	$_1 5.73 \pm 2.60^a$	na	na	$_1 10.42 \pm 2.08^b$
	Night predator	na	$_1 1.56 \pm 0.52^a$	$_1 2.60 \pm 1.56^a$	na	na	$_1 20.31 \pm 1.78^b$
LFν SFνMF	**Day control**	na	$_1 9.90 \pm 3.93^a$	$_1 8.33 \pm 3.90^a$	na	na	$_1 6.25 \pm 0.00^a$
	Night control	na	$_1 1.04 \pm 0.60^a$	$_1 3.12 \pm 1.04^a$	na	na	$_2 20.31 \pm 1.78^b$
	Day Predator	na	$_1 9.37 \pm 4.29^{ab}$	$_1 3.12 \pm 3.12^a$	na	na	$_{12} 12.50 \pm 4.17^b$
	Night predator	na	$_1 3.65 \pm 1.78^a$	$_1 2.60 \pm 1.00^a$	na	na	$_2 18.23 \pm 2.60^b$

Note: Different letters between rows indicate significant differences within rows, and different number between columns indicate significant differences within results for each gender of yabby (P<0.05)

6.3.2.5 Incidence of cannibalism

Twelve incidents of cannibalism occurred when large yabbies shared the experimental units with small and medium yabbies. These incidents only occurred where the crayfish received control solutions. There were 11 incidents involving large yabbies consuming medium size yabbies; however, there was only one incident of a large yabby cannibalising a small yabby. When cannibalism was detected, the carcase was removed, the tank was purged and the animal was then replaced. No repeat incidents were observed.

6.4 Discussion

6.4.1 General

An important characteristic of yabbies appears to be a high degree of effectiveness in responding and adapting to various stimuli. Gherardi (2002) reported successful responses to a variety of threats. Yabbies not only show an ability to detect predator odour or recognize conspecifics with greater RHP (Dingle 1983; Chapter 5), they also show an ability to distinguish between alarm cues (Chivers & Smith 1998), and food and odour from various finfish as reported by Height (2002) and Storer (2006). Many of these characteristics were also observed in the present study. Although Height (2002) and Storer (2006) both reported that yabbies could distinguish food from finfish odour, Hazlett (2002) confirmed this behaviour resulted from associated learning. In the present experiment, yabbies increased time in shelter and reduced active time when exposed to predator odour, thereby demonstrating an appropriate response to potential predator cues. This implies that yabbies are able to adapt using limited resources such as available shelters. Gherardi *et al.* (2002) reported that yabbies responded to both conspecific and heterospecific (marron) alarm odours, while marron did not. This was considered by those authors to indicate that yabbies are highly adaptable in new environments. These instincts enhance the ability of yabbies to safely navigate harsh and hostile environments. In this respect, Gherardi *et al.* (2002) concluded that yabbies hold considerable potential as invaders.

Adaptation, an important strategy in the face of scarce resources, increases the chance of species survival; this includes the ability to respond to threat. Gherardi (2002) reported that yabbies display considerable plasticity, more so than native species in Western Australia. In the present experiment when provided with different sizes of shelter, yabbies exposed to predator odour tended to select the shelter that provided more security (smaller shelter size). In contrast, in the absence of predator or conspecific threat, yabbies did not show the same tendency when choosing particular shelters. In spite of these clear tendencies, shelter preference was variable overall; a number of factors likely affected the activity pattern, shelter preference and shelter selection in yabbies in the present study.

6.4.2 Shelter preferences of crayfish

Many authors have investigated shelter competition between crayfish (Westin & Gydemo 1988, Lamouroux *et al.* 1999, Booth 2001, Atkinson *et al.* 2004). Shelter selection and preference have been investigated by Cobb (1969), Nyström (2002), Tulonen *et al.* (*2006*) and others. Ability to select shelter for refuge is a key to species survival in the first instance (Gilinsky 1984); selection of shelter within a suitable habitat underpins the successful establishment of crayfish populations in nature. In the field, shelter availability is crucial (Vannini & Cannici 1995). In polyculture systems, providing shelter in accordance with species preference should maximise crayfish production. However, determination of shelter preference is affected by many factors such as presence of potential predators (Gherardi *et al.* 2002, Whisson 2004). Although the current body of research into crayfish culture and science is considerable, little work has been conducted that investigates the role and importance of shelter; reports that exist are largely inconclusive (Andersen *et al.* 2006). In the present study where preference for different sizes of shelters was investigated, the inclusion of shelter preferences under a range of natural circumstances (such as predator and conspecific threat, and day and night) add an important dimension to understanding the role of shelter in nature and in mono- and polyculture. In particular, consistent choice of smaller shelters relative to yabby size when exposed to threat suggests that provision of artificial shelter of greater complexity holds promise in crayfish aquaculture. This is supported by research into use of burrows for shelter by yabbies in pond aquaculture where burrow size and morphology was correlated with size of burrow occupant (Lawrence 2002, Verhoef & Austin 1999). Understanding the dynamics of shelter preference in response to predator threat and competition from conspecifics is important in successful management of co-stocking with finfish in polyculture systems.

6.4.3 Factors affecting activity and shelter selection

Medium and large yabbies tended to be more active than small yabbies without predator odour. However, in the presence of predator odour large and medium yabbies reduced their active time significantly. Gender differences were observed in large yabbies where males reduced their active time significantly more than large females with predator odour. This could indicate that larger males may possess a greater ability to learn and adapt than females as well as medium and smaller yabbies. These results support the

finding of Peeke (1998) who reported gender differences in response to intruders of male and female juvenile lobsters (*Homarus americanus* Milne Edwards). Lawrence *et al.* (2002) found that female yabbies tend to burrow more than males, however, that study did not investigate differences in size.

Factors discussed in the following sections are known to affect settlement (homing behaviour) of animals in natural habitats as well as substratum preference (i.e. for shelter or refuge). Many studies in this area are based on field observation, manipulation (Hernkind *et al.* 1997) and experiments in aquaculture including Whisson 1996 and 2000, Lawrence *et al.* 2000, and others. The selection of shelter size when under pressure from predators has not been previously described. The following factors are all known to have an impact on shelter selection and occupation in different habitats.

6.4.3.1 Diurnal rhythm

In this study, shelter selection by yabbies exposed to predator odour followed different patterns during day and night. Lawrence *et al.* (2000) examined type and abundance of yabby burrows on farm dams in various parts of Western Australia, and found gender, type of dam and seasonal changes may influence burrowing behaviour but daylight, day length and diurnal factors were not studied. It appears from the present study that diurnal rhythm affected shelter-related activity of yabbies.

In the present study, yabbies of different size spent significantly different proportions of time active or in shelters. The amount of time spent in each size of shelter also varied according to size of yabby. Small and medium yabbies spent more time in shelter compared to larger yabbies. However, when all data was combined and analysed from the perspective of time of day, the total time that all sizes of yabbies with the control solution spent inside any shelter was 61.3% : 38.7% (day:night) and 37.6% : 62.4% (day:night) outside of shelters. These proportions changed with predator odour, where all yabbies collectively spent 50.9 %: 49.1% (day:night) of time in shelter and 34.7% : 65.3% (day:night) outside shelter. The response to perceived threat appears to be affected by diurnal rhythm. Shelter use during day and night in the present experiment was similar for both genders; this contrasts with the findings of Peeke (1998) who showed a different pattern of shelter use by American lobster according to gender, day and night.

6.4.3.2 Abundance of vegetation and shelter complexity

Vegetation can serve as a substrate for refuge for crayfish. In the wild, crayfish can select the appropriate size and location of shelter according to preference and need. Conversely, size and type of available shelter can limit crayfish populations. Jordan (1996) argued that substrate availability has a great impact on crayfish abundance, and is more important than type of habitat. Moreover, different crayfish species prefer different types of habitat. Peterson *et al.* (1996) found that *Cambarellus shufeldtii* occurred in sites with lower relative abundance of emergent vegetation, while *C. diminutus* preferred submerged vegetation. In the present experiment, the shelter provided included various sizes to emulate shelter options in natural habitat where yabbies can select according to size. Smaller yabbies are more vulnerable to predation and therefore tended to choose the narrow gaps of the shelter bundle when exposed to predator odour or paired with larger yabbies. The propensity of smaller crayfish to seek well-covered refuge is supported by Whisson (2000) who observed that the tape-like leaved aquatic plant *Vallisneria sp.* plays an important role as a nursing ground for juvenile marron. However, this plant is considered a weed and its use in water bodies therefore has limited support from industry and local authorities. Abundance of vegetation is nonetheless important for survival of smaller crayfish. Small crayfish species were reported to spend most of their time under cover of dense vegetation (Whisson 2000). Such behaviour is not limited to crayfish; Eklov & Persson (1996) found that small perch spent more time in refuge when potential predators were present.

Providing different sizes of shelter in this trial served to illustrate that crayfish select the size of shelter for security, namely, where they can escape from interaction with conspecifics, risk of predation and perception of threat. Complexity of available shelter therefore plays an important role in reducing the risk of predation and cannibalism in aquaculture and in the wild. This is maintained by Eklov (1997) who suggested that structural complexity of shelter will both qualitatively and quantitatively alter the interaction between crayfish predators and their prey. This was borne out by results of the present experiment where medium yabbies chose to spend more time in 25mm shelter rather than 50mm or 90 mm when confronted with predator odour. The 25mm shelter likely provided higher security as its small diameter limited access to its conspecifics and predators. Similarly, large yabbies preferred the 50mm shelter over

90mm when exposed to predator odour. Field observations by Cobb (1969) showed that crayfish generally occupied shelters in which the height was less than the width, and that there was a correlation between lobster size and shelter size. In the laboratory in the same study, lobster chose low profile shelters when given a choice of flat (height = ½ width) versus square (height = width) profile openings. This generally agrees with results of the present study, and supports the notion of shelter choice according to perception of the protection and security that it will provide.

6.4.3.3 Factors affecting shelter selection

6.4.3.3.1 Temperature

Warmer water can result in higher activity in crayfish compared to temperatures below the optimal range (Barbaresi & Gherardi 2002). However, crayfish in general are also more active during the night than in day time. Gherardi (2002) stated that both temperature and shelter availability influence habitat selection of crayfish. In the present study, water temperature ranged between 18 and 20°C; this range is considered slightly below optimal for yabbies. Frost (1975) found that crayfish restrict activity when ambient temperatures drop below the optimum range (Abrahamsson 1972). The present experiment was conducted in controlled conditions where all aquaria were maintained at the same temperature; this did not vary significantly over the course of the study.

6.4.3.3.2 Presence of conspecific or predator odour and availability of shelter

The presence of conspecifics has been shown to be an important factor in shelter selection in the present experiment. All small, medium and large yabbies in various combinations of size and gender showed different patterns of shelter selection. The size of this study was insufficient to characterise consistent shelter preferences fully. Nonetheless, it is clear that size of yabby is correlated with preference for shelter size. Further study on the combination of mixed size and gender is recommended.

Predator odour also had an influence on the proportion of active time and shelter occupation of yabbies: the response of smaller yabbies was greater than that of large yabbies with respect to their shelter selection preferences. The response of yabbies when the presence of predator is supported with the optimal foraging and predator-prey interaction in *Orconectes propinquus* by Stein (1977).

6.4.3.4 Visual and chemical cues

Stein and Magnuson (1976) reported that the presence of predators will alter crayfish behaviour. This is supported by observations from the present study where both visual and chemical cues influenced shelter selection as well as activities in Chapters 4 and 5. Furthermore, physical interaction between conspecifics was also observed to influence the selection of shelter in the present study when the shelter was shared between yabbies. Consequently, multiple cues appear to be utilised by yabbies to shape their responses. These observations concur with the premise put forward by Hazlett & Schoolmaster (1998), Bouma & Hazlett (2000) that some crayfish possess the ability to "integrate multiple tactile cues" in a range of circumstances.

As a general rule, crayfish tend to remain in shelter during the day and are active at night. In this experiment, the effect of predator threat was increased shelter use during the day and a similarly significant reduction of activity at night when exposed to predator odour. This was observed in yabbies of all sizes, particularly by the smaller counterpart of pairs of crayfish studied. Smaller yabbies were more responsive to predator odour than larger ones; this could be interpreted to reflect their vulnerability to predation relative to size. The correlation between size and prey selection in crayfish has been demonstrated by Stein (1977). There are other factors that also influence the response of crayfish of disparate size to visual and chemical cues including diurnal rhythms, seasonal change and reproductive state; these are outside the scope of the current study but are examined in other chapters.

6.4.3.4.1 Shelter selection by crayfish under threat from predators

Crayfish possess a highly developed ability to avoid threat, i.e. from predators and cannibalism. This capability had been widely investigated and been shown to vary between crayfish species (Gellar 1982, Dix & Hamilton 1993, Hazlett 1996, 1999, 2002). Courtenay (2001) distinguished this adaptive response behaviour as instinctual rather than a learned survival tactic. Behavioural change by crayfish in response to stimuli had been argued by Hazlett (1995) to depend on learned associations. Courtenay (2001) built on these findings by demonstrating that expression of adaptive change behaviour disappeared over time but re-emerged in later generations in response to novel stimuli. Height (2005) concluded that this adaptive ability of yabbies is a major

driver of this species' ability to thrive compared to other congeners, and appears to be grounded in an exceptional capacity to perceive, interpret and to react to stimuli to survive. This type of learning behaviour was termed by Lee (2002) as "behavioural plasticity". This was clearly demonstrated in the present study. For example, when exposed to predator odour, all sizes and combinations of pairs of yabbies altered shelter selection in favour of relatively smaller, apparently more secure shelter.

6.4.3.4.2 Shelter selection by crayfish under threat from conspecifics

When under threat from conspecific cues, yabbies in the present experiment responded by reducing active time in general, also referred to as "foraging" by many authors (Rahel & Stein 1988, Brown *et al.* 1999, Hazlett & McLay 2000, Bouwma & Hazlett 2001). Smaller yabbies tended to react and respond more consistently than larger crayfish. In this experiment, this behaviour has been clearly distinguished through comparison of various combinations of small, medium and large yabbies where smaller yabbies reduced time outside shelter and spent more time in the more secure (relatively smaller diameter) shelters.

6.4.3.4.3 Interaction of conspecifics

<u>Avoidance of conspecific threat</u>

Verhoef and Austin (1999) suggested that, under most situations the use of shelter is unnecessary for intensive indoor rearing of juvenile yabbies. In contrast, in a polyculture environment, young crayfish have been reported to deplete stored energy faster, become less tolerant to starvation than larger crayfish, and thereby suffer increased susceptibility to predatory interactions (Svensson 1993). Furthermore, juveniles moult more frequently than older crayfish (Merrick & Lambert (1991), Mills *et al.* (1994), Timmermans *et al.* (1995), and Wheatley (1995), thereby also exposing them to predation (Svensson 1993). According to Blake and Hart (1995), smaller crayfish (*Pacifastacu leniusculus*) spent more time in macrophyte as shelter when fish predators (*Perca fluviatilis* and *Esox lucuis*) were present. These authors reported that predators vary in success of predatory behaviour; for example, perch were found to be more effective hunters of crayfish than eel. Effective use of shelter by crayfish clearly also influences the success of predation by other species. Likewise, as shown in the present experiment, effective use of shelter when under conspecific threat also appears

to reduce the risk and incidence of cannibalism. Results in smaller yabbies showed reactions to larger conspecifics were similar to their reaction to predators in that they spent more time in shelter and reduced foraging activity. In this regard, providing size-appropriate shelter could increase survival.

Shared shelter and cannibalism

When yabbies were paired or grouped, a few animals were seen to share shelter during the observation periods. However, some pairing resulted in conflict such as cannibalism. There were 12 recorded incidents in which large yabbies cannibalised either the paired medium or small yabbies even though all crayfish in this experiment were intermoulted, hard-shelled and therefore theoretically not at a vulnerable stage. Given that shelter can increase display of territorial aggression, this level of cannibalism by larger yabbies can be nonetheless seen as highly aggressive. Cannibalism by larger conspecifics has been reported and is discussed in Chapters 7 and 9. Maternal cannibalism was studied by Little (1976) who reported that maternal hormones persisted while the young remained attached to pleopods. However, within a few days after the detachment of larvae, or if larvae numbered less than 10, Little (1976) discovered that females began to eat their young. The present study shows that larger yabbies also have a propensity to cannibalise on juveniles or relatively smaller conspecifics.

6.5 Key findings

1. Yabbies showed an ability to respond to predator odour by reducing their activity time.
2. There were significant changes in shelter selection by yabbies after exposure to predator odour.
3. Study of shelter competition should take into account preference for shelter according to size of crayfish under investigation.
4. The greater plasticity of yabbies enhances their likelihood of success as invaders.
5. Yabbies avoided interaction with larger conspecifics by selecting shelter that afforded maximum protection (i.e. the smallest possible size of shelter).
6. Yabbies always chose the smallest size of shelter when exposed to predator odour.

7. Small and medium size yabbies reduced their active time significantly when exposed to predator odours.

8. Conspecifics and predation affected the behaviour responses of yabbies.

Chapter seven

Combined effect of temperature and shelter availability on survival of famished juvenile yabbies

Cannibalism remains one of the major causes of stock losses in crustacean aquaculture. This occurs mainly during periods of asynchronous ecdysis when post-moult crayfish are vulnerable to cannibalism by conspecific intermoults, particularly when food and shelter are scarce. Shelter is an important factor in providing a safe refuge for crayfish, thereby increasing survival. Water temperature is the key factor controlling metabolic rate and the moulting cycle and growth. Frequent moulting and exposure to predators leads to higher vulnerability. This experiment investigates the effect of temperature on the survival rate of juvenile yabbies with and without shelter

7.1 Introduction

Temperature is the most important abiotic factor governing many activities of crayfish (Nyström 2002). It impacts on mobility, foraging activity, growth such as moulting, distribution, behaviour and survival rate (Westin & Gydemo 1988, Vahoef & Austin 1999). Temperature also triggers the reproductive system in many freshwater crayfish. Suitable ranges of temperature induce mating. For instance, *Astacus astacus* require at least 3 months of temperatures in excess of 15 °C for successful reproduction (Abrahamsson 1972). Temperature tolerance and optimal limits have been reported for several crayfish species (Nyström 2002). Each species has a range of preference. Yabbies can tolerate a temperature range between 1-35°C but the optimum level is 22-30°C (Frost 1975, Semple *et al.* 1995).

Starvation is another factor affecting the frequency of cannibalism in crayfish populations (Hazlett 2003, Kestemont *et al. 2003,* Stenroth & Nyström 2003). Food restriction or food scarcity is the most important ecological factor inducing aggressive behaviour and driving cannibalism (Elgar & Crespi 1992, Thomas *et al.* 2003). It is well documented that cannibalism occurs in both natural populations and crayfish aquaculture (Goddard 1988). In redclaw (*Cherax quadricarinatus*) culture, low survival rates were observed when sizes were different and minimal shelter was provided. Vohoef & Austin (1999) suggest that when providing adequate food with uniforms-sized juvenile yabbies, there was no significant difference in terms of survival rate between sheltered and non-sheltered. However, Crear *et al.* (2000) revealed that shelter plays an important role in reducing cannibalism. Holdich (2002) suggests that very few studies have been conducted relating the frequency of cannibalism to shelter availability and other factors affecting cannibalistic behaviour.

Yabbies can survive with a low level of supplementary foods. In addition, survival rates are better in lower temperatures; although growth rate is not high the cannibalism rate is significantly reduced (Barki *et al.* 2001). However, there was no data on levels of cannibalism at different levels of temperature without food supply (Holdich 2002). This Chapter examines the effect of temperature on famished yabby survival, with and without shelter.

7.2 Materials and methods

7.2.1 Site and culture system

This experiment was conducted at the Curtin Aquatic Research Laboratories (CARL), Curtin University of Technology, Western Australia as described in Chapter 3. Eighteen 300L blue plastic circular tanks fitted to a recirculation system were used (Plate 7.1). When filled to the designated level, each tank contained 300L of water. Water was circulated at a flow rate of 2.8cm/second giving an hourly filtration and recirculation of the entire 300L of water. Prior to the start of this experiment, the system was operated and tested for a period of 6 weeks until the bio-filter performed fully as measured by an absence of nitrite (NO_2) (Boyd 1994). Each tank was aerated continuously. Twenty shelters made from 60mm lengths of 25mm PVC pipe were used in the shelter treatment. Heaters and cooling fans controlled ambient temperature. The sumps contained submersible heaters, which maintained the desired temperature. A single 100w bulb was fitted for the room and set to provide 12 hours of simulated natural light for the duration of the experiment.

Plate 7.1 300L blue tanks used in yabby temperature and shelter trial

7.2.2 Experimental animals

Juvenile yabbies (2.01±0.8g) were sourced from berried female crayfish (*Cherax albidus*) spawned in two circular concrete tanks of 5m diameter that were part of a recirculation system at a commercial hatchery in Parkerville, Western Australia (as described in Chapter 3). The animals were fed regularly with a commercial crayfish pellet (Glen Forrest Stockfeeders). Juvenile yabbies were harvested and transferred to CARL in May 2005.

7.2.3 Experimental design

Twenty juvenile yabbies of mixed sex were placed into the 300L experimental tanks. Three different temperature regimes were used: 20±0.5, 25±0.5 and 30±0.5°C. These temperature levels were selected to encompass the range considered optimal for growth and survival of yabbies. Yabbies at each temperature level were monitored for eight weeks, both with and without shelter, and each regime was replicated three times. The stocking density was 12 yabbies per m^2 and the average initial yabby weight is shown in Table 7.1. Yabbies were not fed during the experiment to create a famished state so that the level of cannibalism could be investigated.

Table 7.1 Treatment allocation

Treatment	Shelter	Temperature (ºC)	Initial wt. of yabby (g)	Sampling
T 1	No	20	1.97± 0.04	Weekly
T 2	Yes	20	1.95±0.01	Weekly
T 3	No	25	2.05±0.03	Weekly
T 4	Yes	25	2.08±0.04	Weekly
T 5	No	30	2.11±0.05	Weekly
T 6	Yes	30	2.11±0.06	Weekly

Note: shelter made of 25mm PVC pipe100mm in length

7.2.4 Data collection

Yabbies were sampled and tanks cleaned weekly. Yabbies were inventoried and survival rates calculated from the total number of live animals remaining. Yabbies from each tank were weighed in total and the average weight per tank calculated; it was

deemed too stressful to include measurement of carapace length or gender in this experiment for yabbies of this size. Gender was considered unlikely to influence the incidence of cannibalism in juveniles due to immature claw size. Any carcases, gastroliths or exuvia were removed weekly. Records from the data sheets were compiled and transferred to a spreadsheet.

7.2.5 Water quality

Water quality parameters including ammonia, nitrite, nitrate and pH were monitored weekly prior to sampling using commercial test kits (Aquarium Pharmaceutical Inc.). Aquarium thermometers were used to monitor water temperature daily.

7.2.6 Statistical analysis

Treatment comparisons of the different temperature levels were analysed using replicated analysis of variance (ANOVA). Post hoc comparisons were performed when there were significance differences. Student's T-test was used to compare mean survival rate and average weights.

7.3 Results

7.3.1 Water quality and temperature

Analyses of temperature and water quality parameters were very consistent between tanks and over time for the duration of the experiment. No significant differences between treatments or sources of samples were observed for any of the parameters tested. Although the water quality in the holding tank and experimental tanks was marginally different, there were no significant differences across treatments. Results were therefore combined and are summarised in Table 7.2.

Table 7.2 Summary of water quality and temperature measurements

Source	Parameter	Treatment		
		20ºC	**25ºC**	**30ºC**
Holding tank	Temperature (ºC)	20.22±0.15	25.22±0.15	30.44±0.18
	Total ammonia (mg/L)	0.12±0.04a	0.16±0.04a	0.18±0.04a
	Nitrite (mg/L)	0.13±0.04a	0.13±0.04a	0.11±0.04a
	Nitrate (mg/L)	0.23±0.04a	0.26±0.02a	0.22±0.04a
	pH	7.80±0.02a	7.92±0.04b	8.02±0.04c
Experimental tanks	Temperature(ºC)	19.67±0.24	24.89±0.11	30.11±0.11
	Total ammonia (mg/L)	0.18±0.04a	0.16±0.04a	0.16±0.04a
	Nitrite (mg/L)	0.13±0.04a	0.13±0.04a	0.11±0.04a
	Nitrate (mg/L)	0.23±0.04a	0.26±0.02a	0.22±0.04a
	pH	7.79±0.03a	7.92±0.04b	8.04±0.05c

Note: ANOVA was used for comparison of weekly results of water quality tests of each tank. Different letters between treatments indicate significantly different means (α =0.05).

7.3.2 Survival rate

Results of weekly sampling show that yabbies held at the three different temperature levels had significantly different survival rates ($P<0.01$). Survival rates at the end of the 8-week period were also significantly different ($P<0.01$). In the 20ºC treatment, survival rates were higher than both the 25ºC and the 30ºC treatments. In the 30ºC treatment, comparison of survival rates of yabbies at different temperatures through weekly sampling for 8 weeks found mortality was total by the seventh week of the experiment in some replicates as shown in Table 7.3.

Table 7.3 Survival rate of yabbies by week

Temperatur		Sampling Interval (weeks)							
ºC	Shelter	1	2	3	4	5	6	7	8
20	No	100±0.0[a]	98.3±1.6[b]	96.6±3.3[a]	95.0±2.9[a]	93.3±4.4[a]	93.3±4.4[a]	93.3±4.4[a]	93.3±4.4[a]
	Yes	100±0.0[a]	100±0.0[a]	100±0.0[a]	95.0±2.8[a]	93.3±4.4[a]	91.6±6.0[a]	90.0±5.7[a]	88.3±4.4[a]
25	No	96.7±1.7[a]	93.3±1.7[c]	88.3±1.7[b]	85.0±2.9[b]	81.7±6.0[b]	78.3±7.3[a]	66.7±7.3[b]	63.3±6.0[b]
	Yes	95.0±5.0	85.0±2.9[d]	78.3±3.3[b]	75.0±2.9[b]	70.0±5.0[b]	66.7±6.0[a]	65.0±5.0[b]	55.0±5.0[b]
30	No	95.0±0.0[b]	78.3±6.7[d]	61.7±10.	48.3±9.3[c]	40.0±10.	35.0±10.	10.0±2.9[c]	1.7±1.7[c]
	Yes	90.0±2.9[c]	71.7±1.7[e]	56.7±6.0[c]	45.0±5.0[c]	38.3±8.8[c]	31.7±10.	8.3±4.4[c]	0.0±0.0[c]

Note: Value %±S.E. Different letters between treatments indicate significantly different means (α =0.05)

7.3.3 Relationship between temperature and survival rate of yabbies

Temperature had a significant impact on the survival rate of famished yabbies. Higher temperature increased the mortality rate. The highest mortality rate occurred in the 30ºC treatment equivalent to a rate of 12.24% and 12.90% mortality per week with and without shelter, respectively. The second highest mortality occurred in the 25ºC treatment with 5.00% and 4.76% mortality per week, with and without shelter. The lowest mortality rate occurred in the lowest temperature treatment of 20ºC, which resulted in 1.88% and 0.99% morality per week with and without shelter, respectively. The temperature and survival rate have a linear, negative relationship; namely, decreased survival of yabbies is a direct function of increasing temperature within the range of temperature investigated. The relationship between temperature and survival of yabbies is shown in Figure 7.1.
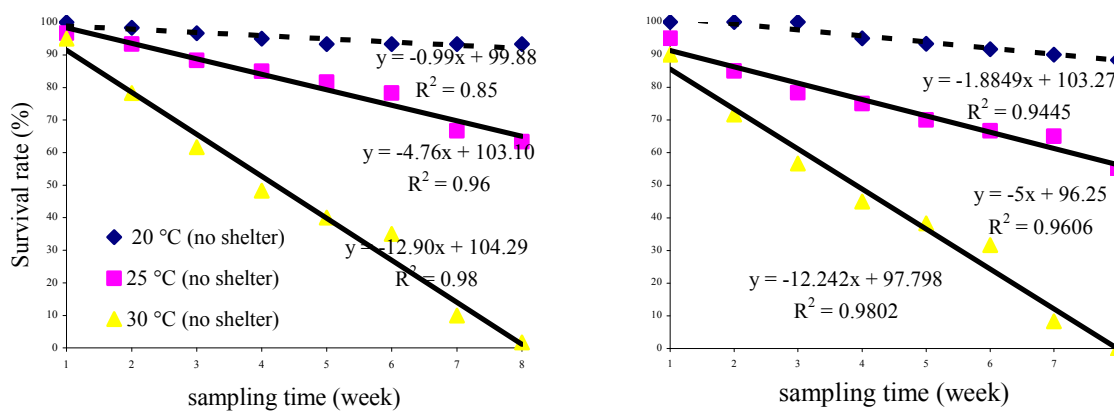
Figure 7.1 Temperature *versus* survival rate for yabbies with and without shelter

7.3.4 Average weight of yabbies

After sorting and weighing, yabbies were randomly allocated to experimental tanks. However, subsequent statistical analysis revealed a significant difference in initial weight between the three treatment groups (P<0.05). For this reason, final weights were not included in the comparison; weight effects were analysed on the basis of trend of weight gained during the experiment.

Final average weight gains were 0.18g in the 20ºC treatment, 0.16g in the 25ºC treatment, and 0.49 g in the 30ºC treatment. These are summarised in Table 7.4. Yabbies in the 30ºC treatment had significantly higher final weights and standard deviation of weight than the other two treatment groups.

All yabbies in all treatments gained weight over the course of the experiment. There was no difference in percentage of weight gained in the 20ºC and 25ºC treatments, but yabbies in the 30ºC treatment did gain a significantly higher percentage of weight than either of the other treatments. Figure 7.2 shows changes in weight (±s.e.) of yabbies over the course of the experiment.

Table 7.4 Summary of the effect of temperature on growth in relation to survival and final weight of juvenile yabbies at the end of the 8-week trial period

Temperature (°C)	N	Initial weight (g)	Survival (%)	Final weight (g)
20	6	1.98±0.02 [c]	90.83±3.52 [c]	2.14±0.02 [c]
25	6	2.05±0.03 [b]	59.17± 3.96 [b]	2.22±0.06 [b]
30	6	2.11±0.04[a]	0.83±0.833 [a]	2.60±0.15 [a]

Values with the same superscript are not significantly different (α =0.05). Final weight for 30ºC treatment recorded at 7th week due to mortality
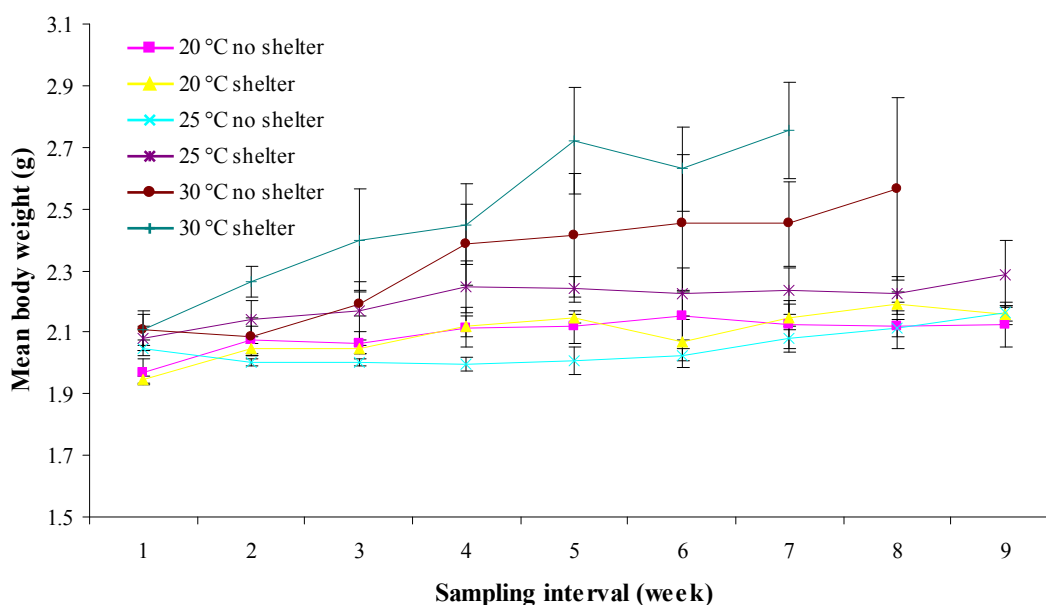
Figure 7.2 Average weights of yabbies at different water temperatures and according to shelter provision (week 1 is initial weight)

Temperature effects on growth, survival and the final weight of juvenile yabbies at the end of this experiment are shown in Table 7.4. There was an inverse relationship between survival rate and final weight. Provision of shelter did not affect survival rate or final weight in this experiment. Results are summarised in Table 7.5.

Table 7.5 Summary of the effect of shelter availability on survival and final weight of juvenile yabbies at the end of the 8-week trial period

Shelter	N	Initial biomass (g)	Survival (%)	Final biomass (g)
No	9	40.84 ± 0.10^a	52.8 ± 26.98^a	68.03 ± 34.20^a
Yes	9	40.88 ± 0.51^a	47.8 ± 25.75^a	63.23 ± 33.53^a

Values with the same superscript are not significantly different ($\alpha = 0.05$).

7.3.5 Effect of starvation on cannibalism and survival

Crayfish in this experiment were provided with neither supplementary nor natural food. The mechanical bio-filter retained all organic matter in the recirculation system thus continuously interrupting nutrient flow through the tanks. The only means of survival was therefore cannibalism. While yabbies in this experiment were deprived of food, they were provided with temperatures within the published optimum range for yabbies.

There was evidence that cannibalism occurred from the outset of the experiment. Gastroliths from moulting yabbies were found in the 25ºC and 30ºC treatment tanks, indicating that some yabbies were eaten during moulting. Towards the end of the trial some intact carcases were found in trial tanks indicating that yabbies died of starvation but were not consumed by others. By this stage, some smaller juveniles exhibited only limited movement at sampling, indicating weakness. Smaller yabbies also showed signs of injury such as loss of antennae and chelae, presumably due to attack from larger contemporaries. Surviving yabbies tended to be larger than carcases removed at weekly sampling. A summary of these findings is shown in Table 7.6.

Table 7.6 Summary of overall findings of weekly sampling by treatment

Temperature (°C)	Shelter	Gastroliths (%)	Carcases (%)	Appendage loss (%)
20	Yes	10.00±5.00[a]	1.67±1.67[a]	6.67±1.67[a]
	No	16.67±4.41[a]	0.00±0.00[a]	5.00±2.89[a]
25	Yes	43.33±4.41[b]	1.67±1.67[a]	11.67±1.67[b]
	No	35.00±5.77[b]	1.67±1.67[a]	8.33±1.67[ab]
30	Yes	76.67±4.41[c]	23.33±4.41[b]	6.67±1.67[a]
	No	83.33±4.41[c]	16.67±4.41[b]	3.33±1.67[a]

Different letters between columns indicate significantly different means (α =0.05). Values are expressed as totals ± S.E.

Based on the remaining inventory and collection of gastroliths and intact carcases, cannibalism accounted for 10.00±5.00% to 16.67±4.41%, 43.33±4.41 to 35.00±5.77% and 76.67±4.41 to 83.33±4.41 % of mortality in the 20, 25 and 30°C treatments, with and without shelter, respectively. Overall, starvation accounted for 16.67±4.41% to 23.33±4.41% of deaths. Although carcases were found in the 20 and 30°C treatments, these were soft and it is assumed they died after moulting.

7.3.6 Effect of shelter on cannibalism and survival

There was no significant difference in survival rates between the treatments with or without shelter (P>0.05). Shelter availability also did not influence growth of yabbies during the 8-week experiment. There was no relationship between temperature and shelter usage in this experiment. The results are summarised in Table 7.5.

7.4 Discussion

7.4.1 Impact of temperature on yabby survival

It is clear that temperature is the most important abiotic factor influencing the distribution of crayfish species (Campbell 1993). Increasing temperature increases metabolic rate and growth rates in poikilothermic species. Fish and crayfish growth rates are governed by water temperature (Paterson & Spanoghe 1997). In crayfish ecosystems, increasing temperature can produce more frequent moulting and therefore increases vulnerability to predation (Stein & Magnuson 1976; Butler & Stein 1985), mortality and incidence of cannibalism (Boulay *et al.* 1993). Moulting is a crucial factor influencing vulnerability to cannibalism (Barki *et al.* 1997). Cannibalism is common in crayfish when food is limited (Hazlett *et al.* 2000).

It has been shown that while growth rate of yabbies improves in higher temperatures, the survival rate drops even when food is not limited (Verhoef & Austin 1999). Yabbies reared in high temperature environments are more likely to be active and fight; increased activity can result in weakness if food is limited (Hird *et al.* 1986). When food is scarce, it has been shown that crayfish display more aggression and fight to gain food and shelter (Usio 2001). Stocking crayfish at high density also increases physical contact with one another (Spanier *et al.* 1998). Increased physical interaction can disrupt social structure and dominance hierarchies among stocked species such as fish (Ajuzie 1988), crustaceans (Winston & Jacobson 1978) and crayfish (Jones 1990). When considering the findings of these authors collectively, it is clear that temperature influences survival in several ways and its effect can be magnified by environmental factors in the wild, or husbandry in artificial rearing.

Yabbies (*C. destructor*) can tolerate an extraordinarily wide range of temperatures from 1-35ºC (Semple *et al.* 1995). Within the range of temperature considered optimal (22-30ºC), Frost (1975) demonstrated that yabbies can maintain a normal metabolic rate and, when required, can conserve some essential amino acids in tail muscle during periods of starvation (Hird *et al.* 1986). Verhoef & Austin (1999) found that maximum yield of juvenile yabbies occurred between 25 and 28ºC, but at 28ºC mortality increased to 43%. In the present experiment, yabbies were subjected to temperatures considered to encompass the optimal range, but without food. It has been shown that food is the most

important limiting factor giving rise to cannibalism (Boulay *et al.* 1993, Hird *et al.* 1986, Hazlett *et al.* 2000, Hazlett 2003).

In the present study, higher temperatures increased mortality significantly, which agrees with the findings of Boulay *et al.* (1993), Verhoef & Austin (1999), and others. Temperature and survival rates were shown to have a direct, linear relationship. Other factors such as stocking density are likely to have an additive effect, which is supported by the findings of Morrissy (1995). Survival was greatest and the incidence of cannibalism was less at the lowest temperature, namely, 19.7±0.2°C. This temperature is slightly below the range currently considered optimal for farming yabbies.

7.4.2 Starvation

Many studies have confirmed that yabbies are omnivorous and can utilise various foodstuffs (Momot *et al.* 1978, Hill & Lodge 1994, Parkyn *et al.* 1997, Duffy, 2006). Studies of stomach contents have revealed a diverse diet of detritus, algae, macrophytes, invertebrates including crayfish, fish and fish eggs (Capelli 1980; Westman *et al.* 1986). Juvenile crayfish have been shown to prefer feeding on macro-invertebrates and other invertebrate families such as Ephemeroptera, Plecoptera, Simulidae and Chiromonidae, compared to adults, which prefer detritus (Stenroth 2003). This fits with the requirements of juveniles for a higher protein diet, and implies that juveniles are more successful predators of smaller prey than adults. Larger crayfish are able to crush hard-shell molluscs for consumption (Nyström & Pérez 1998). Momot (1995) found that crayfish selectively switch mode of feeding in response to competition, predation and cannibalism. There remains some disagreement over preferred modes of feeding of crayfish and their adaptation to various habits. Finally, although many studies have investigated feeding behaviour of *Cherax destructor,* few studies have been carried out with *C. albidus.*

In pond aquaculture without supplementary feed, crayfish can survive and grow on existing alternative sources such as algae, organic matter and periphyton (Azim 2005; Duffy 2006). The object of the present study was to create a state of complete starvation so that cannibalism remained the only potential source of food. Results of water quality analysis confirmed that the mechanical and bio-filtration apparatus successfully removed all organic substrates continuously throughout the experiment.

In the present study, starvation related mortalities occurred from Week 5 of the experiments, indicated by finding and removing intact carcases at weekly sampling. These carcases were not consumed by others and showed little sign of injury. These findings fit with current understanding of juvenile feeding behaviour where cannibalism of others may occur when the carapace is soft (during or after moulting), but that immature claw size and function precludes predation and consumption of other hard-shelled invertebrates including conspecifics.

Starvation as a proportion of total mortality was greatest at 30±0.5°C. This agrees with the work of Paterson & Spanoghe (1997) and others who investigated the effect of temperature and osmoregulation, metabolic rates, stress rates and survival of crustaceans. In the present study, yabbies survived for up to 8 weeks without food, but mortality was almost 100% in the highest temperature treatment. This is more than double the mortality reported by Verhoef & Austin (1999) of *C. destructor* reared at 28ºC when food was not limiting. These finding are consistent with those of Collins & Anderson (1999) who concluded that the absence of food in combination with prolonged starvation at higher temperature has a clearly negative impact on animal conditions. It should be noted that the longest survival reported for wild yabbies was 6 months (Lawrence and Morrissy 2000). This implies that when food is scarce, yabbies may possess a competitive advantage over other species under the range of temperatures investigated in the present study.

7.4.3 Cannibalism

Cannibalism can occur in terrestrial and aquatic ecosystems under a range of circumstances. Population density, competition for food, starvation and relative body size are the main factors generally regarded to govern the frequency of cannibalism in natural populations (Dong & Polis 1992). Of these, availability of food is the most important factor favouring cannibalism (Elgar & Crespi 1992). Cannibalism is believed to play a role in population control relative to available resources, thus it is often observed in dense crayfish populations (Abrahamsson 1966). Cannibalism may play a unique role in crayfish ecology by supplementing or replenishing reserves of the essential amino acid arginine, for which yabbies have a high requirement, but only limited ability to synthesize (Hird *et al.* 1986).

In general usage of the term, cannibalism is presumed to mean conspecific cannibalism. However, in crayfish ecosystems, interspecific predation is common and is regarded as a form of cannibalism (Holdich & Domaniewski 1995). Holdich & Domaniewski found that some crayfish species may cannibalise others, but not their own species. The existence and significance of this behaviour is supported by results reported in Chapter 5 with predation between resident and invader yabbies and marron of disparate sizes. The main concern regarding such interspecies behaviour is the competitive advantage that may confer to the more aggressive population, and the potential consequences for the less aggressive species, particularly when food is scarce.

Yabbies are vulnerable to predation and cannibalism during moulting when the carapace is soft. During moulting, crayfish will abandon shelter in favour of open areas while the exuvia is being sloughed, and also to avoid conspecific cannibalism while the new post moult shell hardens (Whisson 2003 Pers. comm..). This is also commonly observed in yabbies (Wangpen, unpublished). However, pheromones are released during moulting (Hazlett 2000) which may signal opportunistic cannibalism where food is limited (Turvey & Merrick 1997). Given the vulnerability of crayfish to cannibalism during moulting, synchrony of moulting in artificial rearing can have a profound impact on crayfish survival. Crayfish of similar size, as was the case in the present study, would normally moult synchronously and thereby reduce or limit cannibalism (Barki *et al.* 1997; Ranta & Linströem 1993). The effect of starvation on the synchronicity of moulting is not known. Investigation of the synchrony of moulting as a contributing factor fell outside of the scope of the current experiment.

The present study was designed to promote the expression of cannibalism through starvation and to study the effect of temperature and provision of shelter on survival. Presence of gastroliths at the bottom of the experimental tanks during sampling was considered to be evidence that cannibalism had taken place. Cannibalism occurred from the outset and was recorded in all treatments. However, mortality attributed to cannibalism was significantly greater in the two higher temperature treatments, namely 25±0.5 and 30±0.5°C. Smaller yabbies in some tanks also showed signs of injury, presumed to indicate increased aggression and result from predation by larger contemporaries. Cannibalism as a contributor to total mortality was greatest at 30±0.5°C and accounted for 76.67±4.41 to 83.33±4.41% of the deaths in this treatment. This

agrees with the work of Verhoef & Austin (1999) and others who investigated metabolic rates, growth rates and survival with increased temperature. Regardless of the degree of starvation, temperature clearly influences the expression of cannibalism, and therefore survival in yabbies. The present study showed that at 30±0.5°C cannibalism was insufficient to sustain life for longer than 8 weeks.

7.4.4 Weight gain in yabbies

As stated in Section 7.3.1 regarding the role of temperature in crayfish survival, many researchers have investigated the influence of temperature on metabolism and growth. In the present study, the weekly average weights of surviving yabbies in all treatments reflected continuous gain for the duration of the experiment. Final weights were greater at the highest temperature, but this result was confounded by significantly different initial weights. Nonetheless, yabbies in the 30°C treatment also gained a significantly higher percentage of weight over the course of the experiment. Experimental design ensured that any gain beyond existing body reserves at the onset of the experiment could only have been due to cannibalism.

7.4.5 Effect of shelter availability on survival of yabbies

Provision of shelter in this experiment did not affect survival of famished juvenile yabbies at any of the treatment temperatures investigated. Since food and optimum temperature are the primary factors that govern crayfish survival (Milstein 1992, Barki 2001), the results of the influence of shelter in the absence of food are not surprising. Shelter would likely play an important role if food was limited to the point of competition, but not limited to extremes of starvation. Crear *et al.* (2000) found that shelter and substrate as moulting aids availability did not prevent cannibalism in spiny lobster, and concluded that the absence of food together with high temperature was primarily responsible for growth and mortalities in that study.

7.5 Key findings

1. Temperatures used in this experiment were considered to be near optimal, yet resulted in significantly different growth and survival rates. In the face of starvation, the greatest biomass was produced at 20°C (39.63 ± 2.08g and 38.07 ± 2.20g) with a survival rate of 90.83±3.52%.

2. The results of this study showed a direct linear relationship between temperature and survival between 20°C and 30°C.

3. Mortality of famished juvenile yabbies was nearly 100% at 30°C.

4. In this experiment, the 20°C treatment resulted in the greatest survival of juveniles. This lies slightly below the currently recommended rearing temperature range for yabbies. However, this cooler temperature falls within the preferred range for marron and points to the capacity of yabbies to survive well under some similar environmental conditions.

5. Where food is limited, provision of shelter did not enhance survival.

Chapter eight

Effect of spatial and temporal change on marron shelter preference

*This Chapter examines the shelter, selection and shelter
preference of different sized marron exposed to silver perch
odours. Results from this experiment will provide insight into
behavioural plasticity, which will assist aquaculturists
and natural resource managers in understanding the risks
to marron in both aquaculture and the wild.*

8.1 Introduction

Crayfish diurnal activity patterns and shelter utilisation are affected by temporal and spatial variation (Ratchford 2000). These variations that affect crayfish distribution have been studied by many researchers (Jordan *et al.* 1996, Barki *et al.* 2000, Ratchford 2000, Russel *et al.* 2003 and Killingback *et al.* 2006). The effect of species competition in communal culture has been reported by Barki *et al.* (2000), while others consider it to be a factor contributing to survival of crayfish species (Killingback *et al.* 2006). Lodge *et al.* (1998) expressed concerns about spatial distribution, particularly the effect of exotic species on the abundance of native species. In polysystems there are many factors controlling spatial distribution, biomass and production. The issue of most concern is how spatial distribution is affected by exotic predatory species.

The presence of predators and conspecifics impacts significantly on species abundance (Glinski 1984) and crayfish distribution (Jordan *et al.* 1996). Jordan *et al.* (1996) explained that red swamp crayfish (*Procambarus clarkii*) respond to predators by choosing to remain in the vegetative habitat, and by increasing time spent in non-vegetated habitats at night. Provision of complex habitat has been shown to significantly reduced cannibalism and predation rates thereby increasing crayfish survival. In the previous chapters, yabbies were shown to react differently to predator odour and conspecifics; however, there was no information about marron spatial distribution and activity when confronted with silver perch odour.

8.2 Materials and Methods

8.2.1 Location and systems

Experiments were conducted at CARL, Curtin University of Technology, Technology Park, Bentley Campus, described in Chapter 3. Fifty-four aquariums were used for the experimental series, each for a period of 24 hours. Each bundle of shelters used in this experiment was comprised of 25, 50 and 90 mm PVC pipe (described in Chapter 6).

8.2.2 Experimental design

Two trials were conducted. In the first trial, marron of different sizes were observed in a normal temporal cycle with predator odour and control. The second trial was the same

except that the normal day and night cycle was reversed. This is summarised in Table 8.1.

Table 8.1 Treatment allocation of temporal trials 1 and 2

Treatment	Marron size	Trial 1 :Normal temporal		Trial 2: Reversed temporal	
		Block A	**Block B**	**Block A**	**Block B**
1	SMM	Control	Predator	Predator	Control
2	SFM	Predator	Control	Control	Predator
3	MMM	Control	Predator	Predator	Control
4	MFM	Predator	Control	Control	Predator
5	LMM	Control	Predator	Predator	Control
6	LFM	Predator	Control	Control	Predator

Note: SMM=small male marron, SFM=small female marron, MMM=medium male marron, MFM=medium female marron, LMM=large male marron, LFM=large female marron.

8.2.3 Experimental animals

8.2.3.1 Marron (*Cherax tenuimanus)*

All marron used in the experiment were selected from one breeding pond from a commercial farm in Pemberton, Western Australia. The average weights of male and female marron were: small - 3.79±0.24g and 3.64±0.18g; medium - 1.84±1.15g and 44.80±2.09g; and large - 174.41±4.47g and 178.29±5.62g. Carapace lengths were measured from the base of antennae to the end of the rostrum and were 26.41±0.57 and 26.09±0.58, 52.29±0.64 and 53.22±0.92, 84.80±0.83 and 86.73±0.83mm, respectively. The three different sizes of male and female marron were randomly placed individually in the aquaria in the two different temporal experiments.

Large marron were purged in 5000L recirculating holding tanks. Small and medium marron were purged in 300L tanks using a recirculation system similar to that described in Chapter 7. Holding tanks contained several shelters, and were aerated for a period of 6 weeks prior to the experiment for acclimation. Marron were then placed into the experimental aquaria, each equipped with a shelter bundle and aeration, depending on the experimental design.

8.2.3.2 Silver perch (*Bidyanus bidyanus*)

Fish odour was collected using silver perch of various sizes, ranging from 68-201g. These fish originated from CARL, located at Technology Park, Curtin University of Technology, Western Australia. Silver perch were housed in a 300L static system for 2 hours prior to the commencement of the experiment. No feed was provided during the 24 hour holding period prior to the experiment. Fish odour was prepared according to Hazlett (1994) from a density of 5 kg fish /100L water.

8.2.4 Experimental procedure

This trial was conducted in an aquarium-based lab facility where temporal alteration could be controlled. Fifty-four aquariums were divided into 2 blocks to minimise variability (Table 8.1). Aquariums were provided with a shelter bundle, filled with freshwater and then aerated continuously for 24 hours. Marron were placed into aquariums the following day and were allowed one hour to select a shelter before recording commenced. Marron in the normal temporal cycle were placed into the experimental units between 1200 and 1300, while marron in the reversed temporal cycle treatment were placed between 2200 and 2300. The silver perch odour was prepared when the water level in the finfish predator tank was lowered to the concentration of 5 kg/100L. Test solutions were added into the aquariums in the same way as described in Chapter 6. The first recording time began at 1400 of a normal day and night cycle, and then every hour for 24 hours. At the end of the first recording period, 80% of water in aquariums was exchanged. Crayfish were rested for an hour before the next recording period commenced. Test solutions were reversed compared to the first recording period. At the end of the first experiment, silver perch were returned to the rearing tank and marron were returned to the recirculation system where they were prepared for the second trial. This second trial was subjected to altered day: night cycles. The day light cycle for the room was reversed such that the same regime of 12h: 12h dark: light was established for 2 weeks prior to the start of the second experiment. The marron were fed every alternate day using crayfish pellets. Procedure, recording and predator solution were the same as that in the first experiment; however, marron in this treatment were exposed to the opposite daylight cycle for 2 weeks prior to the commencement of the observations.

8.2.5 Data collection

The size of shelter that marron occupied was recorded on the recording sheets (described in Chapter 6). Raw data was tallied into a spreadsheet (Excel™ Microsoft Corporation) before being converted into a database for statistical analysis. Extra hard copies were produced and worksheets were saved on CD Rom for backup.

8.2.6 Water quality

Water quality parameters were tested using test kits for the most common variables, including ammonia, nitrite, nitrate and pH. An aquarium thermometer was placed in the tank to monitor the temperature.

8.2.7 Statistical analysis

Treatment comparisons between the use of different shelters and different temporal cycles were analysed using the one-way analysis of variance (ANOVA). Post Hoc analysis (Tukey HSD) was used when results were significantly different ($P<0.05$). The non-parametric analysis Mann-Whitney U-Test was used to compare treatment means.

8.3 Results

8.3.1 Marron activity and the effect of temporal change

8.3.1.1 Time spent outside shelter

Marron were observed outside shelters in both the normal and reversed day:night trial. Total time spent outside shelters was categorised as either mobile or non-mobile. Different size, gender, day:night regime, control solution and predator odour were compared using one-way analysis of variance (ANOVA). Results of hourly observations for 24 hours showed that there were no significant differences in behaviour during either the normal or reversed day cycle. The preferred time spent outside shelters by marron of all sizes was night, in both the control solution or under the influence of predator odour $df(7)$ f=1.076 P=03.78. A summary of overall time marron spent outside shelter in all these combinations is presented in Table 8.2.

Table 8.2 Mean percentage of time marron spent outside of shelter during the day and night after exposure to control solution or predator odour

Temporal change	Day		Night	
	Control	Predator	Control	Predator
Normal day: night	$_1$41.36±5.20^a	$_1$43.98±5.20^a	$_1$44.75±4.72^a	$_1$47.99±5.26^a
Reversed dark: light	$_1$44.14±4.67^a	$_1$52.78±4.94^a	$_1$41.82±4.50^a	$_1$55.40±5.01^a

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences at p<0.05.

Details of the time spent outside shelter for each size of marron during the normal and reversed temporal cycles are shown in Figures 8.1, 8.2 and 8.3, respectively. Statistical analysis showed significantly different time spent outside shelter in small male, small female and medium female marron: *df*(7) f=2.142 P=0.051, *df*(7) f=2.509 P=0.024 and *df*(7) f=2.215 P=0.044, respectively. However, no significant differences were found among either medium male or large male and female marron (P>0.05).
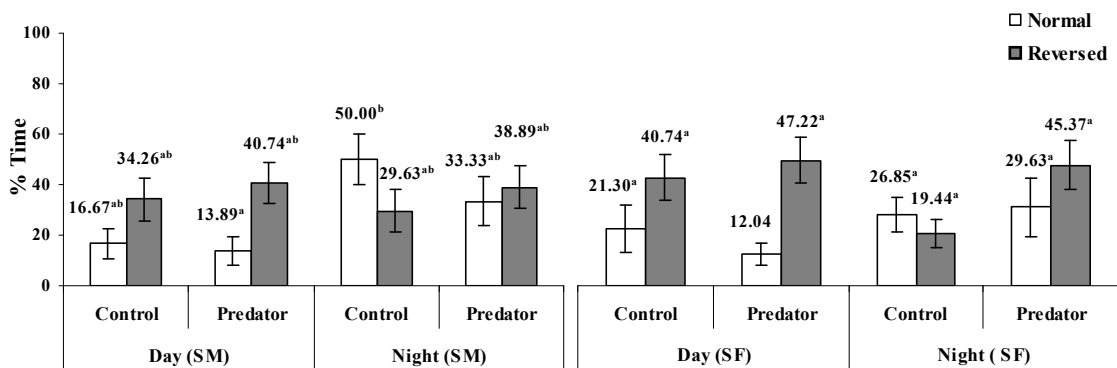
Figure 8.1 Total time small male (SM) and small female marron (SF) spent outside shelter
Note: different letters indicate significant differences (α =0.06), in reversed day=dark, night=light.
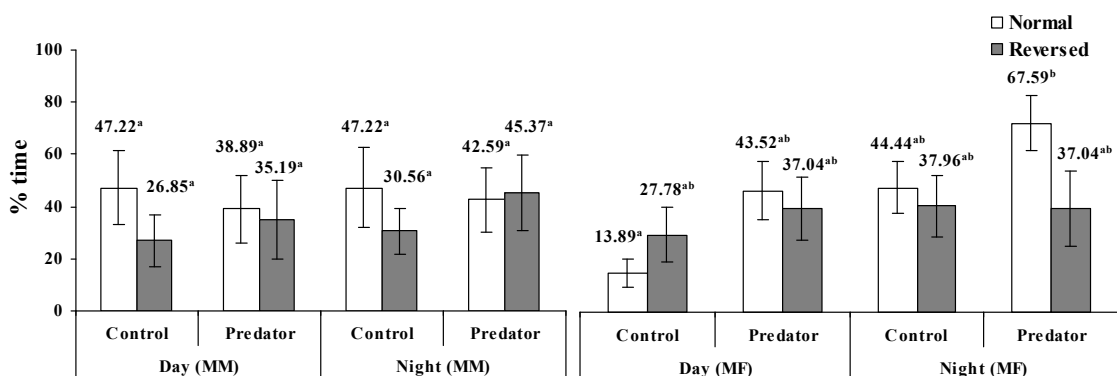
Figure 8.2 Total time medium sized marron spent outside shelter
Note: different letters indicate significant differences (α =0.06), in reversed day=dark, night=light.
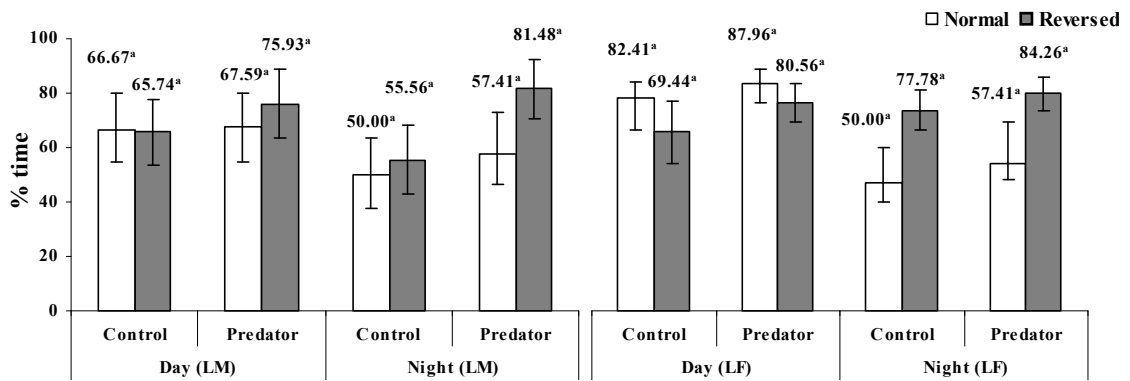
Figure 8.3 Total time large sized marron spent outside shelter
Note: different letters indicate significant differences (α =0.06), in reversed day=dark, night=light.

8.3.1.2 Active, non-active, mobile and non-mobile time spent by marron

In addition to time spent outside shelter, mobile and non-mobile time spent by marron were compared. Results for different sizes of marron during normal and reversed day:night cycles with test solutions were compared. These results are presented in Figures 8.4, 8.5 and 8.6.

8.3.1.2.1 Total mobile time of small marron

There were significant differences in the patterns of mobile time for small male versus small female marron during the day and night when exposed to different test solutions: *df*(7) f=6.821 P=0.000 and *df*(7) f=7.436 P=0.000, respectively. Males and females in general were observed to spend similar percentages of mobile time during the normal temporal cycles when exposed to predator odour (P<0.05). Small males exposed to control solution showed significantly more mobile time at night than during the day (P<0.05). However, small females showed no significant differences in this regard (P>0.05). When both genders were subjected to temporal change, they showed significant differences in active time between day and night (P<0.05) compared to the normal diurnal cycle. However, there were no significant differences in mobile time when they received either control or predator odour (P<0.05). Results are shown in Figure 8.4.
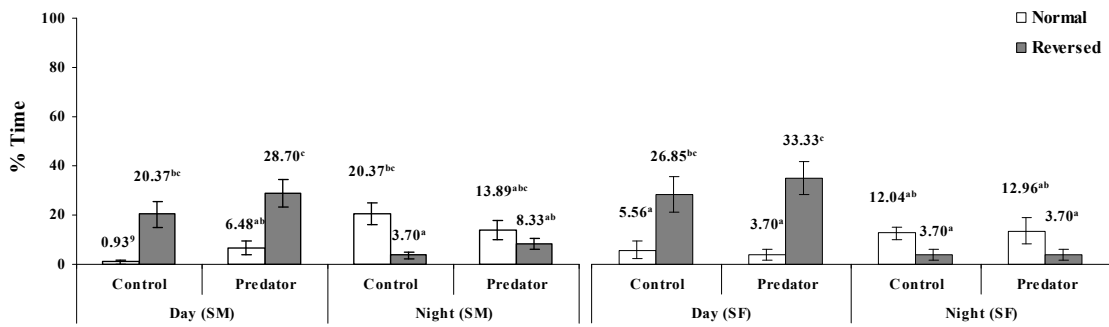
Figure 8.4 Total mobile time of small male marron (SM) and small female marron (SF)
Note: different letters indicate significant differences (α =0.05). in reversed day=dark, night=light.

8.3.1.2.2 Total mobile time of medium marron

No significant differences in percentage of mobile time were found in medium males (P=0.928). However, there were significant differences in the mobile time of medium females *df*(7) f=2.215 P=0.044. No movement by medium females receiving control solution during the day in the normal temporal cycle was recorded; this was significantly different to medium females in the reversed temporal cycle (P<0.05). In contrast, during the normal night, female marron exposed to predator odour showed a higher degree of mobility than marron receiving the control solution in the reversed temporal cycle (P<0.05). Results are shown in Figure 8.5.
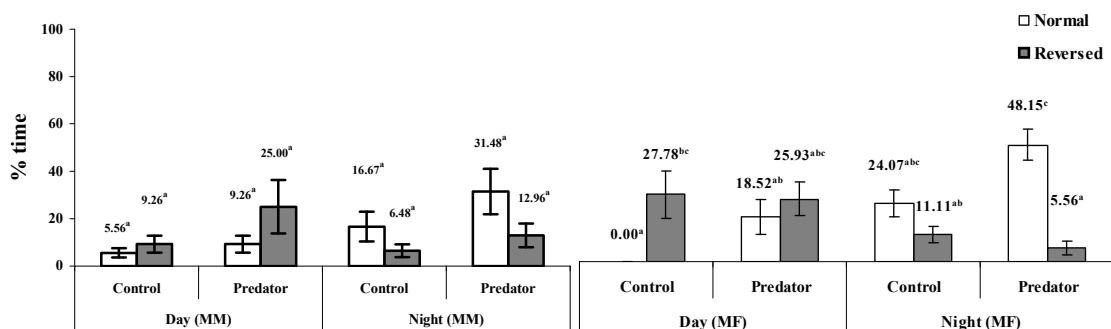
Figure 8.5 Total mobile time of medium male marron (MM) and medium female marron (MF)
Note: different letters indicate significant differences (α =0.05). in reversed day=dark, night=light.

8.3.1.2.3 Total mobile time of large marron

There were significant differences in the percentage of mobile time in both large male and large female marron: *df*(7) f=3.347 P=0.004, and *df*(7) f=3.944 P=0.001, respectively. When compared within gender, there was no significant difference in mobile time of large males in the normal temporal cycle (P>0.05). However, in the reversed cycle, large males exposed to predator odour were significantly more mobile than during the normal day:night cycle (P<0.05).

Large female marron showed patterns similar to large males; when exposed to predator odour they were highly active during the reversed day. However, differences in mobile time during the normal and the reversed temporal cycles were not significant (P>0.05). Results are shown in Figure 8.6.
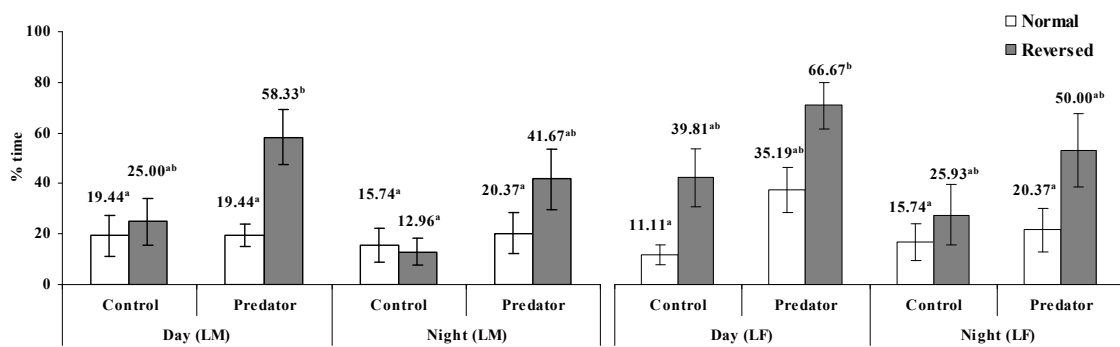
Figure 8.6 Total mobile time of large male marron (LM) and large female marron (LF)
Note: different letters indicate significant differences (α =0.05). in reversed day=dark, night=light.

8.3.2 Effect of temporal change and predator odour on shelter use in marron

Average percentages of time spent in shelter and outside shelter (active) were compared. The results were expressed as percentage of observation time, with control or predator odour solution, during the normal temporal and reversed temporal cycles. Small male and female marron show patterns similar to medium marron. However, large marron show the opposite: when exposed to both control and predator odour, they spent significantly more time outside shelter. Results are summarised in Table 8.3.

Table 8.3 Average time different sized marron spent inside and outside shelter

Temporal	Test solution	Dark : Light	% Time								
			In shelter			Outside shelter			Mobile		
			Small	Med	Large	Small	Med	Large	Small	Med	Large
Normal	Control	Day	$_{12}53.4^{a}$	$_{1}45.0^{a}$	$_{1}31.6^{a}$	$_{12}32.8^{a}$	$_{1}38.2^{a}$	$_{1}54.8^{a}$	$_{234}13.8^{a}$	$_{12}16.8^{a}$	$_{12}13.6^{a}$
		Night	$_{23}78.6^{c}$	$_{1}68.1^{bc}$	$_{1}21.9^{ab}$	$_{12}18.3^{ab}$	$_{1}29.3^{abc}$	$_{1}65.0^{bc}$	$_{3}3.1^{a}$	$_{12}2.6^{a}$	$_{1}13.1^{a}$
	Predator	Day	$_{123}60.4^{b}$	$_{1}32.8^{ab}$	$_{1}25.8^{ab}$	$_{12}27.8^{ab}$	$_{1}39.0^{ab}$	$_{1}57.6^{b}$	$_{123}11.8^{a}$	$_{12}28.2^{ab}$	$_{12}16.6^{a}$
		Night	$_{3}82.9^{f}$	$_{1}51.8^{cd}$	$_{1}18.0^{ab}$	$_{1}12.3^{a}$	$_{1}36.2^{bc}$	$_{1}60.8^{df}$	$_{12}4.8^{a}$	$_{12}12.1^{a}$	$_{123}21.2^{ab}$
Reversed	Control	Dark	$_{12}50.7^{c}$	$_{1}66.0^{d}$	$_{1}24.6^{b}$	$_{12}30.3^{b}$	$_{1}24.8^{b}$	$_{1}51.1^{c}$	$_{34}19.1^{ab}$	$_{12}9.2^{a}$	$_{123}24.2^{b}$
		Light	$_{23}72.7^{d}$	$_{1}60.5^{cd}$	$_{1}28.5^{ab}$	$_{12}23.7^{a}$	$_{1}31.4^{ab}$	$_{1}55.5^{bcd}$	$_{123}3.6^{a}$	$_{12}8.0^{a}$	$_{12}16.0^{a}$
	Predator	Dark	$_{1}42.8^{fg}$	$_{1}50.9^{g}$	$_{1}13.4^{a}$	$_{12}33.5^{cd}$	$_{1}28.8^{bc}$	$_{1}48.1^{g}$	$_{4}23.6^{b}$	$_{12}20.3^{ab}$	$_{3}38.4^{ef}$
		Light	$_{123}54.5^{cd}$	$_{1}54.0^{cd}$	$_{1}11.8^{a}$	$_{2}39.8^{bc}$	$_{1}37.6^{b}$	$_{1}56.8^{d}$	$_{12}5.6^{a}$	$_{12}8.4^{a}$	$_{23}31.4^{b}$

Note: Different letters between rows indicate significant differences within rows, and different numbers between columns indicate significant differences at $p<0.05$.

8.3.3 Effect of temporal change and predator odour on shelter selection

Shelter selection of marron in each size group exposed to test solutions and following the normal day: night cycle was compared. Results revealed that shelter selection differed between size groups. No significant differences between genders and the reversed day and night cycle were observed. Patterns of shelter selection with respect to normal day:night cycles and control:predator odours were similar in the paired figures compared between males and females. Results of observations from all sizes of marron are shown in Figures 8.7 to 8.12.
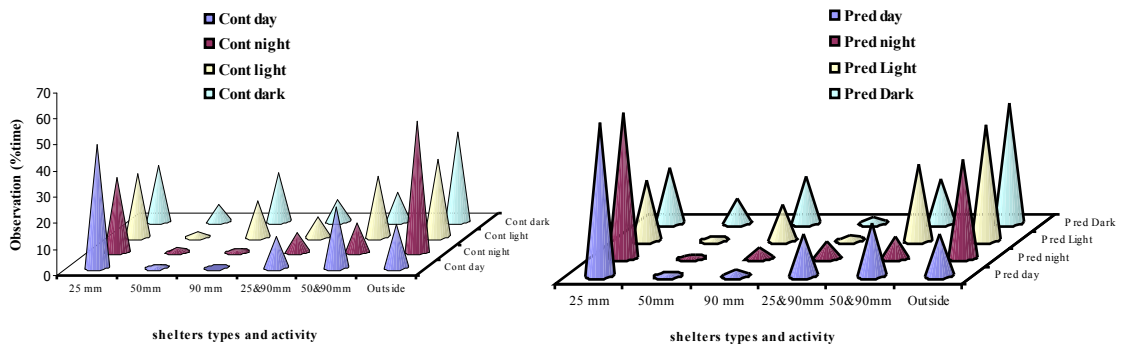
Figure 8.7 Shelter selection pattern of small male marron
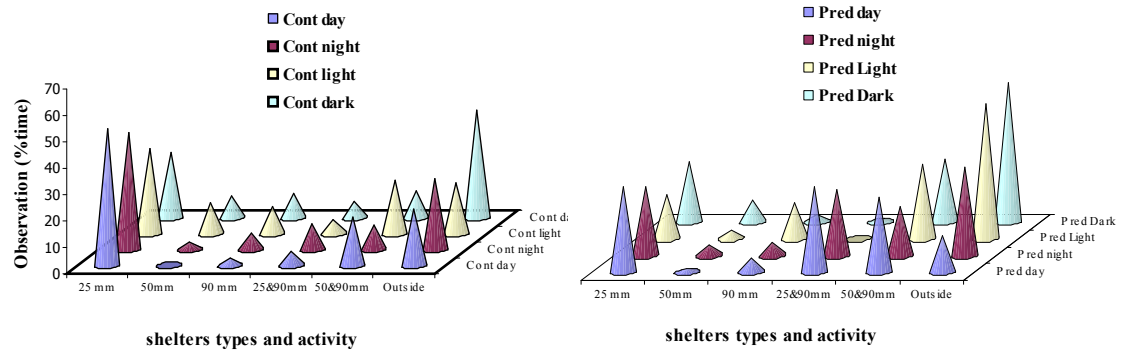
Figure 8.8 Shelter selection pattern of small female marron
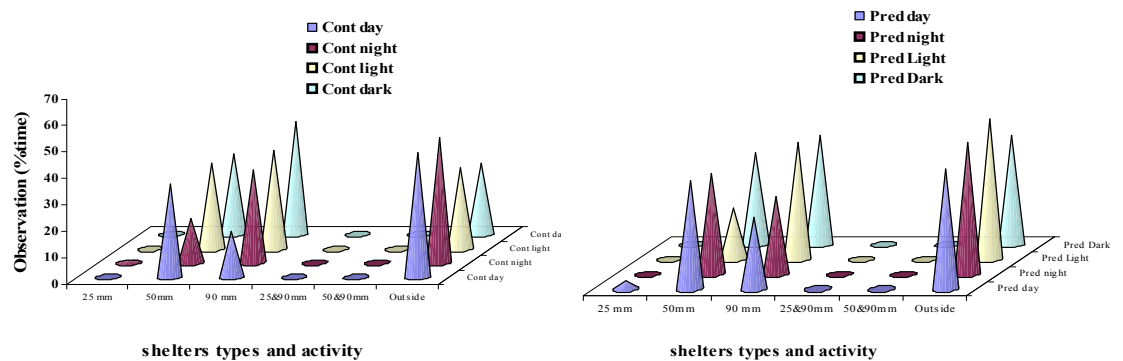
Figure 8.9 Shelter selection pattern of medium male marron
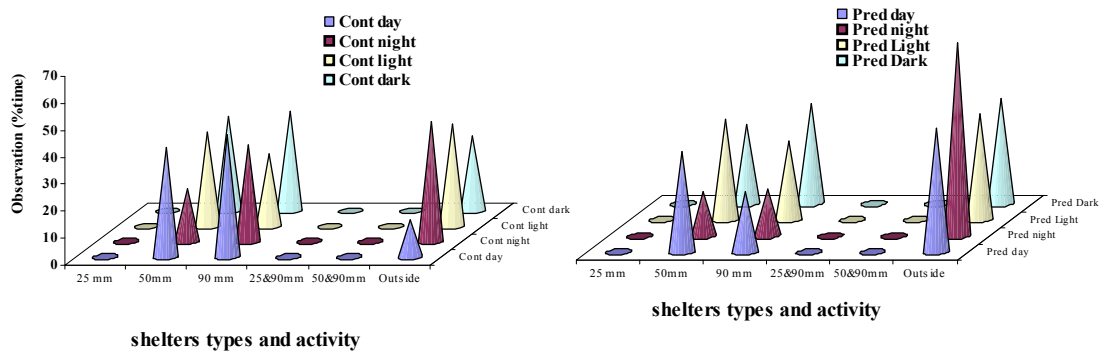
Figure 8.10 Shelter selection pattern of medium female marron
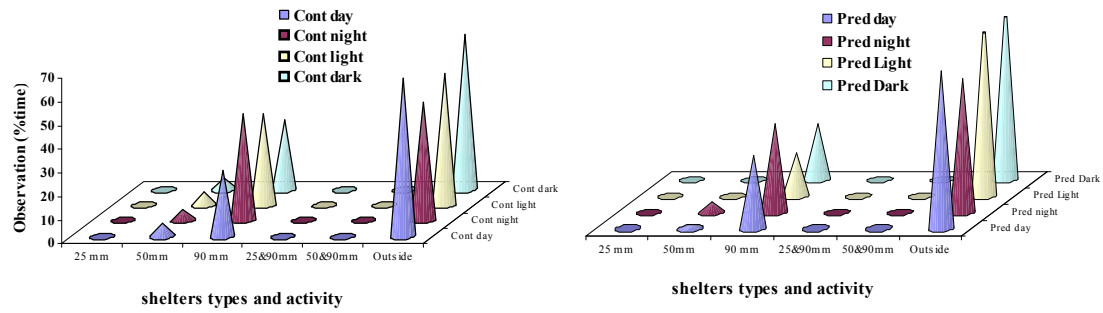
Figure 8.11 Shelter selection pattern of large male marron
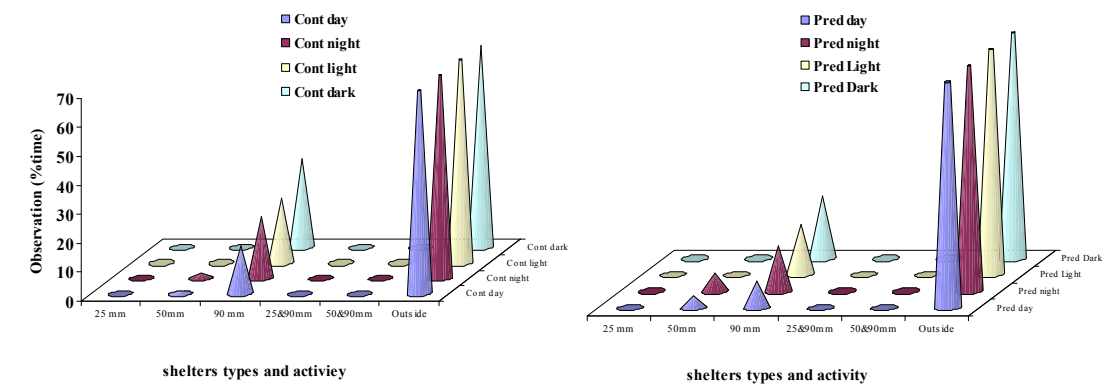
Figure 8.12 Shelter selection pattern of large female marron

8.4 Discussion

8.4.1 Effect of temporal change

Crayfish interact with the surrounding environment in response to many factors: marron are no exception to this. Both abiotic and biotic elements such as temporal change, predation and conspecific interaction affect the behavioural responses of marron directly and indirectly. The influence of environmental factors has been examined in many other species, vertebrate and invertebrate. Gherardi (2002) commented that little research has been conducted on the impact of spatial and temporal change on crayfish and should be explored. In the present experiment, the effect of temporal change on activity of marron was investigated together with the presence of control solution and predator odour. Results show that, in general, marron failed to alter their response to either test solution. According to previous research reported in Chapter 5, marron were unable to discern finfish odour, other chemical stimuli, cobbler (*Tandanus bostocki)* or redfin perch (*Perca fluviatilis,* Height 2002), and conspecific alarm odour (Gherardi *et al* 2002). Marron did show some response when presented with visual cues together with strong chemical cues (Storer 2006). Nonetheless, no significant response of marron to finfish predator odour was observed in the present experiment.

In general, marron are similar to other crayfish with respect to high activity at night with either control solution or when exposed to predator odour. Marron normally leave shelter at dusk to forage, similar to Noble crayfish (*Astacus pallipes,* Barbaresi & Gherardi 2001) and return to shelter before dawn. However, Kanciruk and Herrnkind (1973) reported that this pattern is affected by temperature, and day-length cycle (Herrnkind *et al*. 1975). Lower temperatures were found to reduce the activity of *A. pallipes* activity even at night time (Barbaresi & Gherardi 2001). Alteration of diurnal patterns has been shown to affect foraging activity of other crustaceans such as marine lobster (*Panulirus argus,* Ratchford & Eggleston 2000). In the present experiment, the pattern of diurnal active time of marron was similar to that reported by Barbaresi & Gherardi (2001) at 20 ºC where in the normal temporal cycle, marron were observed to show some degree of response to predator odour. However, these responses were not statistically significant differences. Observations of the diurnal rhythm of small male marron with respect to activity is depicted in Figure 8.13. Understanding the effects of temporal change holds promise in assisting aquaculturists to plan management

strategies to harness the synergy between finfish and crayfish in polyculture (Barki *et al* 2001).
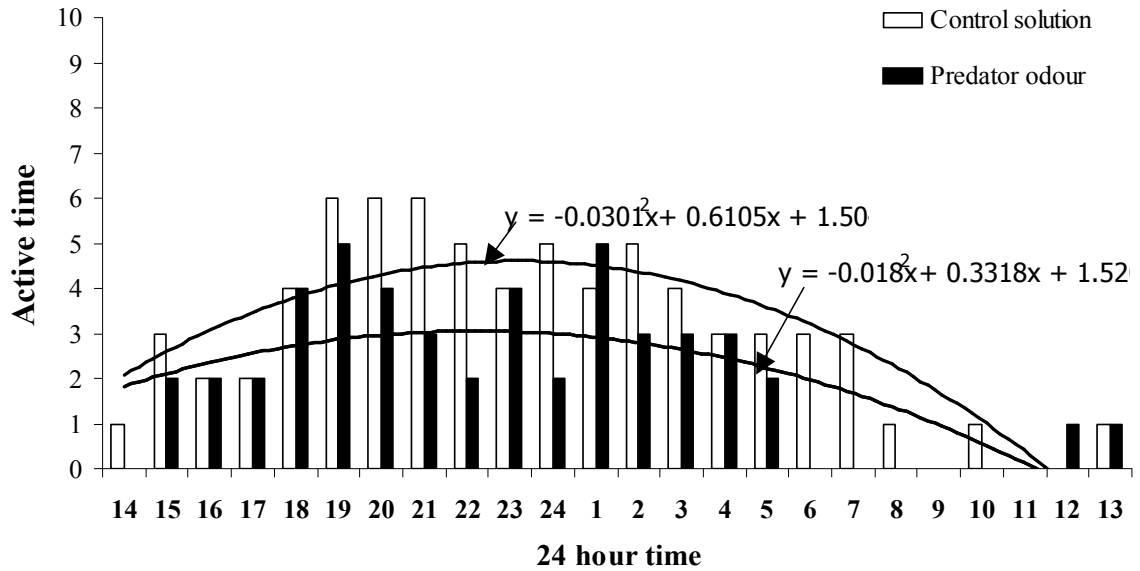
Figure 8.13 Total active time of small male marron in the normal temporal cycle

In contrast to activity during the normal day:night cycle, it was observed that these patterns were disrupted by the reversal of day and night as depicted in Figure 8.14.
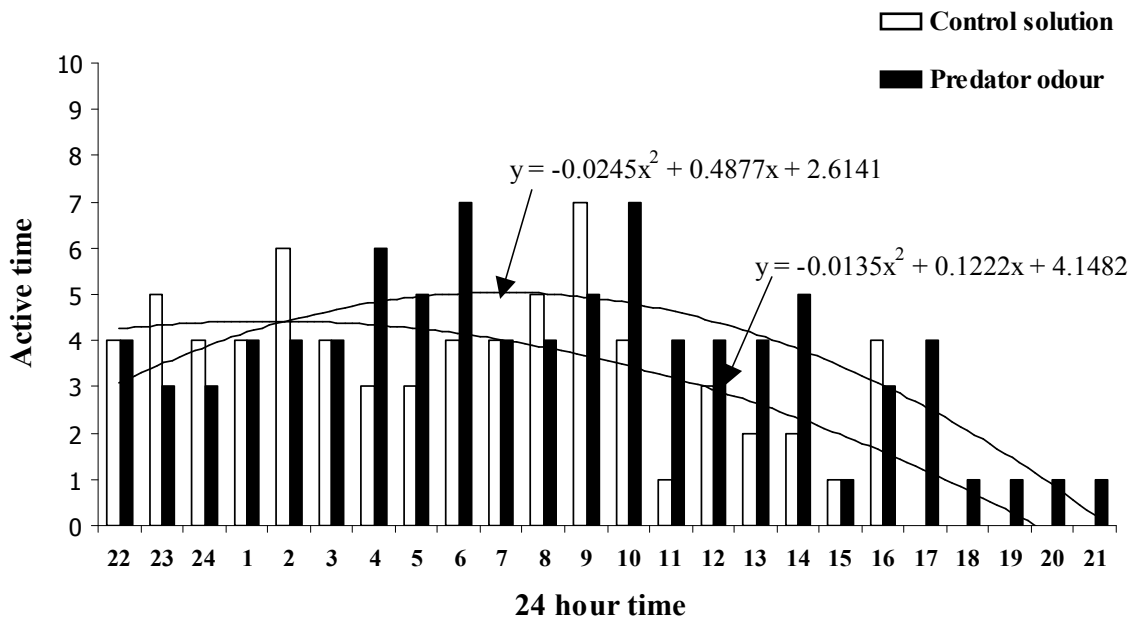
Figure 8.14 Total active time of small male marron in the reversed temporal cycle (0700 to1800 =dark, 1900 to 0600 = light). Note that the x-axis now reflects the reversed cycle.

The effect of temporal change on activity patterns of crayfish was clearly demonstrated for marron in the present experiment. While normal activity was maintained during the normal temporal cycle, behaviour was altered significantly in the reversed cycle even though marron were acclimatised to the new temporal pattern for two weeks before observations were commenced. In the normal temporal cycle group, marron were placed into the experimental units between 1200 and 1300, whereas marron in the reversed treatment were placed between 2200 and 2300. Both groups were allowed an hour to adjust to the experimental units before observation began. Observed active time outside shelter did not show a clear response to predator odour under either temporal cycle even though activity was clearly altered for marron in the reversed day:night treatment. This suggests that the magnitude of response to temporal change was far greater than the expected response to predator odour. The reversal of day and night greatly affected the bio-rhythm of marron in this study.

It should be noted that although marron were acclimatised to the reversed day:night cycle for two weeks prior to the experiment, only an hour was allocated to acclimate to the experimental unit before observation and recordings commenced for both treatment groups. The one hour acclimatisation period following transfer appeared sufficient for the normal temporal group, but not for the reversed temporal group. Furthermore, the two week acclimatization period for the reversed temporal group to adjust to the altered day:night cycle appeared insufficient. Marron in the reversed treatment were active from the start, especially larger marron, even though time of placement into the aquaria corresponded to mid-day of the light/daytime period for this group, as for the normal temporal cycle treatment group. Activity is normally limited during daytime, as was observed for the normal temporal group in spite of transfer to the experimental aquaria. This suggests that change in temporal and spatial circumstances results in stress for marron, and that these two stressors may be additive, as illustrated by the increased activity overall and disrupted pattern of activity following placement into the experimental aquaria. Furthermore, as depicted in Figures 8.13 and 8.14, marron in the reversed temporal group were also more reactive to predator odour. This supports the notion that temporal change plus transfer to the experimental aquaria was stressful, and that the stressors were additive. This effect appeared to be greater for larger marron. These results indicate that temporal and spatial factors can disrupt normal behaviour, likely through stress. These findings are relevant to commercial rearing of marron:

altered behaviour observed in the present study would likely increase vulnerability of marron to predators (i.e. increased activity and reduction in shelter use) and increased susceptibility to disease (i.e. through increased activity, and stress).

Crayfish subjected to various stressors have been reported to show disrupted physiological responses. Fotedar *et al* (2006) found that stress through exposure to inland saltwater reduced the capacity for osmoregulation resulting in a longer recovery time for marron compared to yabbies. Farrell & Leonard (2000) also showed that frequent handling of crayfish affected growth performance. The present study likewise shows that stress from spatial and temporal change results in altered behaviour.

8.4.2 Mobile and stationary time of marron

Although the pattern of activity altered as a result of temporal change, the total amount of observed active time outside shelter and time in shelter did not change significantly. This section is in regard to the proportion of active time outside shelter that marron spent either mobile or immobile in the different tested situations. Results show that smaller marron altered the degree of mobility when the temporal cycle was reversed. In the normal temporal cycle, marron maintained a normal pattern of reduced mobility during the day and increased during the night. However, with the reversed temporal cycle group, small marron changed their mobility pattern accordingly, as depicted in Figure 8.4. Medium and large marron did not display a clear pattern of mobility as did the small marron. This suggests that temporal change affected marron activity, and that smaller marron were able to adjust to the temporal change significantly better than larger marron.

There was no change in mobility pattern of marron exposed to the chemical stimuli from silver perch. This suggests that marron disregarded the silver perch odour as a threat, which agrees with the observations of Height (2002). Storer (2006) indicated that marron began to react only when alarm odour was released by injured conspecifics after the majority of them had been consumed by Murray cod. Gherardi *et al* (2002) also reported poor or no response to such chemical stimuli by marron and yabbies. These authors also noted that behavioural responses of marron were much slower than those of yabbies in general.

In Chapter 5, it was noted that predator odour in that study may have been insufficient to produce a response in marron and yabbies to chemical cues from finfish predators. The present study therefore set out to determine whether marron would alter behaviour in response to more frequent exposure to silver perch odour. Test solution was therefore added hourly to maintain a higher level of exposure. Results of water quality testing revealed that total ammonia in the control aquaria measured 0.25-0.50 ppm while levels of 2-5 ppm were obtained from aquaria that received predator odour solution. This suggests that a high level of predator odour was achieved: this is supported by the work of Boyd and Tucker (1994) that linked high nitrogenous waste and therefore total ammonia with decomposition of fish by-product. Exposure to levels of ammonia higher than 0.5 ppm can cause stress in marron (Morrissy 1996).

8.4.3 Effect of predator odour on shelter selection

Choice of shelter in this study was investigated in order to compare the shelter preference of yabbies when reared singly or paired, and when exposed to predator odour (Chapter 6). Shelter choice under threat from predators has been reported for several species including *A. astacus* and *Pacifastacus leniusculus* (Tulenen *et al.* 2006), other crustaceans such as spiny lobsters *P. interruptus (*Zimmer-Faust *et al.* 1985), *P. argus* (Ratchford & Eggleston 1998) and *J. edwardsii* (Butler 2006). Furthermore, the presence of predators was also found to affect shelter choice (Tulenen *et al.* 2006). In the present study, marron exposed to silver perch odour showed no significant difference in shelter preference in either the normal or reversed temporal cycle, indicating that choice of shelter by marron was not influenced by predator odour or temporal change.

In nature, habitat selection is often based on the suitability of shelter for successful rearing of progeny (Blake and Hart 1993). Shelter selection is therefore considered to be instinctive (Courtenay 2001, Konikoff 1973). Predator avoidance and shelter seeking behaviour in marron have been shown to be influenced by stimuli such as predator alarm cues; marron were shown to adapt responses to such cues following exposure and experience (Storer 2006). Silver and redfin perch are exotic to the natural habitat of marron; it is not known whether marron used in the present study had prior exposure to these finfish predators or not. This could explain the limited response of marron to predator odour in the present study.

Loss of habitat is a major concern for marron populations in the wild. Knowledge regarding the suitability of habitat for marron is scarce. Currently loss of habitat results from human activity, and from naturally occurring degradation, e.g. flooding and erosion (Flannery 2006). Freshwater crayfish including marron are believed to have evolved from marine lobster species, and are distributed throughout the world (Taylor 2002). However, this natural evolution from marine species has not resulted in the establishment of crayfish in tropical river systems. This could be due to the presence of many varieties of predatory fish and competition from other species.

8.4.4 Implications of the predator response for marron in polyculture

The current notion of marron behaviour is that their limited response to the presence of predators in most circumstances represents a competitive disadvantage. However, this could be to the benefit of intensive polyculture of marron with other species. Whisson (2000) and Storer (2006) indicated that marron and silver perch remain the prime candidates for polyculture. Storer (2006) suggests that such polyculture holds promise of synergistic production if silver perch were stocked in cages. Their chemical cues appear to have little or no impact on growth performance. Furthermore, marron also prefer nocturnal activity which reduces the overlap of trophic effect between species. Simple feeding regimes could be adapted to both species (Blake & Hart 2000). Use of cages for silver perch would shade pond bottoms, thereby reducing water temperature during summer; this could reduce seasonal heat stress of marron. Shelter provision for marron would likely reduce the success of avian predators. In order to maintain the DO level preferred by marron, aeration may be required (Boyd & Tucker, 1994). Results of the present study support the suitability of marron for polyculture with silver perch due to the lack of observed response to high levels of odour from this predator. Marron showed signs of stress in response to spatial and temporal changes in this study; taking such factors into account in the commercial husbandry of crayfish will likely improve production. Further investigation of the nature and interaction of factors contributing to stress in crayfish, particularly marron, is warranted.

8.5 Key findings

1. The presence of predator odour from silver perch did not influence the active time of marron outside shelter.

2. Marron responded to temporal change by altering active behaviour outside shelter.

3. Small marron subjected to temporal change appeared more able to adapt to the experimental environment than did larger marron.

4. Temporal change and exposure to predator odour had no impact on shelter selection.

5. A two week period for acclimatisation to the reversed temporal cycle was inadequate, more so for large marron. Marron were stressed by temporal change and responded with varied behavioural changes.

6. Response to predator odour for marron subjected to temporal change was not significantly different from that of marron in the normal temporal group. However, marron in the temporal change group did alter time in shelter, outside shelter and mobile time variably during the respective night and day time.

Chapter nine

Behavioural aspects of crayfish shelter acquisition under predatory threat

Three consecutive trials were conducted to examine the effect of prior residence and shelter use of yabbies and marron under threat from various finfish predators. The first laboratory trial stocked several combinations of marron and yabbies in aquaria with different sizes of shelter. Each species had its turn as the prior resident with subsequent behaviour monitored. The second trial examined the importance of exposure to predator odour on learning behaviour of yabbies and marron. Finally, the shelter utilisation of crayfish in polysystems was tested in earthen ponds where the effect of complex shelter on the growth and survival of small marron, large female marron and silver perch was examined.

9.1 Introduction

As shown in the previous chapters, both marron and yabbies have displayed behavioural modifications in a wide range of situations. Yabbies, with their higher plasticity, have demonstrated a greater capacity for survival. For example; in Chapter 6, large yabbies left their shelter in search of prey, consuming medium-sized conspecifics; however, this was not observed when predator odour was introduced, displaying an ability to make choices that will increase survival. This intelligence is of concern in terms of potential species displacements in the south-west of Western Australia. The role of shelter (and associated issues like prior residency) may become important for managing competition with native marron, the original inhabitants. In this chapter, we try to explore the role of prior residency using combinations of marron and yabbies as either prior residents or intruders in aquaria. Figler (1999) showed that size of *Procambarus clarkii* was more important than gender or prior residency, with larger crayfish winning shelter over smaller crayfish, even when they were not prior residents. Height *et al.* (2004) reported that larger marron spent significantly more time in shelters than yabbies as either prior residents or intruders; however, the researchers did not include predator odours in this experiment. In both studies there were no reported incidences of cannibalism during shelter competition. In the present trial, I will investigate these interactions further and report on the effect of prior residence on cannibalism. The findings of this trial will paint a clearer picture about the asymmetrical hetero-specificity between marron and yabbies. This knowledge could also be useful in the strategic control of introduced species.

The rate of cannibalism between yabbies in the first trial varied with the presence of intruder crayfish, and in the second trial both yabbies and marron became more vulnerable to predation when exposed to predator odour (Söderbäck 1992). This scenario gives another dimension of avoidance behaviour and shelter utilisation in both species (Hazlett 2000). The role of finfish predators also plays an important role in governing crayfish activity (Blake & Hart 1993). Yabbies have shown to possess greater learning capacity than marron and would survive in these environments (Gherardi *et al* 2002). The complex shelter provided in this trial was expected to be adequate in reducing the predation level.

Marron and silver perch polyculture is an economically viable form of farming (Whisson 1997, 2000, 2006). However, productivity of the system could be higher if the survival rates of crayfish were improved. An understanding of crayfish behaviour around shelter when predator intensity is high will provide valuable insight into the habitat mechanisms that will increase survival in polyculture. The second trial reported in this chapter was conducted in CARL's 80t mesocosm, stocked with a high density of predators including Murray cod and silver perch. The aim of this sub-trial is to monitor shelter usage and associated behaviour of marron and yabbies that have not previously been exposed to predator odour.

In the third sub-trial, predation under free-range polyculture conditions is explored further. This follows a previous study by Whisson (2000) in 27m^2 concrete tanks where the complex aquatic plant, *Vallisneria* sp., clearly improved survival of marron stocked with silver perch. The author made recommendations about how these results could be translated to pond systems, including a discussion about problems with the uncontrolled spread of *Vallisneria* in earthen pond sediments. Therefore, a highly complex artificial habitat would be required to afford the same protection in ponds. The aim of this third sub-trial was to compare growth and survival of marron and silver perch in earthen ponds supplied with and without an abundance of complex artificial shelter. Following Whisson's (2000) study, berried females were stocked with perch to give an understanding of survival of post-release (0+) animals, as well as adults, in the presence of an artificial mesh-shelter that is currently used in the marron industry.

9.2 Materials and methods

9.2.1 Experimental systems

9.2.1.1 Laboratory trial

The trial was conducted in aquarium-based facilities described in Section 3.1.1 located at the Curtin Aquatic Research Laboratories (CARL). Thirty-six aquaria were used for this experiment measuring 30x60x25cm, and holding 40L of freshwater. Three different sizes of shelters were used measuring 50mm, 90mm and 25+50+90mm (diameter of PVC pipes) bundled as a single shelter (as described and used in Chapters 6 and 8). These were placed in the centre of each aquarium as shelters. A single airstone was also

provided for continuous aeration throughout the experiment. The experimental room was described in Chapter 3, and was set to provide artificial light to simulate a normal 12h:12h, day/night ratio.

9.2.1.2 Mesocosm trial

The second trial was conducted in an 80-tonne semi-circular concrete mesocosm of 2.2 metre depth (described in Chapter 3), also located at CARL (Plate 3.7). The bottom of the mesocosm was covered with coarse 10mm gravel to a depth of 25cm. Low pressure pumps drew water continuously from the tank bottom to remove suspended solids. Water then passed through a degassing chamber and biofilter, which removed nitrogenous wastes. For this trial, shelter comprised a bundle of 32 x 90mm PVC pipes and four sets of synthetic marron shelters all placed in a 1-m^2 quadrat. Crayfish were placed into the shelter area via a 4m, 90mm diameter PVC pipe which conveyed crayfish to the shelter without exposure to predators already placed in the tank.

Two underwater CCTV cameras were installed (1/3" DSP Colour Underwater/Night-View Camera, PACOM™). These analogue cameras connected to a video capture unit fitted with a four-slot image enhancement unit (Embedded Technology™) to digitally transform images for electronic storage. A video cassette recorder (VCR, AIWA™) and television set were provided for stand-by and fitted with a timer recording programme in case the main recording system failed.

9.2.1.3 Field trial

This sub-trial was conducted at the Collie Aquafarm in Collie, Western Australia (as described in Section 3.5.1). The system consisted of six grow-out ponds constructed adjacent to an acidified, water-filled mine lake, WO-5H, described by Whisson & Storer (2003) and described in Section 3. The ponds were emptied and left to dry for two weeks prior to the start of this experiment. One tonne of chipped lime (CaCO$_3$) was added to each pond to increase calcium levels, to buffer acidity and to control pH.

9.2.2 Experimental designs

9.2.2.1 Aquarium based experimental design (sub-trial 1)

Two different species of prior residents of disparate size (large marron and small yabbies) were used to examine the influence of shelter size on dominant cannibalism in aquaria provided with one of three different types of shelter (Table 9.1). Both yabbies (n=18) and marron (n=18) were randomly placed in combination with one of each of the three different types of shelter. The prior residents were given 24 hours before intruders of the other species were introduced to each aquarium. The number of live small yabbies was observed every four hours for 24 hours.

Table 9.1 Prior residence and shelter size trial for marron and yabbies in aquarium-based trial

Diameter of shelter (mm)	Number of prior residents and intruders	
	Yabbies	**Marron**
50	6	6
90	6	6
25+50+90 bundle	6	6

9.2.2.2 Mesocosm-based experimental design (sub-trial 2)

A time series design was used to investigate shelter utilisation at certain times under predator threat. The first observation of shelter occupation was recorded by VCR and the second set of observations was recorded by divers. There were four shelter treatments in this trial using the two different methods of observation as described in Table 9.2. Each treatment was replicated three times.

Table 9.2 experimental allocations and shelter complexity test design

Treatment	Predator Exposure	Observation details		
		Description	Time (day)	Observation
T1	Free-range	Yabbies	3	VCR
T2	Free-range	Yabbies+marron	3+3	VCR+HK
T3	Caged	Yabbies+marron	3+3	HK
T4	Caged+free-range	Yabbies+marron+predator	3+3+3	HK

Note: VCR= Video Cassette Recording, HK=divers

9.2.2.3 Field study design (sub-trial 3)

This sub-trial was divided into two treatments to test crayfish survival under polyculture conditions with and without complex artificial shelter. Treatments were randomly allocated among the six ponds to give three replicates of each of the two treatments. Treatment 1 was allocated to ponds 2, 3 and 6 with no shelter, while ponds 1, 4 and 5 were provided with 140 commercial loose-mesh synthetic shelters (Custom Networks Co Ltd™) (Plate 3.6) per pond (approx. 1 per 5 m^2). All ponds were stocked with 100 berried marron and 150 silver perch. At the conclusion of the trial ponds were drained and the remaining perch, and adult and juvenile marron were collected, counted and weighed.

9.2.3 Experimental animals

9.2.3.1 Yabbies (*Cherax albidus)*

Yabbies used in the aquarium and mesocosm trials were sourced from a commercial yabby farm, Cambinata Yabbies, Kukerin, Western Australia (32°S, 116°E). The yabbies were sorted by size and then placed in holding tanks at CARL for several months prior to commencement of this experiment (as described in Chapter 7). Mean yabby weights in the aquarium trial were 13.22 ± 0.42g and the carapace length

averaged 33.57 ± 0.35mm. Yabbies were randomly divided into 2 groups of 18 and placed into one of two 100L holding tanks. The mean body weights for yabbies in the mesocosm trial were 32.26 ± 3.42g and carapace length averaged 52.21 ± 3.01mm. The yabbies were fed using crayfish pellets (Glen Forrest Stockfeeders Pty Ltd) every other day, and once a day during the mesocosm trial.

9.2.3.2 Marron (*Cherax tenuimanus)*

All marron used in aquarium and mesocosm trials were sourced from one breeding pond on a commercial farm in Pemberton, Western Australia. Marron were purged in a 5,000L recirculation holding tank for 5 weeks; the tank contained several shelters. Marron were provided with crayfish pellets (Glen Forrest Stockfeeders Pty Ltd) until three days prior to the beginning of the experiment in order to foster a state of starvation. Thirty-six marron were selected for weighing; these averaged 179.08 ± 2.15g with an average carapace length of 82.86 ± 2.27mm. Eighteen marron (prior residents) were then placed into the aquaria individually according to the experimental design. Remaining marron were placed in one of two 300L recirculation tanks (as described in Section 3). The average weight of marron used in the mesocosm trial was 178.33 ± 12.35g and the average carapace length was 95.36 ± 5.23mm. All tanks and aquaria were aerated continuously throughout this experiment.

All marron used in the pond experiment (sub-trial 3) were selected from a 2-year-old cohort raised at the Collie Aquafarm. Mean body weight was 110g. Marron were harvested and transferred into the holding tanks at the South-West Aquaculture and Environment Centre (SWAEC - as described in Section 3.13) for three weeks prior to the experiment while ponds at Collie Aquafarm were prepared for the trials.

Only berried female marron were used for this trial. To protect eggs while held at SWAEC, berried stock were kept at low densities $(1/m^2)$ in 5 tonne flat-bottom fibreglass tanks. Water quality was kept within optimal limits and the holding system was left in darkness to minimise stress. Thirty individuals were sampled and weighed to assess group average weight. However, these sampled animals were excluded from the experiment itself due to the stress of sampling. The assigned marron were transported back to Collie Aquafarm in 600L fish transporters.

9.2.3.3 Silver perch (*Bidyanus bidyanus*)

Silver perch used in the aquarium and mesocosm trials were provided by CARL. These had been relocated from a commercial farm at Parkerville, and reared in the 80-tonne mesocosm at CARL for two years. Approximately seventy fish ranging in size from 120-2500g were made available for this experiment.

Silver perch used in the field trial were reared at Collie Aquafarm and transported to SWAEC (described in Section 3.2.3) three weeks prior to commencement of the trials. One hundred and fifty silver perch averaging 270.9 ± 61.1g in weight were selected and transported back to Collie Aquafarm to stock each pond.

9.2.3.4 Murray cod *(Maccullochella peelii peelii)*

Eight Murray cod were co-stocked in the Mesocosm with the silver perch and averaged between 7kg in size. The natural spawning season of Murray cod coincided with the timing of this experiment and some males did not forage for food because they remained near recently-laid eggs.

9.2.4 Experimental procedures

9.2.4.1 Aquarium experiment (sub- trial 1)

All aquaria were scrubbed and cleaned as described in Section 3.2. Prior residents of each species were placed into aquaria containing one of each of the three shelter types according to the experimental design (Table 9.1). After 24 hours of acclimation of prior residents, intruders were placed into the designated aquaria. Crayfish were observed for evidence of cannibalism, including physical condition of the smaller yabbies and any appendage loss. Observations commenced at 9:30am and were recorded for the first five minutes, and then for five minutes every four hours for 24 hours. When dead crayfish were observed, the carcases were removed and the aquarium was drained and refilled with freshwater. The surviving crayfish were recorded and returned to holding tanks at the conclusion of the trial.

9.2.4.2 Mesocosm-based trial

The first treatment included 10 yabbies only for a 3-day period. The second treatment included 10 yabbies placed in a 90x90x90cm cage to which 10 marron were added. After three days, the cage was removed and the remaining animals were counted. The third treatment used a 90cm^3 cage covering a bundle of complex shelters. Ten yabbies were placed into the cage in the proximity of the shelter area via a 90mm pipe. Yabbies remained inside the cage for three days, after which 10 large marron were added to the cage for an additional three days. Thereafter, the cage was checked and the remaining number of yabbies and marron were counted. In the fourth treatment, ten yabbies were placed in the cage for three days followed by the addition of ten marron, which were also left for three days, as per Treatment 3. The cage was then removed leaving yabbies and marron behind and the shelter area intact. The crayfish were then exposed to predators for three days. Thereafter, divers were used to count remaining crayfish. Each treatment was replicated three times. A summary of the different treatments is shown in Table 9.2.

Observations were recorded in several ways as summarised in Table 9.2. In the first treatment, observations of shelter occupation were recorded by VCR between 1800h and 1900h daily for three days. The second treatment used VCR for the first 3 days; thereafter divers recorded observations for 3 days after marron were added. Treatment three used 10mm mesh cages which precluded use of VCR equipment to record observations. Divers were therefore used for all observations of shelter occupation by yabbies and marron in this treatment. The last treatment also employed only divers for observation, including those made after removal of the cage. All treatments were replicated three times using the same observation regime.

9.2.4.3 Field trial

9.2.4.3.1 Supplemental feed

Silver perch and marron were fed three times per week between 1600 and 1800 using commercial preparations (described in Appendix 3). Feed intake was determined and then checked by feeding to satiation once per month in each replicate (Table 9.3). Submerged feeding platforms (0.75m under the water surface) were used for feeding

silver perch. Satiation levels were initially determined 30 min. after feeding using observation of general feeding behaviour to determine feeding rate. However, as turbidity increased, feeding platforms were no longer visible, and confirmation of perch feed intake thus became difficult. Feeding was stopped 4 days prior to harvest to reduce gut contents. Marron feeding rates were determined using $1m^2$ mesh (300μm) sinking feeding trays placed in each pond. Trays were monitored after 8 hours; feeding rates to the whole pond were then adjusted accordingly.

Table 9.3 Feeding rate (kg) for marron and silver perch per pond per month

Month	Marron	Silver perch					
	All ponds	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6
December	1.9	10.3	10.1	9.7	10.1	10.3	10.3
January	1.9	10.6	11.2	10.8	10.9	10.9	11.1
February	3.3	7.8	7.8	7.5	7.8	7.8	7.3
March	1.5	7.3	7.3	7.3	7.3	7.3	7.3
Total	8.6	36	36.4	35.3	36.1	36.4	36

Note: Trial duration for December and March was 26 and 22 days, respectively.

9.2.4.3.2 Trial duration

Marron were reintroduced to the ponds on 29 November 2004. Silver perch were introduced three days after marron to allow berried females to acclimatise to conditions and to locate and inhabit shelter. Trials commenced on 5 December 2004 and completed at harvest on 26 March 2005. Duration of the trial was therefore 117 days in total.

9.2.5 Water quality monitoring

Total ammonia, nitrite, pH and temperature were monitored in all three trials. In the laboratory trial, these were tested daily. In the field trial, these parameters were tested and recorded weekly from each pond and were compared by treatment (Appendix 1). In the mesocosm trial, water quality monitoring was conducted daily.

9.2.6 Data collection

Red LED spotlights were used to assist observation and recording in the laboratory trial (sub-trial 1). The number of remaining prior residents and intruders were recorded, as well as condition of the remaining live yabbies (i.e. appendage loss). At the conclusion of the recording period, data was tallied in a spreadsheet and then converted to percentages (Zar 1984) before conducting statistical analysis.

At the end of the first treatment of the trial conducted in the mesocosm (sub-trial 2), video footage was retrieved, viewed and observations of the number of yabbies and marron in shelter and the respective times were transcribed. These were tallied into a spreadsheet. At the end of the 2nd, 3rd, and 4th treatments, the number of remaining crayfish were counted by divers who removed remaining yabbies and marron. Data was recorded by divers into log books, after which data was tallied in a spreadsheet.

In the field trial (sub-trial 3), all ponds were checked weekly for the presence of juvenile marron by raising 10 randomly selected shelters and placing a net underneath. At harvest, remaining stocks of perch, and adult and juvenile marron were counted, individually weighed and measured for total length. General observations of physical condition of marron were also recorded, including fouling and appendage loss.

9.2.7 Statistical analysis

9.2.7.1 Laboratory trial

In the laboratory trial (sub-trial 1), treatment comparisons between size of shelter and combination and type of prior resident with intruder with respect to survival of the small yabbies were analysed using two-factor analysis of variance (ANOVA, Selvanathan *et al*. 2000). When comparing between groups of prior residents, the non-parametric Mann-Whitney U-Test (Ott, 1998) was used. Student's *t*-test was performed to compare the mean of cannibalism between different species of prior resident. Post hoc analysis was performed when results showed a significant difference at P<0.05.

9.2.7.2 Mesocosm-based trial

Treatment comparisons for time spent in shelter for both yabbies and marron in the cage were made using one-way analysis of variance (ANOVA, Selvanathan *et al*. 2000).

When the cannibalism rates of yabbies were compared with that of marron, analysis was conducted using the Mann-Whitney U-Test (Ott 1998). Post hoc analysis was performed when results showed a significant difference at P<0.05.

9.2.7.3 Field trial

Comparisons between treatments were analysed for differences in weight and length using a one-way analysis of variance (ANOVA, Selvanathan *et al*. 2000). Tukey and Scheffe's F-test was performed when results showed a significant difference at P<0.05. Marron and perch weights were compared between treatments using Student's *t*-test. When comparing length:weight ratios between treatments for marron and silver perch, the Independent Sample *t*-test was utilised. The Binomial Test was used to examine differences in survival rates of marron and perch, and to compare numbers of juveniles recovered between treatments. Means of water quality parameters were compared using a one-factor ANOVA and for non-parametric analysis, the Mann-Whitney U-Test (Ott 1998) was used.

9.3 Results

9.3.1 Laboratory experiment (sub-trial 1)

9.3.1.1 Dominance cannibalism of small yabbies by large marron

9.3.1.1.1 Marron as prior residents

Large marron in this experiment cannibalised the smaller yabbies heavily when the marron were prior residents, significantly more than when yabbies were established as the prior resident (*df* (1) f=27 P=0.035). Cannibalism occurred immediately after intruder yabbies were introduced. There were no significant differences in the incidence of cannibalism between different types of shelter (P>0.05). Results are summarised in Table 9.4.

Table 9.4 Incidence of cannibalism with different types of prior resident and shelter

Prior resident types	Shelter Types (mm)	Cannibalism at each observation time (%)						Total Incidence (%)
		1.30pm	5.30pm	9.30pm	1.30am	5.30am	9.30am	
Marron	50	50.0	16.7				16.7	83.3[a]
	90	50.0	16.7		33.3			100.0[a]
	25+50+90	50.0	16.7	16.7				83.3[a]
Yabby	50	16.7	16.7					33.3[a]
	90			16.7	16.7			33.3[a]
	25+50+90		33.33	16.7				50.0[a]

Note: different letters within type of prior resident are significantly different ($\alpha=0.05$).

9.3.1.1.2 Yabbies as prior residents

When yabbies were established as prior residents, the rate of cannibalism was significantly less ($P<0.05$). There were no significant differences in the rate of cannibalism between different types of shelters ($P>0.05$).

9.3.1.2 Effect of shelter on incidence of cannibalism

The provision of shelter did not significantly reduce cannibalism of small yabbies by large marron ($P>0.05$). However, when yabbies were established as prior residents, their survival rate was significantly higher than when marron were prior residents.

9.3.1.3 Cannibalism over time

Dominant cannibalism occurred immediately after intruding yabbies were released into the aquaria of prior resident marron. Time from release to observation that cannibalism had occurred was significantly shorter when yabbies were established as prior residents ($P<0.05$). This observation was consistent across all replicates. Results are depicted in Figure 9.1.
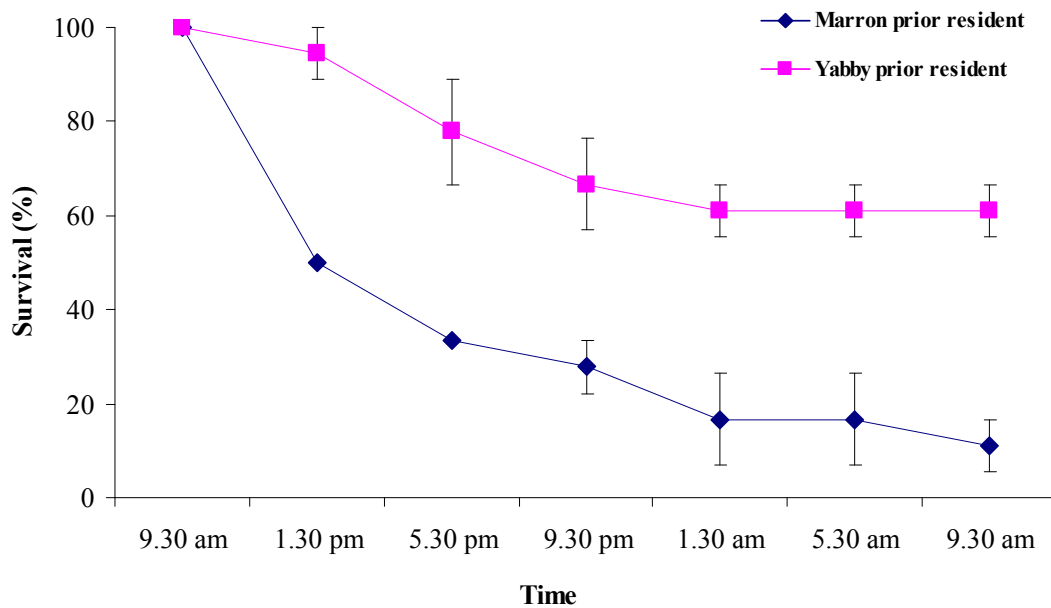
Figure 9.1 Survival rates of yabbies by type of prior resident (±SE) over 24 hours

9.3.2 Mesocosm experiment (sub-trial 2)

9.3.2.1 Avoidance of conspecific cannibalism and finfish predation

Cannibalism and predator avoidance by yabbies were observed in treatments containing complex shelter. Use of VCR footage successfully showed interaction between experimental animals, but visibility was compromised by lens condensation and by the range of the single camera. At first observation, marron and yabbies were found to share the shelters during the day. However, by the end of the recording period only individual yabbies continued to occupy the shelter. Results are shown in Table 9.5.

Table 9.5 Mean survival rate of yabbies and marron across treatments

Treatment	Yabby Survival Rate (%)	Marron Survival Rate (%)
Yabbies only (Free-range)	10.00 ± 5.77^a	na
Yabbies+marron (Free-range)	No survival	No survival
Yabbies+Marron (Caged)	96.67 ± 0.33^b	86.67 ± 0.33^a
Yabbies+Marron+Predator (Caged, then free-range)	No survival	No survival

9.3.2.2 Yabbies under threat from predators in the mesocosm

In one replicate, the 90mm shelters were continually occupied by at least one yabby during the entire VCR recording period (1800h to 1900h). In the other replicates, no yabbies were observed in shelter in the VCR footage, and were therefore assumed to occupy other areas of the mesocosm during the recording period. However, at the end of the experiment, divers found only one yabby remaining in the mesocosm in all three replications (survival rate $10.00 \pm 5.77\%$). Results are summarised in Table 9.5.

9.3.2.3 Yabbies with marron (cannibalism when free-range)

When marron were added to the mesocosm to which yabbies had been placed 3 days earlier, results showed that neither marron nor yabbies survived after three days' exposure to predators (Table 9.5).

9.3.2.4 Yabbies with marron (cannibalism when caged)

When yabbies and marron occupied the cage together in the 3rd treatment, the survival rate of yabbies was $96.67 \pm 0.33\%$, significantly higher than that of marron ($86.67 \pm 0.33\%$) (Table 9.5).

9.3.2.5 Yabbies with marron (caged, then free-range)

The complex shelter used in the present experiment provided some refuge for yabbies and marron. However, after three days of exposure to predators, no surviving marron or yabbies remained in any replicate of this treatment (Table 9.5)

9.3.3 Field experiment (sub-trial 3)

9.3.3.1 Juvenile marron production

A single spawn of juveniles took place during the trial, and was collected from each of the three replicate ponds of each treatment. The number of juveniles harvested in the sheltered ponds averaged 111.67 ± 31.67. This was significantly higher than the ponds without shelter where the number of juveniles averaged only 10.33 ± 2.91 ($P<0.01$). However, the average weight of non-sheltered juveniles was 2.30 ± 1.24g, which was significantly greater than that of juveniles in the sheltered treatment, namely 1.40 ± 0.20g ($P<0.05$). These results are depicted in Figure 9.2. All juvenile marron were harvested with appendages intact and in good condition with no algal fouling evident.
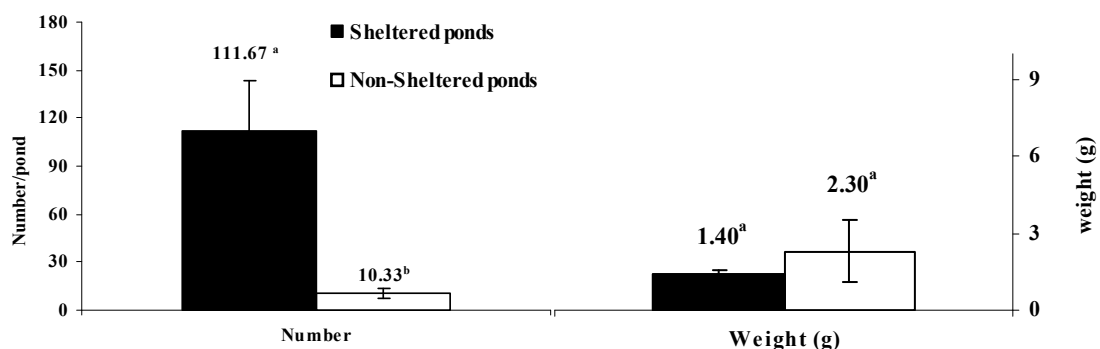
Figure 9.2 Average number and weight (g) of juvenile marron in sheltered and non-sheltered ponds
Significant differences between treatments are indicated by different superscripts.

9.3.3.2 Female marron production

Survival rate and average weight gained over the 117-day trial period is shown in Figure 9.3. There was considerable variation between treatments. Adult female marron in ponds provided with shelters had significantly higher survival rates than females in the non-sheltered treatment (P<0.01). As depicted in Figure 9.6, mean body weight was significantly greater in the non-sheltered treatment ponds (P<0.001). However, the length:weight ratio was significantly higher in marron harvested from non-sheltered ponds compared to the sheltered treatment (2.05 ± 0.18 and 1.81 ± 0.05g, P=0.013). Furthermore, total biomass in the sheltered ponds was 4 times greater than that in the non-sheltered ponds.

Adult marron harvested from the six experimental ponds were vigorous and in good condition. However, approximately 3% of individuals from both treatments had missing claws or legs, a large proportion (>80%) were covered with algae (fouling) and 20% had moulted. As a general observation, marron in the sheltered treatment seemed to present in better physical condition.
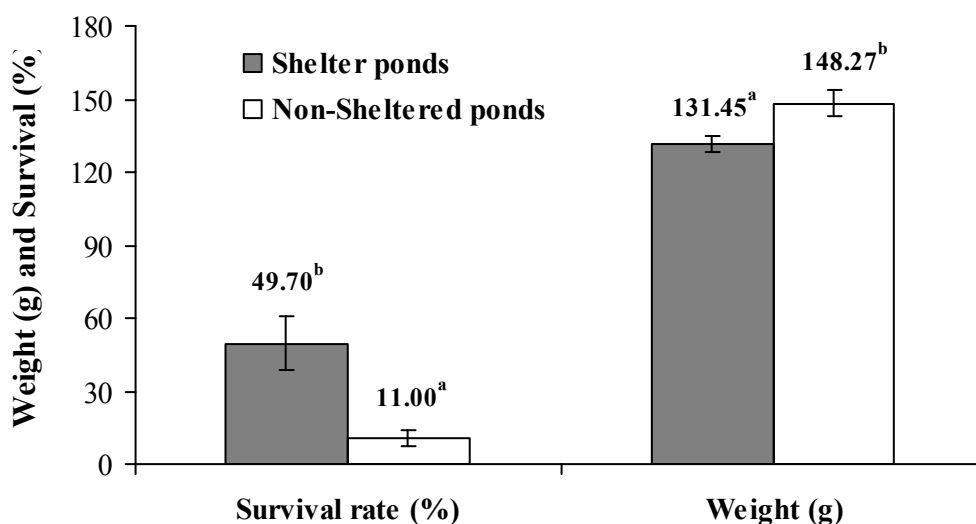
Figure 9.3 Survival rate (%) and mean body weight (g) (±SE) of adult female marron from each treatment after 117 days
Note: significant differences between treatments are indicated by different superscripts.

9.3.3.3 Silver perch production

Silver perch harvested from sheltered ponds recorded an average weight gain (AWG) of 158.00 ± 19.1g, which was significantly higher than that recorded in the non-sheltered treatment; 124.84 ± 18.20g (P=0.013). The higher gain also contributed to higher mean body weight (MBW). Figure 9.4 shows the significantly higher MBW of perch from the sheltered ponds (429.50 ± 9.58g) compared to the non-sheltered ponds (396.05 ± 9.18g, P=0.012). There were no significant differences with respect to survival rate (94 vs 100%), and length:weight ratios found between the sheltered (1.43 ± 0.04) and non-sheltered treatments (1.36 ± 0.18), respectively (P>0.05). However, variability (SE) of length:weight ratios was significantly higher in the non-sheltered treatment (P<0.05).
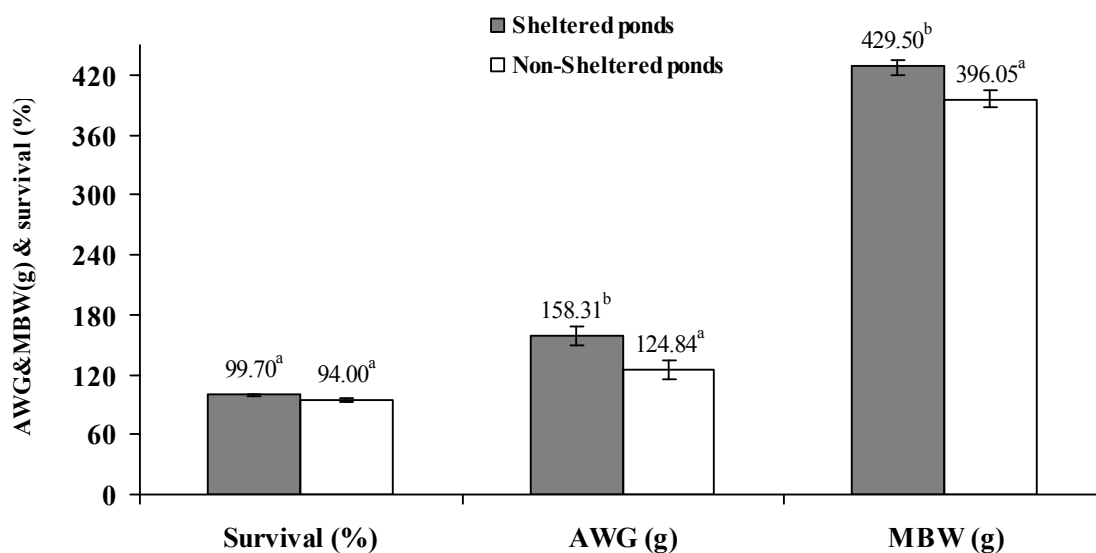
Figure 94 Survival rate (%), average weight gain (AWG, g) and mean body weight (MWB, g) (±SE) of silver perch in sheltered and non-sheltered ponds over 117 days
Note: significant differences between treatments are indicated by different superscripts.

9.4 Discussion

9.4.1 Laboratory experiment (sub-trial 1)

9.4.1.1 Social interaction and avoidance strategies

Social interaction and hierarchy are among the most important factors governing congeneric responses of crayfish (Gherardi *et al.* 2002). The presence of larger crayfish has been shown to suppress the foraging behaviour of smaller crayfish (Karplus & Barki 2004). This effect has also been observed to drive smaller crayfish to seek more secure shelter (discussed in Chapter 6). In the present study, even with the provision of shelter and when small yabbies had the advantage of prior residence, cannibalism by larger marron was high. In the wild, yabbies would likely be able to escape by seeking shelter, burrowing (Bovbjerg 1970, Lawrence *et al.* 2001) or moving to safer refuge (discussed in Chapter 6). However, in the present trial, yabbies were generally unable to avoid attack even when various types of shelter were available. When yabbies were established as prior residents, their success in avoiding cannibalism was somewhat better. Nonetheless, mortality was high.

9.4.1.2 Effect of starvation

Hunger is a major driver of aggressive behaviour in crayfish (Czesny *et al.* 2003, Moutou *et al.* 1998). Marron in the present trial were starved for 3 days prior the experiment, sufficient to create a state of considerable hunger. Beukemal (1968) and Moutou *et al.* (1998) found that increasing feed will reduce agonistic behaviour in finfish. Dispersion of feed in crayfish culture is known to reduce social interaction (Barki *et al.* 2001), which may also reduce aggressive behaviour. Inadequate feed distribution may have the opposite effect. For example, Karplus & Barki (2004) demonstrated that provision of clumped feed increased aggressive behaviour in the Australian redclaw. In the present study, feed restriction was a likely cause of the higher rate of cannibalism.

9.4.1.3 Dominance cannibalism

Gherardi (2002) recommended investigation of cannibalism in traditional contests, namely, between crayfish with different resource holding potential (RHP, Dingle 1983)

and therefore different levels of aggression. The present study was thus conducted to examine this between large marron and small yabbies. In this experiment, marron had the advantage of RHP and displayed highly agonistic behaviour. Mortality reached 100% in one experiment block. The average rate of cannibalism of intruder yabbies by prior resident marron was 88% within 24 hours. Only two prior resident marron were unsuccessful in capturing and eating the intruder; both of these were missing chela. These results indicate that prior resident status of large marron with high RHP evoked a high degree of territorial aggression when smaller intruders were introduced. This resulted in a high level of dominant cannibalism. The influence of prior resident status on dominant behaviour is supported by the findings of Marsh (2004). Crayfish with appendage loss have been shown to be handicapped with respect to their ability to fight and hunt (Gherardi 2000); with respect to cannibalism, results of the present study support this.

9.4.1.4 Interspecific conflict, cannibalism and species displacement

In Chapter 6, larger yabbies were observed to consume smaller yabbies when shelter was shared. That finding agrees with Lynas (2002) who reported that yabbies appear to be aggressive by nature and may successfully out-compete native crayfish of similar size. In contrast, in the present trial where crayfish were of disparate size, the larger marron showed significantly more aggression and cannibalised the smaller yabbies. The risk of species displacement is a bio-security and ecological concern. The intention of the present study was to investigate aspects of congeneric cannibalism in the context of successful interspecies competition. Results of the present study have implications for mechanisms of species displacement as well as their potential for use in biological control.

Abrahamsson (1966) reported that cannibalism generally occurs when larger crayfish target smaller crayfish. Investigations reported in Chapter 7 showed that hunger and high temperature increase the incidence of cannibalism among yabbies. Several studies have explored aspects of the potential for displacement of native crayfish by introduced species (Hazlett *et al.* 1992, Momot 1996, Hazlett 2000, Usio *et al.* 2001 and Paavola *et al.* 2005). Only a few studies have examined the resilience of native species in the face of introduced competitors. One such study (Elvey *et al.* 1996) found that introduced yabbies could not gain access to shelter when competing with *A. franklinii,* a native

Tasmanian crayfish. In Western Australia, no study of cannibalism among native crayfish species has been documented even though it is known to occur widely among crayfish in nature (Goddard 1988). Lodge & Hill (1994) assert that study of true cannibalism is difficult, and suggest that some studies of cannibalism may overestimate this phenomenon. For example, Nyström (2002) found crayfish parts in gut contents in studies where crayfish survival was 100%. Strenroth (2003) and Fotedar (2000) interpreted such findings to mean that crayfish consumed their exuvia. In the present survival study, there is no doubt that smaller yabbies were eaten by larger marron. These findings are important to consider when establishing management strategies for native crayfish populations in their natural habitat as mounting evidence of displacement by introduced species (Morrissy & Cassells 1992, Usio *et al.* 2001 and Paavola *et al.* 2005).

In Western Australia, native marron are relatively larger than introduced yabbies. As illustrated clearly in the present study, marron can be highly aggressive towards intruders, especially when RHP is greater. Ensuring that adequate numbers of larger marron are maintained in natural habitats holds promise as a means to secure native populations under threat from introduced or invading crayfish species. Equally, in commercial settings, restocking juveniles where much larger crayfish have prior residence is not advised. It is recommended that restocking strategies be reviewed to incorporate these findings. Investigation of the factors contributing to the resilience of native species under threat is warranted.

9.4.2 Mesocosm experiment (sub-trial 2)

9.4.2.1 Technical problems and limitations

The mesocosm used three underwater cameras connected to a video capture card (Embeded Technology[TM]) linked to a continuous data recording and storage system (hard drive). Unfortunately, this system failed due to water incursion that lead to an irreparable camera malfunction. This meant resorting to traditional methods of VCR recording and hookah diving half way through the trial.

9.4.2.2 Instant predation

The VCR recording indicated that both yabbies and marron did not occupy the shelter immediately after release into the mesocosm. Practically neither species was attracted to the shelter provided. Marron and yabbies were dispersed through the school of finfish predators. Murray cod simply gulped the smaller crayfish instantly. Silver perch, in contrast, swam around crayfish before lightly pinching the crayfish tail or group attacking. The crayfish on the other hand were seen to raise their chelae and push the finfish predator; in some observations finfish let the crayfish move freely. Other times crayfish were seen to explore their new territory without fear. On several occasions Murray cod swallowed then spat yabbies from their mouths as if they were jammed by crayfish chelae. However, when they managed to catch the tail of the crayfish they merely swallowed them. The battle between Murray cod and large marron in this trial would often last for twenty minutes before the other crayfish became aware of the dangers. Chivers & Smith (1998) and Hazlett (2000) reported that "alarm odour" was released by injured conspecifics and alerted other crayfish. The results from Chapters 6 and 8 indicate that most of the larger crayfish specimens failed to acknowledge the predator odour and did not respond appropriately or somewhat underestimated the threat.

9.4.2.3 Cannibalism

9.4.2.3.1 Conspecific cannibalism

In the cage treatment, the incidence of cannibalism between marron and yabbies under the continuous pressure from finfish predators was tested. There was no evidence of smaller yabbies being cannibalised by larger ones or vice versa in this trial. The uniform size of marron in this trial nullified conspecific aggression. The yabbies in the cage with high shelter complexity successfully utilised the provided shelter to suit their needs. These results indicate that the yabbies (*Cherax albidus*) were tertiary burrowers, behaving similarly to yabbies (*C. destructor*) Gherardi (2002). This supported the finding of Lawrence *et al.* (2001) that yabbies (*C. albidus*) will only burrow when there is a climatic signal from drought or changes in water quality parameters, particularly temperature. In contrast, marron in this trial showed lower survival than yabbies; however, the remaining carcasses were intact and covered with fungus, indicating that

they had been dead for some time. Their soft shells indicated that they died shortly after moulting or had moulted unsuccessfully when surrounded by various finfish predators.

9.4.2.3.2 Heterospecific cannibalism

There was no VCR recorded incident of heterospecific cannibalism between marron and yabbies in this trial. However, based on former observations, it is assumed that the predators consumed most of the crayfish. The only yabby remaining from three replicates clearly demonstrates the pressure from finfish predators is greater than congeneric aggression. The other explanation was that the recording period was only for a short time each day (between 18:00 and 19:00). This was the most active time for the crayfish, nonetheless the only remaining yabby always occupied the same shelter.

9.4.2.3.3 Predation pressure

There was no doubt that there was significant predator pressure in the mesocosm with a total biomass of over 90kg of fish, equating to 5 kg/m^2. This amount of predator pressure would greatly affect crayfish. Murray cod have been tested with juvenile marron by Storer (2006) in aquariums, resulting in 100% crayfish mortality within 13 days. In larger marron (Section 9.3) under free-range polyculture with silver perch, a biomass of silver perch in sheltered and non-sheltered ponds of 0.2 and 0.4 kg/ m^2 was sufficient to reduce marron survival rates down to 49.7 and 11% respectively.

It should be noted that all predators in this trial were introduced species in Western Australia. Marron have not historically interacted with these species, while yabbies have evolved with both silver perch and Murray cod.

9.4.3 Field experiment (sub-trial 3)

9.4.3.1 Marron production

Production of juvenile and adult female marron in terms of total numbers and biomass was higher in the sheltered ponds. This demonstrates that availability of shelter is an important aspect of crayfish and finfish commercial polysystems. This is supported by the findings of Mattila (1991), Whisson (1997) and Storer (2006). Providing shelter in this experimental polysystem resulted in significantly higher recovery of adult and juvenile marron by providing protection from silver perch predators (Figure 9.2).

Shelter has also been shown to provide protection for marron from other finfish with different predatory habits such as Murray cod (Storer 2006). In this study, 2.3 juveniles were recovered per female harvested from sheltered ponds, whilst the non-sheltered ponds produced 0.9 juveniles per surviving female. However, production of juveniles in these trials was well below previously reported figures for marron monoculture. Whisson (1997) recovered 64 juveniles per surviving female, which equates with production of juvenile marron in excess of 6,400 individuals per pond. In this trial, surviving brood females produced the equivalent of 3,200 individuals per sheltered pond and 700 individuals per non-sheltered pond, respectively. There are several factors that may have contributed to these results. Predation by silver perch was considered to be a major factor contributing to low survival rates of both juvenile crayfish (Stein 1977) and broodstock (Whisson 1997, Whisson 2000). Stein (1988) also demonstrated that certain life stages are more attractive to fish predators than others. Storer (2006) concluded that schooling behaviour of silver perch enhances their ability to feed heavily on sub-adult crayfish.

In the present experiment, ponds were stocked with only berried females. If predation of adult females was considerable prior to release of juveniles, this could explain the low harvest of juveniles in this study, particularly since only one spawn occurred in the absence of any males. This could explain the very low recovery of adults and juveniles in the non-sheltered treatment. Furthermore, the threat of predation is believed to influence the quality of incubation and may result in premature release of eggs due to pleopod movement in escape or avoidance behaviour. This could explain the low harvest result of the sheltered treatment.

As discussed in Chapter 6, opportunistic cannibalism is common and is a significant cause of losses in crayfish production. Section 9.1 clearly showed that dominance cannibalism by large marron caused high mortality in juvenile yabbies, up to 80 % within a 24-hour period. This has also been shown to occur within species (Elgar & Crespi 1992, Chapter 6) and could account for some losses in the present experiment. Cannibalism among crayfish of similar size has also been reported during moulting (Hogger 1988). In the present study, 20% of recovered stock was noted to have moulted prior to harvest. Conditions conducive to the occurrence of cannibalism were present in this study, particularly for marron with shelter. Age of crayfish broodstock also

influences the success of spawning. According to Lawrence & Jones (2002), the most successful broods reared usually occur from 3 years of age. Marron in this trial were first-berried and 2 years of age. This may have contributed to the low harvest for both treatments.

Handling technique and frequent sampling of crayfish was likely to have resulted in diminished growth (Farrell & Leonard 2000). Although animals sampled to establish group average weight at the start of experiment were discarded, transport, relocation and other new environmental factors such as temperature and water quality may have resulted in premature release of eggs and pre-juveniles. Similar transport and translocation losses have been reported by others (Huner & Lindqvist 1991).

Water temperature may have played a role in results of the present study. Whisson (2001) reported that high water temperature may increase the frequency of moulting in young crayfish. The relationship between moulting and vulnerability to predation and cannibalism has been reported by Stein (1988) and was discussed extensively in Chapter 6. In this trial, water temperatures exceeded 24ºC for 42 of the 117 days. Morrissy (1990) demonstrated that marron experience stress above this temperature. Continuous exposure to temperatures of 25 to 30ºC was shown in Chapter 6 to result in high mortality in juvenile yabbies (Austin & Verhoef 1999). Extreme seasonal high temperatures were reported to cause the collapse of the marron farming industry in Queensland in 1990 (Morrissy 1990a, Jones & Ruscoe 1996). Sustained water temperatures above levels considered to cause stress in marron likely contributed to losses in the present study.

Villarreal (1990), Boulay *et al.* (1993), and Bennison & Morrissy (1991) reported that, even in grow-out monoculture, 0+ crayfish survival was very poor with minimal shelter. Survival rates have been shown to improve as juvenile marron grow (Morrissy 1992b, Morrissy *et al.* 1995). In the present short-term experiment, newly hatched small juveniles were likely targeted by either larger marron or silver perch and therefore had similarly low recovery rates.

9.4.3.2 Silver perch production

Growth of silver perch in the sheltered treatment was superior to that in the non-sheltered ponds. However, other studies of silver perch in polyculture report higher production even at much higher density (Rowland 1999, Storer 2006). The present study stocked silver perch at 2,083/ha (in a 0.072 ha pond). Fish gained an average of 158.31g (sheltered) and 124.84g (non-sheltered) over the 117-day trial period (Figure 9.4). Roland (1994) stocked juvenile silver perch at 14,500/ha (0.4 ha) in monoculture and reported gains of 202g over 280 days from September to March. Whisson (1997) found that free-range silver perch in marron ponds gained 251g from November to July in 0.1 ha ponds when stocked at 1,800/ha. When silver perch were co-cultured with redclaw (Jones & Ruscoe 1996), a high weight gain of 340g was reported, but fish survival was low (14.3%).

Temperature has been shown to influence growth rate in finfish. Silver perch growing at different sizes would experience a varying range of temperature, thereby affecting their growth performance. In Collie, temperatures average 24.23 ± 0.09, 24.3 ± 0.05, 22.68 ± 0.09 and 22.23 ± 0.09 °C from December through to March, respectively.

Survival of silver perch was high in this experiment, and also in previous experiments by Whisson (1997) and Storer (2006). This species is therefore a prime candidate for polyculture with crayfish species (Whisson 2000). In Chapter 5, the slow response of marron to silver perch odour was discussed; this observation may prove favourable for co-culture of these two species. Storer (2006) found that co-culture of marron with silver perch did not affect growth performance of either species. However, in the non-sheltered treatment of the present study, marron showed a high degree of interaction with the fish, and mortality of marron was high in both treatments. Further study is warranted to explore other ways of optimising performance of both species when reared together.

9.4.3.3 Impact of shelter on finfish and crayfish production

Shelter availability improved recovery of adult marron by 452% (Figure 9.3). Although average adult weight at harvest was significantly lower than that of marron in the non-sheltered treatment, total biomass from the sheltered ponds was greater than non-

sheltered ponds by 400%. Moreover the juvenile marron production in sheltered ponds was 6.5 times greater than the non-sheltered ponds. Shelter may not exert such an influence over the final size of marron when reared for longer periods with shelter provided at different ratios. Lawrence *et al.* (2004) provided loose mesh shelter (0.15 hides/m^2 and 0.30 hides/m^2). After two years, there was no significant difference between the mean weight of marron in 0.15 hides/m^2 ponds (116 ± 5.29g) and 0.30 hides/m^2 ponds (124 ± 4.31g) (P = 0.29).

Other benefits of shelter provision in polyculture include improved physical condition of both marron and perch. The length:weight ratio of both marron (sheltered: 1.81 ± 0.08, non-sheltered: 2.05 ± 0.19, P<0.05) and perch (sheltered: 1.43 ± 0.07, non-sheltered: 1.36 ± 0.25, P>0.05) were superior in the sheltered ponds, although the latter were not statistically significant. These results indicate that marron in sheltered ponds were harvested in significantly better condition than from the non-sheltered treatment. Azim *et al.* (2001) and Wahab (2000) argue that shelters act as substrate for phytoplankton, periphyton and microphytes to establish themselves. These authors believe that such organic material contributes to better food conversion. Filamentous algae were observed in ponds of the shelter treatment only in the present study, located mostly on the submerged shelters. This could be attributed to uneaten feed and resulting organic material supporting this growth in the system. However, feed intake was closely monitored, and some green algae are considered to indicate health of water bodies (Boyd & Tucker 1994) with respect to the absence of toxic gasses and adequacy of dissolved oxygen. This supports the likelihood that algae did contribute to superior gains, and may also have improved survival of marron in the sheltered treatment.

The observation of avian predators seen taking prey during this experiment undoubtedly contributed to low survival in the non-sheltered ponds. Rowland (1999) observed that cormorants or shags prey heavily on juvenile silver perch in nursery ponds and Schmidt (1998) found this bird consumed common carp (*Cyprinus carpio*). Storer (2006) confirmed that cormorants had contributed to some adult marron injuries. The presence of shelters in the treatment ponds may have deterred the bird, thereby enhancing survival and production in that treatment.

9.4.3.4 Recommendations for further research

Growth and survival of marron in polyculture with free-range finfish remain limited by predation and cannibalism even when shelter is provided. Rearing fish in cages holds promise of a viable alternative (Whisson 2006, Storer 2006). Stocking (grading) of crayfish and finfish in polyculture also holds promise for producing superior yields (Lawrence & Bird 2004). Other aspects of management such as feeding regime and shelter types are other avenues of research meriting further investigation.

9.5 Key findings

9.5.1 Key findings from laboratory trial

1. Prior resident effects have a great impact on survival of intruders, with larger sizes of prior residents exaggerating dominance cannibalism.

2. Simple PVC pipe provided in this trial did not improve survival rate of yabby intruders.

3. The prior resident effect should be taken into account when restocking small size crayfish in the wild and in aquaculture.

4. The findings of prior residence and dominance cannibalism can be applied to control introduced species by using larger native species.

5. Further study of shelter manipulation and dominance cannibalism is recommended.

9.5.2 Key findings from mesocosm trial

1. Yabbies in this trial displayed better strategies for utilising shelter for predator and cannibalism avoidance.

2. Shelter complexity in this trial can effectively reduce hetero-specific cannibalism.

3. Murray cod is a natural, solitary predator of crayfish.

4. Silver perch are a schooling species and can display group attacking behaviour, even when the prey is larger than their individual gape size.

5. Under pressure from finfish predators, marron survival rates were lower than those of yabbies.

6. When marron and yabbies were placed in the same cage, they did not display shelter fidelity or predator avoidance behaviour.

9.5.3 Key findings from field trial

1. Shelter provision improved the survival rate of both adult and juvenile marron.

2. Survival of both juvenile and adult marron was affected by silver perch.

3. Marron growth did not improve with the provision of shelter; however, the total biomass of marron in the sheltered ponds was greater than in non-sheltered ponds.

4. Silver perch grew faster and were in better condition in the sheltered ponds.

5. Although shelter provision has improved pond production, juveniles produced per surviving female were well below the industry standard.

6. Shelter provision in this trial did reduce avian predation.

Chapter ten

General discussion

This thesis has explored the role of shelter in freshwater crayfish polysystems. Knowledge gained through this research is relevant to aquaculturists, ecologists and resource managers, through an increased understanding of the factors that shape behaviour in multi-species systems. Review of current scientific literature and new findings from experimental trials conducted in this study provide useful insight into crayfish ecology, behavioural responses and, more importantly, additional strategies for the management of shelter, with a view to enhancing productivity in polysystems.

10.1 Introduction

Shelter is a major factor governing crayfish behaviour in aquatic environments. Shelter can provide protection, food, and a safe rearing ground for young. It can also influence water quality, and forms the basis of community structure. Suitable shelter reduces agonistic behaviour and social conflict through the provision of a better environment and thereby improves aquatic productivity and wellbeing. Under polyculture conditions the critical role of shelter is compounded by other limiting factors such as artificial feeding, water quality management, and control of pests and diseases. This research has increased our understanding of the importance of shelter and has provided a useful insight into contributing factors within crayfish polysystems.

This final chapter discusses the influence of shelter on crayfish behaviour and interaction with finfish, and how commercial productivity can be improved through an improved understanding of how system dynamics are affected by shelter for crayfish. From this research, specific recommendations for commercial polyculture will be proposed and future study areas discussed.

10.2 The role of shelter in crayfish ecosystems

10.2.1 Shelter as a basic requirement for crayfish

Chapters 4 and 5 revealed that marron (*Cherax tenuimanus*) did not alter their behaviour in the presence of silver perch odour, while yabbies (*C. albidus*) responded with reduced locomotion and increased time in shelter. These disparate observations could be associated with adaptation of the respective species. Crayfish can be empirically divided into two groups according to shelter preference, namely; crayfish that are able to construct shelter (Table 10.1), and those that make use of available shelter. Categorized in this way, yabbies are considered as burrowers (Lawrence *et al.* 2001), while marron are a non-burrowing species (Morrissy 2000). Phylogenetically, it could be said that yabbies represent the more adaptive species; when confronted with potential threats, they retreat to shelter (creating shelter where necessary), thereby enhancing the likelihood of species survival (Lawrence *et al.* 2001, Gherardi 2002). In contrast, crayfish that depend on available shelter are relatively less developed; when exposed to various threats, they are more vulnerable. However, to compensate for their

disadvantage, some crayfish have developed a greater body size (e.g. *Astacopsis gouldi, Cherax tenuimanus*). Marron, with their larger size, will risk exposure to seek safer habitat and will engage in avoidance behaviour (e.g. chelae display) instead of retreating to shelter when exposed to odours from predators (Chapter 5). Preference for the most appropriate shelter nonetheless appears to confer a competitive advantage. As shown in Chapters 4 and 6, under normal conditions yabbies preferred a relatively small size of shelter when provided with a range of sizes. However, when exposed to predator odour, yabbies selected the smallest suitable shelter (Chapter 6). This response is consistent with the tendencies of burrowing crayfish when the burrow is by design the most suitable shelter for the size of the animal burrowing (Huner 2000, Lawrence *et al.* 2001).

Table 10.1 Crayfish that burrow (self-constructed shelters)

Common name	Scientific name	Origin	Reference
Prairie [a]	*Procambarus hagenianus*	North America	Hobbs 1942
Robust[a]	*Camburus spp.*	North America	Guiaşu 2002
Red swamp [b]	*Procambarus clarkii (spp.)*	North America	Gherardi *et al.* 2000b
Rusty[c]	*Orcornectes rusticus (spp.)*	North America	Hamr 1997
Yabbies [c]	*C. albidus, destructor*	Eastern Australia	Lawrence & Morrissy 2000
Peruvian	*Virilastacus sp.*	South America	Hobbs 1991
Terrestrial*	*Engaeus,Geocharax (spp.)*	Australia	Suter &Richardson 1977
Signal**	*Pacifastacus lenuisculus*	North America	Guan 1994

Note: a= primary, b=secondary, c=tertiary burrows; after Hobbs (1942), * 14 species found
** constructed simple burrows upon invasion of British rivers (Holdich 2002)

10.2.2 The role of shelter in crayfish distribution

10.2.2.1 Evolution and adaptation

Burrows have been constructed by crayfish of the Family Cambaridae since the Triassic period (Hasiotis 1999). The ability to burrow for shelter has thus been inherent in crayfish for 140 to 200 million years and millions of generations (Hasiotis & Mitchell 1993). Yabbies invaded fresh water from marine origins and became widespread throughout the eastern states of Australia (Scholtz 2002, Taylor 2002). They have

adapted to harsh environmental conditions while co-existing with a multitude of finfish predators. Due to the continent's diverse geography, wide habitat variation, and resulting proliferation of available niches, we find 27 species of the *Cherax* genus (Riek 1969), with more than 120 crayfish species in total (Kibria *et al.* 1997*,* Short 2000, Wingfield 2000).

The distinctive physiology of yabbies, their tolerance of a wide range of temperatures (Chapter 7) and their high behavioural plasticity (Lee 2002) are important traits that enhance survival and distribution. An ability to select the most appropriate shelter (Chapters 5 and 6) serves to protect from threatening behaviour (Chapters 5 and 7). Recognition of potential predators (Chapter 6) and the ability to utilise benthic substances, substrates and vegetation as shelter enables yabbies to establish themselves in the various environments (Duffy 2006). These characteristics obviously make yabbies good candidates for aquaculture. However, not all of these qualities are desirable in natural environments. For example, the introduction of yabbies has resulted in invasion and displacement of native species (Chapter 5, Usio *et al.* 2001, Lynas 2002).

According to Lawrence & Morrissy (2000), rearing of yabbies is restricted to certain regions of the State delineated to avoid territory common with that of the native marron due to concern about species displacement (Morrissy & Cassells 1992, Usio *et al.* 2001). This was deemed sufficient to safeguard marron from the invasive crayfish in the south-west drainage region. However, lack of natural predators of yabbies coupled with their adaptive capabilities has resulted in the widespread establishment of yabbies in Western Australia (Lawrence & Morrissy 2000). Further the misnaming of yabbies as a native species "koonac" (*Cherax preissii*) has unintentionally dispersed them in the south-west region (Morrissy & Cassells 1992). In this circumstance human activity has played an important role in the translocation of crayfish in the region (Horwitz 1994).

10.2.2.2 Global distribution of freshwater crayfish

Not surprisingly, the most successful and widespread crayfish species in aquaculture are the burrowing crayfish (Table 10.1) such as red swamp crayfish, rusty crayfish, robust crayfish, signal crayfish and the yabby. Red swamp and rusty crayfish have been translocated all over the world for aquaculture (Holdich 2002). The signal crayfish has

been introduced to Europe after the noble crayfish population (*Astacus astacus*) was decimated by crayfish plague (*Aphanomyces astaci*) (Taylor 2002). Farmed yabbies in Western Australia (*C. albidus)* have likewise been successfully translocated.

10.2.2.3 Translocation and production of yabbies

Yabbies can change their morphology and modify their behaviour according to soil, water quality and other environmental factors (Lawrence *et al*. 2001). In response to changing conditions (i.e. low water levels) yabbies will burrow (Gherardi 2002). When temperatures rise, yabbies can grow faster (Chapter 7), and above 15 ºC they start to reproduce at a very early stage (Lawrence and Morrissy 2000). Clearly, the ability to adapt to new environments has led to population expansion both in the natural environment and through translocation for the purposes of aquaculture. Yabbies are now widely spread throughout Australia (Morrissy & Cassells 1992, Horwitz & Knott 1994, Morrissy 1994).

Yabbies are considered hardy, able to withstand limited food and temperature variation even without shelter (Chapter 7). Similar to the best-known commercially farmed species (Table 10.1), yabbies are seen as low-cost and simple to rear, which has encouraged farmers to stock farm dams. In Australia's west, the biological advantages of yabbies were seen as a means to diversify farm output (Lawrence & Morrissy 2000).

10.2.2.4 Impact of burrowing crayfish on water sources

In Western Australia, production of yabbies hit a record high in 1994: production fluctuated thereafter due to drought (Lawrence & Morrissy 2000). In the Wheatbelt region, clay type soils were recommended and preferred for successful rearing of yabbies in farm dams. However, in other areas, burrowing of yabbies has resulted in the penetration of clay dam linings and high seepage (summer drain) into underlying sand (Lawrence *et al*. 2000, Wangpen pers. obs.). Stocking of yabbies in such dams is thus not recommended. Alternative species such as marron that do not burrow may prove better candidates in sandy environments, however, they require high water quality and shelter, and therefore greater capital investment, particularly in regions outside their natural range.

10.2.2.5 Spatial distribution and habitat fragmentation

With the exception of crayfish in Madagascar, no freshwater crayfish are found in the tropical river systems of South-east Asia, South America or Central Africa where there are many potential crayfish predators and a high degree of spatial competition (Chapter 8). This type of sensitivity suggests crayfish are good environmental indicators (Boyd 1998, Butler *et al.* 2001, Admowicz & Puvis 2004, Rozas *et al.* 2005). Inability to recognise predators (and to acquire or construct shelter in response) will result in high mortality in the presence of large fish or terrestrial predators as shown with marron (Chapter 9). In contrast, yabbies native to the Murray-Darling river system have co-existed with large finfish (such as Murray cod, golden perch and silver perch). As shown in Chapters 4, 5 and 6, the ability to recognise and respond to predator cues (e.g. silver perch odour) by seeking or creating shelter is paramount to the successful establishment of the species in various environments (Morrissy & Cassells 1992, Lawrence *et al.* 2000, Height 2002).

Native marron flourished in Western Australia prior to the introduction of invasive yabbies. The non-burrowing characteristic of marron has not disadvantaged them due to their greater relative size compared to the main co-existing predator species of the south-west region such as Freshwater Cobbler, Western Hardyheads, Swan River Gobies, South-western Gobies, Mud Minnows and Balston's Pygmy Perch (Morgan & Beatty 2005). Apart from co-existing with these small fish predators in year-round stable, cool temperatures, there are fewer large predators and ample material for shelter such as crevices, logs, rocks and tree roots. During the summer dry period, river fragmentations form along natural drainage systems (billabongs) which may or may not be hospitable for marron. Exceptionally low water levels result in higher water temperatures and attract terrestrial predators. Marron faced with such environmental threats tend to escape to seek new habitat or safer areas (Morrissy 1992, O'Sullivan *et al.* 1994, Beatty & Morgan 2005). This trait was supported by experiments with medium and large sized marron that spent significantly more time climbing aquaria walls when exposed to predator odour and conspecific tactile threats (Chapter 5). This finding concurs with studies by Height (2002) and Storer (2006).

10.2.3 Effect of shelter quality on crayfish behaviour

There is disagreement within the literature over the role of shelter for crayfish. For example, after food, shelter availability is considered by some to be the second most important factor affecting crayfish behaviour (Hazlett *et al.* 1975). However, Bovbjerg (1970), Ameyaw-Akumfi (1976) and Stein (1977) indicate that shelter is the most visible factor influencing agonistic behaviour in crayfish especially in the burrowing species. Further, while many other authors describe the importance of shelter for various species of crayfish in the wild (e.g. Bovbjerg 1970, Ameyaw-Akumfi 1976, Stein 1977, Huolila *et al.* 1997, Figler *et al.* 1999; Lawrence *et al.* 2001, Nakata *et al.* 2001), the importance of shelter is extended to include both monoculture and polyculture systems (Whisson 1997, 2000; Chapters 5, 6 and 9). Providing shelter for crayfish aquaculture is recommended in general, particularly in crayfish populations of mixed size (Chapters 6 and 9). Importantly, shelter type and size should be appropriate to the needs of each species and size of crayfish.

10.2.3.1 Effect of shelter size on marron and yabbies

Yabbies show a clear pattern of preference for shelter size relative to their own size when engaged in shelter competition with conspecifics (Chapter 4) and other species (Chapter 5). Yabbies spent more time in shelter than marron; however, in Chapter 5 it was concluded that residence time increases as the suitability increases; this affected time spent in those shelters. In Chapter 6, yabbies were provided with a shelter bundle made of PVC pipe providing five different shelter sizes. The outcome was clear in that the most common shelter size that yabbies occupied was the smallest diameter or cross-section area of shelter that they could fit into while in the lowered posture, i.e. small yabbies consistently selected the 25mm (4.9 cm^2) shelter. Furthermore, when exposed to predator odour, small yabbies selected even smaller shelters, namely the gap between the 50 and 90mm pipes (4.6 cm^2). Yabbies did not occupy shelters much larger than their own body size. Other factors that influenced shelter seeking by yabbies included the appearance of larger conspecifics and predation.

Medium-sized yabbies also preferred the 25mm shelter over the 50mm or 90mm shelter when presented with predator odour. The 25mm shelter likely provided greater security as its smaller cross-section would limit access by larger predators and larger

conspecifics. These findings agree with Cobb (1969) who showed that spiny lobster generally occupied shelters in which the height was less than the width. This preference is considered to be due to the compressed body form of the most common fish predator of lobster; they are thus unable to turn into a horizontal position to attack sheltering lobster. As for yabbies, the selection of shelter also correlates with size. Results of Chapters 6 and 8 concur with the findings of Cobb (1969) that shelter choice is a function of perceived protection and security.

Like yabbies, small marron also preferred to take shelter in the smallest available shelter relative to their own body size (Cobb 1969, Chapter 8). However, results did not show a clear pattern for medium and large-sized marron. Furthermore, unlike yabbies, marron did not alter shelter preference when exposed to predator odour (Chapter 8). Crayfish are more vulnerable at different life stages, particularly relative to body size. Results of Chapters 6 and 8 suggest that smaller crayfish are more insecure and therefore display a stronger response to potential threat, and seek increased protection from shelter. However, the lack of response from larger marron and diminished response from larger yabbies could be due to inexperience with predators. This possibility is supported by results from Chapter 9 where larger sized marron (180±12.3g) were predated by finfish despite abundant shelter being available. In contrast, some yabbies (50±5.2g) chose to remain in shelters resulting in 10.55% of yabbies surviving in the same trial (Chapter 9).

10.2.3.2 Effect of shelter type on marron and yabbies

It is clear that provision of shelter improves crayfish survival and therefore production in aquaculture. However, it is difficult to differentiate between shelter preferences observed in experimental settings and basal shelter requirements for the particular crayfish species. For example, it is unclear whether substrate or shelter or a combination drives growth, survival and production (Kozak *et al.* 2002, Bolliet *et al.* 2003 and Stazner & Peltret 2006). It follows, then, that alternative shelter types should be assessed in terms of effective increase in crayfish biomass and appropriateness (availability and viability) for specific polysystems.

Selection of suitable habitat is essential for survival (Blake & Hart 1993), including shelter for refuge as well as a base for homing behaviour (Vannini & Cannici 1995). In the natural environment, burrows form the primary shelter for burrowing species and

may be a repository for food and also used for copulation. For example, burrowing wild red swamp crayfish (*P. clarkii)* did not appear to require any additional substrate for shelter beyond self-made burrows (Huner 2002). However, in the culture of *P. clarkii* in rice paddies, production is significantly higher (Huner 1994) when a vegetative biomass is maintained above 1 kg m^{-2} (McClain *et al.* 1998). Similar results found in Western Australia demonstrated that regular provision of hay appears to increase yabby production (Nenke 2000). Noble crayfish (*A. astacus*) production in Europe tripled in ponds containing macrophytes (i.e. *Elodea Canadensis, Chara, Typha*) and supplemented with pellets (Keller 1999), compared with average production in unfed ponds (Piwernetz & Balg 1999). In this respect, high crayfish productivity is clearly influenced by availability of effective and appropriate substrate.

Mills *et al.* (1994) noted that crayfish shelter can take the form of aquatic plants, sticks, submerged logs, rocks, ledges, and other debris; collectively, an abundance of such shelter substrates can minimise exposure to predation and cannibalism by reducing the hunting efficiency of crayfish predators. Natural vegetation, including aquatic macrophytes, has been shown to enhance marron survival (Table 10.2). The survival of free-range marron with silver perch was up to 89.3±3.2% when *Vallisneria sp.* was provided for additional shelter (Whisson 1997). In contrast, survival of marron was only 9.8±3.9% in experiments conducted by Storer (2005) and 11.0±9.2% in the present study (Chapter 9) when only synthetic shelter was provided.

10.2.4 Factors influencing shelter utilisation

There are many factors that influence the use of available shelter by crayfish, as follows:

10.2.4.1 Temperature

Chapter 7 investigated the effect of shelter availability on yabbies at three different temperatures. Results showed that yabbies maintained normal condition at 20ºC but at 30ºC both biomass and survival was diminished. Experimental conditions precluded burrowing; provision of other shelter did not influence production or survival. In the wild, yabbies burrow when temperatures are high and water levels are low; use of burrows appears to be for escape or hibernation. Mundahl (1990) showed that burrow

temperature is approximately 6ºC lower than that at the water surface. In Chapter 7 at 25ºC, considered close to the optimum temperature for yabby growth, the survival rate was 45% regardless of shelter availability; this is similar to work by Verhoef & Austin (1999) who reported 46% survival under similar conditions. While actual shelter use was not directly recorded in Chapter 7, anecdotal observations suggest that shelter use increased with temperature; however, this did not influence survival of yabbies in this study.

Table 10.2 Survival of marron in ponds stocked with silver perch with different shelter regimes

Source	Marron weight (g)	Silver perch weight (g)	Duration (d)	System & Shelter types	Marron survival (%)
Whisson 1997 (Parkerville)	3.54±0.03[a]		288	M only+*Vallisneria*	77.0±11.4[a]
	3.51±0.03[a]	1.03±0.02[a]		M+SP free range+*Vallisneria*	89.3±3.2[a]
	3.51±0.04[a]	1.02±0.04[a]		M+SP caged+*Vallisneria*	75.0±9.7[a]
Whisson 2000 (Jurien)	4.1±0.65[a]		221	M only+SMS	13.8±8.7[ab]
	4.1±0.65[a]	12.3±1.03[a]		M+SP free range+SMS	9.8±3.9[a]
	4.1±0.65[a]	12.3±1.03[a]		M+SPcaged+SMS	31.3±1.0[b]
Storer 2005 (Collie)	71.5±0.4[a]		200	M only+SMS	82.0±11.3[a]
	71.5±0.4[a]	199.4±0.6[a]		M+SPcaged+SMS	80.0±0.1[a]
Chapter 9 (Collie)	110	270.9±61.1	117	BM+SP free range	11.0±9.2[a]
	110	270.9±61.1		BM+SP free range+SMS	49.7±18.1[b]

Note: M= marron, SP=silver perch, BM= berried female marron, SMS=synthetic marron shelter

10.2.4.2 Size of crayfish

Competition for shelter is influenced by relative body size, or resource holding potential (RHP), as described by Dingle (1983). In Chapter 4, often the larger member of pairs or trios of yabbies occupied shelter for a higher proportion of time. Over the course of the experiment, paired yabbies were often observed sharing the shelter. Where shelter was limited, larger marron always gained the privilege of available shelter in the first instance (i.e. initial 5 minutes, Chapter 5). However, when shelter use was observed over 24 hours, it was noted that smaller marron utilised shelter more frequently than larger marron (Chapters 5 and 8). Smaller crayfish are prone to conspecific and heterospecific cannibalism by larger crayfish and predation (Chapter 9). When available shelter does not provide adequate protection, the survival of juveniles is jeopardised. In

this regard, smaller crayfish tend to need shelter more than larger crayfish; the latter still require shelter during reproductive periods, and for avoiding predators, e.g. during moulting. These observations are in line with those of Stein and Magnuson (1976). Relative size of crayfish is a key factor affecting shelter acquisition and the incidence of predation and conspecific cannibalism (Chapters 6 and 9). In this regard, Chapter 9 reported similar findings where stocking large perch reduced survival of small juvenile marron. Likewise, with relevance to rearing finfish in communal culture, Barki *et al*. (1997) found that red claw crayfish growth and survival were suppressed by larger co-stocked tilapia. It follows, then, that the combination of body size and higher complexity of preferred shelter will likely increase the survival of juvenile crayfish (Chapter 9). These results are consistent with and supported by the findings of Figler *et al*. (1999), Vorburger & Ribi (1999) and Nakata & Goshima (2003).

10.2.4.3 Gender and reproductive status

Chapters 4, 5, 6 and 8 of this study investigated competition for shelter. Results indicated that gender alone did not influence the outcome of shelter competition in marron. In Chapter 4, it was found that female yabbies (both berried and non-berried) generally spent more time in shelter than males. This gender effect was not demonstrated in marron investigated in experiments (Chapters 5 and 8). Interestingly, Marriapan & Balasundaram (2003) found that berried female freshwater prawns (*Macrobrachium noblii*) spent more time in shelters than males, but males spent more time in shelters than non-berried females. Clearly, gender combined with reproductive status, and relative size of females, can influence the success of shelter competition in crayfish.

10.2.4.4 Prior residence

The prior resident effect did confer an advantage in shelter acquisition and retention time in all studies examining this aspect (i.e. Chapters 4, 5, and 9). This was also demonstrated in the initial five-minute period of observation in Chapter 4. When shelter is limited, prior residents are motivated to fight even when the relative size of the prior resident is small. Chapter 5 demonstrated this prior residence behaviour by both marron and yabbies. Prior resident status can significantly influence the chance of survival. For example in Chapter 9, large prior resident marron cannibalised 100% of small juvenile

yabby invaders. Conversely, only 20% of the much smaller prior resident yabbies were cannibalised by larger invading marron under otherwise similar circumstances. This aspect of prior resident status may confer an advantage to native marron in competition with introduced yabbies in the wild. For example, this could explain the observed resurgence of the marron population in Lake Moyanup following introduction of yabbies (Campbell & Whisson 2002). This phenomenon could prove useful as an indirect means of biological control of introduced crayfish species and in conservation of larger natives under threat of displacement.

10.2.4.5 Physical condition

Apart from the factors described above, life stage may also influence shelter use. Female marron with advanced reproductive status sought safer shelter to increase the survival rate of offspring, while female yabbies would construct burrows for the same reason (Chapters 4, 5). In many terrestrial and aquatic species, securing suitable shelter may induce reproductive behaviour and related hormones leading to an elevation of reproduction status. Attaining the required reproductive state is known to assist *Orconectes virilis* and *Procambarus clarkii* in shelter and mate selection, which occurs exclusively with Form I males (Guiaşu & Dunham 1997). Such aspects of physical condition then also influence selection of shelter.

Other physical factors may influence behaviour and shelter selection, for example, handicaps (impaired physical functions). Crayfish with single chelae or those without any chelae have impaired fighting ability and reduced ability to defend shelter. In Chapter 9, it was demonstrated that marron with one chela could not harm juvenile intruder yabbies. That finding was supported by Edsman & Jonsson (1996) and Gherardi (2002) who found that loss of appendages resulted in diminished competitive ability or less attractiveness to females for mating (Villianelli & Gherardi 1998).

10.2.4.6 Behavioural factors

There are many behavioural factors that affect shelter use by crayfish. These include activity pattern, foraging, response to reproductive cues, and agonistic behaviour (Chapters 4, 5, 6 and 8). Marron and yabby activity patterns observed during this study were clearly influenced by the type and availability of shelter. The presence of

congenerics, exposure to predator odour and temporal change (Chapters 5 and 8) all influenced behaviour or activity patterns. When exposed to congeneric marron displaying the raised posture, which is considered to be dominant agonistic behaviour and fits the interpretation of a heterospecific cannibalistic response of larger marron to smaller intruder yabbies (Chapter 9), the recipient marron displayed avoidance behaviour. In contrast, when exposed to finfish predator odour in an experimental setting, small, medium and large marron showed increased periods of time spent climbing, which is considered to be escape behaviour (Chapter 5). This tactic was assumed when marron were exposed to finfish predators, which therefore affected both adult and juvenile survival of marron in polyculture (Chapter 9).

Reproductive cues

Guiaşu & Dunham (1997) noted that during the reproductive season, adult male crayfish of the family *Cambaridae* differentiate into non-breeding and breeding forms. The latter form display more agonistic behaviour than others, while Jones (1990) indicated that red claw did not show remarkable aggressive behaviour. This could be due to the ability to distinguish dominance of individuals from the red bright colour patch on their claws (Karplus *et al.* 2003). No such notable observation was made in this study. However, in Chapter 4 the berried female yabbies were shown to use shelter more frequently than males and frequently shared in the provided shelters. This was similar to observations of berried marron in Chapter 9, which explains the significantly higher survival rates of marron in the sheltered ponds compared with those in the non-sheltered ponds in that polyculture study.

Aggression

Aggression is a general behaviour prevalent in all kinds of crayfish; it is difficult to define which species may be more aggressive than others. While the expression of aggressive behaviour depends on many factors, limited availability of crayfish shelter results in clear displays of aggression (Gherardi 2002). Generally male crayfish tend to display aggressive interactions such as fighting and also tend to have a higher rate of appendage loss or single chelae, which impairs fighting ability (Whisson 2004, 2006). Other factors influencing aggression include reproductive status (Chapter 4), and prior resident status (Chapters 4, 5 and 9). Starvation increases agonistic behaviour and

cannibalism (Chapter 7), and environmental factors such as increased water temperature may promote increased moulting frequency and may also provoke the display of agonistic behaviour (Chapter 7). If shelter is intruded upon at any time, both marron and yabbies will leave the shelter to defend their territory by fighting, or will retreat depending upon the outcome of the initiating challenge.

Foraging behaviour

Foraging in crayfish is generally a nocturnal behaviour (Guan & Wiles 1998). Both marron and yabbies are considered to be omnivorous but are also known to display opportunistic cannibalism (Chapters 6 and 9). Shelter utilisation tends to be high when food resources are not limiting. Hunger stimulates foraging behaviour and activity outside of shelter.

In multi-species systems the foraging behaviour of participant species is greatly affected by spatial distribution, food availability (Stein 1977, Barki *et al.* 1997) and population dynamics (Krebs and Davies 1993). In order to be successful in acquiring the necessary resources, each individual exhibits both agonistic and avoidance behaviour during confrontation with competitors (Vahl 2006). Predators, in general, select prey that will maximise their net energy gain or optimal foraging (Stein 1977, Krebs and Davies 1991, Whisson 2000). 'Optimal foraging' in this context is based upon underlying costs and benefits (Mitchell & Hazlett 1996). Stein (1977) observed that predators selected the most vulnerable prey. However, in Chapter 6, in the control treatment, large yabbies cannibalised medium-sized yabbies over small yabbies. This result concurs with high mortality rates observed in red claw polyculture with minimal shelters (Boulay 1993). As shown in Chapter 9, availability of food and spatial distribution are major causes of conflict between prey and predator. In the present study this affected the crayfish through intra-specific cannibalism resulting in high mortality (Chapter 7). These results supported the findings of Hazlett (2003) who observed that starving crayfish could be highly aggressive. After large yabbies were fed, cannibalism ceased following replacement of the medium-sized yabbies. In polysystems, if food is not available, co-stocking fish (i.e. silver perch) can change their foraging to hunting (Kaufamn *et al.* 1996). In contrast, as prey, crayfish can sometimes identify chemical signals of starved predators (Hazlett 2004), therefore spending more time in shelters and reducing

foraging activity (Chapters 4, 5, and 6). This, in turn, influences marron growth (Chapter 9).

Resource Holding Potential (RHP)

The outcome of shelter competition is greatly affected by the relative resource holding potential (RHP) of the rivals (Dingle 1983, Chapters 4 and 5). This suggests that, generally, crayfish with larger physical appearance (body size, carapace length, chelae) are more aggressive and therefore likely to win competition for the limited resource (Mesterton-Gibbons & Dugatkin 1995). However, prediction of the outcome from competition does not rely solely on RHP (Mesterton-Gibbons & Dugatkin 1995, Taylor & Elwood 2003 and Cioni *et al.* 2007). Other factors that affect shelter competition include the prior residence effect (Figler 1999, Chapter 4), different species, and reproductive status. The prior residence effect, demonstrated in results from Chapters 4 and 5 (the 5 minute contest), indicates that prior residents spend longer in shelter than intruders, regardless of higher RHP or gender (Chapters 4 and 5). For the 24 h observation periods conducted in Chapters 5, 6 and 8, shelter retention time appeared to be dependent upon species of crayfish (i.e. yabbies spent longer in shelter than marron). However, comparisons between different sizes of crayfish show that juveniles of both species spent significantly more time in shelter than adult crayfish.

In addition, the time spent in shelter did not increase survival for any size of species of crayfish (Chapters 6 and 9). The ability to remain in shelter during critical periods when predators are present is more important for survival (Chapters 6 and 8). Further, predator odour has an influence on shelter retention time and relative size of shelters selected by yabbies (Chapter 6). In multi-species systems it appears that the most adaptable rather than strongest animal will survive.

10.2.5 Effect of shelter on crayfish survival

10.2.5.1 Conspecific and heterospecific cannibalism

Population density, availability of food (Thomas *et al.* 2003), body size and starvation are the main factors affecting the frequency of cannibalism in natural crayfish populations (Dong & Polis 1992). Combinations of these risk factors can increase the levels of cannibalism (Barki *et al.* 1997, Harzlett 2003, Thomas 2003). Triggers for

cannibalism are sometimes not known, but it often occurs when different sizes of crayfish live together: increased rates of social interaction may create the opportunity for this situation (Chapter 6). The provision of shelter of increased complexity resulted in the reduction of cannibalism between marron and yabbies (Chapter 9). Results showed that marron survival and production rates were higher for groups reared in the pond provided with shelters. However, simple shelter provision did not appear to enhance the survival of yabbies due to the predominating influence of prior resident status of marron (Chapter 9).

10.2.5.2 Dominant cannibalism

In Chapter 9, large prior resident marron had a greater RHP and displayed highly aggressive behaviour which resulted in high mortality of the intruders (yabbies). Physical condition can also greatly impair hunting ability. For instance several marron that were unsuccessful in capturing and eating intruders were found with one claw intact. In contrast, yabbies as prior residents maintained an 80% survival in the same experiment. These results indicate that prior resident status of marron with high RHP evoked a high degree of dominance aggression toward smaller intruding yabbies, which culminated in cannibalism.

10.2.5.3 Predation

In polyculture, co-stocked finfish and crayfish may become predators of one another (Savino & Miller 1991, Mason 1994 and Whisson 2004). In addition they may also be threatened by crustaceans, amphibians, avian and terrestrial predators such as mammals (Beja, 1996, Jones & Ruscoe 1996 and Gherardi 2002). Silver perch are known to prey on various sizes of marron depending on light intensity, life stage and water turbidity (Whisson 1999). In Section 9.2, when marron and yabbies were released from experimental cages, silver perch attacked both species regardless of crayfish size. These observations do not support the findings of Jones and Ruscoe (1996) who stated that perch only consumed <5g crayfish. Other evidence of predation was displayed by Murray cod when the fish simply swallowed >200g marron whole. As summarised in Table 10.2, polyculture of finfish with crayfish of greatly disparate sizes is likely to result in predation by the larger species. Mechanisms to control predation in polyculture include increased turbidity (Whisson 1999) or cage culture of finfish (Storer 2006).

Others have suggested that prevention of predation could be prevented by increasing complexity of shelter (Blake & Hart 1993, Miner & Stein 1996, Whisson 2000).

Increasing habitat complexity in polyculture has a significant impact on crayfish survival (Chapter 9). However the low survival rate of marron in polyculture has been directly attributed to predation by avian species (Chapter 9, Rowland 1995 and Storer 2006). This was observed at the experimental site described in Chapter 9.3 where marron carcases were found following sighting of cormorants. This occurred mainly in the pond without shelters. The sheltered ponds afforded slightly better survival as the shelters were located in the sloping shallow area of the ponds which appeared to reduce consumption of moulting marron from avian predators. In this regard habitat complexity may reduce the intensity of finfish predation but the threat of avian and terrestrial predation remains. The suggestion to cover the pond with bird nets seems more appropriate.

Several finfish species have been reported to feed on crayfish including brook trout (Momot 1967, Faragher 1983), redfin perch, eels (Blake & Hart 1995), and silver perch (Whisson 1999). Momot (1967) concluded, however, that predation does not appear to be an important mechanism of population control of crayfish. However, Faragher (1983) found that while brown trout and rainbow trout were predators of marron, the population of marron rose because larger trout were caught by anglers. In Section 9.2, two finfish species were included in the mesocosm, namely Murray cod and silver perch. The selection of appropriate predators could be a useful tool in controlling unwanted crayfish (Frutiger & Müller 2000). This may apply to controlling the introduced yabby (*Cherax albidus*). Further study is warranted.

10.3 Effects of shelter on marron production in polyculture

Investigating the role of shelter is a cornerstone of this thesis. The intention has been to understand the role of shelter with respect to crayfish polyculture, and specifically the related mechanisms that influence crayfish and finfish production. This is of direct relevance to management strategies and ultimately investment in the crayfish farming industry.

10.3.1 Shelter complexity in polyculture ponds

In response to exposure to predator odour, both yabbies and marron increased time spent in shelters (Chapters 6, 8). However, yabbies spent more time in shelters than marron in these experiments, and consistently selected smaller sized shelters when under threat. When prior resident marron were confronted with congeneric intruders, they increased movement outside the shelter (Chapter 5). This suggests that yabbies prefer smaller shelters closely fitting their body size when in the presence of finfish predators and conspecifics. Provision of shelter complexity in these investigations (Chapters 6 and 9) allowed for the expression of shelter-seeking behaviour and preferences that differed from the studies of single shelters with individual crayfish in Chapters 4 and 5. Yabbies appear to possess a more sophisticated means of perceiving threat or higher plasticity (Lee 2002) compared to marron (Gherardi *et al.* 2002 and Height 2002), and seem able to adapt their response accordingly; this appears to drive the differences in shelter selection observed in these investigations. Commercially, the provision of shelter of greater complexity holds promise of enhanced survival of crayfish in polyculture.

To understand the role of shelter complexity in crayfish polyculture systems, this study used a range of different sizes of shelter (Chapter 8) and synthetic materials (commercial crayfish shelter) (Chapter 9). There were clear differences in shelter selection by marron, but selection preferences differed between genders and across sizes of marron. It is difficult to draw definitive conclusions from these results. Further study is recommended in order to fully understand the factors that affect the behaviour of marron toward predators and congenerics in the context of polyculture.

The provision of shelter in marron aquaculture and polyculture systems has been shown to increase marron survival (Swannell 1994, Whisson 1997, Storer 2006). In Chapter 9, the number of juveniles harvested and the survival of adult marron increased significantly ($P<0.05$) with the provision of a complex, synthetic commercial shelter. This demonstrates that availability of shelter with a degree of complexity is important for both adult and juvenile marron in commercial polysystems. This is supported by the findings of many authors including Mattila (1991), Whisson (1997) and Storer (2006) who agreed that polyculture of marron continues to hold commercial promise. Providing shelter in this experimental system reduced the success of predation (Table

10.2). However, survival and biomass of marron from the polyculture study in Chapter 9 clearly demonstrated that shelter and shelter complexity positively influenced the production of marron in polyculture. Further study of the dynamics of shelter, management strategies and other factors are required to support the commercial viability of the polyculture of marron.

10.3.2 Effect of shelter on marron survival

It was clear that providing shelter improved marron production (Chapter 9); however, prior resident status played an important role (as shown in Chapter 5). Similar results were reported in Section 9.3 where marron were stocked a week before the silver perch were introduced. This sequence of stocking could create a prior resident effect (Section 9.1 and Figler 1999) and contribute to a highly aggressive response by marron, particularly in berried females (Chapter 4). Marron in this study therefore reacted to the co-stocked finfish as intruders, increasing exposure to silver perch. Moreover, the schooling behaviour of silver perch proved to be an effective strategy in hunting crayfish (Section 9.2). If finfish were relatively large compared to resident crayfish, high mortality would likely result. This phenomenon was demonstrated in Section 9.3 where the survival rate of marron in non-sheltered ponds was significantly lower (11%) than that in the sheltered ponds. This underscores the importance of the prior resident effect and sequence of stocking species, regardless of the provision of shelters (Section 9.1).

10.3.3 Effect of shelter on finfish growth and survival

Growth, survival and condition of silver perch in the sheltered ponds were superior to that in the non-sheltered ponds. This agrees with the findings of others (Wahav & Azim 1994) who reported that crayfish shelter can provide a natural substrate for growth of phytoplankton and periphyton, both significant food sources for finfish. Similar findings were reported using car tyres which attracted more microphytes on their rigid surface (Mosig 1996). Some types of shelter available for crayfish are not furnished from substrates suitable for the growth of such foodstuffs, e.g. PVC pipe. This is worthy of further investigation for polyculture.

10.3.4 Shelter manipulation in ecosystems

Avian predation as described in Chapter 9 is one of the major problems of marron aquaculture. Birds often visit shallow areas where marron hide during moulting periods. Total exclusion of avian predators is expensive; a cost-effective alternative could be to improve crayfish habitat complexity. However, the commercial crayfish shelters employed in Chapter 9 did not totally prevent attempts by birds to capture crayfish: some netting was damaged by beaks and some marron were observed with carapace injury consistent with pecking. Reducing avian predation through a combination of water turbidity and shelter provision has been found useful in increasing survival rates in marron polyculture (Storer 2006). A hard shelter substrate was also found to be more suitable for use in shallow water, particularly for smaller crayfish (Englund 2000). For example, the use of bio-filter blocks (Jones 2006) has now largely been adopted by the red claw industry; and car tyres or rocks (McCormack 1996, Mosig 1996) have proven suitable for marron and yabby farming, provided they do not introduce toxic substances into the water, do not block water circulation or reduce oxygen levels, and do not allow the build up of toxic pockets which could harm crayfish.

Large structures such as tyres or synthetic loose nets provide useful shaded areas in crayfish ponds, particularly when water temperatures rise above 24ºC (Morrissy 1990). In experiments where cages are included, Whisson (2000) found that marron survival rates were relatively high compared to no-cage systems. The cages in this case could provide shady and dark cool areas that perform as marron shelters. This system provides a more complex habitat which prevents interactions between co-stocked species. These outcomes could lead to greater stocking densities for marron and silver perch polyculture.

In Section 9.2, combinations of 90mm PVC pipes and commercial crayfish shelter were used in the mesocosm trial. This created more complex shelter options for both marron and yabbies. However, no marron and only 10% of yabbies survived in this study. In this regard yabbies seem to be more adaptable than marron. Even though the gravel substrate prevented yabbies from burrowing, they were able to be use the complex shelter structures more than congeneric marron.

10.3.5 Role of shelter on behavioural integration of crayfish

As shown in Table 10.2, the combination of relative finfish size and the presence or absence of shelter also affects marron survival. It was clearly demonstrated that in polysystems the relative size of predator and availability of shelter are the most important factors determining crayfish survival. The availability of shelter including cages (Whisson 2004, Storer 2006) reduces social conflict and agonistic behaviour, and provides a substrate for food for both crayfish and finfish. Without shelter, the survival of crayfish is particularly low. Remarkably, the experiment with *Vallisneria sp.* as a shelter substrate resulted in marron survival rates as high as 80% (Table 10.2). This is a clear indicator of the multiple roles of macrophytes as shelter, food and as a nursery ground for crayfish.

Production of yabbies in rural Western Australia has been overwhelming successful (Fisheries Western Australia 2000, Nenke 2000). Aquaculture techniques for yabby culture have improved significantly (Lawrence & Morrissy 2000). Behavioural studies clearly show that yabbies provide a perfect model of species survival. Yabbies responded to threats by reducing active time spent outside shelters (Chapters 4, 5, and 6). They also showed an appropriate response toward finfish predators. These characteristics would commend yabbies as good candidates for polyculture. Trials were conducted to investigate their polyculture potential using 300L recirculation tanks. Each tank was stocked with 20(2g) yabbies together with 3 (TL 150-200mm) silver perch. Results showed that yabbies caused serious mortality of silver perch in every tank within a few days (Wangpen 2005). This short free-range trial was aborted due to the condition of the remaining silver perch (torn fins and body wounds etc). Cage culture of finfish could be an alternative system in yabby and finfish polyculture. However, further study is required. This trial clearly demonstrates that yabbies could be harmful to other co-stocked finfish and could become invaders in water bodies.

The issue of the invasion of non-indigenous species has long been of scientific concern (Elton 1958). The displacement of native species has been recently put forward as the most important factor contributing to the loss of biodiversity. Much effort has been directed at controlling or eliminating invasive species by any means including physical, chemical and biological control. Among these, biological control is considered to be a safer method. In Chapter 9, prior resident marron successfully consumed up to 100% of

juvenile intruder yabbies. This is an important finding of this thesis, as it implies that prior resident status together with relative body size are a promising means of resisting successful establishment of introduced species. This identifies a strength of native marron as well as a means of manipulating habitat to support their capacity for resurgence. This simple, low-cost method of safeguarding native marron populations requires public awareness for success to avoid unnecessary removal of valuable larger specimens from their natural habitat.

10.3.6 Additional roles of shelter in polysystems

10.3.6.1 Food source

Shelter can provide substrate for growth of algae as a source of food. Results of Chapter 9 demonstrated that both marron and silver perch were in better condition in the sheltered ponds. The benefit of algae as supplementary feed enhances the potential benefits of polyculture.

10.3.6.2 Reduction of light penetration

Provision of shelter or cage culture (Whisson 2000) reduces light penetration to create shade during summer, thereby reducing exposure to the heat especially when transparency of pond water is high. Reduced light penetration prevents growth of submersible plants; excessive growth of such plants can cause oxygen depletion when decomposing.

10.3.6.3 Suitable substrate

Natural vegetation as shelter provides an alternate food supply. In the pond aquaculture trials where there was a dependency on supplementary feed, aggression increased when food was not available (Chapter 7). That trial sought to explore the relationship between shelter and starvation of yabbies at different temperatures. In the wild or in aquaculture ponds without supplementary feed, yabbies can find alternate food sources from algae, organic matter or other zooplankton present (Section 9.3). Results (Table 10.2) indicated that juvenile marron benefited from vegetation as a nursery habitat; even in unfed marron, the survival rate was over 75% (Whisson 1997). The use of *Vallisneria sp.* for juvenile marron has therefore been recommended by Swanell (1994) and confirmed by Whisson (1997, 2000).

10.4 Conclusions

The provision of shelter in polysystems improves crayfish and finfish survival and production and is therefore universally recommended. The following factors should be taken into account:

1. Identification of species requirements, i.e. body size, burrowing or non-burrowing species, provision of appropriate substrate for shelter (clay, sandy loam, etc.)

2. Crayfish species differ in predator recognition and adaptive behaviour: yabbies in these studies showed more complex and responsive predator avoidance behaviours than marron. Their plasticity status to be determined.

3. Starvation in temperatures above the range currently considered to be optimal (>28°C) will result in high mortality. Regular feeding together with highly complex shelter will enhance survival and productivity at such temperatures.

4. When crayfish are added to ponds or tanks with existing stock, a prior resident effect may result in conspecific cannibalism.

5. Yabby characteristics pose an ongoing high risk of displacement to native marron. However, marron have the capacity to resist when relative body size is greater than yabbies, and prior resident status is established.

6. Polyculture of marron with silver perch continues to hold promise due to the limited reactivity of marron to finfish odour. Provision of shelter in this system is essential.

7. Shelter manipulation and turbidity management can increase the chance of crayfish survival, particularly during moulting.

10.5 Recommendations

10.5.1 Laboratory studies

1. Studies of the behavioural responses of crayfish in competition for shelter need to consider shelter preferences of different species and size (RHP) of crayfish.

2. The high variability observed in crayfish behavioural experiments was attributed to limited replication. It is therefore recommended that such studies of crayfish include at least 18 replicates.

3. The influence of prior residence of larger marron on juvenile yabbies merits further study to investigate whether this phenomenon is a realistic means of controlling invasive yabbies.

10.5.2 Field studies

The expansion of marron polyculture holds considerable promise for the crayfish industry. The following areas are recommended for further study:

1. Investigation of new husbandry and new culture systems including:

   a. Comparison of carrying capacity with increased shelter complexity in cage polyculture;

   b. Comparison of marron in cages stocked with free-range silver perch;

   c. Further investigation of shelter type and arrangement, including recycling of synthetic material in combination with marron shelter and vegetation (e.g. use of car tyres as shelter);

   d. Use of yabbies in polyculture;

   e. Rearing of caged silver perch in purpose-built ponds with monosexed yabbies.

2. The effect of different types of shelter on primary production and water quality in polyculture systems with new species; for example, smooth marron *vs* hairy marron, normal yabbies *vs* hybrid yabbies, or real male *vs* hybrid all male etc.

3. The prior resident effect in marron and yabbies should be further investigated in the field as a potential means of biological control.

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Appendices

Appendix 1 Water quality data

Table 1.1 (Chapter 9.2) Water quality in mesocosm
Role of shelter on prevent the cannibalism from congeneric and finfish predation.

Water quality parameters	Tested weekly Oct - Dec 2006 (±SE)
Temperature (ºC)	22.5 (1.3)
pH	7.6 (0.4)
Salinity (ppt)	0.01 (0.01)
Conductivity (mS/m)	35 (3.6)
Dissolved Oxygen (mg/L)	9.2 (1.4)
Total ammonia (mg/L)	1.6 (0.1)
Nitrite (mg/L)	0.1 (0.0)
Nitrate (mg/L)	0.5 (0.2)

Average data from 10 samplings

Table 1.2 (Chapter 9.3) Water quality in earthen ponds
Role of shelter in marron and silver perch polyculture system
A: Monthly average of all ponds

Water quality parameters	Dec (±SE)	Jan (±SE)	Feb (±SE)	Mar (±SE)
Temperature (ºC)	24.23(0.09)	24.3(0.05)	22.68(0.09)	22.23(0.09)
pH	7.56(0.03)	7.72(0.03)	7.44(0.09)	7.36(0.11)
Salinity (ppt)	0.67(0.00)	0.80(0.01)	0.88(0.01)	0.09(0.01)
Conductivity (mS/m)	1.42(0.04)	1.60(0.02)	1.64(0.01)	1.79(0.02)
Dissolved Oxygen (mg/L)	7.68(0.03)	7.74(0.05)	7.65(0.06)	7.60(0.08)
Total ammonia NH_3 (mg/L)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
Alkalinity	52.1(2.99)	Na	Na	41.67(3.81)
Transparency (secchi-disk cm)	>50	48(0.06)	41 (1.6)	38 (2.9)

Note: Average data from six experimental ponds (0.072 Ha), measured between 1500-1700

B: Pond aver over trial duration

Water quality Parameters	Pond 1 (±SD)	Pond 2 (±SD)	Pond 3 (±SD)	Pond 4 (±SD)	Pond 5 (±SD)	Pond 6 (±SD)
Temperature	23.59(1.72	23.45(1.75	23.53(1.8	23.33(1.7	23.61(1.7	23.80(1.7
pH	7.32(0.52)	7.43(0.48)	7.56(0.03)	7.65(0.24	7.67(0.24	7.68(0.25
Salinity	0.81(0.1)	0.79(0.01)	0.80(0.01)	0.80(0.1)	0.77(0.09	0.78(0.1)
Conductivity	1.60(0.18)	1.58(0.19)	1.58(0.18)	1.58(0.19	1.53(0.15	1.62(0.50
Dissolved Oxygen	7.42(0.4)	7.64(0.3)	7.57(0.4)	7.70(0.03	7.60(0.33	7.56(0.40
Ammonia NH_3	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00	0.00(0.00	0.00(0.00
Alkalinity	45.37(1.67	42.17(4.66	46.88(3.1	45.33(1.9	43.73(2.6	41.22(3.6
Transparency	45.00(2.1)	42.00(5.40	44(2.9)	42(4.10)	43 (4.3)	47 (1.2)

Note: Average data between December and March in all ponds, measured between 1500-1700

Appendix. 2

Figure 2.1 Tank number and treatment allocations

Stand 1 L

Tank 1 **T2**	Tank 2 **T3**	Tank 3 **T1**	Block1
Tank 4 **T3**	Tank 5 **T1**	Tank 6 **T2**	Block2
Tank 7 **T1**	Tank 8 **T2**	Tank 9 **T3**	Block3

Stand 1 R

Tank 10 **T3**	Tank 11 **T1**	Tank12 **T2**
Tank 13 **T1**	Tank 14 **T2**	Tank 15 **T3**
Tank 16 **T2**	Tank 17 **T3**	Tank 18 **T1**

Stand 2 L

Tank 19 **T1**	Tank 20 **T2**	Tank 21 **T3**	Block1
Tank22 **T2**	Tank 23 **T3**	Tank 24 **T1**	Block 2
Tank 25 **T3**	Tank 26 **T1**	Tank 27 **T2**	Block 3

Stand 2 R

Tank 28 **T2**	Tank 29 **T3**	Tank 30 **T1**
Tank 31 **T3**	Tank 32 **T1**	Tank 33 **T2**
Tank 34 **T1**	Tank35 **T2**	Tank36 **T3**

Stand 3 L

Tank37 **T3**	Tank 38 **T1**	Tank 39 **T2**	Block1
Tank 40 **T1**	Tank 41 **T2**	Tank 42 **T3**	Block 2
Tank43 **T2**	Tank 44 **T3**	Tank 45 **T1**	Block 3

Stand 3 R

Tank 46 **T1**	Tank47 **T2**	Tank 48 **T3**
Tank 49 **T2**	Tank 50 **T3**	Tank 51 **T1**
Tank 52 **T3**	Tank 53 **T1**	Tank 54 **T2**

Table 2.1 record sheet	Tank	Resident	Intruder	Time	Day				Test solution	
Crayfish	Rec#	Sec	shelter	movement	posture R/I/L	cleaning	ante/antu 1/2/B	climbing	fighting	appen/mort
Resident	1	0								
Intruder	1	0								
Resident	2	30								
Intruder	2	30								
Resident	3	1:00								
Intruder	3	1:00								
Resident	4	1:30								
Intruder	4	1:30								
Resident	5	2:00								
Intruder	5	2:00								
Resident	6	2:30								
Intruder	6	2:30								
Resident	7	3:00								
Intruder	7	3:00								
Resident	8	3:30								
Intruder	8	3:30								
Resident	9	4:00								
Intruder	9	4:00								
Resident	10	4:30								
Intruder	10	4:30								
Resident	11	5:00								
Intruder	11	5:00								

Table 2.2 Mean values (±) s.e.) Percentage of time(s) spent in shelter of two different test solutions (freshwater and silver perch odour) for small berried females (SB) prior residence and large male yabby (LM) Intruder at 1 and 3 days

Treatment 1	Test type	Prior rest.(SB)	Intruder(LM)	Z (*P*)
(day1,5mins)	Control	90.00±6.01	13.33±9.43	.-3.571(0.000)**
	Predator	92.22±7.78	7.78±6.62	-3.725 (0.000)**
(day1,24hr)	Control	21.11±6.33	43.33±5.77	-2.146 (0.032)*
	Predator	33.33±7.26	38.89±4.23	-0.316 (0.752)
(day3,5mins)	Control	55.56±15.64	66.67±14.04	0.232 (0.816)
	Predator	34.44±15.73	93.33±5.53	-2.675 (0.007)**
(day3,24hr)	Control	33.33±7.26	31.11±4.55	-2.270 (0.787)
	Predator	16.66±8.50	40.00±4.41	-1.905 (0.057)

Table 2.3 Mean vales (± s.e.) of percentage of time(s) spent in shelter of two different test solutions (freshwater and silver perch odour) for large berried female (LB) prior residence and large male yabby (LM) Intruder at first and third days.

Treatment 2	Test type	Prior rest.(LB)	Intruder(LM)	Z (p)
(day1,5mins)	Control	88.89±11.11	3.33±3.33	-3.528 (0.000)**
	Predator	81.11±10.60	2.22±2.22	-3.377 (0.001)**
(day1,24hr)	Control	20.00±5.77	27.78±6.19	- 0.941 (0.347)
	Predator	35.56±8.35	27.78±5.72	-0.628 (0.530)
(day3,5mins)	Control	77.78±14.70	63.33±15.90	-0.511 (0.609)
	Predator	66.67±16.67	56.67±17.16	-0.425 (0.671)
(day3,24hr)	Control	34.44±6.89	35.56±5.30	-0.091 (0.928)
	Predator	34.44±7.29	30.00±6.24	-0.451 (0.652)

Table 2.4 Mean vales (± s.e.) of percentage of the time(s) spent in shelter of two different test solutions (freshwater and silver perch odour) for small berried female (SB) prior residence and large berried yabby (LB) Intruder at 1th and 3nd days

Treatment 3	Test type	Prior rest.(SB)	Intruder(LB)	Z (p)
(day1,5mins)	Control	90.00±6.01	0.00±0.00	-3.902 (0.000)
	Predator	92.22±7.78	7.78±6.62	-3.771 (0.000)
(day1,24hr)	Control	28.89±5.39	28.89±7.54	-0.045 (0.964)
	Predator	25.56±5.80	28.89±6.96	-0.405 (0.685)
(day3,5mins)	Control	75.56±14.35	51.11±16.45	-1.134 (0.257)
	Predator	33.333±16.67	65.56±16.42	-1.193 (0.233)
(day3,24hr)	Control	30.00±9.43	32.22±5.72	-0.270 (0.787)
	Predator	18.89±6.76	35.56±6.69	-1.905 (0.057)

Table 2.5 Mean value of body posture (%) at different test solution and pair of yabbies

Treatment/time	Posture type		Prior resident	Intruder	Z (P)
Treatement 1	L	Control	42.22±13.92	3.33±2.36	-2.241 (0.025)
		Predator	43.33±13.64	3.33±2.36	-2.184 (0.029)
SB vs LM	I	Control	22.22±6.41	44.44±13.24	-1.029 (0.303)
		Predator	34.44±10.42	71.11±7.16	-2.179 (0.029)
1 day 5 mins	R	Control	35.56±12.37	52.22±13.62	-0.982 (0.326)
		Predator	22.22±10.51	25.56±7.84	-0.633 (0.527)
Treatement 1	L	Control	37.78±12.95	45.56±10.56	-0.493 (0.622)
		Predator	72.22±10.11	67.78±10.24	-0.407 (0.684)
SB vs LM	I	Control	36.67±12.80	31.11±10.60	-0.226 (0.821)
		Predator	15.56±7.09	22.22±8.30	-0.571 (0.568)
3day 5 mins	R	Control	25.56±10.29	23.33±7.99	-0.046 (0.964)
		Predator	12.22±8.13	10.00±5.00	-0.526 (0.599)
Treatement 2	L	Control	67.78±1.88	1.11±1.11	-3.357 (0.001)
		Predator	27.28±13.52	2.22±2.22	-1.620 (0.105)
LB vs LM	I	Control	17.78±6.83	61.11±13.49	-2.167 (0.030)
		Predator	57.78±14.60	76.67±1041	-0.678 (0.498)
1 day 5 mins	R	Control	14.44±11.19	37.78±13.82	-1.652 (0.099)
		Predator	14.44±8.99	21.11±9.64	-0.993 (0.320)
Treatement 2	L	Control	41.11±11.95	82.22±8.46	-2.559 (0.010)
		Predator	34.44±16.42	58.89±16.54	-1.020 (0.308)
LB vs LM	I	Control	55.56±11.92	16.67±7.82	-2.380 (0.017)
		Predator	51.11±14.67	35.56±16.25	0.560 (0.575)
3 day 5 mins	R	Control	2.22±1.47	1.11±1.11	-0.615 (0.593)
		Predator	14.44±8.35	5.56±5.56	-1.031 (0.303)
Treatement 3	L	Control	46.67±11.90	12.22±11.03	-2.791 (0.005)
		Predator	64.44±13.24	16.67±11.06	-2.140 (0.032)
SB vs LB	I	Control	33.33±10.80	56.67±14.34	-1.114 (0.265)
		Predator	23.33±10.83	55.56±13.28	-1.534 (0.125)
1 day 5 mins	R	Control	20.00±10.54	31.11±13.28	-0.465 (0.642)
		Predator	12.22±11.03	27.78±12.99	-1.310 (0.190)
Treatement 3	L	Control	60.00±12.58	56.67±12.36	-0.045 (0.964)
		Predator	83.33±6.87	71.11±13.28	-0.236 (0.813)
SB vs LB	I	Control	21.11±10.33	26.67±11.55	-0.324 (0.746)
		Predator	15.56±7.09	6.67±4.71	-1.055 (0.291)
3 day 5 mins	R	Control	17.78±9.83	16.67±7.99	-0.142 (0.887)
		Predator	0.00±0.0	22.22±11.64	-1.835 (0.067)

Table 2.6 Mean value of time in behaviour responses of all paired contest and times

Treatment/time	Flicking		Prior resident	Intruder	Z (P)
Treatement1	Ae	Control	1.11± 1.11	0.00±0.00	-1.000 (0.317)
		Predator	0.00±0.00	1.11±1.11	-1.000 (0.317)
SB vs LM	Au	Control	46.67±11.06	5.56±3.38	-2.829 (0.005)**
		Predator	52.22±11.64	0.00±0.00	-3.166 (0.002)**
1 day 5 mins	Both	Control	22.22±7.22	72.22±8.94	-3.164 (0.002)**
		Predator	24.44±10.15	64.44±8.68	-2.447 (0.014)*
Treatement1	Ae	Control	0.00±0.00	0 .00±0.00	0.000 (1.000)
		Predator	0 .00±0 .00	0 .00±0.00	0.000 (1.000)
SB vs LM	Au	Control	60.00±1106	53.33±11.18	-0.535 (0.592)
		Predator	67.78±9.40	68.89±9.92	-0.090 (0.928)
3day 5 mins	Both	Control	12.22±6.19	17.78±7.78	-0.094 (0.925)
		Predator	17.78±5.21	11.11±8.73	-1.816 (0.069)
Treatement2	Ae	Control	0.00 ±0.00	0.00±0.00	0.000 (1.000)
		Predator	0.00±0. 00	0.00±0.00	0.000 (1.000)
LB vs LM	Au	Control	47.78±14.70	0. 00±0.00	-2.848 (0.848)
		Predator	67.78±13.41	2 .22±2.22	-2.966(0.003)**
1 day 5 mins	Both	Control	27.78±12.99	58.89±10.60	-1.915 (0.055)
		Predator	18.89±10.60	63.33±9.72	-2.773 (0.006)**
Treatement2	Ae	Control	0.00±0.00	0.00±0.006	0.000 (1.000)
		Predator	0.00±0 .00	0 .00± 0.00	0.000 (1.000)
LB vs LM	Au	Control	72.22±9.54	62.22±13.41	-0.224 (0.823)
		Predator	75.56±7.66	36.67±9.57	-2.625 (0.009)**
3day 5 mins	Both	Control	4 .44±2.42	1.11 ±1.11	-1.156 (0.248)
		Predator	13.33±6.01	14.44±7.29	-2.773 (0006)**
Treatement3	Ae	Control	0.00 ±0.00	0.00±0.00	0.000 (1.000)
		Predator	0.00 ±0.00	0.00±0.00	0.000 (1.000)
SB vs LB	Au	Control	62.22±12.22	1.11±1.11	-2.966 (0.003)**
		Predator	70.00±11.18	5.56 ±2.94	-3.145 (0.002)
1 day 5 mins	Both	Control	13.33±4.71	68.68±14.19	-2.425 (0.035)
		Predator	13.33± 5.77	74.44±7.47	-3.471 (0.001)**
	Ae	Control	0.00 ±0.00	0.000±0.00	0.000 (1.000)
		Predator	1.11±1.11	0.00 ± 0.00	-1.000 (0.317)
Treatement3	Au	Control	67.78±9.25	64.44±9.80	-0.223 (0.824)
		Predator	74.44±8.01	68.98±8.24	-0.821 (0.561)
SB vs LB	Both	Control	16.67±6.01	14.44±6.26	-0.413(0.680)
		Predator	5.56 ±4.44	13.33±8.66	-0.949(0.342)

3day 5 mins

Table 2.7 Comparison of aggressive behaviour of prior residents and intruder yabbies in both test solutions

Treatment/time	Behaviour		Prior resident	Intruder	Z (P)
Treatment1	Clim	Control	5.56 ±5.56	23.33±9.13	-1.846 (0.065)
		Predator	0.00 ±0.00	24.44±7.84	-2.846 (0.004)
SB vs LM	Fig	Control	3.33± 2.36	2.22±1.47	-0.122 (0.903)
		Predator	5.56±3.38	5.56 ±3.38	0.000 (1.000)
1 day 5 mins	App	Control	0.00±0.00	0.00±0.00	0.000 (1.000)
		Predator	0.00± 0.00	0.00±0.00	0.000 (1.000)
Treatment1	Clim	Control	0.00 ±0.00	0.000±0.00	0.000 (1.000)
		Predator	1.11±1.11	0.00 ± 0.00	-1.000 (0.317)
SB vs LM	Fig	Control	4.44±2.94	0.00±0.00	-1.458 (0.145)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
3day 5 mins	App	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
Treatment2	Clim	Control	0.00 ±0.00	45.56±10.82	-3.494 (0.000)
		Predator	3.33 ±1.67	26.67±8.16	-2.285 (0.017)
LB vs LM	Fig	Control	4.44±4.44	4.44±4.44	0.000 (1.000)
		Predator	7 .78±7 .78	7.78 ±7.78	0.000 (1.000)
1 day 5 mins	App	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
Treatement2	Clim	Control	1.11±1.11	0.00 ± 0.00	-1.000 (0.317)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
LB vs LM	Fig	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
3day 5 mins	App	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
Treatement3	Clim	Control	6.67 ±1.67	34.00±12.37	1.576 (0.115)
		Predator	4.44 ±1.76	20.00±7.26	-1.377 (0.169)
SB vs LB	Fig	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
1 day 5 mins	App	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
Treatement3	Clim	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
SB vs LB	Fig	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
3day 5 mins	App	Control	0.00±0.00	0.00 ±0.00	-0.000 (1.000)
		Predator	0.00±0.00	0.00 ±0.00	-0.000 (1.000)

Table 2.8 Analysis of variance of behavioural responses of marron and yabby Different letters between groups indicate significantly different means (α 0.05).

		Sum of Squares	df	Mean Square	F	Sig.
% time in shelter	Between Groups	10512.341	6	1752.057	1.781	.105
	Within Groups	170144.712	173	983.495		
	Total	180657.053	179			
% time movement	Between Groups	8154.876	6	1359.146	1.513	.177
	Within Groups	155444.953	173	898.526		
	Total	163599.829	179			
% time cleaning	Between Groups	710.684	6	118.447	1.275	.271
	Within Groups	16070.983	173	92.896		
	Total	16781.667	179			
% time climbing	Between Groups	5511.617	6	918.603	4.988	.000*
	Within Groups	31861.866	173	184.173		
	Total	37373.483	179			
% time Fighting	Between Groups	2204.338	6	367.390	.560	.761
	Within Groups	113419.221	173	655.602		
	Total	115623.559	179			
% time flick antennae	Between Groups	149.304	6	24.884	.368	.899
	Within Groups	11710.017	173	67.688		
	Total	11859.321	179			
% time flick antennules	Between Groups	7087.411	6	1181.235	2.421	.028*
	Within Groups	84422.798	173	487.993		
	Total	91510.209	179			
% time flick both	Between Groups	5340.843	6	890.141	1.129	.347
	Within Groups	136413.891	173	788.520		
	Total	141754.734	179			
% time in Lposture	Between Groups	1848.491	6	308.082	.545	.773
	Within Groups	97758.094	173	565.076		
	Total	99606.585	179			
% time in Iposture	Between Groups	14078.095	6	2346.349	2.439	.027*
	Within Groups	166396.917	173	961.832		
	Total	180475.012	179			
% time in Rposture	Between Groups	10881.178	6	1813.530	2.309	.036*
	Within Groups	135871.723	173	785.386		
	Total	146752.901	179			
% time lost appendage&mortality	Between Groups	.000	6	.000	.	.
	Within Groups	.000	173	.000		
	Total	.000	179			

Table 2.9 Total active time spent by all sizes, genders and combinations of yabbies outside the shelters during the 24 hours

Active time	Control	Predator
Mean	50.16667	38.62500
Variance	567.53623	434.41848
Observations	24.00000	24.00000
Pearson Correlation	0.81386	
Hypothesized Mean Difference	0.00000	
df	23.00000	
t Stat	4.06226	
P(T<=t) one-tail	0.00024	
t Critical one-tail	1.71387	
P(T<=t) two-tail	0.00048	
t Critical two-tail	2.06866	

Table 2.10 Total time spent outside shelter (active time) by small male and small female yabbies with different test solutions during the 24-hour

	SM *Cont*	SF *Cont*	SM *Pred*	SF *Pred*
Mean	5.416667	5.333333	4.083333	3.541667
Variance	25.47101	19.27536	13.38406	11.91123
Observations	24	24	24	24
Pearson Correlation	0.917665		0.905353	
Hypothesized Mean Difference	0		0	
df	23		23	
t Stat	0.20212		1.701254	
P(T<=t) one-tail	0.4208		0.051189	
t Critical one-tail	1.71387		1.71387	
P(T<=t) two-tail	0.8416		0.102378	
t Critical two-tail	2.068655		2.068655	

	MM *Cont*	MF *Cont*	MM *Pred*	MF *Pred*
Mean	10.875	9.625	6.791667	7
Variance	8.983696	12.50543	9.389493	15.13043
Observations	24	24	24	24
Pearson Correlation	0.840396		0.696721	
Hypothesized Mean Difference	0		0	
df	23		23	
t Stat	3.194855		-0.36286	
P(T<=t) one-tail	0.002014		0.36001	
t Critical one-tail	1.71387		1.71387	
P(T<=t) two-tail	0.004028		0.720021	
t Critical two-tail	2.068655		2.068655	

	LM *Cont*	LF *Cont*	LM *Pred*	LF *Pred*
Mean	9	9.916667	7	9
Variance	21.04348	21.99275	31.21739	13.04348
Observations	24	24	24	24
Pearson Correlation	0.933718		0.907109	
Hypothesized Mean Difference	0		0	
df	23		23	
t Stat	-2.65435		-3.54196	
P(T<=t) one-tail	0.007085		0.00087	
t Critical one-tail	1.71387		1.71387	
P(T<=t) two-tail	0.014169		0.00174	
t Critical two-tail	2.068655		2.068655	

	SM *Cont*	SM *Pred*	SF *Cont*	SF *Pred*
Mean	5.416667	4.08333333	5.333333	3.54166667
Variance	25.47101	13.384058	19.27536	11.9112319
Observations	24	24	24	24
Pearson Correlation	0.784543		0.725004	
Hypothesized Mean Difference	0		0	
df	23		23	
t Stat	2.077671		2.891356	
P(T<=t) one-tail	0.024545		0.004118	
t Critical one-tail	1.71387		1.71387	
P(T<=t) two-tail	0.04909		0.008235	
t Critical two-tail	2.068655		2.068655	

	MM *Cont*	MM *Pred*	MF *Cont*	MF *Pred*
Mean	10.875	6.791667	9.625	7
Variance	8.983696	9.389493	12.50543	15.13043
Observations	24	24	24	24
Pearson Correlation	0.569849		0.847094	
Hypothesized Mean Difference	0		0	
df	23		23	
t Stat	7.114553		6.17895	
P(T<=t) one-tail	1.51E-07		1.32E-06	
t Critical one-tail	1.71387		1.71387	
P(T<=t) two-tail	3.02E-07		2.65E-06	
t Critical two-tail	2.068655		2.068655	

	LM *Cont*	LM *Pred*	LF *Cont*	LF *Pred*
Mean	9	7	9.916667	9
Variance	21.04348	31.21739	21.99275	13.04348
Observations	24	24	24	24
Pearson Correlation	0.746393		0.690539	
Hypothesized Mean Difference	0		0	
df	23		23	
t Stat	2.618615		1.315978	
P(T<=t) one-tail	0.007678		0.100574	
t Critical one-tail	1.71387		1.71387	
P(T<=t) two-tail	0.015356		0.201148	
t Critical two-tail	2.068655		2.068655	

Appendix 3 Silver perch pellet feed formulation

Table 3.1 Formulation and biochemical composition of commercial silver perch pellets used for all experiments (Glen Forrest Stockfeeders Pty Ltd)

Ingredients	%
Fishmeal	27.0
Soybean meal	20.0
Blood meal	2.0
Corn gluten meal	4.0
Wheat	28.4
Sorghum	11.0
Milrun	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	35.6
Crude fat	5.5
Linoleic series fatty acid	1.1
Fibre	4.4
Carbohydrate	52.1

	g/kg
Total methionine	7.4
Total lysine	22.6

Table 3.2 composition of crayfish pellet used for experiments (Glen Forrest Stockfeeders Pty Ltd)

Proximate composition	%
Protein	23%
Fat	6%
Crude fibre	8.3%
Calcium	2.9%
Phosphorous	1.0%
Salt	0.3%
Metabolisable energy	9.9 MJ/kg
Vitamin A	1700 IU/kg
Vitamin E	10 mg/kg
Nicotinic acid	33 mg/kg
Folic acid	0.8 mg/kg
Thiamine	2.5 mg/kg
Copper	0.8 mg/kg
Vitamin D	250 IU/kg
Vitamin K	0.3 mg/kg
Calcium pantothenate	21 mg/kg
Riboflavin	3 mg/kg
Biotin	125 mg/kg
Choline	170 mg/kg
Iodine	0.1 mg/kg
Iron	2 mg/kg
Manganese	10 mg/kg
Zinc	4 mg/kg