Translocations of freshwater crayfish: contributions from life histories, trophic relations and diseases of three species in Western Australia

Stephen John Beatty

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DECLARATION

I declare that the information contained in this thesis is the result of my own research unless otherwise cited

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Frontispiece: Elizabeth Gratwick
Abstract

By examining Western Australian freshwater crayfishes, this thesis aims to further our understanding of how life-history strategies, trophic relationships and disease introductions contribute to the threats posed by introduced species. Reproductive and population biology of two species of freshwater crayfish endemic to Western Australia (the marron *Cherax cainii* and gilgie *Cherax quinquecarinatus*) and the introduced yabbie *Cherax destructor* were described. Multiple stable isotope analysis was employed to determine the trophic positions of sympatric populations of *C. cainii* and the invading. A serious microsporidian disease of freshwater crayfishes was also discovered in a wild population of *C. destructor*. These data were used to determine the potential threat that *C. destructor* poses to the endemic crayfishes of Western Australia.

*Cherax cainii* supports an iconic recreational fishery that has been in steady decline for three decades. It is likely that considerable plasticity in the biology of *C. cainii* exists amongst the ca 100 populations and that this may result in the current fishery management regulations being not effective in protecting all stocks. To test these hypotheses, the biology of *C. cainii* were described from populations occurring in an impoundment dam (Lake Navarino) at the approximate centre of its current range and in the Hutt River at the northernmost point of its range and compared with those from a previous study near the southernmost point of its distribution. The study confirmed these hypotheses. For example, the onset of spawning was later in the more southerly Lake Navarino population (August) than in the northerly Hutt River population (July). Furthermore, the respective orbital carapace lengths (OCL) at which *C. cainii* reached maturity in the two populations studied here differed markedly. The lengths at which 50% of female and male *C. cainii* matured in Lake Navarino were 32.1 mm and 28.6 mm OCL for females and males, respectively, compared with 70 mm and 40 mm OCL for females and males in the Hutt River, respectively. Therefore, these data clearly demonstrate that the current minimum legal size limit of 76 mm
CL (~55 mm OCL) is ineffective in allowing females to undertake a spawning event prior to legal capture. It is therefore recommended that the minimum legal size limit be increased to 98 mm CL in the Hutt River to allow 50% of females to reach maturity prior to exploitation. Furthermore, as the spawning rate of mature female *C. cainii* in the Hutt River was low (10%) compared with those mature females in the more southerly Lake Navarino (96%), this increase in minimum legal size of capture is of particular importance should fisheries managers wish this translocated population to be exploited sustainably.

It is proposed that the much larger lengths at first maturity and low spawning rate in the Hutt River were due to faster growth rates likely caused by relatively high water temperatures and in response to competition with the sympatric, introduced crayfish, *C. destructor*, respectively. This highlights the plasticity of the biology of *C. cainii* and has considerable implications for effective management of the size-regulated recreational fishery.

*Cherax quinquecarinatus*, a south-western Western Australian endemic: occupies a broad range of aquatic systems, is likely to be an important component to those aquatic food webs, and is also subject to recreational fishing pressure. *Cherax quinquecarinatus* was found to mature at a relatively small size (*cf* *C. cainii*) with the $L_{50}$s for females and males being 18.8 and 24.5 mm OCL, respectively, with the majority of *C. quinquecarinatus* first spawning at the end of their second year of life. The potential (ovarian) and pleopodal fecundities of *C. quinquecarinatus* were relatively low compared to other freshwater crayfishes, being 81.7 (±5.93 s.e.) and 77.1 (±13.76 s.e.), respectively. *Cherax quinquecarinatus* underwent an extended spawning period, from late winter to late summer (i.e. August to February). Three spawning events were facilitated by short brood and rapid gonadal recovery periods, traits consistent with other crayfish species able to exist in temporary environments.

The seasonal von Bertalanffy growth curve, fitted for the first 14 months of life for female and male *C. quinquecarinatus*, had respective $K$ and $OCL_\infty$s of 0.29 and 59.6 mm
OCL for females, and 0.25 and 73.8 mm OCL for males, respectively. At 12 months of age, the OCLs of females and males were 14.7 and 14.1 mm, respectively. Estimates of total mortality ($Z$) were relatively high at 2.34 and 1.95 year$^{-1}$ based on an age-converted catch curves for females and males, respectively, with a considerable proportion of this attributed to fishing mortality (exploitation rates of 0.76 and 0.75 for females and males, respectively). *Cherax quinquecarinatus* exhibited traits of both an $r$- and a K-strategist, which has likely to have aided the success of this species across a wide range of permanent and temporary systems.

During this study, *C. destructor* was found in many wild aquatic systems in the southern Pilbara and Southwest Coast Drainage Divisions of Western Australia. This is of great concern as all native freshwater crayfishes in Western Australia are restricted to the southwest while the aquatic systems of the Pilbara Division do not naturally house freshwater crayfish.

Despite the reported impacts that invasive freshwater crayfish species may have on native crayfish species and food webs, the biology and ecology of *C. destructor* in wild systems in Western Australia was unknown and therefore an assessment of their potential impact has not previously been possible. *Cherax destructor* was collected monthly from the Hutt River (Pilbara Drainage Division) for determination of life-history and reproductive biology in a wild aquatic system in Western Australia. Proliferation in that system was attributed to specific traits including: a small size at first maturity with 50% ($L_{50}$) of females and males maturing at 21.6 and 26.5 mm OCL, respectively, a size attained at the end of their first year of life; a protracted spawning period (July to January); high mean ovarian fecundity of 210.2 ($\pm 9.24$ s.e.); and a rapid growth rate that was comparable to the larger sympatric *C. cainii* in this system. Life-history characteristics of *C. destructor* in the Hutt River were typical of many other invasive crayfish species and were likely to have aided in its establishment.
This study is the first to examine the diet and trophic position of sympatric populations of two species of freshwater crayfish in Australia. By determining temporal changes in the assimilated diet and trophic positions of sympatric populations of *C. destructor* and *C. cainii*, this study tested the hypothesis that *C. destructor* has the potential to compete with *C. cainii* for food resources. This was tested using multiple stable isotope analyses with samples of *C. cainii*, *C. destructor* and a wide variety of their potential food sources analysed in the Hutt River in summer and winter, 2003. Summer samples indicated that these species occupied similar predatory trophic positions when their assimilated diet consisted of a large proportion of *Gambusia holbrooki* (either when the fish were alive or deceased due to a presumably large natural mortality rate). Although *C. cainii* continued to assimilate animal matter based on winter signatures, those of *C. destructor* appeared to shift towards more of herbivorous trophic position. It appeared that *C. destructor* and *C. cainii* were keystone species in the Hutt River and were likely to be important in the cycling of nutrients and in structuring the aquatic food web that may have been considerably altered by their introduction into this system.

As *C. destructor* has the ability to switch trophic positions, when an otherwise abundant, high protein food sources (i.e. fish) becomes limited (as was the case in winter in the Hutt River), it was able to co-exist with *C. cainii*. Furthermore, the ability of *C. destructor* to switch from a diet of fish in summer to a predominantly herbivorous/detrital diet in winter suggests that it may compete for food resources with the other smaller native freshwater crayfishes (such as *C. quinquecarinatus*) in the small, unproductive lotic and lentic systems common to south-western Australia, which often lack fish during summer.

The recently described *Thelohania parastaci* was identified in *C. destructor* in the Hutt River and *Vavraia parastacida*, previously recorded from *C. cainii* and *C. quinquecarinatus* populations elsewhere in the region, appeared to be infecting *C. cainii*. Although not confirmed to have infected *C. cainii*, the presence of *T. parastaci* in the sympatric *C. destructor* is of serious concern as there is the potential that the disease may be
able to be transmitted to the native congeners of the region, particularly as *C. destructor* establishes itself in other natural waterbodies.

This thesis has addressed major gaps in the understanding of the biology, ecology and threats to the unique freshwater crayfish fauna of Western Australia. The results of this research highlight the plasticity of the biology and ecology of freshwater crayfishes and enabled an initial assessment to be made of the potential ecological impacts of an invading species. Considerable implications for fisheries and other natural resource management agencies ensuing from this research are detailed. The conclusions drawn from this study are also discussed in the broader context of invasive species in general and important future investigations stemming from these results are identified.
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Additional publication produced from this research:

Chapter 1

General Introduction

1.1 Phylogeny and zoogeography of freshwater crayfishes

1.1.1 Origin of freshwater crayfishes

Until recently freshwater crayfishes (Astacida), of which there are more than 540 extant species (Holdich 2002; Taylor 2002), were considered to be the closest relatives of the marine clawed lobsters (Homarida), these two groups together forming the Astacura. However, Scholtz and Richter (1995) and Schram (2001), using contemporary methods of phylogenetic analysis, demonstrated that the characteristics that these two groups share are plesiomorphic (ancestral) and that they are not sister groups. Rather, Astacida are the basal most member of the Fractosternalia, a taxon that also includes the thalassinid, anomalan and brachyuran decapods (Scholtz and Richter 1995; Schram 2001). Fractosternalia can be recognised by a freely moving eighth thoracic sternite that is the most obvious synapomorphy (shared derived character state) of the group (Scholtz and Richter 1995; Scholtz 1999). Within the Fractosternalia the monophyly of the Astacida is supported by the following synapomorphies (Scholtz and Richter 1995; Scholtz 1999): presence of approximately 40 large, specialized growth cells (teloblasts) in the posterior section of the germ band cf 19 in the majority of decapods (Scholtz 1993); hatchlings similar to adults, i.e. there are no free-living larval stages (as widespread dispersal is usually unnecessary and may be detrimental in finite freshwater aquatic systems) (Scholtz 1999; Reynolds 2002); presence of a telson thread that attaches the hatchling to the mother’s pleopods; presence of residual yolk on the anterior of newly hatched individuals; unstalked eyes; and the lack of uropods or first pleopods until after the second moult (Scholtz 1999; Reynolds 2002).
Whilst it is generally accepted that the ancestors of the Astacida had a marine origin, it is believed that, as all recent species live in fresh (or brackish) waters and many of the synapomorphies of the group are adaptations to living in freshwaters, the group’s stem species was already a freshwater form (Hasiotis 1999; Scholtz 1999). Recent fossil finds suggest the origin of freshwater crayfishes to be in the Early Carboniferous during the formation of Pangaea, 350–320 million years ago (Hasiotis 1999).

1.1.2 Interrelationships and evolution within the Astacida

Within the Astacida two superfamilies are recognised, one, the Astacoidea, includes two families (Cambaridae and Astacidae) and is restricted to the Northern Hemisphere, whereas the second, the Parastacoidea, is represented by a single family (Parastacidae) and is restricted to the Southern Hemisphere (Scholtz 1999; Hasiotis 1999).

The Astacoidea and the Parastacoidea are each considered to be monophyletic based on the following synapomorphies. Astacoidea: post-embryos bearing re-curved hooks on their 1st pereiopods for attachment to the pleopodal setae of their mothers and reduced pleurobranchs of the last two thoracic segments. Parastacoidea: first two post-embryonic stages bearing hooks on the dactyl of the 4th and 5th pereiopods rather than on their 1st; lack the 1st pleopods present in Astacoidea (the first two pairs of pleopods in male Astacoidea form a petasma for sperm transfer); non-calcified posterior margin of the tail fan is soft rather than calcified (Scholtz 1999, 2002). Within the Astacoidea, Cambaridae are recognised by: ischia of the 2nd and 3rd or 3rd and 4th pereiopods of males bear hooks used for holding females during copulation (not present in any other decapod taxon); females display an annulus ventralis (spermatheca) at the posterior side of the seventh thoracic sternite and males display a specialised sperm tube on the 1st pleopod (Scholtz 1999, 2002). In contrast to the Cambaridae there are no known synapomorphies for the Astacidae.
The few recent studies discussing the interrelationships of these taxa all considered that the restriction of extant astacids to freshwaters and their current distribution could be most parsimoniously explained by a single invasion of freshwater during the Early Carboniferous with the subsequent evolution of, and adaptive radiation within, the two Northern and Southern Hemisphere superfamilies occurring upon the break-up of Pangaea. Thus, the classifications proposed by these workers mirror their proposed phylogenies and the hypothesis is that the Astacoidea and Parastacoidea apparently evolved independently on the two super-continents Laurasia and Gondwana, respectively (Hasiotis 1999; Scholtz 1999). However, whilst arguing for such an evolutionary hypothesis Hasiotis (1999) made the following statement, “The ancestral stock would have entered freshwater environments via rivers world-wide around Pangaea nearly simultaneously.....” and is therefore arguing for multiple invasions of freshwater. Furthermore, he later argues that the Astacidae gave rise to all other crayfishes and therefore Astacoidea is paraphyletic. In contrast to these contradictory and confusing statements, Scholtz (1999, 2002) states categorically that both superfamilies are monophyletic, but does note that the monophyly of the Astacidae is questionable and the grouping appears to be based on symplesiomorphies rather than synapomorphies. What does appear to be generally accepted is that the Astacidae are the most “primitive” form of freshwater crayfish and it is likely that an “astacid-like” ancestor evolved into freshwater and terrestrial systems by adapting burrowing behaviour that gave rise to the two superfamilies (Scholtz 1999, 2002). Within these superfamilies the Parastacoidea and Cambaridae underwent parallel adaptive radiations, often relating to the development of burrowing habitats and reproductive modes, on the two supercontinents whereas the Astacidae remained relatively similar to the original “astacid-like” ancestor (Hasiotis 1999; Scholtz 1999, 2002).

The above explanation of the diversification of freshwater crayfish families is currently not without speculation, particularly in light of continued debate over the
monophyly of Astacidae (Scholtz 2002). Scholtz (1999) highlighted gaps in the current understanding in crayfish zoogeography, for instance, the absence of freshwater crayfish in Africa or India has not been conclusively accounted for. It has been proposed that these landmasses separated from Gondwana prior to freshwater crayfishes invading them, possibly indicating an uneven distribution on the super continent (Scholtz 1995). Furthermore, the disjunctive distribution of Cambaridae and Astacidae, with the former being found in North America, northern middle America and east Asia (Cambaroides), and the latter being found in Europe and western North America (Pacifastacus). It has therefore been proposed that there is a possibility that two species of Laurasian crayfish were stem species of the two Northern Hemisphere families and that the disjunctive genus of Cambaroides and Pacifastacus are relict species (Scholtz 1995).

1.1.3 Australian freshwater crayfishes

Several recent studies addressing the taxonomy of freshwater crayfishes in Australia have described new species or synonymised others and has resulted in some confusion and disagreement regarding the status of certain taxa (see for example, Austin 1996; Austin and Knott 1996; Horwitz and Adams 2000; Austin and Ryan 2002; Austin et al. 2003). Further discussions regarding the taxonomic inclusiveness of species are provided below and in the appropriate chapters.

The Southern Hemisphere Parastacidae consists of 14 recognised genera and 139 species (Riek 1969, 1972; Crandall et al. 1999; Taylor 2002). Of these taxa, ten genera and 123 species, or ca 33% of all freshwater crayfish genera and 20% of all species, are found in, and are endemic to, Australasia, figures which makes the region second only to the south-eastern United States in terms of numbers of both genera and species (Crandall et al. 1999; Taylor 2002). The remaining four genera and 16 species of parastacids are endemic to South
America (Samastacus, Parastacus and Virilastacus) and Madagascar (Astacoides) (Taylor 2002).

Within Australia, freshwater crayfishes are found in all States and Territories. However, the majority of species are restricted to the temperate regions of south-western, south-eastern and eastern Australia where they occupy a diverse range of aquatic habitats (Riek 1967, 1969, 1972; Austin 1996; Austin and Knott 1996; Crandall et al. 1999; Taylor 2002). Two genera are endemic to Tasmania (Astacopsis and Parastacoides), two occur on both the south-eastern mainland and Tasmania (Engaeus and Geocharax), two are highly restricted to upland regions of south-eastern Queensland (Tenuibranchiurus) and western Victoria (Gramastacus), one is restricted to the south-western corner of Western Australia (Engaewa), the two remaining genera (Cherax and Euastacus) contain ca 60% of all parastacid species. In contrast to Euastacus that is restricted to south-eastern Australia, Cherax is the most widely distributed genus of freshwater crayfish in Australia, with its members being found in north-eastern, eastern, south-eastern and south-western Australia, a further two species are known to occupy the freshwaters of Papua New Guinea (Riek 1972; Crandall et al. 1999; Taylor 2002).

Crandall et al. (1999) utilised mitochondrial DNA sequence data (ca 500 base pairs from the 16S region) to demonstrate that, with the possible exception of Euastacus, the 10 freshwater crayfish genera in Australia and New Zealand were monophyletic. Their study also suggested that Engaewa is sister to the remaining genera that themselves form two major clades. Due to that study not including outgroups, they could only provide unrooted trees. However, they still rooted the trees using Engaewa as the sister taxon that results in it appearing that the genus is ancestral to the other Australian genera, one of only three possible scenarios given the presence of three clades in the family. Although it may be the case, Crandall et al. (1999) stressed that this conclusion should not be drawn from their data (as also discussed in Horwitz and Adams (2000). In one of the three clades, Paraneophrops and
Parastacoides are sister to Euastacus and Astacopsis, whilst in the other Cherax is sister to Tenuibranchiurus, which is sister to Geocharax, which is in turn sister to Engaeus and Gramastacus, i.e. (Engaewa, (((Paranephrops, Parastacoides), (Euastacus, Astacopsis)), (Cherax, Tenuibranchiurus, (Geocharax, (Engaeus, Gramastacus)))))

1.1.4 Taxonomy of Cherax: the current state of play

Cherax has the most widespread distribution of any parastacid genus in Australia (Horwitz and Knott 1995). Members of the genus are found in all mainland States and Territories and also in New Guinea (Riek 1969; Horwitz and Knott 1995). As several Cherax species are heavily targeted by recreational fishers and important to the aquaculture industry, the majority of parastacid research has focussed on this genus. Much of this research has been focussed on the culture of three species, i.e. Cherax destructor Clark, 1936, Cherax tenuimanus (Smith, 1912)/Cherax cainii Austin and Ryan, 2002 (see below for explanation) (Plates 1.1a, c) and Cherax quadricarinatus von Martens, 1868 and resolving their taxonomy.

Indeed, the taxonomy of and number of species in the genus is the subject of continued debate amongst taxonomists. For example, Riek (1967, 1969) recognised 27 species within Cherax, 19 of which were from northern and eastern Australia and eight from the south-western Australia. However, Austin and Knott (1996), who employed morphological and electrophoretic analyses, considered Cherax glabrimanus Riek, 1967 and Cherax neocarinatus Riek, 1967 and Cherax plebejus Hess, 1865 to be junior synonyms of Cherax quinquecarinatus Gray, 1845 (Plate 1.1b) and Cherax preissii Erichson, 1846, respectively, whilst Austin (1996), who utilised electrophoretic as well as morphological analyses, synonymised Cherax gladstonensis Riek, 1969 with Cherax cairnsensis Riek, 1969; Cherax neopunctatus Riek, 1969 with Cherax cuspidatus Riek, 1969; Cherax albidus Clarke, 1936, Cherax davisi Clark, 1941 and Cherax rotundus Clark, 1941 with C.
and Cherax bicarinatus Gray, 1845 with Cherax quadricarinatus. Although Austin (1996) did not include Cherax punctatus Clark, 1936 or Cherax robustus Riek, 1951 in his analysis of electrophoretic data, he did consider them as distinct species based on principal component analysis of morphological data. An additional three species from northern Australia which were described shortly before Austin’s (1996) study were not considered in his study: Cherax nucifraca Short, 1991, C. cartalacoolah Short, 1993, and Cherax parvus Short and Davie, 1993.

Until recently Western Australia was considered to contain five naturally occurring species of Cherax, i.e. Cherax tenuimanus, Cherax quinquecarinatus, Cherax preissii, Cherax crassimanus Riek, 1967 and Cherax glaber Riek, 1967. However, in a recent study utilising morphometric and allozyme data, Austin and Ryan (2002) considered that the small population of C. tenuimanus from Margaret River was distinct from other populations in south-western Australia. As the original description of C. tenuimanus was based on animals collected from Margaret River, they proposed that C. tenuimanus should refer to the species in that system, and proposed the new name C. cainii for the more widespread form. This nomenclature is currently under review by the International Commission on Zoological Nomenclature (ICZN case number 3267, Molony et al. 2003). In the current study C. cainii will be the name used as no verdict has yet been reached on the challenge.

1.1.5 Western Australian freshwater crayfishes

The 11 species of freshwater crayfishes native to Western Australia, i.e. the six species of *Cherax* listed above and five species of *Engaewa* (*E. subcoerulea* Riek, 1967, *E. reducta* Riek, 1967, *E. similis* Riek, 1967, *E. walpolea* Horwitz and Adams, 2000, and *E. pseudoreducta* Horwitz and Adams, 2000), are naturally restricted and endemic to the Southwest Coastal Drainage Division, with no freshwater crayfishes naturally found in the Pilbara and Kimberley Drainage Divisions (Fig. 1.1) (Riek 1967; Austin and Knott 1996; Horwitz and Adams 2000). However, red claw *Cherax quadricarinatus*, native to northern and north-eastern Australia has recently invaded the Kimberley Drainage Division (Morgan et al. 2004). Not only are all of the freshwater crayfishes that naturally occur within the Southwest Coastal Drainage Division endemic to the region, but the genus *Engaewa* is itself endemic, whilst the species of *Cherax* naturally found in Western Australian form a monophyletic group within *Cherax* (Crandall et al. 1999). The lack of permanent river systems east of the Southwest Coastal Drainage Division until eastern South Australia has resulted in the isolation of the freshwater crayfish fauna of south-western Western Australia. Thus, the high rate of endemism of the freshwater crayfish fauna of this region mirrors that of the vascular plants (75%, Hopper 1992) and freshwater teleosts (80%, Morgan et al. 1998). The uniqueness of this region’s biota is believed to be a result of the ancient separation and resultant isolation of the region from the rest of Australia and, based on both this endemic richness and degree of threat to the biota, has led to it being considered one of the world’s endemic hotspots (Figgis 1993; Myers et al. 2000).

1.2 Biology and ecology of freshwater crayfishes

1.2.1 Life-history strategies

Freshwater crayfishes have previously been categorised into two groups based on their reproductive biology and life-history parameters: summer or winter brooders (Honan and
Mitchell 1995a). Although not always the case, summer and winter brooder groups often display characteristics of ‘r-selected’ and ‘K-selected’ species, respectively (Honan and Mitchell 1995a). The summer brooding group (including many cambarids and many members of the parastacid genera Cherax and Geocharax) generally have relatively short brooding periods (3–20 weeks), the ability to undergo multiple spawning during an asynchronous breeding period, attain maturity at a small size, are highly fecund, occupy a diverse range of permanent and temporary systems, undergo continuous and relatively rapid growth, have a short life-span, and a relatively small maximum size (Honan and Mitchell 1995a; Reynolds 2002). In contrast, members of the winter brooding group (including the majority of astacids and members of the parastacid genera Astacoides, Astacopsis, Euastacus, Engaeus, Parastacoides and Paranephrops) brood for longer periods (generally 16–50 weeks); have an annual or biennial synchronous breeding period; mature at a relatively large size; are less fecund; occupy a relatively narrow range of permanent habitats; exhibit markedly seasonal growth (also true for most cambarids); are slow growing; have a long life-span and attain a large size (Honan and Mitchell 1995a; Reynolds 2002). Although many characteristics of summer brooders are associated with r-selection, and those of winter brooders with K-selection, selection pressures within different habitats should also be considered rather than assigning species to these classic life-history groups (Honan and Mitchell 1995a).

1.2.2 Ecological roles of freshwater crayfishes

Freshwater crayfishes possess a range of physiological, ecological and behavioural adaptations that have resulted in them occupying a wide range of environments and assuming a range of trophic roles. They have been described as: omnivores (Growns and Richardson 1988; Lodge et al. 1994; Nyström and Strand 1996; Gutiérrez-Yurrita et al. 1998); detritivores (e.g. Hessen and Skurdal 1986); opportunistic omnivorous, microphagic
detritivores (O’Brien 1995); and selective herbivores (e.g. Chambers et al. 1990). Detritus often constitutes the majority of gut content (e.g. Hessen and Skurdal 1986; O’Brien 1995). However, studies using stable isotope analysis have found that crayfishes mostly rely on animal protein for growth, i.e. having a predatory trophic role with lower protein sources, such as detritus, being used for maintenance, thus their role as a detritivore is believed to be purely functional (Nyström et al. 1999; Parkyn et al. 2001; Hollows et al. 2002).

The wide range of trophic roles that freshwater crayfishes may assume has resulted in them being regarded as keystone benthic members of aquatic ecosystems (e.g. Momot 1995). Furthermore, their consumption of food from many trophic levels has led to the assertion that they play a major role in the structuring of ecosystems and that they can complicate predicted trophic cascades (Lodge et al. 1994; Nyström et al. 1999).

1.2.3 The biology and ecological role of freshwater crayfishes in Western Australia

Although there are 11 species of freshwater crayfishes endemic to Western Australia, the majority of research has been directed at aspects pertaining to the aquaculture of *C. cainii* (e.g. Morrissy 1980; Alon et al. 1990), with relatively little having been conducted on wild populations or ecological roles of these unique species. This lack of knowledge extends to the three most important commercial and/or recreational species, i.e. the endemic *C. cainii* and *C. quinquecarinatus*, and the introduced *C. destructor*. Of particular concern is that *C. destructor* is believed to be becoming established in wild systems in the region with no research yet having occurred on the biology or ecology of those wild populations (see below).

*Cherax cainii* marron

*Cherax cainii* (Plate 1.1a) can attain a carapace length of 200 mm and a weight of over 2 kg; the third largest freshwater crayfish in the world (Riek 1967; Coy 1979; Austin and Knott
While originally restricted to permanent rivers in the forested, high-rainfall region from between just west of Albany and just south of Perth (Riek 1967; Morrissy 1978), its value as a species for recreational fishing and aquaculture has resulted in its translocation into aquatic systems as far north as the Hutt River, near Geraldton, and as far south-east as Esperance (Riek 1967; Morrissy 1978; Lawrence and Morrissy 2000) (see Fig. 1.1). Concomitant with the extension of its range has been a reduction in available habitat within its natural range, largely due to past agricultural practices resulting in aquatic habitat alteration such as salinisation and eutrophication (Morrissy 1978; Molony et al. 2001).

The paucity of research and resultant lack of knowledge regarding such aspects as population structure, feeding biology, reproductive biology and life-history strategy in wild stocks of *C. cainii* was highlighted by Nickoll and Horwitz (2000). Those authors suggested that, as *C. cainii* fulfils the majority of the accepted criteria necessary for it to be regarded and promoted as a flagship species in aquatic ecosystem restoration projects in this region, the collection of these important biological and ecological parameters for wild populations was urgent (Nickoll and Horwitz 2000). Although research into the life-history and reproductive biology of wild populations of *C. cainii* is limited, it indicates that this species exhibits a mix of life-history characteristics common to both winter and summer brooders (Morrissy 1975; Honan and Mitchell 1995a).

The lack of an extensive body of biological data for wild populations of *C. cainii* is surprising considering its aquaculture potential and its iconic stature with recreational fishers in Western Australia (Molony et al. 2001). Indeed, Alon et al. (1990) noted that although a great deal of work had focussed on examining the feasibility of utilising *C. cainii* for aquaculture in eastern Australia and overseas (e.g. Alon et al. 1988, 1990; Shu et al. 1990), a paucity of biological data had hampered culture attempts.

The *C. cainii* recreational fishery has been in general decline in terms of both total catch and catches per unit effort (CPUE) for the past three decades (Molony et al. 2001;
Molony and Bird 2002). This decline is thought to be due to habitat change (e.g. salinisation of water bodies (Morrissy 1978)), predation by introduced fishes (e.g. redfin perch *Perca fluviatilis* Linnaeus, 1758 (Morgan *et al.* 2002; Molony *et al.* 2004)) and recreational fishing pressure (Molony *et al.* 2001). Thus, a better understanding of the plasticity of the biology of *C. cainii* in wild aquatic systems is necessary not only to better understand its ecological importance in the wild, but to more efficiently utilise this species in aquaculture enterprises and also to more effectively manage the recreational fishery and assess reasons for its decline.

*Cherax quinquecarinatus* *gilgie*

*Cherax quinquecarinatus* (Plate 1.1b) is not only a relatively widespread species of freshwater crayfish in south-western Western Australia; it also inhabits a wide range of habitats in the region (Riek 1967; Austin and Knott 1996). For example, although found in permanent lakes, rivers and streams it is also common in the ephemeral waterbodies of the southern peat flat region that also contain teleost species able to aestivate and are often dry for up to seven months of the year (Morgan *et al.* 1998, 2000).

The larger size and consequent recreational and commercial importance of *C. cainii* has seen it overshadow its smaller congeners in terms of research effort. This is despite the fact that other species such as *C. quinquecarinatus* occupy a wider range of habitats (with greater burrowing tendencies) (Austin and Knott 1996) and often grow to a size that has resulted in them forming an important traditional food resource for the traditional owners of the region (with the common names of the freshwater crayfish species of the region being derived from the respective Aboriginal names, e.g. marron, gilgie and koonac (see Meagher 1974; McGuire 1996; Bindon and Chadwick 2002)) and which has also seen them being subjected to recreational fishing. Given the lack of specific fishery regulations pertaining to this species, there is a need for a comprehensive study of the reproductive and population
biology of *C. quinquecarinatus* to provide managers with the data necessary for the development of sound fishery regulations.

*C. destructor* yabbie

*C. destructor* (Plate 1.1c), native to eastern Australia, was first introduced into farm dams in south-western Australia in 1932 (Austin 1985; Morrissy and Cassells 1992). The *C. destructor* complex has been the subject of considerable aquaculture effort both in south-western Australia and in the east and south-east of Australia (Lawrence and Morrissy 2000). The ability of the *C. destructor* to tolerate a wide range of physicochemical conditions has enabled it to inhabit a wide range of aquatic habitats, including both temporary and permanent systems (reviewed by Horwitz and Knott 1995).

In an attempt to prevent the uncontrolled spread of the species into wild aquatic systems (those aquatic systems that are not constructed by humans or those impoundment reservoirs that connect to such systems), the Department of Fisheries, Government of Western Australia currently only allows the culture of *C. destructor* in the region to the east of Albany Highway and only in artificial waterbodies. Despite these legislative restrictions, *C. destructor* is widespread in the region between the Hutt River, in the north, to the Esperance region, in the south-east (Morrissy and Cassells 1992; Horwitz and Knott 1995) (Fig. 1.1).

1.3 Impacts of introduced species on freshwater crayfishes

1.3.1 Worldwide aquatic introductions

There has been a worldwide increase of exotic species introductions; the regrettable endpoint being global ecological homogenisation with a corresponding, catastrophic loss of species (Ruesink *et al.* 1995; Lodge *et al.* 1998). Aquatic ecosystems, both marine and freshwater, are particularly vulnerable to exotic introductions for two reasons: firstly, due to the relatively high number and diversity of vectors that exist to transport aquatic species, relative
to terrestrial species, outside of their range (e.g. ballast water, aquarium hobbyists, aquaculturalists) (Lodge et al. 1998); and secondly, relative to terrestrial systems, fewer barriers exist that may prevent the dispersal of aquatic exotic species (Lodge et al. 1998). It has therefore been suggested that the linkage of waterways (e.g. the connecting of adjacent river systems by extreme flooding events) and water movement facilitates the dispersal of aquatic exotics (Lodge et al. 1998). The above exotic species transportation vectors all relate to the anthropogenic requirement for and enjoyment of water (e.g. domestic water supply and transport).

Once established in a waterway, the presence of exotic species of fish or invertebrates can be viewed as permanent with eradication difficult if not impossible (Horwitz 1990). The introduction of exotic aquatic species can have severe consequences on the structure and function of receiving ecosystems (Holdich 1988; Nyström et al. 1999). Aside from direct predation on and competition with native aquatic species, nutrient and energy flow may be altered in the receiving environment due to changes in the structure of aquatic food webs (Horwitz 1990).

1.3.2 Impacts of freshwater crayfish introductions

Ecological impacts

Despite the best intentions of fisheries managers, the translocation and escape of aquatic species outside their natural geographic range for the purpose of aquaculture will almost inevitably result in the establishment of self-sustaining wild populations (Hobbs et al. 1989). The wide range of trophic roles that freshwater crayfishes assume in aquatic systems, coupled with their burrowing behaviour, results in their translocation often having serious and wide-ranging impacts on receiving ecosystems (Holdich 1988; Arrignon 1997; Bohl 1999). As they often constitute the greatest biomass of benthic invertebrates within aquatic systems and display a diverse and opportunistic mode of feeding, they can structure aquatic communities
in general (e.g. Olsen et al. 1991; Momot 1995; Nyström and Strand 1996; Usio 2000; Stenroth and Nyström 2003) and in particular those based on macrophyte communities (e.g. Lodge and Lorman 1987; Lodge et al. 1994; Nyström and Strand 1996).

The translocation of exotic freshwater crayfishes has been to the detriment of native species in Europe. Two North American crayfishes, *Pacifastacus leniusculus* (Dana, 1852) and *Procambarus clarkii* (Girard, 1852), have caused major perturbations to food webs and replaced populations of native species, in particular *Astacus astacus* (Linnaeus, 1758) (Holdich 1988; Chambers et al. 1990; Nyström and Strand 1996; Gutiérrez-Yurrita and Montes 1999) (has also resulted in the introduction of the crayfish plague *Aphanomyces astaci* Schikora 1906: see section on Disease Introductions). In North America, *Orconectes rusticus* (Girard, 1852) is believed to be replacing the native resident congener *Orconectes virilis* (Hagen, 1870) and even another introduced species *Orconectes propinquus* (Girard, 1852) in Wisconsin (United States) lakes (Hill et al. 1993). This replacement has been facilitated by the higher growth rate of *O. rusticus* relative to its congeners that results in superior reproductive success and a competitive advantage in foraging (Hill et al. 1993). In addition, the ability of *O. rusticus* to exclude congeners from suitable shelter increases predation by teleost fishes on the two endemic congeners (Didonato and Lodge 1993; Hill et al. 1993). *Orconectes rusticus* was also shown to be replacing the native *Orconectes sanbornii* (Faxon, 1884) in central Ohio streams (Butler and Stein 1985; Mather and Stein 1993). This replacement was also attributed, at least in part, to the superior growth rate and aggressive nature of *O. rusticus* that allowed them to displace *O. sanbornii* from shelter; again resulting in increased predation by teleosts on the native species (Butler and Stein 1985; Mather and Stein 1993).

**Genetic implications**
The discontinuous nature of some freshwater habitats often results in the formation of isolated, genetically distinct faunal populations enhanced by the brooding behaviour of females that results in limited dispersal of offspring and the (likely) self-recruitment of populations. Thus a major implication of any introduction is the potential reduction in genetic and morphological variation through: the loss of native species or sub-species via direct predation or competition; via indirect food-web interactions; and through hybridisation via the interbreeding of introduced crayfish with genetically distinct native populations (Horwitz 1990; Perry et al. 2002).

**Disease introductions**

In addition to the direct effects of predation and competition, the introduction of aquatic species almost invariably results in the simultaneous introduction of other species (Holdich 1988). Symbionts, parasites and pathogens can be introduced via the translocation of freshwater crayfishes and they may have considerable ecological and economic consequences (reviewed in Horwitz 1990). The fact that cultured individuals are usually kept at high densities to increase productivity presumably results in them being more prone to disease as there is increased contact between individuals and greater stress levels.

Introduced diseases have resulted in major reductions in the range and abundance of the indigenous freshwater crayfish species of Europe (Holdich 1988). The crayfish plague *A. astaci*, a fungal disease, is responsible for the decline of many stocks of native freshwater crayfish species throughout Europe and is a prime example of unintentional introduction of other organisms accompanying translocated crayfishes (e.g. Holdich 1988). *Aphanomyces astaci* was believed to be introduced from North America in the 1860s (Holdich 1988). As the native crayfish stocks declined in Europe, North American species were introduced to replace them, namely *P. leniusculus*, *P. clarkii* and *Orconectes limosus* (Rafinesque, 1817). These North American cambarid species are resistant to *A. astaci* compared with the native

1.3.3 Threats posed by the yabbie _Cherax destructor_ in Western Australia

Ecological impacts

The introduction of _C. destructor_ into Western Australia poses a serious risk to aquatic ecosystems, and in particular endemic freshwater crayfishes (Austin 1985; Horwitz 1990; Morrissy and Cassells 1992; Jasinska _et al._ 1993). There is an urgent need to verify largely anecdotal reports of the spread of this species into wild aquatic systems of Western Australia in order to ascertain its current distribution. Furthermore, research into the viability of co-occurring populations of _C. cainii_ and _C. destructor_ in wild systems in this State is required, specifically, by comparing life-history and reproductive strategies of sympatric populations.

As noted earlier, _C. destructor_ co-exists with the northern-most wild population of _C. cainii_ in the Hutt River (Indian Ocean Drainage Division, Fig. 1.1). It is believed that _C. cainii_ were introduced to the Hutt River in 1972 and yabbies to the lower reaches of the river in 1977 (Morrissy and Cassells 1992). This system therefore provides an excellent opportunity to assess the viability of established, sympatric populations in a wild system by describing and comparing their reproductive biology and life-history strategies. This research will be important in predicting future impacts of _C. destructor_ on other native freshwater crayfish species as its apparent inevitable spread into the biotically unique aquatic systems of this State continues.

In addition to gaining an understanding of its life-history and reproductive biology, an understanding of the ecological role of translocated populations of _C. destructor_ in aquatic
ecosystems of Western Australia systems is of considerable importance in clarifying their potential impact on other aquatic organisms and ecosystem functioning. Stable isotope
analysis has been used effectively in determining the ecological roles of freshwater crayfishes (Beatty 2000; Parkyn et al. 2001), in particular, by determining the sources of their assimilated energy. This technique can therefore be used to determine whether there is resource overlap between introduced and native crayfishes.

**Disease introduction**

Little is known about the disease status of Australian freshwater crayfishes. However, a major threat is the microsporidian *Thelohania parastaci* Moodie, 2002 (Microspora, Thelohaniidae). Later stages of infection by species of *Thelohania* are typified by a pale, streaky appearance of the muscle tissue that has resulted in the infection being known as ‘porcelain’ disease. Species of *Thelohania* have been responsible for infections of many freshwater crayfish species both overseas (e.g. *Austropotamobius pallipes* (Lereboullet, 1858) in O’Keefe and Reynolds 1983; *Astacus astacus* in Burba and Bucinskiene 1998) and within Australia (e.g. *C. destructor* in Carstairs 1979, Moodie et al. 2003a, 2003b; and red claw *C. quadricarinatus* in Herbert 1987, 1988). Where present, the rate of *Thelohania* infection of crayfish populations varies (e.g. 0.9% in *A. astacus* populations in Lithuania; 7.8% in a population of *C. quadricarinatus*, Herbert 1987; up to 20% of individuals of *C. destructor*, Carstairs 1979).

*Vavraia parastacida* is a microsporidian species that is of similar appearance to *Thelohania* spp. and is known to infect *C. cainii* and *C. quinquecarinatus* in this region (Langdon 1991a, 1991b). Although *Thelohania* infections of Australian freshwater crayfishes have been previously been recorded, the species responsible have only recently begun to be described (e.g. Moodie et al. 2003a, 2003b). *Thelohania parastaci* has recently been recorded from *C. destructor* in farm dams in Western Australia (Moodie 2003a). Whilst *T. parastaci* has not yet been reported from wild populations of *C. destructor* in
Western Australia (or in any wild or aquacultured populations of *C. cainii*), the threat of infection of the native freshwater crayfish in Western Australia remains as *C. destructor* apparently continues to spread into wild aquatic systems. Given the coexistence of established populations of *C. cainii* and *C. destructor* in the Hutt River, it provides an ideal opportunity to determine whether of *T. parastaci* has infected these populations.

### 1.4 Aims of this thesis

The overall aim of this thesis is:

*Using Western Australian freshwater crayfishes, further the understanding of how life-history strategies, trophic relationships and disease introductions contribute to the threats posed by introduced species.*

- As freshwater crayfishes have been demonstrated to be flexible in terms of life-history, it is hypothesised that populations of *C. cainii* will exhibit considerable variations in their biology that will have direct implications for the effective sustainable management of its recreational fishery. Therefore, the first aim of this thesis was to compare the biology of a northern, riverine population of *C. cainii* to a more southerly lentic population (Chapters 2-3).

- The second major aim of this thesis was to, in describing its biology, test the hypothesis that as the gilgie *C. quinquecarinatus* is present in almost all types of freshwater aquatic systems within its natural range, this species will exhibit the life-history categories described by Honan and Mitchell (1995a) as typical of summer brooders (Chapter 4).

- Increasing anecdotal evidence suggests that *C. destructor* is rapidly invading many wild freshwater systems of south-western Australia. It is hypothesised that *C.
The destructor will display life-history traits associated with successful invasive species, i.e. *r*-selected species (Pianka 1970). Thus, the present study aimed to test these hypotheses by documenting the current distribution of *C. destructor* in aquatic systems in Western Australia and describing the life-history characteristics of a successful population from the wild. These life-history parameters will be compared with those of common endemic congeners (such as *C. cainii*) to further our understanding of the threat this species poses to sympatric native crayfishes (Chapter 5).

- By determining temporal changes in the assimilated diet and trophic positions of sympatric populations of *C. destructor* and *C. cainii*, this study aims to test the hypothesis that *C. destructor* has the potential to compete with *C. cainii* for food resources (Chapter 6).

- As *Thelohania parastaci* is a known parasite of *C. destructor* in aquaculture facilities it is highly likely that this pathogen will also be present in wild populations of this species. It is also possible that *C. destructor* will act as a vector for transmission of this disease to native crayfishes. This study aimed to test the hypotheses that *C. destructor* in the Hutt River is infected with *T. parastaci* and that *C. cainii* has also been infected with this disease (Chapter 7).

- Use the data collected in the previous Chapters to compare the biology of the three species and assess the threat that *C. destructor* poses to the crayfishes and aquatic systems of south-western Australia and determine whether Western Australian freshwater crayfishes subscribe to previously described life-history groups (Chapter 8).
Chapter 2

Reproductive biology of the large freshwater crayfish *Cherax cainii* in south-western Australia

2.1 Introduction

The marron, *Cherax cainii*, can attain a carapace length of 200 mm and a weight of 2 kg (Coy 1979) and is the largest species of freshwater crayfish in Western Australia, and the third largest in the world (Riek 1967; Austin and Knott 1996). While originally restricted to permanent rivers in the forested, high-rainfall region between Albany and Perth (Riek 1967; Morrissy 1978), it has been translocated as far north as the Hutt River (Morrissy and Cassells 1992) and as far east as the Esperance region (Riek 1967; Morrissy 1978; Lawrence and Morrissy 2000) (Fig. 1.1). Recently, increased salinity and eutrophication, due to extensive land-clearing for agriculture, has resulted in its native range being greatly reduced (Morrissy 1978).
The large size attained by *C. cainii*, together with a rapid growth rate, reputed non-burrowing behaviour, distinct breeding period, high tail meat content and excellent palatability has led to the development of a burgeoning aquaculture industry and large recreational fishery for this species in south-western Australia (Riek 1967; Hogger 1988; Austin and Knott 1996; Lawrence and Morrissy 2000). Furthermore, its potential for aquaculture overseas has also been investigated (e.g. Alon *et al.* 1988, 1990; Shu *et al.* 1990). While research into this species has concentrated on aspects pertaining to either its domestication, e.g. rearing, artificial feed development and growth studies under intensive and extensive aquaculture conditions (e.g. Morrissy 1980; Alon *et al.* 1990) or on the annual monitoring of recreational stock sizes and catch characteristics (Molony and Bird 1999; Molony *et al.* 2001), a paucity of biological data has previously hampered culture attempts both in Australia and overseas (Alon *et al.* 1990).

Historically, studies involving the reproductive biology of freshwater crayfishes have generally relied on the macroscopic descriptions of ovarian development, such as their colour, weight and texture (e.g. McRae and Mitchell 1995; Gutiérrez-Yurrita and Montes 1999; Whitmore and Huryn 1999). These macroscopic descriptions of gonadal development, as opposed to histological examination utilised in descriptions of reproductive cycles of teleost fishes (e.g. Pen *et al.* 1993; Gill *et al.* 1996; Morgan *et al.* 2000), do not consider intracellular oocyte development and thus may not accurately describe the reproductive status of individuals.

Some aspects of the reproductive biology of *C. cainii* (e.g. size at sexual maturity, potential and effective fecundity, factors influencing spawning rates, and factors influencing juvenile survival) have previously been examined for farm dam populations (Morrissy 1973, 1976a, 1980). To date, the most comprehensive study of the reproductive biology of wild *C. cainii* was undertaken by Morrissy (1975). However, and in accord with most other studies, the description of ovarian development used by Morrissy (1975) was macroscopic.
The aim of this study was to describe, for the first time, the reproductive biology of *C. cainii* based on histological techniques that provide a detailed description of ovarian development. The trends in gonadosomatic indices and oocyte maturation are described and biological parameters such as length at first maturity and effective and potential fecundity versus size are determined.

### 2.2 Materials and methods

#### 2.2.1 Study sites

Lake Navarino, also known as Waroona Dam (32°50.73'S, 116°00.05'E), was constructed in 1966 and currently serves as an irrigation water supply dam. (Fig. 1.1, Plates 2.1a, b, 2.2). The reservoir has a catchment of approximately 47 km², a surface area of 144 ha and a maximum depth of 36 m. Due to its proximity to Perth, Lake Navarino is extremely popular with recreational fishers, e.g. 11000 legal sized *C. cainii* were taken in the 2000 season (which lasts for two months during summer, i.e. January and February) and the system received ca 5.5% of the estimated total fishing effort in Western Australia (Molony and Bird 2002). Lake Navarino is located at the northern-most point of pre-European distribution of marron, i.e. the Harvey River Catchment (Morrissy 1978).

#### 2.2.2 Environmental variables

On each sampling occasion water temperature, pH and salinity were recorded from the bottom of the water column (at a depth of 1.3 m) at three locations at the study site and a mean calculated. Day-lengths for the region were obtained from the Perth Observatory and mean monthly maximum and minimum air temperatures (for the previous year and also for the past 50 years) were obtained from the Western Australian Bureau of Meteorology’s nearby Wokalup Research Station.

#### 2.2.3 Sampling

Sampling took place for two hours after dusk with up to 60 *C. cainii* collected from a 300 m length of shoreline in Lake Navarino in most months between August 1999 and July 2000. In order to collect the entire size range of *C. cainii*, the following sampling equipment was used: a 40 m seine net (wing mesh of 15 mm and bunt mesh of 3 mm fished to a depth of 1.5 m); a 9.5 m seine net (3 mm mesh and fished to a depth of 1.5 m); SCUBA, scoop netting and manual scoop netting from impoundment banks. All *C. cainii* were immediately
placed in ice slurry for transport to the laboratory. In order to obtain a wider size range to describe the
morphological relationships between wet weight, carapace length (CL) and orbital carapace length (OCL),
marron were also sampled, using the 40 m seine net, from the nearby Willowdale marron farm (owned by
ALCOA) and Harvey Dam (where a larger size range of marron was more readily obtained).

2.2.4 Relationships of orbital carapace length, carapace length and weight
As both OCL and CL are freshwater crayfish measurements utilised by scientific researchers and the
Department of Fisheries, Government of Western Australia, the relationship between the two measurements
was determined. The OCL was measured to the nearest 1 mm from the base of the orbital region to the
posterior margin of the branchiostegite, whilst CL, which is the measurement currently employed by the
Department of Fisheries, Government of Western Australia, for the recreational size limit of 76 mm (Molony
and Bird 1999), was measured from the tip of the rostrum to the posterior edge of the branchiostegite (e.g.
Morrissy 1975; Molony and Bird 1999). For the relationships of both CL to OCL and OCL to wet weight the
suitability of a number of growth functions was tested for each sex and the function giving the greatest
Pearson’s correlation coefficient adopted as the best fit of the data. A likelihood-ratio test (Cerrato 1990) was
used in order to determine if significant differences existed between the relationships of OCL to CL and CL to
wet weight of female and males. If no differences in the functions of two growth relationships between sexes
were found, sexes were pooled and common growth functions were adopted for both relationships. The SPSS
statistical package was used in defining both relationships (Saila et al. 1988).

2.2.5 Gonadosomatic indices (GSIs)
The ovaries of 20-30 females in each month were removed under a dissecting microscope, excess moisture
removed and weighed to the nearest 0.01 g. The GSI was calculated using the equation:

\[
GSI = 100 \left( \frac{W_1}{W_2} \right)
\]

where \(W_1\) is wet weight of the gonad and \(W_2\) is total wet weight of the individual \(C. cainii\).

2.2.6 Macroscopic and histological descriptions of gonad development
Gonads were assigned, on the basis of their macroscopic appearance, to one of the following seven stages: I,
virgin (immature); II, maturing virgin/recovering; III, developing; IV, developed; V, mature (gravid); VI, ripe
(spawning), and; VII, spent (adapted from Laevastu 1965; Johnson 1979; Aitken and Waddy 1980). Gonads
from all females, and a random subsample of 10 eggs from all berried (i.e. those females with eggs, embryos or hatchlings attached to pleopods) *C. cainii*, were placed in Bouin’s fixative for 24 hours, washed in water and dehydrated in 70% ethanol. Gonads and eggs were embedded in wax, sectioned transversely at 6 μm and stained with Mallory’s trichrome.

In order to verify the allocation of gonadal stages based on macroscopic criteria, histological sections of female gonads and individual eggs were examined under a compound microscope at 40x magnification. The maximum and minimum diameters of up to 30 randomly selected oocytes of each sectioned gonad, and also a number of attached eggs were measured through the nucleus and the mean diameter calculated. The proportions of oocytes at different stages of development and within the different months were also determined.

2.2.7 Potential and effective fecundities

The potential fecundities of female *C. cainii* were determined for 19 females immediately prior to spawning via manual counts of oocytes in mature ovaries. The effective fecundities were determined by counting the eggs, embryos or hatchlings attached to berried females.

2.2.8 Size at first maturity

The lengths at first maturity of female and male *C. cainii* were determined by fitting a logistic equation, using a non-linear sub-routine in the *SPSS* package (Saila et al. 1988), to the percentages of *C. cainii* in sequential 5 mm OCL increments, classed as either immature (stages I/II) or developing/mature (stages III-VII). The logistic equation is:

\[
P_L = \frac{1}{1 + e^{-\ln 19 \left(\frac{L - L_{50}}{L_{95} - L_{50}}\right)}}
\]

where \(P_L\) is the proportion of *C. cainii* with mature gonads (see below) at OCL length interval \(L\) and \(L_{50}\) and \(L_{95}\) are the lengths at which 50 and 95% of the population mature, respectively. The females used for this part of the study were restricted to those caught between August and December, i.e. the peak breeding period (see Results).

Fifty male *C. cainii* were measured to the nearest 1 mm OCL, their gonads were removed and the testes were macroscopically staged for the determination of length at first maturity using the same techniques described for the females. Maturity determination was verified via the sectioning of the testes of 10 males with
vas deferens in different stages of development. The presence of spermatophores and sperm tubes within the
vas deferens of opaque appearance (stages III–V) confirmed the maturity of those males, which contrasted with
the lack of spermatophore development in the clear vas deferens (stages I–II), as described in Aitken and
Waddy (1980) and Hamr and Richardson (1994). For determination of length at first maturity, male *C. cainii* at
gonad stages III–V were considered mature and a logistic equation was fitted as for the females.

2.3 Results

2.3.1 Environmental variables of Lake Navarino

The rainfall for the study area followed a seasonal pattern typical of the Mediterranean
climate of south-western Australia, i.e. high winter and low summer rainfall (Fig. 2.1a). The
day lengths for the region declined progressively from a maximum of 865 minutes in mid
summer to 599 in mid-winter (Fig. 2.1b). The mean maximum air temperatures during the
same period also showed a highly seasonal pattern (Fig. 2.1c). Mean monthly water
temperatures have been shown to be strongly correlated to ambient air temperatures and
therefore follow a similar seasonal pattern. The water temperatures peaked in February
(25°C) and were lowest during August (12°C) (Fig. 2.1d).

2.3.2 Sex ratios, OCL versus CL and weight versus OCL

The sex ratio of *C. cainii* in Lake Navarino was 1.61 females : 1 male. A likelihood-ratio test
(Cerrato 1990) revealed that the relationships between the OCL and CL for female and male
*C. cainii* were not significantly different (*P < 0.05; df = 2) and therefore the sexes were
pooled. The relationship between the OCL and CL of 200 *C. cainii* was OCL = 0.7402CL -
2.0552 (*r* = 0.9959). Similarly, a likelihood-ratio test revealed that the relationships between
the wet somatic weight and OCL for female and male *C. cainii* were not significantly
different (*P < 0.05; df = 2). The relationship between the wet somatic weight and OCL of
200 *C. cainii* was \( W = 0.0006OCL^{3.0462} \) (*r* = 0.9978)

2.3.3 Histological and macroscopic gonad descriptions
There was a close association between the macroscopic appearance of female gonads at the various developmental stages and their histological characteristics, however, on a number of occasions gonads were shown to be incorrectly staged macroscopically following histological examination. For example, ovaries that were in the early developmental period of a new stage were often macroscopically classified at the previous stage. Oogonia (< 100 µm) were present in all months and at all stages of gonad development (Table 2.1, Figs 2.2, 2.3 and 2.4) but were proportionally most prevalent in stage I/II and VII gonads where they represented 19.4 and 17.0% of oocytes, respectively. Perinucleolar oocytes represented the greatest proportion of oocytes in stage I/II female gonads (75.5%) with a size range of between 100–400 µm and a modal oocyte diameter of 100–200 µm; however, they were also present in all other stages. When stained, oogonia and perinucleolar oocytes had a light-blue cytoplasm and dark blue cell nuclear membranes with the latter also showing red perinucleolar bodies (Fig. 2.2).

Early yolk vesicle (stage III) oocytes (400–800 µm) represented the greatest proportion of the oocytes in stage III gonads with a modal diameter of 500–600 µm (Fig. 3). At this stage, distinct cohorts of oocytes were apparent. The maximum oocyte diameter in stage III gonads was 1000 µm (Table 2.1). The oocytes stained blue with yolk vesicles appearing in the mid cytoplasmic region of larger oocytes (Fig. 2.2). Stage III ovaries also had nutritive follicle cells (< 100 µm diameter) that were concentrated in monocellular layers around the oocytes and stained light blue with a dark blue nucleus (Fig. 2.2).

Late yolk vesicle (stage IV) oocytes had a size range of 700–1100 µm, with a modal diameter of 700–800 µm (Fig. 2.3). Whilst their size range overlapped with the early yolk vesicle stage oocytes that dominated stage III gonads, it was separate from the oogonia/perinucleolar oocyte size cohort. Stage IV oocytes were characterised by the increased prevalence of yolk vesicles (within a darker blue band) and also orange-staining
yolk granules starting to appear on the periphery of the yolk vesicle band (Fig. 2.2). Follicle cells continued to envelop the oocytes (Fig. 2.2).

The yolk granule (stage V) oocytes had a diameter range of between 800–1700 µm and a mode of 1000–1200 µm and were characterised by a high proportion of yolk granules within the cytoplasm (Fig. 2.3). These yolk granules stained orange/red whilst the nucleus and a thick band of yolk granules and vesicles around the nucleus stained dark blue (Fig. 2.2).

Ripe (stage VI) oocytes had a size range of 1200–3100 µm with a mode of 1900–2000 µm (Fig. 2.3). The cytoplasm of ova was dominated by yolk vesicles (Fig. 2.2).

Spent (stage VII) ovaries contained oogonia and oocytes with a range of diameters of 1–1600 µm with a mode between 200–300 µm (Fig. 2.3), the majority of which were oogonia and perinucleolar oocytes (Fig. 2.2). The majority of spent ovaries were in individuals that were also berried, with eggs ranging in diameter from 2500–3300 µm, with a mean of 2844 µm (±19.75 µm s.e.) (Fig. 2.3).

The macroscopic description of the stages of male gonad development is shown in Table 2.2. The major distinguishing macroscopic characteristic of mature (stages III–V) male gonads was the thickened, opaque vas deferens (Table 2.2) that was indicative of the presence of spermatophores, which was verified by the sectioning of male gonads.

### 2.3.4 Temporal descriptions of female gonadal development

The temporal development of oocytes in *C. cainii* is typical of a batch spawner possessing a synchronised spawning period. The greatest proportion of yolk granules and ripe ova (ranging from 1100–3100 µm) was recorded in August, with a small number occurring in September and November (Fig. 2.4). In December, the oocytes began to separate into two distinct size/stage cohorts, indicating commencement of oocyte maturation, with the diameter of the larger oocyte cohort possessing a mode of 500–600 µm (Fig. 2.4). The bimodal
separation of oocyte diameters continued through to July when the diameter of the larger oocyte cohort had a range of 1100–2500 µm with a mode of 1700–1800 µm (Fig. 2.4). The presence of larger oocytes (up to 2100 µm) in the January sample highlighted the presence of a single individual that had yet to release her full complement of eggs (see Fig. 2.5).

The monthly proportions of different female gonad stages shown in Fig. 2.5 support this pattern of temporal oocyte development. While stage VI (i.e. ripe/spawning) gonads were found in June, July, August, September, November and January (Fig. 2.5), the majority of females in September, October and November were spent (i.e. stage VII). Thus, the percentage of stage VII (spent) gonads increased from 0 in August to 53.1, 66.7 and 83.3% in September, October and November, respectively (Fig. 2.5). Furthermore, monthly proportions of the different gonad stages mirrored the monthly proportions of females that were berried (Fig. 2.6). The percentage of mature (i.e. gonads of recovering stage II, or III-VII) females that were berried increased from 0% in August to 50, 66.7 and 95.6% in September, October and November. The proportion then declined to 10.7% in December when most young had been released. No berried females were caught in any subsequent months (Fig. 2.6).

The percentages of females with gonads at stage I/II and III increased between November and December from 4.2 and 0%, respectively to 32.4 and 40.5%, respectively (Fig. 2.5). The recovery of gonads during this period is further highlighted by the presence of developed stage IV gonads in December (13.5%).

Development of gonads continued between January and March when the proportions of females that exhibited gonads at stage IV increased from 15.4 to 81.5% (Fig. 2.5). Whilst the first stage V gonad was found in February, by May it was the most prevalent stage (32.1%) (Fig. 2.5). The proportion of females with stage V gonads was greatest in June with 81.3%. Ripe gonads (stage VI) were dominant in both July and August with 70 and 69.2% of females exhibiting that stage in those two months, respectively.
2.3.5 **Gonadosomatic indices**

The mean GSI of mature females declined precipitously from 4.1 to 1.4 between August and September, respectively, indicating that peak spawning occurred between late August and September (Fig. 2.7). The GSI of mature females continued to decline between September and November (mean GSI in November was 0.7 indicating that spawning was continuing in at least some of the females (Fig. 2.7). The temporal trend in the GSI of mature females mirrored the increase in the percentage of spent gonads (stage VII) and is also supported by the temporal trend in the percentages of females that had attached eggs (cf Figs 2.5 and 2.6). The GSI of mature females then remained relatively low between November and January before increasing progressively from 0.7 in January to 3.4 in July (Fig. 2.7).

2.3.6 **Potential and effective fecundities**

The potential fecundities (PF) of female *C. cainii* ranged from 201 to 521. The mean PF for 18 *C. cainii* was 374 oocytes. The effective fecundity (EF) ranged from 71 (with an OCL of 40 mm) to 707 (with an OCL of 70 mm). The mean EF for 47 *C. cainii* was 286 eggs.

The correlation between PF and OCL (PF = 10.892OCL - 152.673, \( r = 0.809 \)) of *C. cainii* was found to be significant (\( F_{1,16} = 30.30, P < 0.01 \)). There was also a significant correlation (\( F_{1,45} = 35.39, P < 0.01 \)) between EF and the OCL of *C. cainii* (\( EF = 11.005OCL - 260.244, r = 0.664 \)). An analysis of covariance, with OCL as a covariate, revealed that mean PF was significantly greater than the mean EF (\( F_{1,62} = 13.62, P < 0.01 \))

2.3.7 **Size at first maturity**

The logistic curve, fitted to the percentage contribution of *C. cainii* with gonads of stages III–VII in sequential 5 mm OCL increments over the spawning period, yielded a \( L_{50} \) of 32.1 and 28.6 mm OCL for female and male *C. cainii*, respectively (Fig. 2.8). The \( L_{95} \) for females and males was 37.9 and 38.8 mm OCL, respectively (Fig. 2.8). The smallest mature female and
male *C. cainii* caught during the breeding period measured 32 and 25 mm OCL, respectively (Fig. 2.8).

2.4 Discussion

2.4.1 Sex ratios, carapace length, orbital carapace length and length-weight relationships

Due to a simple sex determination mode based on sex chromosomes (Austin and Meewan 1999), the sex ratios of freshwater crayfishes are generally 1 : 1 (Reynolds 2002). Therefore, the ratio recorded here of 1.61 females to 1 male *C. cainii* appears to be unusual compared with other freshwater crayfish populations (cf. Chapters 3 and 5). It may have been influenced by preferential retainment of males by recreational fishers (as anecdotally occurs in other crustacean fisheries); however, this requires further research.

While the strong correlation between the OCL and CL (the current measurement for the recreational size limit) for both sexes over a wide size range enables the use of a regression equation to reliably predict OCL from CL, the fact that *C. cainii* can damage their rostrums leads to the conclusion that OCL is the most appropriate measure for use in biological studies, as is generally the case. However, the retention of CL for fisheries purposes results in the release of *C. cainii* that possess damaged rostrums that may otherwise be of legal size, which may help reduce the impact of recreational fishers on *C. cainii* populations.

2.4.2 Seasonal breeding cycle

*Cherax cainii* were shown to have a distinct breeding period in this study with spawning occurring at the onset of water temperature rise and increasing day-length between August and September. The majority of females released their brood between mid-November and mid-December, as indicated by the sharp decline of the percentages of females that were berried between these months (i.e. 95.6% in November to 10.7% in December, respectively). This represented a period of pleopodal attachment of eggs and juveniles to females of
approximately 12 weeks, a similar period of attachment to that previously recorded for this species (Morrissy 1975). This study recorded far higher spawning rates for mature female *C. cainii* at Lake Navarino (95.6% of mature females were berried in November) than Molony and Bird (1999) who recorded less than 15% of female marron being berried in this impoundment previously. However, the latter study only sampled the population pre and post recreational season and was therefore outside of the peak spawning as recorded in this study’s more rigorous monthly sampling regime.

The above estimate of the reproductive season was also supported by the trends in gonad stages since the spent (stage VII) female gonads increased in prevalence from September to November, after which ovaries showed a rapid recovery and were dominated by stage II and III in December and January, stages IV and V in May and stage V and VI in June and July, respectively. Given the presence of (albeit a very low percentage) stage VI female *C. cainii* in January, some late spawning may have also occurred. These trends in gonadal development based on macro and microscopic examination of the gonads, were mirrored in the trends of the GSIs of females.

This study shows that in female *C. cainii* gonadal recovery is initiated during summer soon after spawning. The period of rapid female gonadal development occurs during the autumn and early winter. The initial period of gonadal recovery corresponds with the highest water temperatures when *C. cainii* feeding rates are greatest (Morrissy 1976b). The rapid gonadal recovery and development recorded during summer, autumn and early winter allows spawning to occur in early spring at the onset of rising water temperatures (Morrissy 1970, 1976b) allowing juveniles the opportunity to grow rapidly during the warm summer months.

The peak release of the majority of juvenile *C. cainii* was found to be mid-November to mid-December, which was marginally earlier than that recorded for *C. cainii* from the more southerly (cooler) Warren River (Morrissy 1975) (Fig. 1.1), where the release of juveniles occurred between late December and late January. In addition to the earlier spawning period
found in this study, the onset of post spawning gonadal development was also subsequently earlier, as indicated by a bimodal size-frequency of ova, i.e. in December compared to the late January to late February period previously recorded by Morrissy (1975, 1976b).

The earlier spawning period for *C. cainii* in this study was likely to be due to the earlier onset of rising water temperatures in Lake Navarino compared to the more southerly and cooler Pemberton region used in previous studies (Morrissy 1973, 1975, 1976b). In addition, dams generally have increased surface area, slower flows and relatively little shade compared to rivers that would all facilitate higher water temperatures.

It is noteworthy that the hormones that control ovarian development in freshwater crayfish are influenced by temperature and photoperiod (Dendy 1978; Westin and Gydemo 1986; King 1993), and a correlation between juvenile pleopodal release and mean water temperatures has previously been recorded for *C. cainii* (Morrissy 1976b). Furthermore, since *C. cainii* were shown to have a lower temperature limit for growth of ca 12°C with optimum growth at 24°C (Morrissy 1990), the growth period and rates in Lake Navarino are also likely to be greater than those for the aforementioned previous studies.

The earlier breeding period in this study compared with that described by Morrissy (1976b) may also be a result of the lower densities of *C. cainii* at Lake Navarino (Beatty unpublished data). Lower densities may be due to high recreational fishing effort coupled with predation by the introduced teleost *Perca fluviatilis* (Plate 2.3) that would be exacerbated by the lack of shelter in this aquatic system *cf* the riverine system in Morrissy (1976b) (Morgan *et al.* 2002) (see Plate 2.2). A paucity of heterogenous habitat may also increase cannibalism by larger *C. cainii* on juveniles further reducing densities. The low densities may allow greater per capita food supply through decreased intra-specific competition that may then allow increased energy allocation to reproductive development and rate of post-spawning gonadal recovery, should food be limiting. Furthermore, post-spawning mortality rates of females due to resources competition at higher densities may have been reduced in
the current reservoir population. The influence of resource availability on gonadal development and reproductive success has been examined for marron and other freshwater crayfish species elsewhere and has highlighted the great energetic costs of gonadal development (e.g. *C. cainii* in Morrissy 1975; *Cherax quadricarinatus* in Jones 1995). The importance of resource availability for lipid storage, which is important to future subsequent reproductive success of spawning females, has been shown by Gutiérrez-Yurrita and Montes (1999).

2.4.3 Size at first maturity

The $L_{50}$ for female *C. cainii* of 32.1 mm in Lake Navarino was consistent with the findings of Morrissy (1975) in the Warren River. Furthermore, the maturation of males at a smaller size ($L_{50} = 28.6$ mm) has been reported in other parastacids, where it has been suggested that a greater energetic cost is incurred in female reproduction that results in females maturing at a larger size (Johnson 1979; Turvey 1980; Hamr and Richardson 1994). Furthermore, the larger female size results in greater fecundity (see below).

2.4.4 Factors influencing effective and potential fecundity

The potential fecundity, as determined by the number of pre-spawned ova present in ovaries in prior to spawning, had a significantly greater mean (373) than the actual or effective fecundity (mean of 286 berried eggs or juveniles). The full reproductive potential of *C. cainii* was not attained as is the case for the majority of wild freshwater crayfishes (Lowery 1988). It is likely that these differences in fecundities may be due to the following factors:

1. The loss of eggs, embryos and juveniles during the period of pleopodal attachment.

   Effective fecundity was determined in the present study using all berried females without differentiating between development stages, therefore dislodgment during the berry period would likely account for at least part of the differences in the two fecundity measures;
2. Possible loss of eggs/juveniles from the pleopods during capture via tail flicking. This however was minimised during capture by placing individual berried females immediately in individual plastic bags to ensure that all dislodged eggs were counted;

3. Not all oocytes are released as evidenced by the presence of large atretic oocytes in many spent gonads;

4. The unsuccessful attachment of eggs to the pleopodal endopodites;

5. Insufficient space on pleopodal plumose setae for initial attachment of all eggs.

The latter barrier to full attainment of potential fecundity has been noted for *C. cainii* and other parastacids (e.g. Woodland 1967; Morrissy 1970 1975; Hamr and Richardson 1994). Furthermore, Morrissy (1975) concluded the loss of eggs for *C. cainii* occurred largely during spawning (and not during the carrying period), due to the fact that there was no difference detected between the numbers of newly spawned eggs and further developed young that were still attached to the pleopods.

Both the potential and effective fecundities of *C. cainii* were positively correlated with OCL. A positive correlation between both fecundities and OCL has been also found for other freshwater crayfish species (e.g. *Austropotamobius pallipes* in Rhodes and Holdich 1982). This lends support to the theory that pleopodal setae space limits the number of eggs that are able to attach. Furthermore, anecdotal evidence from *C. cainii* aquaculture facilities suggests that two broad groups of females exist: those that initially grow rapidly and breed later in life at a larger size and therefore have greater batch fecundity (i.e. direct energy towards growth rather than reproduction), and those that initially grow slowly and breed earlier at a smaller size with smaller batch fecundity (i.e. allocate energy into reproduction, some breeding as early as one year of age) (personal communication, Brett Molony, Department of Fisheries, Government of Western Australia). It is unclear as to whether these strategies are strictly dependant on resource availability, however, it may be expected that greater resource availability may encourage the former reproductive strategy whereby the population benefits
from females reaching a large size prior to spawning to increase individual pleopodal
fecundities.

This study represents the first comprehensive study of the reproductive biology of a
wild population of *C. cainii* and the first histologically based study of the reproductive
biology of a freshwater crayfish species. *Cherax cainii* were shown to have discrete
spawning and juvenile release periods. The period of peak spawning occurred at the onset of
rising water temperatures from late August to September with female gonadal recovery
initiated immediately following spawning, however, the period of most rapid ovarian
development occurred during autumn and early winter. This study highlights the need for
histological verification of macroscopically assigned ovarian development stages in
accurately describing the reproductive biology of freshwater crayfishes.
Chapter 3

Biology of a translocated population of the large freshwater crayfish *Cherax cainii*, in a Western Australian river

3.1 Introduction

Freshwater crayfishes often exhibit marked intra-specific biological plasticity as a result of exposure to different environmental conditions, and population genetics (e.g. Austin 1998). However, such variation in reproductive parameters of the Southern Hemisphere parastacids has historically received little attention (Honan and Mitchell 1995a; Austin 1998). This paucity of research is surprising, considering that there are more than 140 freshwater crayfish species currently recognised in Australia, many of which support commercial and/or recreational fisheries (Crandall *et al.* 1999; Austin and Ryan 2002). The Australian freshwater crayfish fauna is represented by nine endemic genera with the majority of species belonging to *Cherax*, *Euastacus*, and *Engaeus*. *Cherax* is the widest distributed Australian crayfish genus with its members found in all mainland states and territories (Horwitz and
Knott 1995) but not all drainage divisions. The reproductive biology of the vast majority of *Cherax* species has not been described in wild systems.

Within Western Australia, all parastacids are entirely endemic and restricted to the Southwest Coast Drainage Division, with none found naturally in the other two drainage divisions (i.e. the Kimberley and Pilbara) (Riek 1967; Austin and Knott 1996) (Fig. 1.1). The marron, *Cherax cainii* is the third largest freshwater crayfish in the world and supports a recreational fishery and aquaculture industry (Lawrence and Morrissy 2000; Molony and Bird 2002). Populations of marron from different rivers have been shown to be genetically distinct (Henryon 1996), and the originally described population of *Cherax tenuimanus* was found to have sufficient genetic differences from the widespread marron (the subject of the current study) for the latter to be described as a new species i.e. *C. cainii* (Austin and Ryan 2002). Relative to the body of research into their aquaculture (e.g. Fotedar et al. 2004), there has been a paucity of research into the biology of *C. cainii* in the wild. Chapter 2 described the reproductive biology of *C. cainii* in Lake Navarino, an impoundment reservoir near the northern limit of its natural range, and approximately 600 km to the south of the translocated population (Hutt River, 28°14'22"S 114°21'55"E, Pilbara Drainage Division) reported in this chapter (Fig. 1.1, Plates 3.1, 3.2a and 3.2b).

In Chapter 2 it was demonstrated that the spawning period of a population in an impoundment (Waroona Dam) occurred about two months earlier than that reported by Morrissy (1975) for a population in a more southerly (and cooler) lotic system (Warren River, Fig. 1.1). Thus, variation in biological parameters (such as spawning periods and growth rates) are exist among populations of *C. cainii* throughout its geographical range, likely as a result of intra-specific genetic variation and prevailing environmental variables (particularly temperature and productivity).

The paucity of information regarding the biology of *C. cainii* populations across its range is surprising, given its iconic status as a recreational species. This is particularly true
when it is realised that the recreational fishery for *C. cainii* has been in decline in terms of total catch and catches per unit effort (CPUE) over the last decade, that the recreational season has been reduced from 55 to 16 days, and that major gear restrictions have also been imposed in many areas (Molony and Bird 2002). Thus, there is an urgent need for a comparative study that determines the biological plasticity (i.e. considerable variability in its morphological, physiological or behavioural traits between or within populations) of the species across its range so that managers can assess the appropriateness of current management strategies to help ensure sustainable exploitation of the species.

The northernmost population of *C. cainii* in Western Australia is a result of translocation into the Hutt River in 1972 (Morrissy and Cassells 1992). This population is exposed to a substantially warmer and drier climate than more southerly populations and, by comparing its biology to those more southerly populations within its natural range, (Morrissy *et al.* 1975; Chapter 2) be used as a model to test the hypothesis that considerable plasticity in the biology of *C. cainii* exists between populations.

### 3.2 Materials and methods

#### 3.2.1 Sampling

On each monthly sampling occasion, three replicate measures of water temperature were recorded at the bottom of the water column. The instantaneous rate of discharge was also determined. Day-lengths at Geraldton (Fig. 1.1), located ca 65 km south of the study site were obtained from the Perth Observatory.

A maximum of 141 *C. cainii* were captured from the Hutt River on each monthly sampling occasion between January and December 2001. On each occasion approximately 250 m² of the river was sampled using 5 and 10 m seine nets (both of 3 mm mesh and fished to a depth of 1.5 m) and random manual scoop netting (nets of mesh sizes 250 μm and 10 mm) to obtain a representative sample of the population. A random sub-sample of animals were immediately euthanized in ice slurry for transport to the laboratory and remaining animals were sexed, measured to the nearest 1 mm and released at the site of capture. All *C. cainii* were dissected within 48 h of capture.
3.2.2 Gonadal development

The gonads of female and male *C. cainii* were macroscopically assigned to developmental stages following the staging outlined in Chapter 2. Those stages were, for females: I, virgin (immature); II, maturing virgin/recovering; III, developing; IV, developed; V, mature (gravid); VI, ripe (spawning); and, VII, spent; and for males: I, virgin (immature); II, maturing virgin; III, mature; IV, gravid; V, spawning; VI, spent. The sixth male stage was added to that in Chapter 2 and displayed well developed, swollen testes and proximal region of the vas deferens and a clear, flattened, distal region that lacked spermatophore bundles, i.e. stage VI.

3.2.3 Gonadosomatic indices (GSI)

The ovaries or testes of individual *C. cainii* in each month were removed, padded dry and weighed to the nearest 0.01 g and GSI calculated using the equation:

\[
\text{GSI} = 100 \left( \frac{W_1}{W_2} \right)
\]

where \(W_1\) is wet weight of the gonad and \(W_2\) is wet weight of the individual crayfish. The GSI of mature (stage III–VII for females and III–VI for males) and immature (stages I/II) gonads were plotted separately as the inclusion of gonads that are not likely to spawn, and thus do not undergo a weight increase prior to spawning, would bias the trends in GSI. As stage II female gonads include both those having recently spawned and virgins at early stages of maturity, female *C. cainii* at stage II were classified as mature if larger, orange oocytes were sighted in the ovary indicating post-spawning resorption was occurring.

3.2.4 Size at first maturity

The OCL at which 50% (*L_{50}* and 95% (*L_{95}* (including their 95% confidence limits) of the populations of *C. cainii* mature in the Hutt River were determined by subjecting the percentage contributions made to each length class by mature females and males to logistic regression analysis, using bootstrapping of 1000 random samples.

\[
P_L = \frac{1}{1 + e^{-\ln 19 \left( \frac{L - L_{50}}{L_{95} - L_{50}} \right)}}
\]

where \(P_L\) is the proportion of *C. cainii* with mature gonads at OCL interval \(L\), and \(L_{50}\) and \(L_{95}\) are the lengths at which 50 and 95% of the population mature, respectively. Maturity was assumed to have been attained in
those crayfish that contained developing/mature gonads (stages III–VII and stages III–VI for females and males, respectively). The females and males used for this relationship were those caught between May and December (i.e. immediately prior to and during the breeding period (see Results)).

3.2.5 Length-frequency and growth rates

Examination of length-frequency distributions of female and male *C. cainii* revealed that there was considerable overlap in the monthly size cohorts of *C. cainii* in the Hutt River. Initially, single normal distributions, as well as a mixture of two and three normal distributions were fitted to length-frequency distributions, in 2 mm OCL increments, in each month. The most appropriate description of the data for *C. cainii* was then determined using the chi-square method (Schnute and Fournier 1980) with the modification described in de Lestang *et al.* (2003).

September 1st was assigned as the birth (hatching) date as the main spawning period of *C. cainii* in the present study was found to occur between July and September (the major decline in mean female GSI occurred between July and September; see Results). The location of the OCL distribution of a cohort within the total frequency distribution of each month and the relationship of the OCL distribution in the adjacent months were used to assign the cohorts to 0+, 1+ or 2+ age classes (de Lestang *et al.* 2003).

As the moult frequency of freshwater crayfish is greatest in the first few months of life (Reynolds 2002), a modified version of the von Bertalanffy growth curve of Hanumara and Hoenig (1987) was fitted to the mean OCL distributions of the one, two, or three age cohorts of female and male *C. cainii* present monthly in the Hutt River, that assumed that the maximum growth rate occurred in *C. cainii* < ca 5 months (de Lestang *et al.* 2003):

\[
OCL_t = \begin{cases} 
OCL_\infty \left[ 1 - \exp \left( - \left\{ \frac{K(t-t_0)}{12} + \frac{CK}{2\pi} \sin \frac{2\pi (t-t_3)}{12} \right\} \right) \right] & \text{if } t < t_0 + 3 \\
OCL_\infty \left[ 1 - \exp \left( - \left\{ \frac{K(t-t_0)}{12} + \frac{CK}{2\pi} \sin \frac{2\pi (t-t_3)}{12} \right\} \right) \right] & \text{if } t \geq t_0 + 3
\end{cases}
\]

(3)

where *OCL_\infty* is the estimate of orbital carapace length at age *t* months, *OCL_\infty* is the asymptotic orbital carapace length, *K* is the curvature parameter, *t_0* is the theoretical age at which the estimated orbital carapace length is zero (*t_0 = t_0' - (6C/\pi)\sin(0.5\pi)\), *C* is the relative amplitude of the seasonal oscillation (where 0 ≤ *C* ≤ 1) and *t_3* is the phase of seasonal oscillation relative to *t_0*. Growth curves were fitted to the length-frequency data using Solver in *Microsoft Excel™*. As a likelihood-ratio test (Cerrato 1990) determined that there was no significant
difference between the growth curves of female and males, length-frequency data for the sexes were pooled and the above procedure was employed to determine the growth parameters for the entire population.

3.2.6 Mortality

A catch curve, which plotted the natural logarithms of numbers of *C. cainii* surviving over age (Beverton and Holt 1957; Ricker 1975), using the growth parameters estimated from the pooled data, was employed to determine the instantaneous mortality rate ($Z$, 1year$^{-1}$). In order to provide an estimate of rates of fishing and natural mortality, estimates of $Z$ were determined separately using size classes that were unexploited (i.e. $Z_u$, an estimate of natural mortality) by recreational fishing (i.e. $< 54.2$ mm OCL (Chapter 2)) and those that were legally subject to exploitation (i.e. $Z_e > 54.2$ mm OCL, an estimate of total mortality). As length-frequency data precluded accurately identifying frequency at age for older animals, the data were used to create an age-frequency distribution via the generation of a length-converted catch curve (Pauly 1983; King 1995):

\[
\ln \left( \frac{N_i}{\Delta t} \right) = \alpha - Z t_i
\]

where $N_i$ is the number of individuals in a size class, $\Delta t$ the time taken to grow through the size class $i$, $t_i$ is the relative age of the size class $i$ (ages determined using the inverse of the modified seasonal von Bertalanffy growth equation with $t_0 = 0$ as only relative ages are required), $\alpha$ is a constant, and $Z$ is either the total rate of instantaneous total mortality of those that were not subject to recreational fishing ($Z_u$, 1year$^{-1}$) or those that were exploited ($Z_e$, 1year$^{-1}$).

For comparative purposes, a further estimate of instantaneous natural mortality rate ($M$, 1year$^{-1}$) was determined using the empirical equation of Pauly (1980):

\[
\ln(M) = -0.0152 - 0.279 \ln(OCL) + 0.6543 \ln(K) + 0.463 \ln(T)
\]

where $OCL$ and $K$ are the growth parameters of the modified seasonal von Bertalanffy growth equation and $T$ is the average mean annual water temperature ($^\circ$C) in the Hutt River. Subsequently, two estimates of the instantaneous rate of fishing mortality ($F$, 1year$^{-1}$) were determined using modified versions (i.e. using the estimate of $Z_e$ and $Z_u$) of the King (1995) equation:

\[
F_1 = Z_e - Z_u
\]
\[ F_2 = Z_c - M \quad (7) \]

where \( F_1 \) is the estimate using the above defined measures of instantaneous total (\( Z_c \)) and natural (\( Z_u \)) mortality and \( F_2 \) is the instantaneous rate of fishing mortality estimated using the empirical estimate of Pauly (1980) (\( M \)).

Two estimates of the exploitation rate (\( E_1 \) and \( E_2 \)) were then determined using modified versions of the Quinn and Deriso (1999) equation:

\[
E_1 = \frac{F_1}{Z_c} \quad (8)
\]

\[
E_2 = \frac{F_2}{Z_c} \quad (9)
\]

where \( E_1 \) and \( E_2 \) are the exploitation rates determined using \( F_1 \) and \( F_2 \), respectively.

Mean life span often approximates 95% of the \( OCL \), of a species (King 1995). Therefore, the age at which \( C. \text{cainii} \) in the Hutt River attained this length was determined using the inverse of the modified von Bertalanffy growth curve (King 1995).

### 3.3 Results

#### 3.3.1 Environmental variables and catch data

The water temperatures and instantaneous rate of discharge for the Hutt River were reflective of the Mediterranean climate of the region and mean water temperatures ranged from a minimum of 16.3°C in August to a maximum of 27.7°C in February (Fig. 3.1a). Instantaneous rate of discharge ranged from a minimum of \( ca \) 0.01 m\(^3\)s\(^{-1}\) in March (early autumn, Plate 3.2a) to a maximum in July (mid-winter, Plate 3.2b) of \( ca \) 37.50 m\(^3\)s\(^{-1}\) (Fig. 3.1b). Day-lengths for nearby Geraldton increased from a minimum of 618 minutes in mid-winter to a maximum of 840 minutes in mid-summer (Fig. 3.1c).

A total of 1275 \( C. \text{cainii} \), at a mean density of 0.4 m\(^{-2}\) (±0.03 s.e.), were caught during the current study, the least number caught was 54 in January (0.2 m\(^{-2}\)) and the maximum was 141 in October (0.6 m\(^{-2}\)). Additionally, 1171 feral yabbies \( Cherax \text{destructor} \), at a mean density of 0.4 m\(^{-2}\) (±0.03), were captured during the current study (see Chapter 5).
3.3.2 Temporal descriptions of gonad development of *Cherax cainii*

The temporal pattern in the ovarian developmental stages of *C. cainii* in the Hutt River suggested that there was a relatively low spawning frequency and a single spawning period between July and September. The low spawning frequency was highlighted by the low proportion of ovaries that had undergone secondary vitellogenesis during this period (stages IV–VI) and the lack of oviigerous females (spent ovaries at stage VII) (Fig. 3.2). Mature ovarian stages (>stage III) were found from March through to October (and also in December) although only in a low proportion of females (Fig. 3.2). Females with stage V (mature) ovaries were first recorded in May (10.0% of females) after which they were found in progressively fewer females until October (1.4%) (Fig. 3.2). Stage VI (gravid) ovaries were only recorded in August and December, when they were found in 3.0 and 4.0% of females, respectively (Fig. 3.2).

Whilst the trend in the temporal pattern in gonadal development in male *C. cainii* was less distinct, the overall pattern was similar to that observed in females (Fig. 3.2). Males with spent testes (stage VI) were found in relatively low proportions in the period between August and December where they were recorded in between 2.4 and 5.3% of males (Fig. 3.2).

3.3.3 GSI

The trend in the mean GSI of female *C. cainii* indicated that peak spawning occurred between July and September, as indicated by the major decline from the maximum of 2.39 (±0.60) in July to 1.83 (±0.69) and 0.89 (±0.32) in August and September, respectively (Fig. 3.3).

Although the trend in GSI of mature male *C. cainii* was not as clear as that in females (Fig. 3.3), the GSI of mature males increased from January to September with means of 0.18
and 0.39 (±0.08), respectively (Fig. 3.3). The mean GSI of mature males then declined progressively to 0.25 (±0.03) in December (Fig. 3.3).

3.3.4 Size at first maturity

The logistic regression analysis yielded a \( L_{50} \) and \( L_{95} \) for females of 70.4 (95% confidence limits = 67.3, 75.6 mm OCL) and 92.9 (95% confidence limits = 82.0, 107.4 mm OCL) mm OCL, respectively (Fig. 3.4). The \( L_{50} \) and \( L_{95} \) for males were 39.6 (95% confidence limits = 37.9, 41.1 mm OCL) and 54.5 (95% confidence limits = 50.9, 58.7 mm OCL) mm OCL (Fig. 3.4). However, the smallest mature female and male \( C. cainii \) captured during the spawning period measured 25 (stage IV) and 26 (stage III) mm OCL, respectively (Fig. 3.4).

3.3.5 Growth

The overall sex ratio of \( C. cainii \) in the Hutt River was 1 female : 1.02 males. However, the ratio of mature crayfish (individuals with gonad stages ≥III) was considerably biased to males (1 female : 5.28 males) (see Fig. 3.3). Newly released individuals were first captured in December (Fig. 3.5); further indicating that spawning occurred from July to September. The very high coefficient of determination (\( r^2 = 0.99 \)) for the growth curve of \( C. cainii \) indicated that these length-at-age data fitted the model well (Table 3.1). The newly- released juvenile (0+) \( C. cainii \) cohort in December had a size distribution from 4 to 8 mm OCL, with the mode of the normal curve fitted to the OCL of the 0+ cohort being 6.1 mm OCL (Figs 3.5 and 3.6). Predicted OCLs then rose to 14.8, 27.9, and 53.3 mm OCL in March, September, and the following September, at approximate ages six, 12, and 24 months, respectively (Fig. 3.6). The seasonal von Bertalanffy curve therefore revealed that the approximate age at which \( C. cainii \) attained the minimum legal fishing size of 76 mm CL (which equates to ca 54 mm OCL (Chapter 2)) was ca 24 months of age.
The $L_{50}$s for females and males (70.4 and 39.6 mm OCL, respectively) equated to approximate ages of 36 and 16 months, respectively, indicating that the majority of females and males would first breed towards the end of their third and second year of life, respectively.

3.3.6 Mortality

The instantaneous total rate of mortality ($Z_e$) of *C. cainii* in the Hutt River was 1.79 year$^{-1}$ (Table 3.1, Fig. 3.7). The initial estimate of the instantaneous natural mortality determined from the slope of the regression line fitted to the length-converted catch curve of unexploited size classes (i.e. $Z_u$), was 0.41 year$^{-1}$, and, using this estimate and $Z_e$, the rates of instantaneous fishing mortality ($F_1$) and exploitation ($E_1$) were 1.38 year$^{-1}$ and 0.77, respectively (Table 3.1). The empirical equation (Pauly 1980) estimated that the instantaneous natural mortality ($M$) was 0.60 year$^{-1}$ (Table 3.1, Fig. 3.7). Using this estimate, the rates of instantaneous fishing mortality ($F_2$) and exploitation ($E_2$) were 1.19 year$^{-1}$ and 0.66, respectively (Table 3.1). *Cherax cainii* in the Hutt River had an approximate mean life span (i.e. age at 95% of the $OCL_\infty = age at 96.82$ mm OCL) of *C. cainii* in the Hutt River was approximately 7.16 years.

3.4 Discussion

Previous studies have demonstrated that freshwater crayfishes may have considerable intra-specific variation in life-history parameters (e.g. Payne 1996; Savolainen et al. 1996; Austin 1998), and that these have often been attributed to genetic (e.g. Austin 1998) and/or environmental (e.g. temperature, Parkyn et al. (2002), and flooding regimes, Gutiérrez-Yurrita and Montes (1999)). The biological traits exhibited by the northernmost population of *C. cainii* outside its range and described in the current study, compared with those
described from more southerly populations, indicates that considerable variation exists between these populations.

3.4.1 Reproductive Biology

As with previous studies of *C. cainii* populations (Morrissy 1975; Chapter 2), the species displayed a distinct spawning period in the Hutt River undergoing a single batch spawning (Figs 3.2, 3.3 and 3.5). However, in the current study the onset of peak spawning (July) was slightly earlier (August) than that recorded in a higher latitude reservoir population (*ca* 600 km south) (Chapter 2) that was, in turn, marginally earlier than in a population further to the south in the Warren River (September/October) (see Fig. 1.1) (Morrissy 1975). The earlier spawning period at lower northerly latitudes is most likely due to higher water temperatures, *i.e.*, *ca* 16–28°C in the Hutt River *cf* 12–25°C in a population 600 km to the south (Chapter 2).

A similar relationship with temperature was found for *Paranephrops planifrons* White 1842 by Parkyn *et al.* (2002), who noted that juveniles in streams that were surrounded by pasture and were exposed to warmer water were released two months earlier and grew faster than those in forested streams that were shaded and had lower temperatures.

3.4.2 Biological plasticity

**Growth rate and length at first maturity**

In contrast to Parkyn *et al.* (2002), who also noted that juveniles in warmer waters reached maturity earlier, the current study clearly demonstrated that female *C. cainii* in the warmer waters of the Hutt River reached maturity at the same age as most of those in the more southerly populations that inhabited cooler waters (Morrissy 1975). However, the relatively rapid growth rate of *C. cainii* in the Hutt River also facilitated the relatively large lengths at first maturity in this system compared with the majority of populations examined in Morrissy (1975). The seasonal von Bertalanffy growth curve revealed that the growth rate of *C. cainii* (*K* = 0.42) in the Hutt River was comparable to those recorded for other fast growing
freshwater crayfish species such as that reported for the fast growing *Procambarus clarkii* by Gutiérrez-Yurrita and Montes (1999) (*K* ranged from *ca* 0.30–0.40), but was greater than that previously recorded by Morrissy (1970) for a population of *C. cainii* inhabiting much cooler waters (*K* = 0.2). Although greater than the majority of growth rates estimated for those southern populations in Morrissy (1975), the size of *C. cainii* 12 months following pleopodal release (i.e., November, release 2 months after hatching) in the present study (33 mm OCL), was comparable to that previously recorded for a fast growing reservoir population of this species (*ca* 34 mm OCL in Wellington Dam, Morrissy (1975)).

Temperature is positively correlated with growth rates of freshwater crayfishes (e.g. *Paranephrops zealandicus* (White 1947) in Jones 1981; *Cherax tenuimanus* (i.e., *C. cainii*) in Jussila and Evans (1996); Whitmore and Huryn (1999)). Faster growth of *P. planifrons* in warmer pasture streams compared with cooler forested streams was believed to be due to both a greater moult increment and higher moult frequency of small-sized crayfish in the pasture streams (Parkyn *et al.* 2002). The Hutt River population of *C. cainii* experiences higher annual temperatures relative to the more southerly populations. The mean monthly temperatures recorded in the Hutt River were often optimal for growth of *C. cainii* (*24 °C*) and did not fall below the minimum temperature for growth (i.e. 11–13 °C, see Morrissy (1990)) (Fig. 3.1a). Therefore, based solely on temperature regime, the growth rate of *C. cainii* in the Hutt River would have been expected to be greater than those estimated in populations in cooler waters. This was the case for the two riverine populations and also for two of the four dam populations that Morrissy (1975) investigated. However, in the case of the remaining two dam populations, the growth rate recorded in the Hutt River was similar to that of one, but considerably less than the other.

In addition to temperature, growth rates in freshwater crayfishes have also been shown to be inversely related to density (e.g. Morrissy 1975; Mitchell and Collins 1989; Verhoef and Austin 1999). For example, Morrissy (1975) found that density (a function of
habitat availability, introduced teleost predation, cannibalism and fishing) was the main factor influencing differences in growth rates in southern populations of *C. cainii*. Thus, *C. cainii* populations in the reservoirs in southern Western Australia generally have decreased densities and high growth rates as these systems are usually devoid of complex benthic habitats that afford cover and protection from larger con specifics but also house the introduced predatory teleosts, such as *Perca fluviatilis* (Morrissy 1975; Morgan *et al.* 2002; Chapter 2). Conversely, natural riverine systems typically contain an abundance of in-stream cover (particularly woody debris), resulting in reduced intra- and inter-specific predation, increased densities, lower growth rates, and smaller sizes at first maturity (Morrissy 1975).

As *C. cainii* in the Hutt River experienced comparatively high temperatures, the growth rate and length at first maturity would have been expected to be greater compared with more southern populations, yet the density of this species and also that of the other introduced crayfish *Cherax destructor* (a species not native to Western Australia, see Chapter 5), were considerable in this system, presumably facilitated by a relatively complex habitat which, as previously noted, typifies riverine environments. Therefore, although relatively fast growing when compared with more southern riverine populations, the high densities of these two crayfish species in the Hutt River and have resulted in the growth rate and length at first maturity of *C. cainii* in this northernmost population being comparable, and in one instance even less than, those recorded in the relatively low density, southern reservoir populations in Morrissy (1975).

**Spawning frequency**

There was a low spawning frequency of *C. cainii* in the Hutt River as indicated by a low proportion of gravid animals (stage VI ovaries), relatively low peaks in mature GSI during the spawning period, and a lack of ovigerous (stage VII ovaries) females being recorded (*cf* Chapter 2). The population continues to survive despite this low spawning frequency and it
is likely that females spawn every other year (or even less frequently) enabling continued existence of the population. This low spawning frequency may have been attributable to density dependent competition with the introduced congener *C. destructor* in the Hutt River, which may have limited the amount of energy available for mature females to direct to the energetically costly process of ovarian development. The recent rapid spread of *C. destructor* into wild aquatic systems in the region (Chapter 5) may therefore have serious implications for the management of the declining recreational *C. cainii* fishery. Thus, controlled investigations examining the influence of sympatric populations of these species on reproductive parameters of *C. cainii* such as growth, size at first maturity, spawning frequency, and diet are required.

**Mortality**

The $Z_e$ value determined from the length converted catch curve fitted to those individuals subject to fishing exploitation (1.79 year$^{-1}$) was moderately high compared with those reported for other species (e.g. 0.998 and 1.603 for *Procambarus digueti* (Bouvier, 1897) and *Procambarus bouvieri* (Ortmann, 1909), respectively (see Gutiérrez-Yurrita and Latournerié-Cervera 1999). Lower natural mortality rates ($M$) are believed to be more typical of K-selected species that are long-lived with larger body sizes (Gunderson 1980). The two estimates of natural instantaneous mortality recorded in the present study using the length-converted catch curve excluding that part of the catch subject to recreational fishing, and Pauly’s (1980) empirical equation, were slightly different ($Z_u = 0.41$, $M = 0.60$). However, both the subsequent estimates of the exploitation rates suggested that the majority of the total mortality of *C. cainii* of legal recreational size was attributed to fishing mortality ($E_1 = 0.77$, $E_2 = 0.66$). The relatively small Hutt River (catchment of 1080 km$^2$) supported *ca* 300 licensed recreational *C. cainii* fishers in the 1986/87 recreational season that had increased to *ca* 400 fishers based on catch effort data in the 2000 season (personal communication, Brett
Molony, Department of Fisheries, Government of Western Australia). This fishing pressure would have also been relatively concentrated in this small, coastal river due to the limited distribution of *C. cainii* in this system (as the upstream reaches were ephemeral and the downstream reaches estuarine), and limited number of accessible sites. The von Bertalanffy growth curve was based only on the modes of size-frequency cohorts of the first 27 months of life (of an estimated 7.16 year mean life span) and, therefore, there may exist some bias in the growth parameters used in determining the relative ages of larger individuals. However, the length-frequency cohort modes fitted the growth model well as indicated by a high coefficient of determination ($r^2 = 0.99$) and an $OCL_\infty$ (101.9 mm OCL) that approximated the maximum size of *C. cainii* captured during the study (93 mm OCL).

### 3.4.3 Management implications

The considerably larger length at first maturity for female *C. cainii* in the Hutt River, compared with those previously reported and the current minimum legal size, coupled with the substantial fishing mortality and low spawning rate in the Hutt River, have considerable implications for the effective management of the recreational fishery. With the exception of a single reservoir (Harvey Dam) where a larger length at first maturity has been recorded and similarly attributed to a rapid growth rate, and where the legal size has been set at 90 mm CL (Department of Fisheries, Government of Western Australia), the legal minimum size for the recreational *C. cainii* fishery is 76 mm CL (equating to 54.2 mm OCL, Chapter 2) in all waterbodies. Therefore, the $L_{50}$ for female *C. cainii* in the Hutt River (70.4 mm OCL or 97.9 mm CL) results in the current legal minimum size being inadequate to allow at least one spawning event of female *C. cainii* prior to exploitation. Should fisheries managers wish to better ensure the sustainability of this translocated population, the minimum legal size for females should be increased in the Hutt River to 98 mm CL to allow ~50% of the female population to mature prior to exploitation.
3.4.4 Conclusions

The results presented in this Chapter and also those of Morrissy (1970, 1975) and in Chapter 2 indicate that *C. cainii* has a life-history strategy that lay between that typical of both winter and summer brooding groups (Honan and Mitchell 1995a). Typical winter brooder characteristics displayed by *C. cainii* are synchrony of breeding period, longevity of life with multiple overlapping age classes in the population, large maximum size, large size at first maturity, and reliance on permanent aquatic habitats (see also Morrissy 1970, 1975; Chapter 2; the current study). The characteristics of the summer brooder group displayed by *C. cainii* are: relatively short brooding period during summer (although with a brooding duration of between *ca* 12 (Chapter 2) to 15 (Morrissy 1970) weeks is one of the longest of the summer group); high egg number per brood, and rapid growth rate (Morrissy 1975; Chapter 2). The rapid growth rate to a large size renders this large species unique amongst those species of freshwater crayfish that have received research attention.

The intra-specific plasticity of the biology of *C. cainii*, particularly with regard to growth rates and length at maturity, further complicates the classification of this species to either kind of the possible life-history groups. Although I have demonstrated that plasticity exists between populations of *C. cainii*, the basis of this variability is not known and may be a result of genetic and/or environmental factors. However, quantifying and determining the role of genetics and environment in this plasticity is crucial for effective fisheries and other natural resource managers. This is particularly important given that this species is declining throughout much of its range as a result of habitat change (e.g. salinisation of water bodies, see Morrissy (1978)), predation by introduced fishes (Morgan *et al.* 2002), and recreational fishing pressure (Molony *et al.* 2001). Based on the results of the current study, the minimum legal size for females should be increased in the Hutt River to 98 mm CL. In light
of these results, it is necessary to further assess the variability in biological parameters among other populations in order to allow appropriate management of the entire fishery.
Chapter 4

Life-history and reproductive biology of the south-western
Australian endemic gilgie *Cherax quinquecarinatus*

4.1 Introduction

Despite the conservation and ecological importance of the freshwater crayfish species of Western Australia, little is known about the biology of wild populations, aside from those of the larger, recreationally and commercially important marron *Cherax cainii* (*sensu* Austin and Ryan 2002) (Morrissy 1975; Chapters 2 and 3). This is surprising as these other endemic species are often locally abundant and grow to sizes that allow them to be targeted by recreational fishers. Furthermore, these endemic species are likely to be as ecologically important as other freshwater crayfishes (e.g. Momot 1995; Rabeni *et al.* 1995).

The gilgie *Cherax quinquecarinatus* is a relatively widespread species of freshwater crayfish in Western Australia being found from the Moore River to just east of Albany (Fig. 1.1). *Cherax quinquecarinatus* exhibits a large intra-specific genetic and morphological
variation that has been shown to be as great as that exhibited between different freshwater crayfish species (Austin and Knott 1996). Furthermore, *C. quinquecarinatus*, which has a propensity to burrow, occupies an extremely wide range of habitats in the region (Austin and Knott 1996). These include permanent rivers, lakes and streams and naturally ephemeral habitats (Riek 1967; Austin and Knott 1996) inundated for only 5–7 months of the year (Morgan *et al.* 1998, 2000). Although *C. quinquecarinatus* is relatively small compared with *C. cainii*, it is comparable in size with other crayfishes consumed by humans, e.g. *Pacifastacus leniusculus* (Lewis 2002) and *Procambarus clarkii* (Huner 2002). The relatively large size, wide distribution and occurrence in a wide range of habitats (where it is often locally abundant), resulted in it being an important component of the diet of the traditional owners of the region (Meagher 1974) and also being the subject of recreational fishing. Currently, there is no closed season and no minimum size limits on this species. The only fishery regulation pertaining to this species is a mixed bag limit of 4L (*ca* 20-50 individuals depending on their size) of any other species aside from *C. cainii*, with no closed seasons applying to those other species.

Through the description of the biology of *C. quinquecarinatus*, the hypothesis that this species will exhibit the life-history categories described by Honan and Mitchell (1995a) as typical of summer brooders, will be tested. These biological data will also provide a basis for the development of sound fishery regulations for this species.

### 4.2 Materials and methods

#### 4.2.1 Sampling Regime

Bull Creek (32º02'57.4''S, 115º52'20.4''E) ([Fig. 1.1](#)), selected as the study site, is an urban drain (previously a natural stream) containing relatively high numbers of *C. quinquecarinatus* that are recreationally fished. Samples of *C. quinquecarinatus* were collected over a 1 km stretch of Bull Creek each month between May 2002 and April 2003 using a variety of methods to capture a representative sample of the population. Sampling was carried out with a back-pack electrofisher (*Smith Root Model 12-A*), box-style freshwater crayfish traps (mesh width 10 mm set overnight for 14 h), and an invertebrate sweep net with a mesh size of 500 μm. Upon
capture, animals were placed in a plastic box and a random sub-sample of up to 93 individuals taken with a fine mesh scoop-net. The sub-sample was then anaesthetised in ice slurry and transported back to the laboratory for dissection. The orbital carapace length (OCL) of the remainder of captured individuals was measured to the nearest 1 mm and the animals were released at their site of capture. The water temperature, salinity and pH were recorded at a depth of 20 cm at three locations in Bull Creek on each sampling occasion.

4.2.2 Morphological Relationships

In order to determine the relationship between the OCL and the carapace length (CL) of *C. quinquecarinatus*, 437 animals were measured to the nearest 1 mm OCL and CL. To determine the relationship between the OCL and wet weight, individuals retained for further dissection were padded dry and weighed to the nearest 0.01 g. Subsequently, a number of regression models were tested for both sexes for the relationship between both OCL and CL and OCL and wet weight using the *SPSS* statistical package (Saila *et al.* 1988). The growth function that produced the highest co-efficient of determination was used to describe those relationships. In order to determine whether differences existed between the sexes for those relationships, likelihood-ratio tests (Cerrato 1990) were used. If no significant differences between sexes were revealed, sexes were pooled and the model re-fitted.

4.2.3 Reproduction

Reproductive cycle

In order to describe the temporal trend in the reproductive biology of *C. quinquecarinatus*, monthly samples of up to 61 and 38 females and males of *C. quinquecarinatus*, respectively, were weighed to the nearest 0.01 g, their gonads removed, and weighed to the nearest 0.01 g. The monthly gonadosomatic indices (GSI) of mature and immature female and male *C. quinquecarinatus* were determined using the equation:

\[
GSI = 100 \left( \frac{W_1}{W_2} \right)
\]

where \(W_1\) is wet weight of the gonad and \(W_2\) is the wet somatic weight.

Ovaries were initially assigned, on the basis of their macroscopic appearance using the verified staging for the congener *C. cainii*, to one of seven stages (see Chapter 2). The seven stages were: I, virgin (immature); II, maturing virgin/recovering; III, developing; IV, developed; V, mature (gravid); VI, ripe (spawning) and; VII, spent. Similarly, the testicular stages were described as per Chapter 2, i.e. I, virgin (immature); II, maturing virgin; III, mature; IV, gravid; V spawning and; VI spent.
Subsequent histological verification of the macroscopically assigned ovarian stages immediately prior to and during the spawning period (June to February, see Results) was undertaken via fixing a sub-sample of up to 30 ovaries of each stage in Bouin’s fixative for 24 h and dehydrating in 70% ethanol. All fixed gonads were then embedded in wax and sectioned transversely at 6 μm, stained with Mallory’s trichrome solution and examined for intracellular development (see Chapter 2). The oocytes were examined at 100x magnification under a compound microscope and diameters of oocytes in each ovarian stage were measured on a viewing screen and size-frequencies for each stage plotted.

Size at first maturity

In order to accurately determine the OCL at which 50 ($L_{50}$) and 95% ($L_{95}$) of C. quinquecarinatus matured in Bull Creek, only those individuals captured immediately prior to, and during, the major spawning period, from June to February (see Results), were used. Logistic regression analysis, using bootstrapping of 1000 random samples, was undertaken on the percentage of mature females (ovarian stages III–VII) and males (testes stages III–VI) in 2 mm OCL increments. The logistic equation is:

$$P_L = \frac{1}{1 + e^{-\ln19 \cdot (L - L_{95})/(L_{95} - L_{50})}}$$

(2)

where $P_L$ is the proportion of C. quinquecarinatus with mature gonads during the reproductive period at OCL interval $L$, and $L_{50}$ and $L_{95}$ are the OCLs at which 50% and 95% of the population mature, respectively.

Fecundity

The relationships between the ovarian ($OF$) and pleopodal ($PF$) fecundity and OCL of female C. quinquecarinatus were determined as follows: for OCL versus $OF$, manual counts of oocytes in un-spawned gonads of ovarian stages IV–VI immediately prior to and during the spawning period from June to February (see Results); and by manual counts of eggs, larvae or hatchlings attached to the pleopods of ovigerous individuals (captured during the spawning period from August to February) for OCL versus $PF$. In order to count unreleased oocytes, ovaries were placed in Bouin’s fixative for 24 h and then counted under a dissecting microscope at 6.4x magnification. The numbers of un-spawned oocytes (for $OF$) or released eggs, larvae and hatchlings (for $PF$) of each individual were then plotted against their OCL and a number of regression equations fitted for each relationship with the one that maximised the coefficient of determination ($r^2$) selected.
The length and width of a random sub-sample of 50 eggs from five ovigerous females were measured to the nearest 0.1 mm and the mean diameter determined.

4.2.4 Temporal Pattern in Hepatosomatic Indices

Hepatosomatic indices of freshwater crayfishes reflect the nutritional status of individuals and populations and therefore have been used to describe life-history strategies (e.g. Fotedar et al. 1999; Lindqvist et al. 1999). Therefore, up to 58 female and 46 male *C. quinquecarinatus* captured monthly from Bull Creek were weighed to the nearest 10 mg, their hepatopancreas removed, weighed, placed in individual foil cups, dried at 80°C for 24 h and reweighed. The dry hepatosomatic index and percentage of hepatopancreatic moisture were determined using the equations:

\[
H_d = 100 \left( \frac{W_{dh}}{W_s} \right) \tag{3}
\]

\[
H_m = 100 \left( \frac{W_h-W_{dh}}{W_s} \right) \tag{4}
\]

where \(H_d\) is the dry hepatosomatic index, \(H_m\) is the percentage of hepatosomatic moisture, \(W_h\) is the wet weight of the hepatopancreas (g), \(W_{dh}\) is the dry weight of the hepatopancreas (g), and \(W_s\) is the wet somatic weight of the crayfish (g).

4.2.5 Growth

The monthly length-frequencies of female and male *C. quinquecarinatus* were plotted in 2 mm OCL increments for the duration of the study period. Those juveniles that were unable to be confidently sexed (ca < 10 mm OCL) were randomly assigned to either the female or male length-frequency distributions. Upon examination, one or two normal distributions could be fitted in each month and a modified version of the chi-squared method of Schnute and Fournier (1980) (as described in de Lestang et al. 2003) was used to fit the most appropriate normal distributions to the data.

Spawning initially occurred from August with the progeny of this initial event subsequently being the dominant 0+ cohort (see Results and Discussion). Therefore, allowing a short ca one month, incubation period (an estimate based on the short period between multiple spawning events, see Results section), the 1st of September was assigned as the birth date. Cohorts were assigned as 0+ and 1+ based on the size distribution within the month relative to previous and subsequent months (i.e. by following the two smallest size cohorts over subsequent months).

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A modification of the von Bertalanffy growth curve equation (Hanumara and Hoenig 1987, equation 5) was applied to the mean OCL distributions of the curves of the cohorts fitted to monthly length-frequency distributions. The moult frequency of freshwater crayfish is greatest in the first few months of life (Reynolds 2002) therefore, the modification of the equation was undertaken so that is assumed that the maximum growth rate occurred in young *C. quinquecarinatus*, i.e. < ca 5 months (de Lestang *et al*. 2003):

\[
OCL_t = \begin{cases} 
OCL_\infty \left(1 - \exp \left[ - \left( \frac{K(t - t_0)}{12} + \frac{CK}{2\pi} \sin \frac{t}{12} \right) \right] \right) & \text{if } t < t_s + 3 \\
OCL_\infty \left(1 - \exp \left[ - \left( \frac{K(t - t_0)}{12} + \frac{CK}{2\pi} \sin \frac{t - t_s}{12} \right) \right] \right) & \text{if } t \geq t_s + 3
\end{cases}
\tag{5}
\]

where $OCL_t$ is the OCL estimate at age $t$ months, $OCL_\infty$ is the asymptotic OCL, $K$ is the curvature parameter, $t_0$ is the theoretical age at which the estimated OCL is zero ($t_0 = t_0 - (6C/\pi)\sin(0.5\pi)$), $C$ determines the relative amplitude of the seasonal oscillation in growth (where $0 \leq C \leq 1$) and $t_s$ determines the phase of seasonal oscillation (i.e. start of the convex segment of the sinusoidal oscillation) relative to $t_0$. A likelihood-ratio test (Cerrato 1990) was used to determine whether the growth functions of females and males were significantly different. Growth curves were fitted to the length-frequency data using Solver in *Microsoft Excel*.

4.2.6 Mortality

The instantaneous mortality rate ($Z$) was determined for female and male *C. quinquecarinatus* in Bull Creek using a catch curve (the natural logarithms of numbers surviving over age) (Beverton and Holt 1957; Ricker 1975). However, as length-frequency data precluded accurately identifying frequency at age, an age-frequency distribution was created via the generation of a length-converted catch curve (Pauly 1983; King 1995):

\[
\ln \left( \frac{N_i}{\Delta t} \right) = \alpha - Zt_i
\tag{6}
\]

where $N_i$ is the number of individuals in each 2 mm size class, $\Delta t$ is the time taken to grow through the size class $i$, $t_i$ is the relative age of the size class $i$ (ages determined using the inverse of the modified seasonal von Bertalanffy growth equation with $t_0 = 0$ as only relative ages are required), $\alpha$ is a constant and $Z$ is the instantaneous total mortality rate (1year$^{-1}$). The regression lines were fitted ignoring the ascending data points,
as they represent younger age groups that were not fully recruited to the population, and the eldest two data points on each curve as they had very low frequencies (i.e. < 10) (King 1995).

The instantaneous natural mortality rate ($M$) was estimated using the empirical equation of Pauly (1980), who examined 175 fish stocks of 84 species in a wide variety of marine and freshwater waters and found a highly reliable equation to predict $M$ based on von Bertalanffy growth parameters and mean water surface temperature, the equation is:

$$\ln(M) = -0.0152 - 0.279 \ln(OCL_\infty) + 0.6543 \ln(K) + 0.463 \ln(T)$$  \hspace{1cm} (7)

where $OCL_\infty$ and $K$ are the growth parameters of the modified seasonal von Bertalanffy growth equation and $T$ is the average mean annual water temperature Bull Creek. Subsequently, the instantaneous rate of fishing mortality ($F$, 1year$^{-1}$) was determined using the equation of King (1995):

$$F = Z - M$$  \hspace{1cm} (8)

The exploitation rate ($E$) was determined using the equation (Quinn and Deriso 1999):

$$E = \frac{F}{Z}$$  \hspace{1cm} (9)

As mean life span often approximates 95% of the length of infinity (King 1995), the approximate life spans of female and male $C. quinquecarinatus$ in the Hutt River were estimated using the inverse of the modified von Bertalanffy growth curve to determine the age of female and males at 95% of their respective $OCL_\infty$s (King 1995).

4.2.7 Density

In order to provide an estimate of density of the trappable population of $C. quinquecarinatus$, a mark-recapture program was undertaken in November 2002 whereby 65 $C. quinquecarinatus > 20$ mm OCL (the minimum size of individuals captured in traps) were captured using eight baited (commercial poultry pellets) opera-style crayfish traps (base 65x50cm, mesh width 1 cm) distributed in the middle of the channel evenly (every ~20 m) along 160 stream metres of Bull Creek for 16 hours overnight. Each individual was sexed, measured to the nearest 0.1 mm OCL, numerically tagged via clipping the uropods using the pattern described in Abrahamsson (1965) which allows up to 1000 crayfish to be individually numbered, and released at the site of capture. An
identical trapping regime was employed in December 2002 and those previously tagged animals that were recaptured were again measured to the nearest 0.1 mm.

The size of the trappable population of *C. quinquecarinatus* (greater than 20 mm OCL) in the stream section was then estimated using the Peterson mark-recapture method:

\[
N = \frac{TC}{R}
\]

where \( N \) the size of the population of *C. quinquecarinatus* within the trapped area, \( T \) is the number of marked *C. quinquecarinatus*, \( C \) total number of captured *C. quinquecarinatus* in the recapture effort, and \( R \) is the number of recaptured marked *C. quinquecarinatus*. The method has a number of assumptions: tagged individuals must be distributed randomly over the population; there was no tag-induced mortality, no recruitment, and no migration before re-sampling occurred (see Results).

The density of *C. quinquecarinatus* at the study site was approximated using the equation:

\[
D = \frac{N}{A}
\]

where \( D \) is the density of *C. quinquecarinatus* in the area of stream sampled, \( N \) is the population abundance in the stream section estimated by the mark-recapture method, and \( A \) is the area of the stream section (320 m²).

### 4.3 Results

#### 4.3.1 Environmental variables

The water temperatures in Bull Creek fell from a maximum of 24.2°C in January to 17.8°C in September (Fig. 4.1). During the study period, conductivity of the stream did not exceed 661.3 μScm⁻¹ and pH ranged from 6.6–7.4. Sudden increases in water levels were only experienced in Bull Creek during and immediately following rainfall events.
4.3.2 Morphological relationships

The sex ratio of the 2316 *C. quinquecarinatus* captured from Bull Creek during the current study was 1 female : 1.43 males. There was a significant difference (likelihood-ratio test, $P < 0.05$) between sexes for the relationship between the OCL and wet weight. The relationships between OCL and wet weight of *C. quinquecarinatus* were $W = 6 \times 10^{-4} OCL^{3.0282}$ for females, and $W = 4 \times 10^{-4} OCL^{3.2000}$ for males. There was, however, no significant difference between the sexes for the relationship between OCL and CL (likelihood-ratio test, $P < 0.05$). Thus, the relationship between the OCL and CL for *C. quinquecarinatus* was: $OCL = 0.6308 CL^{1.0663}$.

4.3.3 Reproductive biology

Macroscopic and histological description of ovarian development

Histologically verified ovarian developmental stages closely corresponded to macroscopic stages based on intracellular development, in particular the presence of vitellin globules within the cytoplasm of oocytes of mature ovarian stages III–VI (Table 4.1, Fig. 4.2). Perinucleolar oocytes at the end of primary vitellogenesis (300–600 μm) were present in all mature stages of ovarian development including within ovigerous females (Figs 4.2 and 4.3).

Temporal pattern in reproductive biology

The mean GSI of mature female *C. quinquecarinatus* suggested that this species underwent a prolonged spawning period in Bull Creek between August and February and appeared to undergo three peaks in spawning during this period. In May, at the start of the study, the GSI of mature females was 0.48 (±0.03), increased slightly in July (0.66 ±0.08) before increasing considerably to 1.95 (±0.51) in August (Fig. 4.4). The mean GSI of mature females then declined to 1.20 (±0.20) in September, increased to 1.95 (±0.33) in October, then declined to 1.27 (±0.16) in November before rising to 1.62 (±0.3) in December (Fig. 4.4). The GSI was
1.56 (±0.27) in January, which then declined to 0.44 (±0.13) in February and then remained relatively constant thereafter. Therefore mean GSI of males followed a similar pattern (Fig. 4.4).

Stage V ovaries (mature) were present in most months, i.e. June (2.9%), July (6.5%), August (42.9%), September (26.9%), October (17.9%), Nov (24.6%), December (15.4%), January (35.0%) and April (2.4%). However, stage VI (spawning) ovaries were first recorded in 3.2% of females in July, increased to 7.1% of females in August and 11.5% in September, with none recorded in subsequent months (Fig. 4.5). The greatest proportion of ovigerous (stage VII) females was found in August (14.3%) before declining markedly in September (3.8%) and then increasing again in October (10.7%) and November (13.1%). While this suggests that some spawning had occurred between the July and August samples, the July sample was taken relatively late in the month, thus, it is most likely that spawning occurred from early August. Ovigerous females continued to be captured in December (7.7%) and a single ovigerous female was captured in February (Fig. 4.5). The above data suggest a protracted spawning period between August and February in Bull Creek.

The frequencies of testicular stages also indicated that the spawning period occurred from August to February and paralleled the trend of ovarian stage frequencies. For example, mature (stage V) testes occurred in 58.3% of males in August, declined to 2.6% of males in November before increasing to 14.4% in December and continued to be present in January and February where they were present in 27.3 and 15.4% of males in those months, respectively (Fig. 4.5).

Size at maturity

The length at which 50% of females attained sexual maturity (L_{50}) was 18.8 mm OCL (95% confidence limits 17.9 and 19.7 mm OCL), and for males the L_{50} was 24.5 mm OCL (95% confidence limits 23.3 and 25.6 mm OCL) (Fig. 4.6). The lengths at which 95% of females
and males attained maturity (L_{95}) were 24.9 and 33.9 mm OCL, respectively (Fig. 4.6). The smallest mature female and male captured measured 16 and 18 mm OCL, respectively.

**Fecundity**

The ovarian fecundity (OF) of 31 female *C. quinquecarinatus* (OCL 21–37 mm) in Bull Creek ranged from 39–149 with a mean of 81.7 (±5.93). The relationship between the OCL and OF was: \[ OF = 0.3515OCL^2 - 13.469OCL + 172.1, (r^2 = 0.759) \] (ANOVA \( F = 43.79, P < 0.01 \)). The pleopodal fecundity (PF) of eight ovigerous females (OCL 24–33 mm) ranged from 40–147 with a mean of 77.1 (±13.76). The relationship between the OCL and PF was: \[ PF = 0.0237OCL^3 - 0.8466OCL^2 + 208.9, (r^2 = 0.876) \] (ANOVA \( F = 17.66, P < 0.01 \)). Analysis of co-variance (OCL as co-variant) revealed that there was no significant difference between mean OF and PF (\( F_{1, 39} = 0.008, P = 0.929 \)). The mean diameter of 50 eggs attached to the pleopods of five female *C. quinquecarinatus* was 2600 µm (±10µm).

4.3.4 **Temporal pattern in hepatosomatic indices**

The overall mean dry hepatosomatic indexes of female and male *C. quinquecarinatus* were 2.5 (±0.06) and 2.3% (±0.05), respectively, which were significantly different (ANOVA, \( F_{1,634} = 9.79, P < 0.01 \)). The mean dry hepatosomatic indices of both sexes reached a minimum of 1.8 (±0.14) and 1.5% (±0.08) in October and August for females and males, respectively (Fig. 4.7). The mean dry hepatosomatic indices then rose progressively to reach a maximum in February being 3.5 (±0.22) and 3.3% (±0.17) for females and males, respectively (Fig. 4.7). The mean dry hepatosomatic indices of both sexes then generally experienced a progressive decline (with the exception of a slight rise in June). The temporal trend in the percentage of hepatopancreatic moisture was effectively the inverse of the trend in the dry hepatosomatic indices for both females and males (Fig. 4.8).
4.3.5 Growth

Due to overlap of older length-frequency cohorts, the seasonal von Bertalanffy growth curves of female and male *C. quinquecarinatus* could only be confidently fitted up to age 14 months (Figs 4.9 and 4.10) with the growth curves providing a good fit to these data given the high coefficients of determination \( r^2 = 0.99 \) (Fig. 4.10, Table 4.2). The likelihood-ratio test revealed that the growth curves for females and males were significantly different \( P < 0.01 \).

The seasonal von Bertalanffy growth curves estimated growth coefficients \( (K) \) for females and males of 0.29 and 0.25, respectively (Table 4.2). Considering that the von Bertalanffy growth curve could only be confidently fitted up to 14 months, the \( OCL_\infty \)s of 59.6 and 73.8 mm OCL for females and males, respectively, were relatively close to the maximum observed in the field with maximum sizes of 43 mm and 48 mm OCL for females and males, respectively.

Juvenile (0+) *C. quinquecarinatus* were first captured in Bull Creek in December with a size range of 2–6 mm OCL (mean of 4.6 mm OCL) (Fig. 4.9). The mean length of the 0+ cohort then progressively increased in size in January and February to 6.4 and 7.4 mm OCL displaying a size range of between 2–12 and 2–14 mm OCL in those two months, respectively (Fig. 4.9). Based on the seasonal von Bertalanffy growth curve, the 0+ cohort of females and males reached 14.7 and 14.1 mm OCL in September at about one year of age, respectively (Fig. 4.10). By November, at 14 months of age, females and males had reached 15.5 and 15.3 mm OCL, respectively (Fig. 4.10).
4.3.6 Mortality

The $Z$ value for females and males were 2.34 and 1.95 year$^{-1}$ whilst the empirical estimates of $M$ for female and male *C. quinquecarinatus* were 0.55 and 0.48 year$^{-1}$, respectively (Fig. 4.11, Table 4.2). The estimates of $F$ and $E$ were 1.78 year$^{-1}$ and 0.76 for females and 1.47 year$^{-1}$ and 0.75 for males, respectively (Table 4.2). The life spans of female and male *C. quinquecarinatus* in Bull Creek was estimated (age at 95% of their $OCL_{\infty}$) to be 10.55 and 11.97 years, respectively.

4.3.7 Density

A total of 65 *C. quinquecarinatus* (size range of 21.5-37.1 mm OCL) were captured, numerically marked and released in Bull Creek in November 2002. Of the 33 individuals captured in December, seven were recaptures (with a size range of 26.8-37.1 mm OCL). The mark recapture estimate of the number of trappable *C. quinquecarinatus* in ∼320 m$^2$ of Bull Creek was 306, equating to a density of 0.96/m$^2$. Only one individual that was recaptured had increased in size during this period, an increase of 2.2 mm OCL. All recaptured individuals were recaptured at the same location and it was therefore assumed that migration into or out of the site was minimal. There may have been some recruitment into the trappable population during this time, however, this was assumed to be minimal given that only one of the seven recaptured individuals had moulted during this time (14.3%).

4.4 Discussion

Riek (1967) and Austin and Knott (1996) considered that *C. quinquecarinatus* (*sensu* Austin and Knott 1996) had the widest natural distribution of all of the crayfishes endemic to the south-west of Western Australia. Furthermore, these authors reported that this species occurred in almost all types of aquatic system that contained water for at least part of the year. Indeed, Austin and Knott (1996) noted, “In comparison with the other species *C.
*C. quinquecarinatus* occurred at sites that have scores that span almost the full range of variation depicted on this first axis, which reflects that over its distribution this species can be found in habitats that range from semi-permanent swamps to deep rivers.” The axis they refer to is the principal component axis that represents the physical dimensions of the water-body, which accounted for the majority (39.8%) of the distribution of the six *Cherax* species examined.

The utilisation of unpredictable, temporary environments or stable, permanent environments is associated with *r*- and *K*-selected species, respectively (MacArthur and Wilson 1967; Pianka 1970). Furthermore, summer brooding crayfishes often display traits typical of *r*-strategists while winter brooding crayfish traits typical of *K*-strategists (Honan and Mitchell 1995). However, as mentioned, a species may display traits intermediate between these life-history strategies or display a mixture of traits typical of one group or the other. For example, *C. cainii*, occupies permanent aquatic systems (Austin and Knott 1996), has a long, synchronised brooding period (Morrissy 1975; Chapters 2 and 3) and a large maximum size, weighing up to at least 2 kg (Coy 1979); characteristics typical of a *K*-strategist and winter brooding crayfish, however, also broods in spring and summer and has a rapid growth rate, which are traits typical of a summer brooder (Morrissy 1975; Chapters 2 and 3).

The biological traits of the *C. quinquecarinatus* population in Bull Creek supports the theory that a crayfish species may display characteristics that are typical of either life-history or brooding groups or intermediate between them. This helps explain the success of this species within its natural range. That is, the combinations of *r*-selected and *K*-selected life-history strategies exhibited by this species are particularly well suited for life in the variety of aquatic habitats found in south-western Western Australia. However, it must be noted that many crayfish species show inter-population variation in many of their traits and that Austin and Knott (1996) commented on the large degree of genetic and morphological variation.
exhibited by *C. quinquecarinatus*. It is therefore likely that the life-history characteristics described in this study, and summarised below, will also vary between populations, and in particular with those populations occurring within temporary habitats. Such plasticity would further increase this species ability to successfully occupy a range of aquatic systems.

4.4.1 Reproductive biology

**Age at first maturity**

The young age and small size at first maturity of *C. quinquecarinatus*, i.e. at the end of their second year of life (at 18.8 and 24.5 mm OCL for females and males, respectively), would undoubtedly be advantageous in highly seasonal waterbodies that annually dry for extended periods. This is typical of summer brooding crayfish species (Honan and Mitchell 1995a) and *r*-strategists (Pianka 1970). The relatively rapid attainment of maturity and the resultant short generation times enable *r*-strategists to increase rapidly in number and thereby take advantage of temporary habitats (Pianka 1970). Whilst particularly suited for life in ephemeral systems, the *r*-strategy of multiple spawning ensures that this species can produce large numbers of offspring in permanent water bodies, many of which contain the larger, more fecund *C. cainii* (Chapter 2).

**Spawning regime**

*Cerax quinquecarinatus* in Bull Creek had an extended spawning period (*r*-strategist) ranging from early August until the end of summer (February) with clear peaks occurring in August, October and December/January. These peaks were indicative of multiple spawning events as evidenced by peaks in GSIs and histological staging that showed the perennial presence of oocytes at the end of primary vitellogenesis in mature females. A prolonged summer breeding period is typical of Australian summer brooding species (Honan and Mitchell 1995a) and would allow *C. quinquecarinatus* to successfully occupy the wide
variety of aquatic habitats found throughout its range including both those that undergo relatively short periods of inundation or are permanent.

The relatively short length of time between multiple spawning events during the breeding period is comparable to that previously recorded for the summer-brooding congener *Cherax destructor* (*ca* 60 days under laboratory conditions, Mitchell and Collins, 1989). In that study the short period between spawning events in *C. destructor* was facilitated by the perennial presence in mature gonads of oocytes that had undergone the relatively slow process of primary vitellogenesis that then underwent a rapid one-two week period of secondary vitellogenesis following moulting or the release of brood (McRae and Mitchell 1995). Similarly, the constant presence of oocytes (*ca* 300–600 μm) in mature (stage III–VII) *C. quinquecarinatus* that had undergone the relatively slow process of primary vitellogenesis would have allowed relatively rapid post-spawning ovarian development, a reduced inter-spawning period, and multiple spawning in Bull Creek.

**Fecundity**

Relative to body size, the mean pleopodal fecundity of *C. quinquecarinatus* recorded during the current study was relatively low (K-strategist) and ranged from 40–147 with a mean of 77 (female size range of 30–41 mm CL). This compares with: size adjusted (weight of 39.5 g) mean pleopodal fecundities of populations of *C. destructor* ranging from 360.3–593.4 eggs (Austin 1998); mean pleopodal fecundities ranging from 110–190 for *Pacifastacus leniusculus* in various studies (summarised in Lewis 2002); 35-351 pleopodal eggs on female *Orconectes rusticus* (Corey 1988); and 50–600 eggs on female *P. clarkii* ranging from 60–120 mm TL, respectively (Huner 2002). The mean diameter of pleopodal eggs recorded here (2600 μm) is moderately large (K-strategist) compared with similar sized species, e.g. *ca* 2400 μm for *O. rusticus* (Corey 1987, 1988), a mean of 1900 μm in *P. clarkii* (Noblitt and Payne 1995) and maximum of 2000-2500 μm for *C. destructor* (Johnson 1979). Lower
fecundities and larger eggs are typically associated with freshwater crayfish species in cooler, more energetically demanding climates (Noblitt and Payne 1995; Eversole et al. 2002), whereas summer brooders generally produce many small sized eggs relative to their body size (Honan and Mitchell 1995a). The freshwater ecosystems of south-western Australia generally have low productivities compared to those in eastern Australia systems (Bunn and Davies 1990). The low productivity of aquatic systems of the region may have resulted in the relatively low fecundity and moderately large size of the eggs of C. quinquecarinatus, paralleling the situation in oligotrophic systems in Tasmania (Hamr and Richardson 1994). However, while the batch fecundity of C. quinquecarinatus is relatively low, the ability to undergo several spawnings in a season in conjunction with relatively large eggs may result in higher survival rates of juveniles and therefore rapid increase in population densities in temporary environments.

The strong positive correlation between size and fecundity for C. quinquecarinatus is consistent with other freshwater crayfish species and has been attributed to pleopodal attachment space and egg size (e.g. Taugbøl et al. 1988; Austin 1998; Gutiérrez-Yurrita and Montes 1999; Eversole et al. 2002; Muck et al. 2002; Chapter 2). The minimum size of ovigerous female freshwater crayfishes is close to the minimum estimated size at first maturity (Reynolds 2002). During the current study, the smallest ovigerous C. quinquecarinatus captured was 23 mm OCL (29 CL), higher than the estimated $L_{50}$ (18.8 mm OCL, 24.1 CL) and more closely approximating the $L_{95}$ (24.9 mm OCL, 31.4 mm CL). However, relatively few ovigerous females were captured in the current study, which also presumably contributed to the skewed sex-ratio of 1 female : 1.43 males. N.B. The sex ratios of freshwater crayfishes are generally 1 : 1 (Reynolds 2002), a reflection of a simple sex determination mode based on sex chromosomes (Austin and Meewan 1999). Reduced locomotor activity of ovigerous females, associated with lowered metabolism, may account for reduced catch-ability relative to non-incubating individuals that exhibit higher metabolic
rates and greater foraging activity (see also Huner 1988; Gutiérrez-Yurrita and Montes 1999). It is also likely that the large numbers of burrows observed in Bull Creek were being utilised by relatively inactive ovigerous females, thereby reducing their chance of capture. Thus, the use of temporal patterns in the frequencies of female gonadal development stages, and not frequency of ovigerous females per se, is probably more appropriate in accurately describing the spawning frequency and timing, particularly for burrowing species.

4.4.2 Temporal pattern in hepatopancreatic indices

The relative size and moisture content of the hepatopancreas have previously been used as condition indices to reflect the nutritional status of individuals and populations and aid in determining life-history strategies (e.g. Fotedar et al. 1999; Lindqvist et al. 1999). Species occupying temporary habitats (typically summer brooders) generally have a greater reliance on these energy reserves due to prolonged periods in burrows (Lindqvist et al. 1999). There was an obvious temporal trend in the dry hepatopancreas index of *C. quinquecarinatus* in Bull Creek. This results from the transfer of organic reserves from the hepatopancreas to the ovaries, for the energetically costly process of secondary vitellogenesis, such as occurs in female *C. quinquecarinatus* during the peak spawning period in Bull Creek (see also Lindqvist et al. 1999). Given the intra-specific morphological and genetic variation and the wide range of habitats occupied by it, *C. quinquecarinatus* occupying temporary systems (Lindqvist et al. 1999) should exhibit even greater temporal variation in indices than those recorded in Bull Creek.

4.4.3 Growth and mortality

Growth

The growth rates of female and male *C. quinquecarinatus* in Bull Creek were relatively low (more typical of a K-strategist and winter brooding freshwater crayfish) with $K$ for females
and males being 0.29 and 0.25, respectively (cf Gutiérrez-Yurrita and Latournerié-Cervera 1999; Gutiérrez-Yurrita and Montes 1999). However, the $OCL_c$ and the maximum size recorded were moderate (i.e. between the sizes typical of r- and K-strategists and summer and winter brooding species) and the life span was estimated to be relatively long at 10.55 and 11.97 for females and males, respectively.

Faster growth by male relative to female freshwater crayfish has previously been attributed to higher energetic cost of female ovarian development and the lack of moulting of berried females (Sokal 1988). The similar values of $K$ for female and male $C. quinquecarinatus$ in Bull Creek may be explained by the limited size-range/age to which the growth curves could be fitted, i.e. up to 14 months, at a time when maturity had not been attained. Consistent with the present study, female freshwater crayfishes usually have a smaller maximum size due to: the lack of moulting (and reduced feeding activity) when ovigerous (Reynolds 2002) and a smaller moult increment due to greater energy required for ovarian development (e.g. Pursiainen et al. 1988).

Mortality

Although the von Bertalanffy growth curve provided a good description of modal progression as indicated by a high coefficient of determination ($r^2 = 0.99$), it was only fitted to the modes of size-frequency cohorts of the first 14 months of an estimated 10.55-11.97 year life span. Thus, some bias in the growth parameters, subsequently used in the length-converted catch curve to determine the relative ages of larger individuals, may exist. However, the data suggested that total mortality was relatively high in this population (females 2.34 year$^{-1}$ and males 1.95 year$^{-1}$) (Table 2). As no teleost predators of $C. quinquecarinatus$ were present in Bull Creek (Beatty unpublished data) much of the mortality was presumably due to density-dependant intra-specific interactions such as competition and cannibalism or through fishing.
The capture of *C. quinquecarinatus* is not specifically governed by fisheries regulations. However, the level of recreational fishing activity observed during the study period (with a number of traps observed in the stream on various occasions) results in the high exploitation rate (0.76 and 0.75 for females and males, respectively). Obviously, this species is heavily fished in the relatively small and easily accessible Bull Creek. Possibly, a proportion of the instantaneous fishing mortality was attributable to the removal of specimens during the current study. Despite the apparent heavy fishing pressure, the species was very abundant in Bull Creek (~0.96/m²) suggesting resilience to fishing.

Due to their life-history strategies, many winter brooding freshwater crayfish species are not resilient to even low levels of fishing mortality (Honan and Mitchell 1995a, b). Conversely, the replacement of natural mortality by fishing mortality in *Orconectes virilis*, a member of the summer brooding group displaying many *r*-strategist traits, increased yields by reducing the density-dependant restriction by larger individuals on juvenile production (Morgan and Momot 1988). Similarly, the separation of broodstock from offspring following pleopodal release in *C. cainii* aquaculture ponds is designed to reduce the intraspecific competition for resources (and predation) thus increasing production. The life-history parameters of *C. quinquecarinatus* allow it to inhabit a wide variety of habitats. Such parameters also provide its resilience to the considerable fishing exploitation in Bull Creek. *Cherax quinquecarinatus* is resilient to fishing pressures and fishery regulations are not required in such permanent systems. However, further assessment of the life-history strategy of *C. quinquecarinatus* in temporary systems is required to determine its resilience throughout its wide habitat range. This is particularly so in light of the low fecundity and slow growth rate typical of a winter brooding species making it more vulnerable to exploitation in variable systems.
4.4.4 Conclusions

This study represents the first research on the population biology of *C. quinquecarinatus*. Traits that were described for *C. quinquecarinatus* in Bull Creek that are typically associated with summer brooders (and often *r*-strategists) included; early maturation, an extended late winter-summer spawning period (with the likelihood of multiple annual spawning events) and high mortality rates. In contrast, the relatively slow growth rate, low fecundity, moderately sized eggs and long life span are traits generally associated with winter-brooding species (often displaying *K*-selected life histories). The life-history strategy of *C. quinquecarinatus* described here would enable rapid population recovery in the event of a sudden decline. Furthermore, it is apparent that although this species underwent considerable fishing exploitation in Bull Creek, its life-history parameters appear to enable this species to be resilient to exploitation, at least in this permanent aquatic system.
Chapter 5

Role of life-history strategy in the colonisation of Western Australian aquatic systems by the introduced crayfish *Cherax destructor*

5.1 Introduction

Once established in an aquatic system, the presence of exotic species is often viewed as permanent, with eradication difficult or almost impossible (Horwitz 1990; Lodge *et al.* 1998). The omnivorous nature of freshwater crayfishes allows them to occupy many trophic levels where they contribute to the structuring of ecosystems and may complicate predicted trophic cascades (Lodge *et al.* 1994; Nyström *et al.* 1996). Thus, introductions of non-native freshwater crayfishes are likely to alter the ecology of freshwater systems, and in particular the structure of food-webs (e.g. Hanson *et al.* 1990; Olsen *et al.* 1991; Momot 1995; Nyström and Strand 1996; Nyström *et al.* 1999; Stenroth and Nyström 2003). Furthermore, introductions of non-native crayfishes have been shown to cause a decline of native
freshwater crayfish species or sub-species via predation and/or competition (e.g. Butler and Stein 1985; Horwitz 1990; Hill et al. 1993), the loss of genetic and morphological variation as a result of hybridisation (Horwitz 1990), and/or the introduction and spread of disease (Holdich 1988; Horwitz 1990). Therefore, the translocation of non-indigenous freshwater crayfishes has often been detrimental to both naturally occurring crayfish species in particular and the receiving aquatic ecosystem in general.

_Cherax destructor_ was first introduced into Western Australian farm dams for aquaculture in 1932 (Austin 1985; Morrissy and Cassells 1992). In an attempt to prevent the spread of the species into wild aquatic systems of the naturally forested, higher rainfall region of the Southwest Coast Drainage Division that is home to all 11 endemic freshwater crayfish species of this State (Austin and Knott 1996; Horwitz and Adams 2000), the Department of Fisheries, Government of Western Australia only allows the culture of _C. destructor_ east of Albany Highway between Perth and Albany (see Fig. 5.1). Inevitably, and despite this state legislative restriction (_Fish Resource Management Act 1994_), _C. destructor_ was first collected with a native freshwater crayfish species (_Cherax quinquecarinatus_) in a wild aquatic system (defined here as a non-human constructed water body) in Western Australia in 1982 (Austin 1985).

Since that first record in a natural system, Morrissy and Cassells (1992) and Jasinska _et al._ (1993) reported the occurrence of _C. destructor_ in natural watercourses of the southern Pilbara, i.e. the Irwin, Chapman, Bowes and Hutt Rivers (Morrissy and Cassells 1992) and in streams of the Aiyennu and Beekeepers Caves, Hill River catchment (Jasinska _et al._ 1993) (Fig. 5.1). Whilst the latter two studies did not record it from the 26 sites that they sampled in natural watercourses throughout the Southwest Coast Drainage Division, they documented a considerable increase in its occurrence in farm dams west of Albany Highway, and suggested that the risk of further escape into natural river systems and coastal wetlands in the region was likely (Fig. 5.1).
Many biologists (i.e. Austin 1985; Horwitz 1990; Morrissy and Cassells 1992; Jasinska et al. 1993) consider that the invasion of *C. destructor* into natural Western Australian aquatic systems poses a serious threat not only to the endemic freshwater crayfishes, but to the freshwater ecosystems of the region. Many of these concerns are based on the ability of *C. destructor* to tolerate variable physicochemical conditions thereby allowing it to inhabit a wide range of aquatic habitats, including temporary and permanent systems (Austin 1985; Jasinska et al. 1993; Horwitz and Knott 1995). Of particular note is its tolerance of low oxygen levels (Morris and Callaghan 1998) and ability to burrow and survive in ephemeral aquatic habitats; environmental conditions that are common to many natural aquatic systems and farm dams in Western Australia (Morrissy 1978). The success of *C. destructor* in colonising the natural systems of the southern Pilbara Drainage Division may also be attributable to it possessing life-history traits that are commonly displayed by other invasive species e.g. rapid growth rate, young age at first maturity and high fecundity (see, for example, Honan and Mitchell 1995).

Thus, the present study aimed to document the current distribution of *C. destructor* in aquatic systems in Western Australia and to describe the life-history characteristics of a successful population from the wild. These life-history parameters will be compared with those of endemic congeners, such as *Cherax cainii*; furthering our understanding of the threat this species poses to sympatric native crayfishes.

5.2 Materials and methods

5.2.1 Distribution of *Cherax destructor* in Western Australia

Between 1996 and 2003 over 1300 sites were sampled for fish and decapods in all of the major drainage systems between the Fitzroy River in the north and the Thomas River in the south-east of Western Australia. Freshwater crayfish were captured using a back-pack electro-fisher (*Smith Root* Model 12-A); seine nets of 5 and 10 m lengths (mesh width of 3 mm) that fished to a depth of 1.5 m, manual scoop netting (nets of mesh sizes 250 μm and 10 mm); and box-style crayfish traps baited with poultry pellets. The latitude and longitude
of each site as recorded using a hand-held Global Positioning System (GPS) and the MapInfo™ program was used to produce a distribution map for *C. destructor* (see Fig. 5.1).

### 5.2.2 Hutt River study site and sampling regime

The Hutt River (Fig. 5.1) drains a catchment of ca 1080 km² that has been extensively cleared for agriculture. In order to obtain a representative sample from the entire river system, *C. destructor* were captured from both the main channel of the Hutt River (28.2395°S, 114.3654°E) and the tributary Yerina Springs (28.1059°S, 114.3449°E) that were approximately eight and 25 river km from the coast, respectively (Fig. 5.1). Between 50–182 *C. destructor* were captured from these sites each calendar month between January and December 2001 in the two hours immediately after dusk. Sampling involved those methods described above. Considerable effort was employed to ensure that all life-stages were captured. Animals were placed immediately in an ice slurry for transport to the laboratory. All crayfish were dissected within 48 h of capture.

### 5.2.3 Environmental variables

During the distributional survey of *C. destructor*, the mean salinity was recorded at three locations at each site where the presence of (alive or deceased) *C. destructor* was recorded. Water temperature was recorded from the bottom of the water column at three locations at each site in the Hutt River. Day-lengths for Geraldton were obtained from the Perth Observatory.

### 5.2.4 Reproductive biology

The orbital carapace length (OCL) of each individual was measured to the nearest 1 mm. The OCL at which 50% (*L₅₀*) and 95% (*L₉₅*) of individuals of female and male *C. destructor* matured in the Hutt River was determined by undertaking logistic regression analysis of the percentage contributions made to each length class by individuals that contained developing/mature gonads (stages III–VII and stages III–VI for females and males, respectively, see below for staging protocol). Data were randomly re-sampled and re-analysed to create 1000 sets of bootstrap estimates. The medians of the bootstrap estimates were used as the point estimates of the parameters and the probability of maturity at each length category. The bootstrap estimates also determined the 95% confidence limits of the parameters. The logistic equation is:

\[
P_L = \frac{1}{1 + e^{-\ln19 (L - L_{50})/(L_{95} - L_{50})}}
\]

where *Pₖ* is the proportion of *C. destructor* with mature gonads (see...
below) at length interval $L$. Only those individuals captured between May to January (i.e. immediately prior to and throughout the breeding period, see Results) were used in the analysis.

The gonads of female and male *C. destructor* were initially macroscopically assigned to developmental stages by following the descriptions of Johnson (1979), McRae and Mitchell (1995) and those in Chapters 2 and 3. As the macroscopic staging of the ovaries of *C. destructor* has not previously been verified histologically, and such an account of ovarian development has recently been used to provide a more comprehensive description of the reproductive biology of a sympatric freshwater crayfish (Chapters 2 and 3), the ovaries of up to 22 randomly selected female *C. destructor* in each month, and a sub-sample of up to three of each stage of testicular development, were prepared for histological examination using the protocol used in Chapter 2.

Histological sections of female and male gonads and individual eggs were examined under a compound microscope at 100x magnification. The maximum and minimum diameters of up to 30 randomly selected oocytes of each sectioned gonad, and also a number of attached eggs, were measured through the nucleus and the mean diameter calculated. The proportions of oocytes at different stages of development and within the different months were also determined. The ovarian stages were: I, virgin (immature); II, maturing virgin/recovering; III, developing; IV, developed; V, mature (gravid); VI, ripe (spawning) and; VII, spent. If larger, orange oocytes were present in stage II ovaries, they were classified as mature as it was indicative of post-spawning resorption (see Results). The testicular stages were: I, virgin (immature); II, maturing virgin; III, mature; IV, gravid; V, spawning and; VI, spent.

The gonads of up to 116 *C. destructor* were removed in each month under a dissecting microscope. The excess moisture was removed and the ovary or testes weighed to the nearest 0.01 g. The GSI was calculated using the equation:

$$GSI = 100 \left( \frac{W_1}{W_2} \right)$$

where $W_1$ is wet weight of the gonad and $W_2$ is total wet weight of the animal. The GSIs of both mature (stage III–VII for females and III–VI for males) and immature (stages I/II) gonads were plotted separately so as not to bias the trend in the GSI of those that had spawned with those that had not (i.e. with immature gonads).

There was a single ovigerous *C. destructor* captured during the study period. The ovarian fecundity ($F_o$) was determined using ovaries at stages V and VI of 21 female *C. destructor*. Ovaries were removed and fixed in Bouin’s fixative (Chapter 2). Fixed gonads were padded dry, weighed to the nearest 0.01g, and a sub-
sample of oocytes removed and counted under a dissecting microscope. The ovarian fecundity was then determined using the following formula:

\[
F_o = \frac{N_s W_g}{W_s}
\]  

where \( F_o \) is the potential fecundity of the individual, \( N_s \) is the number of oocytes in the sub-sample of the ovary, \( W_g \) is the weight of the entire ovary and \( W_s \) is the weight of the sub-sample of oocytes.

The relationship between the \( F_o \) and OCL of \( C. destructor \) was determined by testing a number of regression equations, and that which displayed the highest coefficient of determination was used to describe the relationship.

5.2.5 Growth and mortality

The most appropriate description of the growth data for \( C. destructor \) was initially determined utilising the chi-squared method described in Schnute and Fournier (1980) and using the modification described in de Lestang et al. (2003). Although the Schnute and Fournier (1980) method fitted two or three normal distributions to the monthly length-frequencies, it was decided that age cohorts could only confidently be discerned in the first 12 months of life (see below), and there was considerable overlap in the larger age cohorts of \( C. destructor \). A single or two normal distributions were fitted to length-frequency distributions in 2 mm OCL increments in each month.

Peak spawning activity during the extended breeding period of \( C. destructor \) was found to be July to August (see Results for rationale), however, the most easily followed 0+ length-frequency cohort was recorded for a 12 month period from January, as a result of an apparent second spawning event between October and November. Therefore, December 1st was assigned as the hatching date; allowing for embryo development. The relationship of the OCL range of a cohort, within each monthly length-frequency distribution, to the previous and subsequent months was used to assign that cohort as either 0+ or 1+.

A modified version of the von Bertalanffy growth curve of Hanumara and Hoenig (1987) was fitted to the mean OCL distributions of either the one (0+) or two (0+ and 1+) age cohorts of \( C. destructor \) present in each month in the Hutt River. As growth is most rapid in the first few months of life in freshwater crayfishes, due to greater moult frequency (Reynolds 2002), the modification of the equation was undertaken so that it was assumed that the maximum growth rate occurred in young \( C. destructor \) i.e. < approximately 5 months (de Lestang et al. 2003):
\[ OCL_t = \begin{cases} OCL_\infty \left(1 - \exp\left[-\frac{K(t-t_0) + CK\sin2\pi \left(\frac{t-t_0}{12}\right)}{2\pi}\right]\right) & \text{if } t < t_s + 3 \\ OCL_\infty \left(1 - \exp\left[-\frac{K(t-t_0) + CK\sin2\pi \left(\frac{t-t_0}{12}\right)}{2\pi}\right]\right) & \text{if } t \geq t_s + 3 \end{cases} \]

where \( OCL_t \) is the OCL estimate at age \( t \) months, \( OCL_\infty \) is the asymptotic OCL, \( K \) is the curvature parameter, \( t_0 \) is the theoretical age at which the estimated orbital carapace length is zero \( (t_0 = t'_0 - (6C/\pi)\sin(0.5\pi)) \), \( C \) determines the relative amplitude of the seasonal oscillation \((0 \leq C \leq 1)\) and \( t_s \) determines the phase of seasonal oscillation relative to \( t_0 \).

The instantaneous total mortality rate \((Z, \text{1year}^{-1})\) of \textit{C. destructor} in the Hutt River was determined by employing a catch curve that plotted the natural logarithms of numbers surviving over age (Beverton and Holt 1957; Ricker 1975). An age-frequency distribution was generated via the creation of a length-converted catch curve (Pauly 1983; King 1995):

\[ \ln \left( \frac{N_i}{\Delta t} \right) = \alpha - Zt_i \]

where \( N_i \) is the number of individuals in size class \( i \), \( \Delta t \) the time taken to growth through the size class \( i \), \( t_i \) is the relative age of the size class \( i \), \( \alpha \) is a constant and \( Z \) is the instantaneous total mortality rate (1year\(^{-1}\)). The relative ages were determined using the inverse of the modified seasonal von Bertalanffy growth equation with \( t_0 = 0 \) as only relative ages were required (King 1995).

The empirical equation of Pauly (1980) was used to estimate the instantaneous natural mortality rate \((M, \text{1year}^{-1})\).

\[ \ln(M) = -0.0152 - 0.279 \ln(OCL_\infty) + 0.6543 \ln(K) + 0.463 \ln(T) \]

where \( OCL_\infty \) and \( K \) are the growth parameters of the modified seasonal von Bertalanffy growth equation and \( T \) is the average mean annual water temperature in the Hutt River. The instantaneous rate of fishing mortality \((F, \text{1year}^{-1})\) was then determined using the equation (King 1995):

\[ F = Z - M \]

The exploitation rate \((E)\) was then determined using the equation of Quinn and Deriso (1999):
The age at which \textit{C. destructor} in the Hutt River attained 95\% of its \( OCL_\infty \) (approximating the life span of a species (King 1995)) was determined using the inverse of the modified von Bertalanffy growth curve.

5.3 Results

5.3.1 Present distribution in Western Australia

\textit{Cherax destructor} was recorded at 15 sites in the main channel and/or tributaries of the following major rivers of the Pilbara Drainage Division: Hutt, Bowes, Chapman, Greenough and Irwin rivers (Fig. 5.1). In the Southwest Coast Drainage Division, it was captured at 26 sites in the main channel, tributaries and/or water-supply dams constructed on the tributaries of the: Arrowsmith, Hill, Avon, Canning, Murray, Harvey, Vasse, Gunyulgup, Blackwood, Warren, Kalgan, Gairdner, Fitzgerald and Phillips rivers (Fig. 5.1). \textit{Cherax destructor} was also captured in Canegrass Swamp, a natural water body, and the Malcolm, Niagra and Bromus public water-supply reservoirs; located on natural watercourses in the Zone of Uncoordinated Drainage (see Fig. 5.1).

5.3.2 Environmental variables

During this study, \textit{C. destructor} were found alive in a range of conductivities up to 35.7 mS\text{cm}^{-1} (ca 20 ppt) with only dead \textit{C. destructor} observed in waters > 20ppt. It must be noted that there may have been other variables, particularly water temperature and dissolved oxygen concentration that may have contributed to part or all of the observed mortalities.

The mean water temperatures and instantaneous discharge for the Hutt River were reflective of the Mediterranean climate of the region with mean water temperatures ranging from a minimum of 16.3\°C in August to a maximum of 27.7\°C in February. Day-lengths for
nearby Geraldton increased from a minimum of 618 minutes in mid-winter to a maximum of 840 minutes in mid-summer.

5.3.3 Reproductive biology

The smallest mature female and male *C. destructor* captured during the spawning period measured 17 and 20 mm OCL, respectively (Fig. 5.2). The logistic regression analysis yielded an \(L_{50}\) of 21.6 mm OCL (95% confidence limits = 20.7, 22.4 mm OCL) and 26.5 mm OCL (95% confidence limits = 25.8, 27.1 mm OCL) for female and male *C. destructor*, respectively (Fig. 5.2). The \(L_{95}\) of female and male *C. destructor* was 28.0 mm OCL (95% confidence limits = 26.6, 29.6 mm OCL) and 31.2 mm OCL (95% confidence limits = 29.7, 32.8 mm OCL), respectively (Fig. 5.2).

Immature ovaries (stages I and II) were dominated by oogonia and chromatin nucleolar oocytes (stage I), with stage II ovaries also containing perinucleolar oocytes, that had a size range of 100–600 \(\mu\)m with a modal ovarian diameter of 100–200 \(\mu\)m (Table 5.1, Fig. 5.3). Perinucleolar oocytes continued to be present in stages III, IV, V, VI and VII ovaries with a bimodal distribution of mean oocyte diameters developing from stage IV. There was only one ovigerous female (stage VII) captured and it contained all developmental stages of oocytes (Fig. 5.3), including an apparent resorbing un-released yolk granule oocyte (mean diameter 1180 \(\mu\)m) (Table 5.1).

Virgin (immature, stage I) testes were very small, with a clear testicular mass and a thin, clear thread-like vas deferens that did not contain spermatophores. Maturing virgin testes (stage II) were semi-opaque and slightly thickened with discernable spermatocyte bundles, the vas deferens was thin and clear and lacking spermatophores. Mature stages (III–VI) contained bundles of spermatozoa within spermatophores in the vas deferens, with stages V and VI also displaying clear distal regions of vas deferens that lacked spermatophores (Table 5.2).
The temporal pattern in the mean oocyte diameter frequency distribution of *C. destructor* in the Hutt River was indicative of a protracted spawning period from July to January. Oocytes that had undergone primary vitellogenesis (100–600 μm) were found in all months of the year whereas oocytes greater than 1000 μm (late yolk vesicle and yolk granule, Table 5.1) were found in females in all months aside from February and March. The onset of spawning occurred in July with the maximum oocyte size of *C. destructor* being 900, 1200, 1400, 1800 and 1650 μm in March, April, May, June and July, respectively.

The temporal trend in ovarian maturation also suggested a prolonged spawning period occurred (Fig. 5.4). Females with stage III gonads, which had completed primary vitellogenesis and contained late perinucleolar oocytes, were present in all months (Fig. 5.4). The percentage of stage VI (ripe) ovaries increased from 8.3% in May to 13.1 and 28.6% in June and July, respectively (Fig. 5.4). The single ovigerous individual (stage VII) was captured in August (Fig. 5.4). Stage VI ovaries continued to be recorded until January and were found in 6.7% of females (Fig. 5.4).

The temporal pattern in the developmental stages of the testes of male *C. destructor* in the Hutt River also suggested an extended spawning period had occurred (Fig. 5.4). Between July and August, the percentage of male *C. destructor* with stages V increased from 0 to 3.7, respectively, and those with stage VI rose from 0 to 18.5%, respectively (Fig. 5.4). The percentage of spawning (stage VI) testes then declined progressively to be present 2.2% of male *C. destructor* in December (Fig. 5.4).

The trend in the mean GSI of mature *C. destructor* also supports the spawning period elucidated by the analysis of the temporal patterns in oocyte development and gonad stage development (Fig. 5.5). The mean GSI of mature female *C. destructor* increased from a minimum of 0.65 (±0.18 s.e.) in February to a maximum of 2.69 (±1.15 s.e.) in July before generally declining (apart from a slight increase in October, suggesting a second, minor
spawning event, see Growth and Mortality section) to be 1.14 (±0.39 s.e.) in January (Fig. 5.5).

A similar temporal trend was found in the mean GSI of mature males during the spawning period (Fig. 5.5). The major rise in the mean GSI of mature males occurred simultaneously with the rise in female GSI from May to July (Fig. 5.5). The gradual decline in the GSI of mature male *C. destructor* following the period of peak spawning was again indicative of spawning occurring between July and January.

The one ovigerous *C. destructor* captured during the study in the Hutt River, which was recorded in August, had an OCL of 37 mm and a pleopodal fecundity of 263. The ovarian fecundity of 21 female *C. destructor* (size range of between 32 and 39 mm OCL) ranged from 149 to 295 with a mean of 210.2 (±9.24 s.e.). The relationship between the $F_0$ and OCL of 21 *C. destructor* was best described by the quadratic equation: $F_0 = 0.698OCL^2 - 36.751OCL + 671.056$, R = 0.627.

5.3.4 Growth and mortality

The sex ratio of *C. destructor* in the Hutt River was 1 female: 1.066 males. The length-frequency distributions of *C. destructor* largely supported the spawning periods ascertained by the above analysis of the temporal trends in gonadal and oocyte development with the newly-released individuals first captured in November (size range of between 6 and 14 mm OCL and a mode of 10.6 mm OCL) estimated to be two months old, allowing for a relatively short, *ca* one month, incubation period prior to hatching, i.e. based on birth (hatching) in September (Figs 5.6 and 5.7). The estimation of the incubation period is based on the study of Mitchell and Collins (1989) who found a short brooding period (i.e. egg and hatchling attachment) of 60 days between previous mating and re-spawning for *C. albidus* (now synonymised with *C. destructor*) and also that of (Rouse and Yeh 1995) who found a one
month incubation period for *C. quadricarinatus* von Martins, 1968, a similar-sized species also being able to spawn multiple times over the reproduction period.

Consistent with this short brooding period, there appeared to be a second, more minor spawning event in November (see GSIs), with newly released individuals being captured from this spawning event in January (size range of between 6 and 11 mm OCL and a mode of 9.0 mm OCL) at age *ca* one month (based on December hatching) (Fig. 5.6). Although apparently a minor spawning event (based on GSIs and early length-frequencies), the length-frequency cohort of the minor spawning event in November was actually more easily discerned in subsequent months than that which dominated the length-frequencies in November and December (Fig. 5.6). Therefore, the growth curve was fitted to the subsequent modes of the monthly normal distributions of OCL cohorts from ages one (January) to 12 (December) months (Figs 5.6 and 5.7). The mode of the normal distribution of this subsequent 0+ cohort increased progressively to 13.9, 25.2 and 29.7 mm OCL in March, September and December at ages three, 9 and 12 months, respectively (Fig. 5.6).

The modified seasonal von-Bertalanffy growth equation displayed a good fit to the modes of the monthly normal distributions of OCL cohorts (coefficient of determination 0.9989) (Fig. 5.7). Using the growth equation, the predicted OCLs of *C. destructor* at ages 6 and 12 months were 18.5 and 29.0 mm OCL, respectively (Fig. 5.7).

The length-converted catch curve revealed a total instantaneous mortality rate for *C. destructor* in the Hutt River of 2.91 year\(^{-1}\) (Fig. 5.8). From the empirical formula of Pauly (1980), the instantaneous natural mortality was 1.09 year\(^{-1}\) and therefore fishing mortality and exploitation rates were 1.82 year\(^{-1}\) and 0.62, respectively. The mean life span (i.e. age at 95% of the *OCL_\infty* = age at 48.69 mm OCL) of *C. destructor* in the Hutt River was approximately 3.86 years.
5.4 Discussion

5.4.1 Distribution of *Cherax destructor* in Western Australia

This study documents the presence of *C. destructor* in a number of wild aquatic systems in the Pilbara Drainage Division, in the Zone of Uncoordinated Drainage and, for the first time, in the yabbie exclusion zone in a number of systems in the Southwest Coast Drainage Division of Western Australia. Austin (1985) collected *C. destructor* in two natural water bodies, however, the water bodies did not lie within the ‘yabbie exclusion zone’ west of the Albany Highway. The studies of Morrissy and Cassells (1992) and Jasinska *et al.* (1993) recorded *C. destructor* in the wild aquatic systems of the Hutt, Bowes, Irwin and Chapman rivers, all of which are in the Pilbara Drainage Division and do not naturally contain freshwater crayfishes. Morrissy and Cassells (1992) did not record *C. destructor* in natural water bodies that contained endemic freshwater crayfish species. However, they did record its expansion into farm dams to the west of Albany Highway.

The current study thus documents the invasion of *C. destructor* into numerous wild systems in the Southwest Coast Drainage Division within the ‘yabbie exclusion zone’. Mills and Geddes (1980) proposed that *C. destructor* field salinity tolerance was limited to that which behaviour was unaffected (12 ppt), however, they found that the 96h SL50 for juvenile and adults were 25.8 and 29.9 ppt, respectively. The results of the current study suggest that the chronic salinity tolerance of *C. destructor* (at ca 20 ppt) was higher than the field salinity tolerance predicted by Mills and Geddes (1980). Many of these aquatic systems of the Southwest Coast Drainage Division have become salinised or are naturally saline, which has resulted in the reduction in inland range of native fish and crayfish species (Morrissy 1978; Morgan *et al.* 2003). The relatively high field salinity tolerance of *C. destructor* recorded here, marginally higher than the endemic *C. cainii* (ca 17 ppt, Morrissy (1978)), coupled with their tolerance of hypoxic conditions (Morris and Callaghan 1998), allows them to occupy a number of these water bodies. The potential impact on the structure and function of these
ecosystems and particularly those in which endemic freshwater crayfish species are still found is of major concern given the successful proliferation and life-history strategy of *C. destructor* in the Hutt River (see below).

### 5.4.2 Reproductive biology

Abdu *et al.* (2000) and Chapters 2 and 4 clearly demonstrated that histological examination provides a very precise description of ovarian development in freshwater crayfishes. Furthermore, Chapter 4 showed that patterns in the frequencies of female gonadal development stages (based on histological examination) provided an accurate description of their reproductive biology. Thus, although only one ovigerous female was captured in the current study, presumably as a result of the burrowing behaviour of ovigerous females (Chapter 4), the use of the above techniques is likely to provide the most accurate description of the reproductive biology of *C. destructor*. The capture of juveniles in every month further supports the descriptions of the reproductive biology of this species provided below.

The spawning period of *C. destructor* in the Hutt River occurred between July and January (Figs 5.4 and 5.5) and was similar to that recorded in farm dams in south-western Western Australia where newly released juveniles were found throughout the period between October and February (Morrissy and Cassells 1992). Given the prolonged spawning period, it is likely that *C. destructor* underwent multiple spawning events as evidenced by GSIs and length-frequency distributions with newly released juveniles being captured in November and again in January (Fig. 5.6).

The initiation of secondary vitellogenesis in the current study occurred from stage III as revealed by the presence of early yolk vesicles (Table 5.1). Of particular note was the presence of this stage in all months, this is in contrast to their absence in *C. cainii* in all months from June to November, inclusive (Chapter 2). Based on specimens of *C. albidus* (*C. destructor sensu* Austin *et al.* 2003) held in aquaria McRae and Mitchell (1995) proposed
that, following spawning, ovaries were held in a constant state of readiness, with oocytes present at the end of primary vitellogenesis able to undergo secondary vitellogenesis (increase in mean size from 400 μm to 2000 μm) rapidly. In a later study, McRae and Mitchell (1997) noted that, provided the female *C. destructor* was not ovigerous and had sufficient nutritional reserves, the presence of males was a cue for rematuration. Thus, the demonstrated ability of *C. destructor* to undergo rapid ovarian rematuration and the fact that males were always present, further supports our argument that this species underwent multiple spawning in the Hutt River, and is likely to be able to spawn multiple times in other south-western Australian systems given such favourable conditions.

Multiple spawning has previously been documented in the invasive freshwater crayfish, *Procambarus clarkii* (Girard 1852) (Gutiérrez-Yurrita and Montes 1999). Those authors considered that such a reproductive strategy allowed it to become established in new and varied environments. At high densities *P. clarkii* was found to have the ability to alter the structure and function of ecosystems, becoming an ecosystem engineer (keystone) species (Gutiérrez-Yurrita and Montes (1999) and references therein). Given the previously mentioned wide range of impacts that introduced species of freshwater crayfish may have, the establishment of *C. destructor* in the Hutt River would have likely resulted in it altering the structure and function of this ecosystem and others in which it has become established (Fig. 5.1).

Life-history strategies of freshwater crayfishes have previously been placed into two categories; winter and summer brooders. Many crayfishes that are considered invasive species (e.g. *P. clarkii*, Gutiérrez-Yurrita and Montes 1999) are summer brooders. Summer brooders generally have strategies that include: short brooding periods in summer; an a-synchronous spawning regime within the breeding period (that may include multiple spawning events); the ability to exist in a wide range of permanent and temporary aquatic systems; relatively high fecundities with small eggs; rapid growth rates and a short life span.
(Honan and Mitchell 1995). Many of these traits are typical of those generally associated with \(r\)-selected life-histories. *Cherax destructor* in the Hutt River therefore displayed life-history traits consistent with a summer-brooding, \(r\)-selected species (Table 5.3).

The ability to undergo multiple spawning when conditions are suitable undoubtedly aids in the ability of *C. destructor* to occupy temporary habitats and rapidly colonise a range of new aquatic environments. This strategy is also exhibited by the endemic congener *C. quinquecarinatus*, which also occupies a wide variety of temporary and permanent habitats in this region (Table 5.3, Chapter 4). By contrast, the large sympatric endemic *C. cainii* only spawns once during its breeding period occupying permanent aquatic systems (Table 5.3, Chapters 2 and 3). The reproductive strategy exhibited by *C. destructor* would have enabled it to rapidly proliferate in the Hutt River; attaining a similar density to that of *C. cainii* (Chapter 3) that was translocated into this system prior to the introduction of *C. destructor* (Morrissy and Cassells 1992). Similarly, its establishment in other aquatic systems in Western Australia would be aided by its reproductive strategy; potentially enabling it to become the most abundant crayfish species in many of those systems.

5.4.3 Growth

The length-frequency distribution of *C. destructor* in the Hutt River revealed the main recruitment period (juvenile release) was spring/early summer as supported by initial capture of 0+ individuals in November. However, the protracted spawning period of *C. destructor* in the Hutt River resulted in another recruitment event occurring in January, which subsequently became the most easily discerned cohort thereafter until the following December, at 12 months of age. The cause of the apparent higher survivorship of the progeny of the second spawning event in November than the initial, more major event that occurred in August is unknown but a period of high juvenile mortality appears to have occurred in early summer.
Extended hatching periods and highly variable individual growth rates, resulting in a considerable overlap of successive age groups, complicate size-frequency analyses in freshwater crayfishes (e.g. Morrissy 1975; Jones 1981; Gutiérrez-Yurrita and Latournerié-Cervera 1999). Whilst there was a high degree of overlap in cohorts in the Hutt River population of *C. destructor*, the extremely high coefficient of determination estimated for the modified seasonal von Bertalanffy growth curve suggest a good description of growth for the first 12 months (Fig. 5.6).

Although the growth rate of *C. destructor* in the Hutt River was comparable to that recorded for *C. cainii* in that system (Chapter 3), the $OCL_\infty$ was less, i.e. ca 51 mm OCL for *C. destructor* cf ca 102 mm OCL for *C. cainii* (Table 5.3). However, as the $K$ was greater for *C. destructor* (0.78 cf 0.42 for *C. cainii*), its length at 12 months of age (29 mm OCL) was as great as that of *C. cainii* in the Hutt River (ca 28 mm OCL, Table 5.3, Chapter 3). Furthermore, based on the lengths at first maturity estimated in this study, the majority of the population of *C. destructor* will have reproduced by this time, whereas female *C. cainii* matured much later and at a much larger size (i.e. $OCL_{50}$ for female *C. cainii* in this system was ca 70 mm OCL equating to an age of ca 36 months (Table 5.3, Chapter 3)). *Cherax destructor* in the Hutt River also had a much greater growth rate compared with that recorded for the endemic *C. quinquecarinatus* in a permanent stream in southwestern Western Australia ($K$ for *C. destructor* and *C. quinquecarinatus* were 0.78 and 0.25-0.29, respectively) and attained a far larger size after one year (29 and ca 14 mm OCL for *C. destructor* and *C. quinquecarinatus*, respectively) (Table 5.3).

Size is a major factor determining the outcome of competitive interactions between individual freshwater crayfish (e.g. Momot and Leering 1986; Pavey and Fielder 1996). Chelae size is also important in determining the outcome of agonistic encounters (Gherardi *et al.* 1999). As *C. destructor* is adapted to burrowing in temporary environments, it possesses chelae of considerable size (see also Austin and Knott 1996), and, based on size attained in
its first year, it would be capable of competing with *C. cainii* and out-competing the relatively small *C. quinquecarinatus* for resources (Table 5.3).

### 5.4.4 Mortality

The estimate of total mortality of *C. destructor* in the Hutt River was 2.91 year⁻¹, relatively high compared to that for *C. cainii* in the Hutt River (1.79 year⁻¹) (Table 5.3, Chapter 3). *Cherax quinquecarinatus* was also found to have a relatively high total mortality rate in a permanent Western Australian stream (2.34 and 1.95 year⁻¹ for females and males, respectively (Table 5.3, Chapter 4)). The exploitation rate recorded here (0.62) suggests that most of the total instantaneous mortality of *C. destructor* in the Hutt River was due to fishing mortality. The main sampling site had very easy public access relative to the remainder of the Hutt River, which largely passes through privately owned land. Therefore, a relatively high level of fishing pressure may be expected at this site, particularly in light of the good palatability of this species and the fact that its capture in Western Australia is not restricted by fishery regulations (*cf* the three weeks allowed for the recreational capture of the highly sought after *C. cainii*).

### 5.4.5 Conclusions

*C. destructor* has become established in many wild aquatic systems in Western Australia in the past decade. The ability of *C. destructor* to withstand relatively extreme physicochemical conditions would help facilitate this establishment. Life-history traits described for *C. destructor* in the Hutt River are typical of an invasive, *r*-strategist crayfish species, i.e. an extended breeding period with multiple spawning events, a high spawning frequency, a rapid growth rate; the attainment of maturity at the end of its first year of a relatively short life (Table 5.3). These traits are likely to have facilitated the proliferation of this species in the Hutt River and also aided its establishment in other Western Australian systems. Furthermore, the comparison of life-history traits of *C. destructor* determined in the
present study with those recently described for the endemic congers *C. cainii* and *C. quinquecarinatus*, suggests that it has the potential to become the most abundant species of crayfish once established in an aquatic system that houses these endemics. Given the uniqueness of the freshwater crayfish fauna of the Southwest Coast Drainage Division of Western Australia, the recent spread of *C. destructor* into wild aquatic systems in the region is of serious concern.
Chapter 6

The diet and trophic positions of sympatric populations of *Cherax destructor* and *Cherax cainii* in the Hutt River, Western Australia: evidence of resource overlap

6.1 Introduction

Freshwater crayfishes are able to consume food from a diverse range of trophic levels, e.g. primary producers and invertebrate grazers (Hanson *et al.* 1990; Nyström *et al.* 1996), leaf litter and invertebrate predators and gatherers (Usio 2000), and fish (Rubin and Svenson 1993). Utilisation of food from many trophic levels results in freshwater crayfishes being able to decouple trophic cascades and structure aquatic systems (Nyström *et al.* 1996, 1999; Usio 2000). These abilities have led to them being considered keystone species (Momot 1995; Nyström *et al.* 1996). Whilst a large body of research has explored the ecological roles
of freshwater crayfishes in lentic systems (e.g. Nyström et al. 1996, 1999), far less attention has been given to their role in lotic systems (but see Stenroth and Nyström 2003).

The introduction of non-native crayfishes may affect lotic communities both directly (e.g. reduction in macroinvertebrate richness (Stenroth and Nyström 2003)) and indirectly (e.g. increased benthic algal production through physically removing surface sediment deposits (Stenroth and Nyström 2003)). Furthermore, invading freshwater crayfishes often pose a serious threat to resident freshwater crayfishes. This threat may be due to the introductions of disease (Holdich 1988; Chapter 7), competition for space and shelter that may increase the susceptibility of the native species to teleost predation (Butler and Stein 1985; Garvey et al. 1994, Chapter 8), and competition for food (Hill et al. 1993). These factors have been implicated in the displacement of native crayfishes in Australia (Austin and Ryan 2002), the United States (Butler and Stein 1985; Garvey et al. 1994; Hill et al. 1993), and in Europe (Söderbäck 1995).

Dietary studies have often been used in investigations of the likely effects that the introduction of a species may have on the receiving community through either predation or competition (Olsen et al. 1991; Nyström and Strand 1996). Traditionally the description of a consumer diet has most commonly involved the removal of stomach/gut contents and direct assessment (e.g. O’Brien 1995), or an assessment of food item abundance prior to and following exposure to consumption by the species in question (Olsen et al. 1991; Nyström and Strand 1996; Nyström et al. 1996).

Such direct methods have several drawbacks. For example, in gut content analysis, food that has undergone major processing (either before entering the stomach), through mastication for example, or because they have been in the stomach for some time before capture, may limit identification of a proportion of the diet to unidentified material (Morgan et al. 2002). Furthermore, incidental ingestion of matter whilst feeding may lead to inaccurate descriptions of consumer diets. Perhaps a greater problem is the inability of gut
content analysis to differentiate between food items important merely as an energy source for physiological maintenance (such as detritus or vegetative matter, Nyström et al. 1999), and those items that are primarily assimilated for growth (that may be volumetrically unimportant animal matter). Therefore, a consumer may in fact occupy a predatory trophic position despite consuming large amounts of detrital and vegetative matter, as the relatively small amount of animal matter ingested may be assimilated for growth (Parkyn et al. 2001; Hollows et al. 2002).

Stable N and C isotope analysis can be used to examine trophic relationships in ecosystems and/or focus on relative importance of assimilated food items of consumers (Gearing 1991). The use of stable C analysis is based on the theory that different types of plants (e.g. C₃ and C₄) are distinct in terms of their δ¹³C:δ¹²C ratio due to fractionation during carbon fixation (i.e. photosynthesis). As animals ingest food without significantly altering the C isotope ratios (aside from a 1-2‰ enrichment from food item to consumer due to assimilation or respiration (Fry and Sherr 1984; Peterson and Fry 1987), examination of their δ¹³C:δ¹²C ratio allows determination of their major sources of assimilated carbon. Determination of the shifts in δ¹⁵N:δ¹⁴N ratios between organisms allow comparisons of trophic levels with each positive increase of up to 4‰ an indication of an increase in trophic position. This fractionation (or enrichment) results from there being differences in the rate of fractionation during assimilation into the biomass relative to that which occurs during excretion (Ponsard and Averbauch 1999; Post 2002) (see section 6.2.3).

In contrast to stomach content analyses, models that utilise stable isotope analysis are not biased by the inclusion or exclusion of unidentifiable food stuffs and provide estimates of the relative importance of assimilated food items of consumers (Parkyn et al. 2001; Hollows et al. 2002). Such analyses therefore, may often provide better indications of trophic position than the more traditional analyses (Post 2002). Thus, in the case of freshwater crayfishes, the heavy processing of food before it reaches the stomach and the wide range of food stuffs
ingested means that gut content analysis is unlikely to identify those food stuffs important for biomass gain. However, stable isotope analysis can be employed to determine the relative assimilated importance of ingested food items, and to develop and test hypotheses regarding trophic relationships (e.g. O’Brien and Davies 2002; Parkyn et al. 2001; Hollows et al. 2002).

Given the continued spread of *Cherax destructor* into wild systems of Western Australia, it is of considerable importance to determine whether this species poses a threat to native species. As part of this assessment, it is necessary to determine whether there is the potential for competition for food resources.

By determining temporal changes in the assimilated diet and trophic positions of sympatric populations of *C. destructor* and *C. cainii*, this study aims to test the hypothesis that *C. destructor* has the potential to compete with *C. cainii* for food resources. I employed multiple stable isotope analyses to determine the assimilated diet and trophic position of populations of this species and *C. cainii* from sympatric populations in the Hutt River (Figs 1.1 and 5.1, Plates 3.1 and 3.2). Furthermore, *C. destructor* and *C. cainii* may have both had considerable impact on the existing ecosystem in the Hutt River (that formerly did not house crayfish) and determination of their current trophic roles may provide insight on their overall impact.

### 6.2 Materials and methods

#### 6.2.1 Sampling regime

As stable isotope signatures may vary temporally (e.g. Boon and Bunn 1994), *C. destructor* and *C. cainii*, and their potential food sources were collected in winter (July) and summer (December). On each sampling occasions, between 30 and 58 *C. destructor* (size range of 7–42 mm OCL) and *C. cainii* (size range of 22–70 mm OCL), and up to 44 *Gambusia holbrooki* (Teleostei) (11–50 mm total length TL) and eight *Pseudogobius olorum* (Teleostei) (40–62 mm TL) were collected using a 10 m seine net (3 mm mesh width). Approximately 30 gastropods (*Plotiopsis* sp.) were also collected in each season using a triangular 250 µm sweep net. The small fishes and gastropod were sampled as they were the most conspicuous potential animal prey items.
available in the Hutt River. Although benthic insects were sampled, insufficient numbers of specific taxa were collected for isotopic analysis to occur.

Leaves from the most conspicuous primary producers in the Hutt River, i.e. *Melaleuca* spp., *Casuarina* sp., *Eucalyptus* sp. and *Juncus* sp., were collected by hand. A conspicuous green filamentous alga that was only present in winter was also collected. Sediment core samples were taken to a depth of 5 cm using a stainless steel corer (10 cm diameter).

Animal and plant samples were washed in distilled water, placed in separate bags and kept on ice until they could be frozen (no longer than two hours). Sediment core samples were placed directly in individual bags and kept on ice until frozen.

### 6.2.2 Sample preparation

Each replicate freshwater crayfish sample consisted of between three and five randomly selected individuals. Three or four replicates of both adults (*C. cainii* >30mm OCL, *C. destructor* >20mm OCL) and juveniles (*C. cainii* <30mm OCL, *C. destructor* <20mm OCL) of both species were prepared in both winter and summer; with the exception of juvenile *C. destructor* in winter, which were not sourced as the majority were mature at that time (see Chapter 5). Approximately 5 g of abdominal muscle tissue (without carapace or intestine) was removed from each individual and placed in 1 M HCl for 24 h to remove inorganic carbonates. Up to four replicates of *G. holbrooki* and *P. olorum* consisting of between 3 and 5 individuals per replicate were taken in each season (*P. olorum* were not sampled in summer) and a sample (0.5–2 g) of dorsal musculature removed (the scales and bones removed) from the trunk of each individual. The gastropod *Plotiopsis* sp. was randomly assigned into two replicates consisting of approximately 15 individuals and the muscle flesh of each snail was removed under a dissecting microscope and placed in 1 mol/L HCl for 24 h. Samples were then rinsed in distilled water and dried at 60°C for 48 h and finely ground with a mortar and pestle.

Three replicates of each species of vegetation were taken. In order to obtain organic detrital samples, the three replicate sediment cores from each season were rinsed several times through a 150μm sieve. The large material was decanted several times to remove heavy inorganic material and the remaining coarse particulate organic matter (CPOM) was retrieved. The fine sieved material was washed and decanted several times in distilled water to obtain sufficient fine particulate organic matter (FPOM). All vegetation and detrital samples were dried at 60°C for 48 h and finely ground with a mortar and pestle.
6.2.3 Sample analysis

Isotope analysis (carbon $\delta^{13}$C:$\delta^{12}$C and nitrogen $\delta^{15}$N:$\delta^{14}$N) of samples was undertaken using a Tracermass Iron Ratio Mass Spectrometer (Europa PDZ, UK) fitted with a Roboprep combustion system to oxidise the samples. The ratios of $\delta^{13}$C:$\delta^{12}$C and $\delta^{15}$N:$\delta^{14}$N are presented as the relative part per thousand (‰) difference between the sample’s signature and that of the international standards, which are derived for Pee Dee Belemite for $\delta^{13}$C and atmospheric nitrogen for $\delta^{15}$N. The precision of the analytical equipment was ±0.1‰ for $\delta^{13}$C and ± 0.3‰ for $\delta^{15}$N. Means of each sample category (e.g. each crayfish species, maturity stage and season) are expressed throughout ±1 s.e.

In order to test whether differences existed in the mean $\delta^{13}$C and $\delta^{15}$N signatures between adults and juveniles of each species, within and between seasons, Levene’s tests for equality of error variance were first performed on the un-transformed data. If the data were heteroscedastic, mean $\delta^{13}$C and $\delta^{15}$N signatures were In-transformed prior to one-way ANOVAs being performed. If the data became homoscedastic, Scheffe’s post-hoc tests were subsequently performed to determine which groups were significantly different. If the data remained heteroscedastic, the more conservative Tamhane’s post hoc test was employed. A probability level of ($\alpha = 0.05$) was used to test all null hypotheses.

6.2.4 Determination of trophic position

The relative trophic positions of *C. cainii* and *C. destructor* to the base level primary producers were estimated using the following formula (Post 2002):

$$ TL_{sc} = 1 + (\delta^{15}\text{N}_{sc} - \delta^{15}\text{N}_{ref}) / \Delta_0 $$

(1)

where $TL_{sc}$ is the trophic level of the consumer (*C. cainii* or *C. destructor*), $\delta^{15}\text{N}_{sc}$ is the mean stable nitrogen ratio (‰) of *C. cainii* or *C. destructor*, and $\delta^{15}\text{N}_{ref}$ is the mean stable nitrogen ratio (‰) of the base of the food web, in this case the overall mean $\delta^{15}$N signature of the primary producers in each season. Trophic fractionation of $\delta^{15}$N may vary between single trophic transfers (Vanderklift and Ponsard 2003). This enrichment results from differences in fractionation during assimilation into the biomass relative to fractionation during excretion, resulting in differences between body $\delta^{15}$N and dietary $\delta^{15}$N (Ponsard and Averbauch 1999; Post 2002). A mean enrichment of 3.4‰ has been widely observed (e.g. Post 2002). However, a recent meta-analysis of 134 estimates calculated a lower overall mean enrichment of 2.54‰ (±0.11‰ s.e.) (Vanderklift and Ponsard 2003). Furthermore, the latter study found crustaceans were, along with molluscs, among the two taxa with the least degree of enrichment having a mean of ca 2‰ (an estimate
used in the present study) based on 21 estimates in seven publications (Vanderklift and Ponsard 2003). It was suggested that the low enrichment between trophic levels of these groups was due mainly to them being ammonotelic (i.e. mainly excreting ammonia) or detritivores (Vanderklift and Ponsard 2003), as organisms with these traits were shown to have relatively low enrichment values. Therefore, in calculating the trophic position of *C. cainii* and *C. destructor* in the current study, 2‰ was selected as the fractionation level.

### 6.2.5 Mixing model: IsoSource

Mixing models in isotopic studies provide estimates of the proportions of potential food items that contribute to a consumer’s assimilated diet. The major limitation of previous mixing models is that they could only estimate contributions of \( n + 1 \) sources (where \( n \) is the number of isotopic tracers, i.e. C and N in the present study). In ecological studies, where generally only one or two elements are traced, the mass balance linear equations widely used in these previous models allowed estimation of proportions of only two or three sources. These models estimate proportions of sources contributing that conserve the mass balance of the isotopic tracers (Schwarcz 1991; Phillips 2001; Phillips and Gregg 2003). For the example of a single tracer, \( \delta_m \) is the observed signature of the mixture (e.g. a consumer) and \( f_A \) and \( f_B \) are the proportions of two sources with isotopic signatures \( \delta_A \) and \( \delta_B \) contributing to the observed signature, then the following equations can be solved to determine those contributing proportions:

\[
\delta_m = f_A \delta_A + f_B \delta_B \tag{2}
\]

\[
1 = f_A + f_B \tag{3}
\]

However, when there are more than two sources with one tracer (i.e. \( > n + 1 \)), the resulting system of equations, i.e.:

\[
\delta_m = f_A \delta_A + f_B \delta_B + f_C \delta_C \tag{4}
\]

\[
1 = f_A + f_B + f_C \tag{5}
\]

obviously have no unique solution as there are three unknown entities within two equations. Even the use of solvable linear equations (i.e. \( n + 1 \) source) has potential errors.

The method of Phillips and Gregg (2003), employed in the current study, determines every possible solution for the proportions of sources that could result in the observed mixture. It achieves this by examining small increments of the proportions of each source to iteratively create all possible combinations that sum to 100%. The predicted mixture signatures are then compared with the observed mixtures and if they are within a certain tolerance (known as a “mass balance tolerance”) the combination is considered feasible and is recorded. The data set that results represents all feasible solutions and the distribution of proportions is thus determined (Phillips and Gregg 2003).
The number of potential source combinations increases exponentially with the number of sources examined, which can create computational limits. Therefore, as relatively large numbers of potential food sources were examined in the present study, a relatively large increment of 2.5% was used to avoid impractical levels of computation (Phillips and Gregg 2003). The use of such an increment in IsoSource has been shown to result in adequate precision in determining ranges of source contributions (Phillips and Gregg, 2003). The present study used the minimum mass balance tolerance that resulted in a feasible solution. This was achieved by initially using a mass balance tolerance of 0.1‰ and, should no feasible solution occur, increasing this value in 0.1‰ increments until an adequate level of tolerance occurred. Although Phillips and Gregg (2003) found that altering the mass balance tolerance did not affect the medians of the feasible distributions, the range of the distributions increased with larger tolerance levels. Therefore, some biases may exist in the width of ranges of the source contributions in the present study. However, the means of those ranges used in the classification of freshwater crayfish groups and examination of differences between those groups would be less affected by the use of higher mass balance tolerances.

Initially, the mean $\delta^{13}$C and $\delta^{15}$N signatures of each of the seven crayfish groups (adult and juvenile *C. cainii* in winter and summer, adult and juvenile *C. destructor* in summer and adult *C. destructor* in winter) were analysed using IsoSource in order to characterise the relative proportions of potential food sources to the diets of the groups. Ranges in source contributions provided are presented as 1–99th percentiles.

6.2.6 Comparison of assimilated diets between species, season and maturity

To further explore whether differences existed in the assimilated diets of *C. destructor* and *C. cainii* both within and between seasons and stages of maturity, the assimilated diet of each crayfish sample was characterised using the mean proportion of each of the 10 conspicuous potential food items contributing to each diet as determined from the frequency distributions generated by IsoSource. It should be noted that, although Phillips and Gregg (2003) recommend that the possible range (e.g. 1st and 99th percentile) of proportions of each food item assimilated by the consumer be considered in discussions of food utilisation, an almost infinite number of proportional dietary solutions could be produced, resulting in an infinite number of tests of similarity having to be performed. The mean proportion that each food item contributed to each crayfish sample was deemed the most appropriate single proportional value of that potential range and thus used for exploring dietary differences/similarities.

Initially, a similarity matrix of the assimilated proportions was constructed using the Bray-Curtis similarity coefficient. This was graphically represented in a classification that used hierarchical agglomerative cluster analysis with group-average linking, and an ordination, using non-metric multidimensional scaling.
(MDS), in the PRIMER v5.0 package (Clarke and Gorley 2001). The similarity matrix for the assimilated proportions of both species was then subjected to one-way analysis of similarity (ANOSIM) (Clarke and Gorley 2001), which is a non-parametric test that utilises a permutation procedure. This procedure is applied to a ranked similarity matrix, which is constructed from the original similarity matrix. It involves the calculation of a test statistic, R, which is a measure of the average rank similarities of replicates within a priori designated groups compared with the average rank similarity of all replicates among these groups. Thus, R is a measure of the discrimination between groups: a value of 0 indicates that there are no differences between a priori groups, i.e. similarities between and within a priori groups are, on average, the same; whereas a value of 1 indicates that all replicates within each a priori designated group are more similar to other members of that group than they are to any other replicate from a different group.

The significance of the R value is tested by arbitrarily re-assigning the group labels in the ranked matrix for a maximum of 1000 possible permutations. The R statistic is then recalculated for each permutation and a distribution of those values produced. If the original R value is unlikely to have come from this distribution by chance (i.e. < 5% of the randomly generated R values being greater than the original R value) then the null hypothesis (that there are no differences between a priori groups) is rejected.

6.3 Results

6.3.1 Summer δ13C and δ15N signatures

Consumers

Teleost fishes were relatively enriched in δ15N in summer (Fig. 6.1) with G. holbrooki adults being the most enriched in δ15N (16.4‰ ±0.12) followed by juvenile G. holbrooki (15.5‰) and P. olorum (14.7‰ ±0.26). It should be noted that due to erroneous isotopic signatures (possibly due to contamination of samples) being recorded for the Swan River goby during summer, and algae (for both δ13C and δ15N) and Casuarina sp. (for δ15N) during winter, the mean isotopic signatures recorded in winter for the P. olorum (δ13C of -24.9‰ ±0.57 and δ15N of 14.7‰ ±0.26) and summer for algae (δ13C of -26.8‰ ±0.37 and δ15N of 11.12‰ ±0.13) and Casuarina sp. (δ15N of 4.6‰ ±1.61) were included in examining the trophic the relationships in the opposing season (Fig. 6.1). The next most δ15N enriched consumers in summer were: juvenile C. cainii (13.8‰ ±0.22), adult (13.6‰ ±0.05) and juvenile (13.5‰
±0.10) *C. destructor*, adult *C. cainii* (12.8‰ ±0.07) and the gastropod *Plotiopsis* sp. (12.2‰ ±0.06) (Fig. 6.1).

Adult *Cherax destructor* had the most enriched mean δ¹³C signature of any consumer during summer in the Hutt River (-23.2‰ ±0.21) (Fig. 6.1). As with δ¹⁵N, adult (-23.5‰) and juvenile (-23.9‰) *G. holbrooki* were also relatively enriched in δ¹³C during summer (Fig. 6.1). The next most δ¹³C enriched consumers in summer were adult *C. cainii* (-24.3‰), juvenile *C. destructor* (-24.6‰ ±0.14), juvenile *C. cainii* (-24.6‰ ±0.14), *P. olorum* (-24.9‰ ±0.57) and *Plotiopsis* sp. (-25.3‰ ±0.03) (Fig. 6.1).

**Primary producers and detritus**

All primary producers and detrital fractions, with the exception of the δ¹³C of algae (-26.8‰ ±0.37), were considerably less enriched than any consumer during summer (Fig. 6.1). Algae also had the most enriched δ¹⁵N (11.12‰ ±0.13) signatures of any primary producer in summer. The δ¹³C signatures of both FPOM (-27.4‰ ±0.03) and CPOM (-27.4‰ ±0.10) were very similar to that of *Juncus* sp. (-27.5‰ ±0.61) during summer suggesting that it was a major component of the detrital fractions at that site (Fig. 6.1). *Casuarina* sp. was the most depleted in both δ¹³C (-28.1‰ ±0.05) and δ¹⁵N (4.6‰ ±1.61) compared with any other producer during summer (Fig. 6.1).
6.3.2 Winter $\delta^{13}C$ and $\delta^{15}N$ signatures

Consumers

Both adult (14.4‰ ±0.11) and juvenile (14.7‰ ±0.39) *G. holbrooki* became less enriched in $\delta^{15}N$ during winter compared with summer. Of the freshwater crayfishes, juvenile (13.1‰ ±0.15) and adult (12.9‰ ±0.43) *C. cainii* continued to be relatively enriched in $\delta^{15}N$, however, *C. destructor* became considerably depleted (9.1‰ ±0.19) (Fig. 6.1). *Plotiopsis* sp. was slightly less enriched in $\delta^{15}N$ during winter (11.5‰ ±0.13) relative to summer (Fig. 6.1).

All consumers were more depleted in $\delta^{13}C$ in winter compared to summer (see below for freshwater crayfishes) (Fig. 6.1). *P. olorum* (-24.9‰ ±0.57) was the most $\delta^{13}C$ enriched consumer in winter, slightly more than adult (-24.9‰ ±0.30) and juvenile (-24.9‰ ±0.76) *C. cainii* and adult *G. holbrooki* (-25.00‰ ±0.06) (Fig. 6.1). *Plotiopsis* sp. was the next most $\delta^{13}C$ depleted consumer in winter (-25.3‰ ±0.23) followed by juvenile *G. holbrooki* (-25.5‰ ±0.04). The $\delta^{13}C$ signature of *C. destructor* in winter (-28.3‰ ±0.12) was the most depleted of any consumer (Fig. 6.1).

Primary producers and detritus

No primary producer in winter had a more enriched isotopic signature than that of algae recorded in summer ($\delta^{13}C = -26.8‰ ±0.37$, and $\delta^{15}N = 11.12‰ ±0.13$) (Fig. 6.1). As with summer, the $\delta^{13}C$ signature of *Juncus sp.* during winter ($\delta^{13}C = -27.3‰ ±0.21$) were similar to that of both FPOM ($\delta^{13}C = -27.4‰ ±0.24$) and CPOM (-27.1‰ ±0.28) suggesting a major contribution of *Juncus sp.* to the detrital fraction at this site in winter. *Melaleuca* sp. became relatively depleted in both isotopes during winter ($\delta^{13}C = -28.7‰ ±0.15$, $\delta^{15}N = -6.2‰ ±0.12$).
6.3.3 Differences in the $\delta^{13}C$ and $\delta^{15}N$ signatures between groups of freshwater crayfishes

There was an overall highly significant difference in the mean $\delta^{13}C$ ($F = 77.65, P < 0.001$) and $\delta^{15}N$ ($F = 103.73, P < 0.001$) signatures of adult and juvenile *C. cainii* and *C. destructor* within and between seasons. There was no significant difference in the mean $\delta^{13}C$ or $\delta^{15}N$ between juveniles of either species within or between seasons (Fig. 6.1).

The mean $\delta^{13}C$ signature of adult *C. destructor* in summer was significantly more enriched than that of juvenile *C. destructor* and adult ($P < 0.1$) and juvenile *C. cainii* in summer. Furthermore, the mean $\delta^{15}N$ signature of adult *C. cainii* in summer was significantly depleted relative to that of juvenile ($P < 0.1$) and adult *C. destructor* in summer.

Within winter, the mean $\delta^{13}C$ signature of adult *C. destructor* was significantly more depleted than that of adult *C. cainii* ($P < 0.01$). Furthermore, the mean $\delta^{15}N$ signature of adult *C. destructor* in winter (similar to $\delta^{13}C$) was significantly more depleted than the signatures of both adult and juvenile *C. cainii* (Fig. 6.1).

Between seasons, the mean $\delta^{13}C$ signature of adult *C. destructor* in winter was significantly more depleted than that of all crayfish groups in summer ($P < 0.01$) (Fig. 6.1). The mean $\delta^{13}C$ signature of adult *C. destructor* in summer was also significantly more enriched than that of adult *C. cainii* in winter (Fig. 6.1). As with $\delta^{13}C$, the mean $\delta^{15}N$ signature of *C. destructor* in winter was significantly more depleted than the signatures of adult and juvenile *C. cainii* or *C. destructor* in summer (Fig. 6.1).

6.3.4 Trophic position of *Cherax cainii* and *Cherax destructor* in the Hutt River

The trophic position of juvenile *C. cainii* was similar between summer and winter and they appeared to occupy the highest trophic level of any crayfish group in both seasons (Table 6.1). The trophic position of adult *C. cainii* increased slightly between summer (3.53) and winter (3.86) (Table 6.1). *Cherax destructor* appeared to have a trophic position between that of juvenile and adult *C. cainii* during summer with juvenile and adults having similar
positions of 3.85 and 3.92, respectively (Table 6.1). The most notable shift in trophic position occurred during winter, when adult *C. destructor* had a substantially reduced position of 1.96 (Table 6.1).

6.3.5 Assimilated diet of *Cherax cainii* and *Cherax destructor* in the Hutt River

The most important dietary source of both juvenile and adult *C. cainii* during summer appeared to be adult *G. holbrooki*, which contributed between 0–70% (mean = 39.8%) and 13–70% (mean = 53.7%) of the diets of juvenile and adults in summer, respectively (Fig. 6.2). The next most important food sources to both juvenile and adult *C. cainii* during summer appeared to be juvenile *G. holbrooki* that contributed 0–75% (mean = 28.8%) and 0–60% of the diet of juvenile and adult *C. cainii*, respectively (Fig. 6.2). *Plotiopsis* sp. (0–38%, mean = 9.7%) appeared to also be an important component of the diet of juvenile *C. cainii* in summer, with CPOM being the next most important component to the diet of adult *C. cainii* (10–30%, mean = 21.7%) (Fig. 6.2).

Adult *G. holbrooki* also appeared to be the major component of the diet of juvenile (0–70%, mean = 43.1%) and adult (35–83%, mean = 69.3%) *C. destructor* in the Hutt River during summer (Fig. 6.2). Furthermore, juvenile *G. holbrooki* appeared to be the next most important component to the diet of both juvenile (0–75%, mean = 25.6%) and adult (0–50%, mean = 12.8%) *C. destructor* during summer (Fig. 6.2). Similar to *C. cainii*, *Plotiopsis* sp. (0–33%, mean = 8%) and CPOM (0–18%, mean = 10.4%) appeared to be the third most important food source to juvenile and adult *C. destructor* during summer (Fig. 6.2).

During winter, *Plotiopsis* sp. appeared to be the most important food source for both juvenile (25–53%, mean = 40.3%) and adult (23–86%, mean = 42.7%) *C. cainii* with *P. olorum* also being of similar importance (3–63% with a mean of 39.3% for juveniles and 0–63% with a mean of 33.7% for males) (Fig. 6.3). Adult *G. holbrooki* appeared to be of
similar importance to both juvenile and adult *C. cainii* in winter ranging from 0–58% for both with means of 18.1 and 19.3% for juveniles and adults, respectively (Fig. 6.3).

The major assimilated food source of *C. destructor* in winter appeared to be *Melaleuca* sp. with a range in contribution from 60–73% (mean = 68.5%) (Fig. 6.3). The other important component appeared to be juvenile *G. holbrooki* with a contribution ranging from 8–30% (mean = 20.7%) (Fig. 6.3).

### 6.3.6 Classification and ordination of the dietary data

Classification (Fig. 6.4) clearly demonstrated the presence of two major groupings in the dietary data. The first of these groups (I) comprised all of the adult *C. destructor* samples collected in winter. The second group comprised three sub-groups, of which sub-group IIa comprised all of the four samples of *C. destructor* adults from summer, three of the four samples of adult *C. cainii* from summer and one each of the samples of juvenile *C. destructor* and *C. cainii* collected in summer; sub-group IIb comprised the remaining samples of *C. destructor* juveniles and *C. cainii* adults and juveniles from summer, two samples of *C. cainii* juveniles from winter and a single sample from adult *C. cainii* in winter; the final sub-group (IIc) comprised the remaining samples of *C. cainii* adults and juveniles from winter.

The result of the ordination (Fig. 6.5) mirrors that of the classification with Group I, i.e. all of the adult *C. destructor* samples collected in winter, clumped together in the bottom right-hand corner of the plot, whilst sub-groups IIa, IIb and IIc are situated in the middle-top, middle and lower left of the plot, respectively.

### 6.3.7 Similarities and differences in the assimilated diets of freshwater crayfishes

The results of ANOSIM for the dietary composition of adult and juvenile *C. cainii* and *C. destructor* in summer and winter were consistent with both the distribution of samples on the classification and ordination plots and with the results of the analyses of variance performed
on the $\delta^{13}$C and $\delta^{15}$N signatures of the tissue from. Thus, the high Global R statistic = 0.805 ($P << 0.001$) suggested that there were highly significant differences between the assimilated diets of some of the a priori designated groups.

Within the seasons no differences were detected between the diets of any of the a priori designated groups in summer, whereas in winter, the assimilated diet of adult *C. destructor* was significantly different ($P < 0.01$) to that of both juvenile and adult *C. cainii*, however, there was no significant difference between the *C. cainii* groups (Table 6.2).

A number of differences in the assimilated diet of the crayfishes existed between seasons. In winter, adult *C. destructor* and *C. cainii* were significantly different to all groups in summer ($P < 0.01$ and $P < 0.05$ for *C. destructor* and *C. cainii*, respectively), whilst, juvenile *C. cainii* were significantly different to adult *C. cainii* and adult *C. destructor* in summer ($P < 0.05$), but not significantly different to juvenile *C. cainii* or juvenile *C. destructor* in summer (Table 6.2).

### 6.4 Discussion

#### 6.4.1 Assimilated diets and trophic positions of *Cherax cainii* and *Cherax destructor* in the Hutt River

It should be noted that the time-lag between consumption of food items and subsequent expression of those isotopic signatures in the flesh of *C. cainii* and *C. destructor* is unknown in the Hutt River. Some time-lag may have existed in these expressions and thus the timing of isotopic testing in the present study (i.e. summer and winter) may not strictly correspond to the assimilated diet and trophic roles of the crayfishes in those specific seasons, however, does account for some potential temporal variation.

*Cherax cainii* and *C. destructor* appeared to assimilate similar food sources and have similar trophic positions in summer. The trophic positions of adult (3.92) and juvenile (3.85) *C. destructor* approximated that of adult (3.53) and juvenile (4.03) *C. cainii* (Table 6.1).
Although ANOVA revealed differences between the mean δ\(^{13}\)C of adult *C. destructor* (-23.2‰ ±0.21, the most enriched of any consumer in the study) to that of juvenile *C. destructor* and adult and juvenile *C. cainii* and in the mean δ\(^{15}\)N between the *C. cainii* and *C. destructor* groups (Fig. 6.1), ANOSIM of the *IsoSource* of all these groups revealed no significant differences in the proportions of assimilated food sources within summer (Table 6.2, Figs 6.4 and 6.5). This suggests that although the mean crayfish signatures varied slightly in summer, considerable dietary overlap existed between these species during summer. The assimilated diet of both species in summer was dominated by *G. holbrooki* suggesting that both species primarily occupied a predatory trophic position. However, it should also be noted that *G. holbrooki* have a short life-cycle (Pen and Potter 1991) and it is therefore likely that at least part of the assimilation of this fish by the two crayfish was via consumption of dead individuals.

In winter both juvenile and adult *C. cainii* again appeared to maintain their predatory trophic positions as suggested by the similar trophic levels to those recorded in summer. The relatively depleted mean δ\(^{13}\)C and δ\(^{15}\)N signatures of adult *C. destructor* in winter resulted in a decline in their trophic position from 3.92 in summer to 1.96 in that season (Table 1). This decline in trophic position appeared to be due to a decrease in the consumption of *G. holbrooki* and a marked increase in the consumption of *Melaleuca* sp. by adult *C. destructor* in winter, suggesting a shift towards a herbivorous role. The propensity of *C. destructor* to burrow to escape unfavourable conditions may result in it burrowing to escape the high flows pulses experienced during winter in the Hutt River, thereby reducing its ability to forage for animal food sources.
6.4.2 Trophic and functional roles of freshwater crayfishes

Molony et al. (unpublished data) considered that *C. cainii* had an omnivorous or micro-predatory role in aquatic systems within its natural range, and that invertebrates associated with detritus or aquatic vegetation were likely to be of considerable importance for growth. In contrast, O’Brien and Davies (2002) used stable isotope analysis to propose that C3 carbon derived from riparian vegetation (or at least the biofilm that it supported) represented a large component of assimilated carbon in stream-dwelling *C. tenuimanus* (i.e. *C. cainii*). The results of the current study suggested a primarily predatory role for both species of crayfish in summer, when small species of fish appeared to be of great importance, but that only *C. cainii* maintained this role in winter (Figs 6.3 and 6.4). Although not generally thought to be consumers of relatively active prey, Rubin and Svenson (1993) showed that freshwater crayfishes have the ability to consume juvenile fish, which led to Nyström et al. (1999) noting that they could be considered as actively foraging predators.

As is the case for many of the aquatic systems in south-western Australia (Morgan et al. 1998), the Hutt River contains very high numbers of *G. holbrooki* during summer. During this period, high water temperatures and low flow rates provided ideal conditions for the successful breeding of this introduced teleost, which results in massive increases in the population size (see Pen and Potter 1991) (Fig. 3.2). Predation on *G. holbrooki* by *C. cainii* and *C. destructor* during summer would be further facilitated by the marked reduction in stream size due to evaporation rates far exceeding rainfall in this arid region and also by the reduced activity of *G. holbrooki* during the evening, the period of peak freshwater crayfish foraging, increasing both the likelihood of encounters between predator and prey and also the ease of capture.

Studies of the diets and ecological roles of freshwater crayfishes that have directly quantified gut contents have generally reported that although the major proportion (volumetrically) of the diet was allochthonous detritus, a wide range of food items were
consumed, (O’Brien 1995; Whitledge and Rabeni 1996; Parkyn et al. 2001). As a consequence of such studies, freshwater crayfishes have generally been classified as functional omnivores that convert a range of plant and detrital materials into animal tissue (e.g. O’Brien 1995; Whitledge and Rabeni 1996; Parkyn et al. 2001).

In contrast to gut content descriptions, stable isotope analyses suggest that freshwater crayfishes occupy a rather different trophic position. For example, in the case of the endemic New Zealand species *Paranephrops zealandicus*, volumetrically, detritus was generally the most important food item, with invertebrates generally contributing < 4% of the gut content (Hollows et al. 2002). However, stable isotope analysis of this species suggested that the invertebrate prey was the most important food source in terms of contributing to crayfish biomass and assimilation (i.e. growth) (Parkyn et al. 2001; Hollows et al. 2002). Hollows et al. (2002) further proposed that, rather than being a directly assimilated food source, allochthonous material may be more important to the diets of stream populations of *P. zealandicus* as a substrate for microbial growth. This species therefore assumed both a predatory trophic role and an omnivorous functional role and contributes to the processing of detritus (Parkyn et al. 2001).

The current study is consistent with the findings of Parkyn et al. (2001) and Hollows et al. (2002), clearly demonstrating that although *C. cainii* and *C. destructor* were apparently primarily top order predators in the Hutt River, detritus and terrestrial vegetation (particularly for *C. destructor* in winter) were also assimilated, and would therefore likely be volumetrically important food items. Therefore, as shown for *P. zealandicus* (Parkyn et al. 2001; Hollows et al. 2002), it is likely that these species have functional roles as omnivores in the Hutt River. As trophic predators and functional omnivores, the large sizes and relatively high densities of *C. cainii* and *C. destructor* suggest that they are keystone species in this system; structuring the food web and being important in terms of the cycling of nutrients. It is also likely that, as has been shown elsewhere for other introductions of non-
native species of freshwater crayfishes (see for example Lodge et al. 1994; Nyström et al. 1999), the introduction of *C. cainii* and *C. destructor* into the Hutt River, a river that did not previously contain any crayfishes, would have considerably altered the pre-existing aquatic food web. Given the predatory *G. holbrooki* has also been introduced into the system, it would undoubtedly have also contributed to the alteration of the ecosystem. The difficulty in obtaining adequate amounts of potential aquatic invertebrate prey for isotopic analysis may also suggest that the introduction of these crayfishes (and also the carnivorous *G. holbrooki*) have had a negative impact on the smaller invertebrate fauna of the river. Such as a decline in benthic invertebrate insect larvae.

### 6.4.3 Conclusions

This study is the first to examine the diet and trophic position of sympatric populations of two species of freshwater crayfish in Australia. Furthermore, it is the first to use stable isotope analysis to determine the assimilated diet of wild populations of two translocated crayfish species. The study employed the use of an isotopic mixing model *IsoSource* that allowed the examination of a wide range of potential food sources in determining the diet of *C. cainii* and *C. destructor*.

In summer, when *G. holbrooki* densities were highest, isotope analyses suggested that fish provided the major source of the assimilated nitrogen and carbon for both species, i.e. they occupy a primarily predatory trophic role. In winter, when the densities of fish are low, *C. cainii* maintains this predatory role, whereas *C. destructor* assumes a more omnivorous role. The general lack of large predatory fishes in the freshwaters of south-western Australia may have resulted in *C. cainii* assuming the role as the top-order aquatic predator in these systems. Detritus and/or terrestrial vegetation were assimilated to some degree by both species in both seasons; likely being a volumetrically important component of their diets. Thus, *C. cainii* fulfils the roles of a trophic predator and a functional omnivore in both
seasons, whilst *C. destructor* is a trophic predator and a functional omnivore in summer it becomes a trophic, as well as a functional, omnivore in winter. This duality of trophic and functional roles means that these translocated populations of *C. cainii* and *C. destructor* are likely to have played an important part in cycling nutrients and structuring the aquatic food web since they were introduced to the Hutt River, i.e. they are keystone species in this system.

As *C. destructor* has the ability to switch trophic positions, when an otherwise abundant, high protein food sources (i.e. fish) becomes limited (as was the case in winter in the Hutt River), it was able to co-exist with *C. cainii*. Furthermore, the ability of *C. destructor* to switch from a diet of fish in summer to a predominantly herbivorous/detrital diet in winter suggests that it may compete for food resources with the other smaller native freshwater crayfishes (such as *C. quinquecarinatus*) in the small unproductive lotic and lentic systems common to south-western Australia, which often lack fish during summer (Bunn and Davies 1990; Pen et al. 1993; Morgan et al. 2000; Gill and Morgan 2003).

It is likely that the predatory role and subsequent assimilation of protein-rich food has resulted in the relatively fast growth rate of *C. destructor* in the Hutt River (Chapter 5). Should this also occur within the natural range of endemic crayfishes further south, its growth rate is likely to exceed that of the smaller endemic crayfishes if they do not also have the capacity to assume such a top-order predatory role. The resultant disparity in growth rates would then be likely to provide a size advantage when competing for resources (i.e. food and space) with the smaller endemic species particularly in the smaller waterbodies that drastically reduce in size during summer. Furthermore, the introduction of this large, omnivorous crayfish species into other aquatic systems in this region may have other unforeseen ecological impacts such as the introduction of disease (see Chapter 7) and altering benthic communities and detrital-based food webs.
Chapter 7

First evidence of microsporidian infection of sympatric wild populations of *Cherax cainii* and *Cherax destructor* in Western Australia

7.1 Introduction

Microsporidians are unicellular, obligate, intracellular, parasites that may infect muscle tissue of decapods, including freshwater crayfishes. Nine *Thelohania* species (Microspora, Thelohaniidae) are known to infect crustaceans (Sprague 1950; Knell et al. 1977; Lom et al. 2001; Moodie et al. 2003a). The streaky appearance of the muscle tissue of the host has resulted in the disease been known as ‘porcelain’ disease (Plate 7.1; Evans and Edgerton 2002). Infected crayfishes may also show signs of reduced locomotor activity, such as a slow tail flicking response, and mortality may result from progressive destruction of muscle tissue (Henneguy and Thélohan 1892; Cossins and Bowler 1974; Quilter 1976).
Thelohania contejeani Henneguy, 1892 is the most common and widespread of the Thelohania species that are known to infect freshwater crayfishes (Cossins and Bowler 1974; Alderman and Polglase 1988; Moodie et al. 2003a, 2003b) and was originally described from infected Astacus fluviatilis (Linnaeus, 1758) (Henneguy and Thélohan 1892). Thelohania contejeani has since been reported in a number of other freshwater crayfishes found in Europe, i.e. Austropotamobius pallipes (e.g. Maurand and Vey 1973; Cossins and Bowler 1974; O’Keefe and Reynolds 1983), Astacus astacus (e.g. Burba and Bucinskiene 1998), Astacus leptodactylus (Eschscholtz, 1823) (Krucinska and Simon 1968), Cambarus affinis (= Orconectes limosus) (Krucinska and Simon 1968), and also in New Zealand in Paranephrops zealandicus (Quilter 1976) and Paranephrops planifrons (Jones 1980). Thelohania cambari Sprague, 1950, another Thelohania species known to infect freshwater crayfishes, was described from infected Cambarus bartonii (Fabricius, 1798) in North America (Sprague 1950).

The lifecycle and structural characteristics of Thelohania species are considered to be similar to other microsporidians (Henneguy and Thélohan 1892; Vey and Vago 1973; Cossins and Bowler 1974; Quilter 1976), however, there is still conjecture pertaining to some aspects of lifecycle and taxonomy (Evans and Edgerton 2002; Moodie et al. 2003a). The transmission of the spores of microsporidians that infect freshwater crayfishes is generally thought to occur by consumption of infected tissue (Cossins and Bowler 1974; Evans and Edgerton 2002; Moodie et al. 2003a) as no evidence of a secondary host has been found (Alderman and Polglase 1988). Spores have been found developing in the ovary of hosts which led to the theory of trans-ovarian (vertical) transmission (Voronin 1971), however, other workers have not been able to show this (Vey and Vago 1972; Moodie et al. 2003a). Thelohania spores are released from muscle cells upon death of the host either as individual spores or within the sporophorous vesicles, the form of which is diagnostic of the Thelohaniidae (Quilter 1974; Moodie et al. 2003a).
Until recently, reports of *Thelohania* in Australian freshwater crayfishes have been from an undescribed species that was found infecting *Cherax destructor* (Carstairs 1979; Mills 1983; Jones and Lawrence 2001) and *Cherax quadricarinatus* (Herbert 1987, 1988). Lom *et al.* (2001) first described the dimorphic sporogony pathways of *T. contejeani* and also its ribosomal DNA (rDNA) sequence. Subsequently, Moodie *et al.* (2003a) described the new species *Thelohania parastaci* from *C. destructor* and found the parasite to have a widespread geographical distribution that included Western Australian farm dams. Furthermore, Moodie *et al.* (2003b) described *Thelohania montirivulorum* Moodie, 2002 infecting a highland population of *C. destructor* in eastern Australia.

Although the recent identification of *T. parastaci* as a widespread Australian species represents a considerable advancement in knowledge, there remains a dearth of information on the distribution of microsporidians infecting freshwater crayfishes in Australia, as is the case with the distribution of other freshwater crayfish diseases (Edgerton 1999). Identification of microsporidian genera has traditionally been achieved by viewing smears of infected tissue under a compound microscope and measuring spore size and number within sporophorous vesicles. Species identification has been more difficult and has been responsible for often erroneous data on distribution of these parasites (Evans and Edgerton 2002).

Moodie *et al.* (2003a) noted that it is possible that the host range of *T. parastaci* includes species other than *C. destructor* and that there was a need to further investigate the biogeography and transmission pathways of the species and develop prevention measures in cultured populations. *Cherax destructor* aquaculture dams in Western Australia were found to be free from *Thelohania* sp. infection in 1990 (Langdon unpublished data), however, the percentage of sites (that were suspected to be infected based on aquaculture processors advice) positive for the disease in a 1999 survey was ca 30% (Jones and Lawrence 2001). The absence of *T. parastaci* in Western Australia prior to 1990 has been attributed to the
original translocation of a singular batch of *C. destructor* from South Australia in 1932 (Jones and Lawrence 2001) and the subsequent introduction of the disease into the State has been blamed on illegal importation of *C. destructor* from eastern Australian States since 1990 (Jones and Lawrence 2001). There are, however, other possibilities such as a lack of historical widespread surveillance (P. Horwitz, personal communication).

The spread of disease and the presence of *T. parastaci* in captive *C. destructor* populations in the south-western region of Western Australia is of great concern given *C. destructor* has recently invaded many aquatic systems, including those in the south-western region that also contain the entirely endemic crayfish fauna of this State (Austin 1985; Jasinska *et al.* 1993; Horwitz 1995; Chapter 5). *Thelohania* sp. (presumably *T. parastaci*) has been recorded in wild populations of *C. destructor* in Victoria and New South Wales but is as yet unknown from wild aquatic systems in Western Australian (Carstairs 1979; Mills 1983; Moodie *et al.* 2003a). Of further concern is the potential for *T. parastaci* to infect the recreationally and commercially important *Cherax cainii*. Furthermore, *Vavraia parastacida* is another microsporidian species that is of similar appearance to *Thelohania* spp. and is known to infect *C. cainii* and *Cherax quinquecarinatus* in this region (Langdon 1991a, 1991b). This presumably naturally occurring microsporidian species has not resulted in any known wide-scale deaths of *C. cainii* of *C. quinquecarinatus* populations, however, its existence highlights the fact that these two native crayfish species have the potential to be infected by microsporidians, including possibly *T. parastaci*.

*Cherax destructor* in the Hutt River (Chapter 4) appeared to show signs of microsporidian infection, i.e. white streaking appearance of the muscle tissue on the underside of the abdomen. This study aimed to test the hypotheses that this infection in *C. destructor* is *T. parastaci* and that *C. destructor* has acted as a vector for transmission of the disease to *C. cainii*. 
7.2 Materials and methods

7.2.1 Study site

The Hutt River (Figs 1.1 and 5.1, Plates 3.1 and 3.2) represents the northernmost range of *C. cainii*, which was translocated from its natural range in the Southwest Coast Drainage Division into this river in 1972 and also supports a sympatric population of the introduced *C. destructor* (introduced to the Hutt River in 1977) (Riek 1967; Morrissy 1978; Morrissy and Cassells 1992; Jasinska *et al.* 1993; Chapters 3 and 5).

7.2.2 Sampling regime

A total of 98 *C. cainii* and 70 *C. destructor* were captured in April, 2001 as part of the study examining the population and reproductive biology of these sympatric populations in the Hutt River (see Chapters 3 and 5). Animals were captured using a 5 m seine net that had a 3 mm woven mesh width and fished to a depth of 1.5 m. Twenty five *C. cainii* (10 females, 15 males) and 27 *C. destructor* (17 females, 10 males) that appeared to show signs of microsporidian infection (i.e. white streaky muscle tissue on the underside of the abdomen and reduced tail-flicking response (Henneguy and Thélohan 1892; Cossins and Bowler 1974; Quilter 1976)) were selected for analysis. Animals were individually bagged and placed immediately in an ice slurry.

7.2.3 Laboratory techniques

Spore concentration technique

Although the latter stages of microsporidian infection are typified by the white, streaky appearance of muscle tissue (Plate 7.1) (O’Keefe and Reynolds 1983; Evans and Edgerton 2002), early stages of infection lack this characteristic and therefore simple macroscopic visual inspection of the individuals may be inadequate for comprehensive screening for infection (Herbert 1987; Evans and Edgerton 2002). Therefore, a spore concentration protocol was used in order to test for microsporidiosis (Langdon 1991a). All individual crayfish were measured to the nearest 1 mm and a 0.5 g sample of muscle tissue was removed from the cephalothorax and tail of each animal (approximately 0.25 g from each region) and homogenised with 4.5 ml of distilled water using a glass on teflon homogeniser. The homogenate was added to a 10 ml centrifuge tube containing 5 ml of a 1% HCl 2% pepsin solution (desiccated pepsin at a concentration 1:10 000) and mixed well. The tube was then placed in a 37°C water-bath for 1 h and centrifuged for 10 minutes at 4000 rpm. The supernatant was then poured off and 50 μl of pellet was transferred onto a glass slide and cover-slip added. Slides were examined for the presence of microsporidian spores under a light microscope at 400x magnification with the aid of a strongly positive *T. parastaci* reference slide supplied by the Fish Health Section of the Department of Fisheries, Government of Western Australia. A chi-squared test ($\chi^2$) was used to test whether the rate of microsporidian
infection differed between *C. cainii* and *C. destructor* and also to determine whether infection rate differed between sexes of each species.

The length and width of spores from positive *C. cainii* and *C. destructor* were measured under phase contrast Normaski microscope at 1000x magnification using a micrometer eyepiece (mean ±1 s.e. is expressed throughout). A Levene’s test for homogeneity of variance was performed on mean diameters of the microsporidian spores in *C. cainii* and *C. destructor* prior to an ANOVA being performed to test whether there was a significant difference in mean spore diameter between the species.

Photographs of spores were also taken from both *C. cainii* and *C. destructor* slides under phase contrast Normaski microscope at 1000x magnification and scanning electron microscope (SEM) photographs were taken at 3600x magnification.

**Genetic technique**

To determine whether the *Thelohania* species present in *C. destructor* and *C. cainii* in the Hutt River was *T. parastaci* a further random sub-sample of flesh from 14 *C. destructor* and three positive *C. cainii* (as determined by the spore concentration technique described above) were tested using the PCR protocol described in Moodie *et al.* (2003a) by the Fish Health Section at the Department of Fisheries, Government of Western Australia.

### 7.3 Results

**7.3.1 Spore concentration technique**

**Infection rates**

The 10 female and 15 male *C. cainii* that appeared to show macroscopic signs of microsporidian disease had a size range of between 18–85 mm OCL with a mean of 50.5 mm OCL (±0.64). Two of these males and three females (of a total of 98 *C. cainii* captured) were subsequently found to be infected using the spore concentration technique (the accuracy of the macroscopic diagnosis of microsporidian infection rate was 20.0%) representing an overall infection rate of 5.1%. There was no significant difference in the rate of infection of female and male *C. cainii* ($\chi^2_{[1]}$, P = 0.256).
Of the 17 female and 10 male C. destructor that showed macroscopic signs of infection (overall size range of 22–39 mm OCL with a mean of 29.7 mm OCL (±0.18)), eight females and three males (of a total of 70 C. destructor captured) were subsequently found to be infected (a macroscopic diagnosis accuracy of 40.7%) representing an overall infection rate of 15.7%. There was no significant difference in the rate of infection of female and male C. destructor ($\chi^2_{[1]}$, P = 0.256). The overall rates of microsporidian infection of C. cainii was significantly lower than for C. destructor ($\chi^2_{[1]}$, P = 0.011).

Microsporidian spore abundances on positive slides ranged from six, for a weakly positive C. cainii sample to 200, for a strongly positive C. destructor. It appeared that, based on the density of spores on positive slide preparations, the strength of infection of C. cainii samples was less than that of the C. destructor samples.

**Spore descriptions**

When viewed under phase contrast Normaski microscope (1000x) (Plate 7.2) and SEM (3600x) (Plate 7.3), the external morphology of spores found in both C. cainii and C. destructor samples were generally pyroid to oval in shape, smooth walled and a refractive vacuole was visible at one pole.

Microsporidian spores in C. cainii muscle tissue had a mean length and width of 4.44 (±0.096) and 2.95 μm (±0.074), respectively, and an overall mean diameter of 3.70 μm (±0.07) (Table 7.1, Plates 7.2a, b). The size range of spores found in C. cainii was 3.22–5.64 μm and 2.42–4.03 μm in length and width, respectively (Table 7.1, Plates 7.2a, b).

The mean length and width of the microsporidian spores in C. destructor muscle tissue were 3.98 (±0.005) and 2.78 μm (±0.005), respectively, and an overall mean diameter of (3.37 μm ±0.004) (Table 7.1, Plates 7.2c, d, 7.3). The length and width of spores in C. destructor ranged from 2.42–4.84 and 2.02–3.63 μm, respectively (Table 7.1, Plates 7.2c, d, 7.3).
Levene’s test for homogeneity of the variances of the mean diameters of microsporidian spores in *C. cainii* (3.70 μm ±0.07) and *C. destructor* (3.37 μm ±0.004) determined that they were not significantly different (*P* = 0.51) and an ANOVA of the un-transformed data revealed that the mean spore diameters were significantly different between the species (*F* (1, 145) = 14.69, *P* ≤ 0.01).

7.3.2 Genetic testing

The PCR testing for the presence of *T. parastaci* in both *C. destructor* and *C. cainii* using the protocol of Moodie *et al.* (2003a) was inconclusive.

7.4 Discussion

7.4.1 What species of microsporidians are infecting *Cherax cainii* and *Cherax destructor* in the Hutt River?

The spore size of *T. contejeani* (the most common and widespread species of *Thelohania*) has been reported as being variable, therefore it is possible that the spore size of the recently described *T. parastaci* is also variable (see Table 7.1, cf Jones 2001). However, the spore size and morphology of the microsporidian found in *C. destructor* in the Hutt River is consistent with that reported for *T. parastaci*, which has infected *C. destructor* in farm dams of this region (Moodie *et al.* 2003a). It is therefore proposed that the microsporidian species recorded from *C. destructor* in the Hutt River was *T. parastaci*.

The mean size of the spores found in *C. cainii* in this study were significantly different to those found in *C. destructor*, they were also smaller than the size range previously reported for *Vavraia parastacida*, a microsporidian species that is of similar appearance to *Thelohania* spp. and is known to infect *C. cainii* and *Cherax quinquecarinatus* in this region (Langdon 1991a, 1991b). The mean length +1 s.d. of spores in *C. cainii* in the current study do not overlap with the mean -1 s.d. of *V. parastacida* (mean length of 5.49
±0.36 s.d.) as described by Langdon (1991b). However, the length range of the spores in *C. cainii* here do overlap with that recorded by Langdon (1991a) with the longest spore in this study being 5.64μm cf the 5.00μm shortest spore recorded by Langdon (1991a).

It is therefore possible that the microsporidian in *C. cainii* in the Hutt River was either *T. parastaci* sp. or *V. parastacida* given:

- The larger spore sizes recorded in *C. cainii cf C. destructor*;
- The previous known infection both by *V. parastacida* (Langdon 1991a, 1991b) and possibly *Thelohania* sp. of *C. cainii* (personal communication, Brian Jones, Department of Fisheries, Government of Western Australia; Pearce 1990);
- The considerable range in spore sizes previously recorded for *Thelohania* sp. depending upon the treatment of spores (Table 7.1, Jones 1980).

Although the study cannot provide 100% certainty, I believe that the microsporidian infecting *C. cainii* was most likely *V. parastacida* (see below); confirmation requires the development of an additional genetic test for this species. There exist a number of possibilities to explain the lack of conclusive results from the genetic testing for *T. parastaci* in the Hutt River (Moodie *et al.* 2003a). Firstly, it is possible that the DNA used was not of adequate quality and thus the PCR failed to amplify the rDNA. However, the musculature tissue used was frozen promptly following capture and it was therefore unlikely that the quality of the genetic material was insufficient. Secondly, it was possible that the random sample of 15 *C. destructor* tested using PCR may have all been negative for the disease, certainly a possibility given the 15.7% infection rate of *C. destructor* ascertained using the spore concentration technique. The third possibility was that the microsporidian infecting either *C. destructor* or *C. cainii* was not *T. parastaci*. This is more probable in the case of *C. cainii* given that the mean spore diameter in this species was significantly greater than those measured in *C. destructor* (that closely approximated those previously described for *T. parastaci*). The spores in *C. cainii* more closely approximated those sizes reported for *V. parastacida*.
(Langdon 1991b). Testing of a large sample of *C. destructor* is required in the Hutt River using the PCR protocol for *T. parastaci* (Moodie *et al.* 2003a) to conclusively identify this species as *T. parastaci* (re-testing using the genetic technique of Moodie *et al.* 2003a). Furthermore, in order to confirm the infection of *C. cainii* by *V. parastacida* in the Hutt River genetic tests need to be developed to positively identify *V. parastacida*.

7.4.2 Microsporidian infection rates

The overall microsporidian infection rates recorded in this study were 5.1 and 15.7% for *C. cainii* and *C. destructor*, respectively (Table 7.1). Determination of infection rates by *T. contejeani* using either macroscopic or microscopic observations has previously been shown to be similar and therefore gross observations alone have often been used (O’Keefe and Reynolds 1983). The accuracy of macroscopic diagnosis of infection in the present study was 20.0 and 40.7% for *C. cainii* and *C. destructor*, respectively. Therefore, macroscopic diagnosis in the current study overestimated the infection rates in both *C. cainii* and *C. destructor*. The overestimation of infection rate using gross observations here were due to initially classifying as positive individuals that displayed only minor symptoms such as apparent reduced locomotor activity, in order to ensure that the true infection rate was not underestimated by the spore concentration technique. The spore concentration technique used here has successfully been applied previously in testing for microsporidiosis (e.g. Langdon 1991a, 1991b; Jones and Lawrence 2001) and also as part of the PCR protocol developed by Moodie *et al.* (2003a) to purify spores. In addition to using the latter technique to determine *T. parastaci* infection of *C. destructor* in Western Australian (Moodie *et al.* 2003a), the spore concentration technique is used to diagnose these infections by the Fish Health Section of the Department of Fisheries, Government of Western Australia.

Infection rates of freshwater crayfishes by *Thelohania* have been shown to vary considerably with host species and between populations (e.g. 1.8% in a population of
Paranephrops planifrons in New Zealand, Jones (1980); 7.8% in a population of Cherax quadricarinatus, Herbert (1987); up to 20% in a population of Cherax destructor in Australia (Carstairs 1979); and 30% in a population of Austropotamobius pallipes in Germany, Schäperclaus (1954)). A number of reasons for the variation in infection rate have been postulated including: cannibalism, variations in water flow, population density and sub-lethal stress (Cossins and Bowler 1974; Carstairs 1979; France and Graham, 1985). However, there has been continuing conjecture regarding a definitive factor that influences infection rate (Alderman and Polglase 1988; Moodie et al. 2003a).

Although there remains conjecture as to the mode of transmission of species of Thelohania, including that of T. parastaci (Moodie et al. 2003a), most research attributes transmission to the consumption of infected flesh (e.g. Cossins and Bowler 1974; Evans and Edgerton 2002). It is likely that density, and the associated level of individual interactions, (in particular cannibalism) is a major factor influencing the infection rates of crayfish populations. Semi-intensive freshwater crayfish aquaculture farms generally have high densities with associated increases in intraspecific interactions (and increased stress levels) such as competition and predation, which may result in high rates of population infection of diseases such as Thelohania sp. (Alderman and Polglase 1988; Horwitz 1990). The high level of infection in farm dam populations of C. destructor in the south-western region of Western Australia (personal communication, Brian Jones, Department of Fisheries, Government of Western Australia), along with the apparent rapid spread of the disease in this State in less than a 10 year period (the disease was absent in 1990 and subsequently ca 30% of suspect sites tested positive for the disease in 1999, Jones and Lawrence (2001)), is consistent with this theory.

Densities of C. cainii and C. destructor were similar in the Hutt River, however, the estimate of the rate of natural mortality of the latter species was greater (see Chapters 3 and 5). This may be due, in part, to a greater mortality rate of C. destructor due to T. parastaci
infection compared to that resulting from the microsporidian (probably *V. parastacida*) infection of *C. cainii*. The greater natural mortality of *C. destructor* may itself facilitate a higher intraspecific transmission rate of the disease (via consumption of dead, infected individuals) thus further contributing to this relatively high mortality rate.

7.4.3 The introduction and spread of *Thelohania parastaci* in Western Australia

The timing of the initial introduction of *T. parastaci* into the Hutt River is unknown. *Cherax destructor* was believed to have been introduced into the Hutt River in 1977 (Morrissy and Cassells 1992). Given the fact that *T. parastaci* presumably arrived into Western Australia from illegal importations of *C. destructor* from the eastern states of Australia in the nine years prior to 1999 (Jones and Lawrence 2001) two scenarios exist that would explain the introduction of *T. parastaci* into *C. destructor* and possibly *C. cainii* in the Hutt River: firstly *Cherax destructor* in the Hutt River may have been already infected with *T. parastaci* either upon introduction in 1977 or from subsequent introductions up until ca 1990 (that would mean the disease was present in this State earlier than reported in Jones and Lawrence 2001); or subsequent introductions of *C. destructor* have occurred after ca 1990 when the disease prevalence apparently increased dramatically in Western Australia (Jones and Lawrence 2001). Both of these theories are plausible; however, the latter may be the more likely given the complete lack of detection of the disease in Western Australia during a comprehensive farm dam survey in 1990 (Jones and Lawrence 2001). Moodie *et al.* (2003a) proposed that the high similarities in the rDNA of *T. parastaci* from *C. d. rotundas* and *C. d. albidus* (*C. destructor sensu* Austin *et al.* 2003) was due to continued mixing of *T. parastaci* populations due to migratory birds (in their faeces) or translocation by humans. Therefore, either of these vectors may have also been responsible for the introduction of the disease into the Hutt River post 1990.
The risks associated with introduction of *C. destructor* include direct competition with other native freshwater crayfish species, food-web perturbations, habitat alterations (particularly due to the effects of burrowing), co-introduction of other organisms, and spread of disease (Austin 1985; Horwitz 1990, 1995; Jasinska *et al.* 1993; Chapters 5 and 6). The results of this study suggest that the latter risk has now become even greater in this State; particularly in light of the fact that the disease is thought to have only relatively recently been introduced to this State and the infection of *C. destructor* by *T. parastaci* in the Hutt River. The likelihood of the microsporidian disease in *C. cainii* in the Hutt River being *V. parastacida* does not lessen the concern of subsequent infection of this species by *T. parastaci* because, as mentioned, it is likely that the disease in only a recent introduction into the Hutt River and *C. cainii* itself was only introduced in 1972 (Morrissy and Cassells 1992).

7.4.4 Potential impacts of *Thelohania parastaci*

The implications of potential infection of the endemic freshwater crayfish fauna of south-western Australia by *T. parastaci* in Western Australia are enormous. The general symptoms of *Thelohania* are brought about, as mentioned, by destruction of striated and cardiac muscle tissue. Reports of survival time of infected individuals vary and may be species and system dependant with reports ranging from a few months (*P. zealandicus* in Quilter 1976) to two years (*A. pallipes* in Brown and Bowler 1977). Moodie *et al.* (2003a) cautioned against assuming that death from infection by *T. parastaci* was a *fait accompli* before transmission experiments are carried out. However, aside from the potential for direct mortality in native freshwater crayfish populations, the reduced locomotor activity of individuals may also have indirect effects on infected individuals. In particular, there may be a reduced ability to avoid predators due to reduced tail-flicking response (see Quilter 1976). Predation by the introduced teleost redfin perch on *C. cainii* and other decapods has been reported in south-
western Western Australia (e.g. Beatty 2000; Morgan et al. 2002) and its impact may be exacerbated should *T. parastaci* infection of endemic crayfish populations occur.

Infected crayfish are not marketable (Alderman and Polglase 1988) and this has had a considerable effect on the farming of *C. destructor* in Western Australia. Aside from supporting a very important inland recreational fishery (*ca* 15 000 licences in the 2002 season), *C. cainii* also forms the basis of a semi-intensive aquaculture industry in south-western Western Australia. There is a serious threat to the industry if infection by *T. parastaci* of *C. cainii* occurs and subsequently undergoes a similar proliferation as experienced by farm dam *C. destructor* populations between 1990 and 1999. There is no treatment available for microsporidiosis aside from monitoring stock and removing infected animals (Schäperclaus 1954; Alderman and Polglase 1988).

### 7.4.5 Conclusions

This study has described for the first time, the possible presence of *T. parastaci* in a wild population of *C. destructor* in Western Australia and the presence of a microsporidian that was probably *V. parastacida* in a sympatric population of *C. cainii*. Due to the inconclusive results of the newly established genetic testing technique, further testing of *C. cainii* and *C. destructor* in the Hutt River is required to conclusively determine whether the microsporidian in *C. destructor* is *T. parastaci*. There is also need for testing for microsporidiosis in both the recently established wild populations of *C. destructor* in Western Australia (documented in Chapter 5) and of sympatric endemic crayfishes, particularly *C. cainii*, *C. quinquecarinatus* and *Cherax preissii* in those systems. Controlled experiments are also required to determine whether infection of the endemic crayfishes in south-western Australia by *T. parastaci* is possible. Following the assessment of the disease status of those endemic populations that co-occur with *C. destructor*, it will then be possible to determine whether control measures are required, such as the quarantining of certain infected populations.
Chapter 8

Summary and General Conclusions

Based on the considerable richness of the endemic biota and the degree of threats to it, south-western Western Australia is recognised as one of only 25 biodiversity hotspots in the world (Myers et al. 2000). Although the teleost and freshwater crayfish faunas are relatively depauperate representing between 5 and 7% of Australia’s total species, respectively (cf 40% of Australia’s vascular plants being found in a region that occupies only 5% of the continent’s area, with 75% of those endemic species), the rate of endemism of the freshwater teleost and crayfish fauna actually exceeds that of vascular plants (80% and 100% for freshwater teleosts and crayfishes, respectively) (Hopper 1992; Austin and Knott 1996; Morgan et al. 1998; Crandall et al. 1999; Horwitz and Adams 2000; Austin and Ryan 2002). This high degree of endemism has led to the recognition that the crayfishes of the region are of considerable conservation importance (e.g. Whiting et al. 2000).

The aquatic fauna in this region has also come under serious human-induced threats, in particular through habitat change (particularly salinisation of aquatic systems, Morgan et al. 2003, clearing of riparian vegetation and damming of rivers, Molony et al. 2001) and predation by introduced teleosts (Morgan et al. 2002, 2004). Despite these threats, a paucity of research has been conducted on the biology and ecology of wild populations of freshwater crayfishes in Western Australia. The present study has addressed major gaps in the understanding of the biology and ecology of two of the most widespread freshwater crayfish species in the region: *Cherax cainii* and *Cherax quinquecarinatus*. It has also increased the understanding of the biology and ecology of the widely introduced *Cherax destructor*, which potentially poses a serious threat to native freshwater crayfishes and aquatic food webs of this region. These data also allow an assessment of the roles life-history strategies, trophic
relationships and introduction of disease contribute to the threats posed by species introduction.

8.1 Plasticity of the biology of C. cainii

This study employed contemporary techniques to accurately determine the biology of two populations of C. cainii, one from a lentic system and the other from a lotic system, within its current geographical range and has revealed considerable plasticity in the biology of this species between these sites. These results have considerable implications for the management of the declining recreational fishery.

The relatively high growth rates of C. cainii in the Hutt River compared with that previously recorded in the Warren River (Morrissy 1975) was likely due to the warmer temperature regime of the Hutt River (at the northernmost extent of its range). The rapid growth rate probably resulted in the relatively large lengths at first maturity ($L_{50}$ for female = 70.4 mm OCL or 97.9 mm CL) and as the minimum legal length of female C. cainii (in all but one reservoir population, i.e. Harvey Dam) is 76 mm OCL, the current legal minimum size is inadequate to allow at least one spawning event of female C. cainii prior to exploitation. Therefore, the legal size for females should be increased in the Hutt River to 98 mm CL to allow ~50% of the female population to mature prior to exploitation.

As C. cainii is an introduced species in the Hutt River, management efforts to protect this population stem solely from a social and economic basis (i.e. sustaining the recreational fishery). From an ecological perspective, both C. cainii and C. destructor should be viewed as exotic species and would have undoubtedly altered this ecosystem (see section 8.3) since their introduction in the 1970’s. However, the eradication of C. cainii and C. destructor from the Hutt River would be nearly impossible to achieve (as with the vast majority of aquatic introductions) and as the system is relatively small and has been subjected to anthropomorphic alterations such as riparian degradation, it is unlikely that eradication plans
would result in significant ecological benefit. Furthermore, given the social value of these crayfishes as recreationally fished species, such an attempted eradication would be contrary to community wishes; vital in the success of any such attempt.

The results of, and methodologies developed in, this study, are currently being incorporated into the major review of the iconic recreational fishery of this keystone species (Fisheries Research Development Corporation Project No. 2003/027). As part of this review, and in order to further understand the plasticity of this species so that the management of the fishery can take into account this intra-specific variation, the biology of other lentic and lotic populations will be described.

8.2 Comparison of the biology of wild populations of *C. cainii*, *C. destructor* and *C. quinquecarinatus* in Western Australia

*Cherax destructor* and *C. quinquecarinatus* displayed life histories more typical of *r*-strategists, strategies that allow species to rapidly colonise new environments and exploit periodically inundated water bodies (Tables 5.3 and 8.1). For example; both are likely to spawn multiple times throughout their extended breeding periods (August to February for *C. quinquecarinatus* and July to January for *C. destructor*); they mature at either a relatively small size (*C. quinquecarinatus*) or at the end of their first year of life (*C. destructor*) compared with *C. cainii* that spawns at the earliest at the end of its second year of life by which time it has attained a substantial size (Tables 5.3, 8.1). *Cherax cainii* is more typical of a *K*-selected species in terms of these traits, as it: only occupy permanent systems, have a distinct spawning period in which they spawn only once, and as noted mature at a relatively large size (particularly in the Hutt River at 70.4 and 39.6 mm OCL for females and males, respectively); and often attain a very large maximum size over a relatively long life span (Tables 5.3 and 8.1).
However, not all of the observed biological traits were consistent with either $r$- or K-strategies that are associated with summer or winter brooding freshwater crayfishes, respectively (Honan and Mitchell 1995a). For example, although $C. cainii$ grew to a large maximum size more typical of a winter brooding crayfish species, it had a spring/summer brooding period, had a relatively rapid growth rate and was relatively fecund, traits more typical of summer brooding crayfishes (Honan and Mitchell 1995a). Furthermore, although $C. quinquecarinatus$ and $C. destructor$ displayed many summer brooding characteristics, $C. destructor$ grew to a relatively large size and $C. quinquecarinatus$ had a relatively low batch fecundity and slow growth rate and was estimated to have a long life span (more commonly associated with winter brooding species) (Tables 5.3 and 8.1).

The rapid growth rate of $C. cainii$ is likely reflective of its position as an opportunistic omnivore and the general lack of competitors for larger, energy rich food stuffs, e.g. fish and macroinvertebrates. Although the batch fecundity of $C. quinquecarinatus$ is relatively low, the moderately large eggs and ability to undergo several spawnings in a season may result in higher survival rates of juveniles and therefore rapid increase in population densities in temporary environments. This parallels the situation in the small, introduced live-bearer $Gambusia holbrooki$ that undergoes multiple spawnings of low numbers of well developed young and also thrives in a wide range of aquatic habitats.

### 8.3 Trophic positions of translocated populations of $C. cainii$ and $C. destructor$

The determination of the diets of $C. cainii$ and $C. destructor$ in the Hutt River employed multiple stable isotope analyses and a mixing model that allowed many potential food sources of $C. cainii$ and $C. destructor$ to be incorporated in the analyses. These analyses demonstrated that, during summer, both species had primarily predatory roles, and that animal food sources were more important for growth than detritus or plant material. While $C. cainii$ maintained this diet in winter, terrestrial vegetation became the primary food source of $C.$
destructor in that season. Although C. cainii and C. destructor in summer and C. cainii in winter occupied primarily predatory trophic positions, both species also assimilated detritus and vegetation in both seasons. Therefore, and as found in other studies, it is likely that, although fulfilling the roles of trophic predators, both species also act as functional omnivores, consuming multiple levels of the aquatic food web. The rapid growth rate of both C. cainii and C. destructor in the Hutt River is likely to reflect the ingestion of a high protein diet.

As these crayfishes occur at relatively high densities, are the largest aquatic organisms, are top level consumers and also consume items from multiple trophic levels in the Hutt River, they are considered as keystone species that structure the food web and will have influenced the flow of nutrients and energy in that system since their introduction in the 1970s. Although this study suggests that C. destructor is unlikely to out-compete the fast growing C. cainii, its rapid growth rate fuelled by the ingestion of ‘high quality’ food, gives it the potential to out-compete the small endemic freshwater crayfishes, particularly in small aquatic systems of south-western Australia.

8.4 The threat of Cherax destructor to the aquatic fauna and ecosystems of Western Australia

This study has documented the recent spread of C. destructor into the aquatic systems of a number of the catchments of south-western Australia (Chapter 5). The apparent relatively recent establishment of these populations (within the last two decades, since the studies of Austin (1985) and Morrissy and Cassells (1992)) compared with the length of time the species has been in this State (i.e. 1932, Austin (1985)) suggests that the rate of spread has increased. However, aside from the latter two studies, which surveyed only limited wild systems, there has been a paucity of previous research documenting the establishment of C. destructor into Western Australia and thus they may have been present in many of these
systems for a far greater period of time. Indeed, a similar lack of knowledge of the
distribution and impacts of introduced teleosts in south-western Western Australia has only
recently begun to be addressed (Morgan et al. 2002, 2004).

The life-history strategy of *C. destructor* elucidated in the present study, coupled with
its tolerance of relative extremes of environmental conditions, suggests that it is likely that *C.
destructor* could occupy all of the types of waterbodies that house the other, relatively small
endemic congeners, and has the potential to be the most wide-spread freshwater crayfish
species in wild systems in Western Australia. Furthermore, *C. destructor* appeared to
consume multiple trophic levels, thus it is likely that it is having considerable impacts on the
existing structure and function of the aquatic systems in which it is becoming established. As
found for invading freshwater crayfishes elsewhere, decoupling of trophic cascades by such
an invader may result in serious, unpredictable alteration of south-western Australian aquatic
food webs.

8.5 Future research arising from this thesis

- This current study has revealed extensive variability in the biology of *C. cainii* that
  should be recognised when formulating fishery regulations. Therefore, the
  reproductive and population biology of other key stocks of *C. cainii* need to be
described in order for fishery regulations to better protect those populations from
over-exploitation. This will occur as part of an ongoing Fisheries Research
Development Corporation (Australia) project (2003/027).

- Determine the biology of *C. destructor* and sympatric populations of *C.
  quinquecarinatus*, and another ubiquitous endemic species *Cherax preissii*, in both
temporary and permanent aquatic systems in south-western Australia. This research
should involve examination of the biology of these species alone compared with their
biology’s in sympatry. This will allow an evaluation of the potential for *C. destructor* to displace the relatively small (*cf* *C. cainii*) endemic congeners of the region.

- The relative contributions that genetic and environmental factors play in the biological plasticity of the endemic freshwater crayfishes of south-western Western Australia should be determined.

- Conduct controlled feeding trials involving *C. cainii*, *C. quinquecarinatus* and *C. destructor* to determine the level of $^{15}$N trophic enrichment from their diets (it was assumed to be 2‰ in the present study) to allow a more accurate assessment of their trophic positions in future multiple stable isotope studies. These studies will also allow an assessment of the rate of assimilation of food sources, and thereby allow a more accurate determination of temporal changes in their diets and trophic positions.

- Undertake multiple stable isotope analyses to determine and compare the trophic relationships, within both permanent and temporary aquatic ecosystems, with and without established *C. destructor* populations. This research will allow an evaluation of the extent to which the ecological role of *C. destructor* may vary between aquatic habitats, and an assessment of the impact they are having on the structure and function of those systems.

- Conduct *in situ* enclosure/exclosure experiments in areas where *C. destructor* have become established to further determine the impact that this species has on aquatic food webs, particularly invertebrate and fish communities.

- Develop a genetic test for the microsporidian *Vavraia parastacida* in order to conclusively differentiate between the microsporidian species infecting wild populations of native and introduced freshwater crayfishes in Western Australia.

- Undertake comprehensive disease testing (including those for fungi, protozoans and viruses) of wild populations of *C. destructor* and co-existing endemic congeners in Western Australia to determine: whether other freshwater crayfish diseases have been
introduced to this State by *C. destructor*; the extent of the geographical range of *T. parastaci* in wild aquatic systems in Western Australia; whether introduced diseases have infected native crayfish species.

- As *C. destructor* is only recently believed to have become established in many of those wild aquatic systems, transmission of *T. parastaci* from *C. destructor* may not have yet occurred or infection rates may be low in the native species. Therefore, intensive conditions should be used to determine whether future infection of those species is possible. Experiments should therefore be conducted to test whether transmission of *T. parastaci* from infected *C. destructor* to native congeners can occur via consumption of deceased, infected *C. destructor* in intensive conditions.

- The impacts of the artificially stocked, large introduced teleosts *Oncorhynchus mykiss* and *Salmo trutta* on aquatic food webs of south-western Australia urgently needs to be determined. As these introduced species are known to predate on native top consumers elsewhere (including fishes and freshwater crayfishes), they undoubtedly have a considerable impact on those ecosystems, and represent a threat to the endemic aquatic fauna of the region. Stable isotope and gut content analysis should accompany aquatic invertebrate community studies, to compare the structure and function of those food webs in which these species have been introduced with those in which they are absent. The results of this research should be considered in the management of these fisheries.

The plasticity in the biology and ecology of freshwater crayfishes highlighted in the current study demonstrates the need for caution before the introduction of species into areas outside their native range, or indeed the movement of individuals of a species between areas within their natural range. The unpredictability of how a species will ‘behave’ in new environments resulting from this plasticity and the overall complexity of ecosystems is likely to result in
erroneous conclusions being drawn as to the nature and levels of ecological impacts of any introduction. For example, the significant predatory trophic role occupied by *C. cainii* and *C. destructor* in the Hutt River was unexpected and suggested that these species could not only alter benthic invertebrate communities, but considerably alter the structure of fish communities if they were translocated to smaller waterbodies where they do not naturally occur. In the case of south-western Western Australia, many of these smaller waterbodies, in which due its ability to occupy a wide range of temporary aquatic systems *C. destructor* could survive, are home to rare endemic teleosts and crayfishes.

Despite the great deal of historical data documenting the negative and often unforeseen impacts that many of aquatic exotic species have both in ecological and monetary terms, their continued deliberate introduction, both legally or otherwise, is very concerning and somewhat perplexing. Compared to terrestrial exotics, aquatic exotic species tend to be relatively ‘invisible’ and therefore detection is delayed and eradication nearly impossible. It is paramount that adequate research attention is given to exotic aquatic species, particularly into their impacts on receiving ecosystems in order to better understand the structure and function of ecosystems. Of equal importance is ensuring the extension of this research to natural resource managers so that balanced decisions relating to the translocation of aquatic species are able to be made not based almost entirely on their social and economic benefits.

It is very unfortunate that the many lessons learnt from past exotic introductions (e.g. species for aquaculture or biological controls) appear to be ignored by many seeking further introductions for reasons that cannot possibly be based on ecological benefit. Given the often unpredictable impacts that exotic species may have (due to the complexity of the structure and function of ecosystems), the precautionary principle of Ecological Sustainable Development needs to be employed in more cases. This is perhaps best expressed by the title of Ruesink *et al.* (1995) *Reducing the risk of nonindigenous species introductions: Guilty until proven innocent.*
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