

**PARASITES OF NATIVE AND EXOTIC FRESHWATER FISHES
IN THE SOUTH-WEST OF WESTERN AUSTRALIA**

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D.V.M., M.V.Sc.



**This thesis is presented for the degree of Doctor of Philosophy of
Murdoch University, 2008.**

DECLARATION

I declare that this thesis is my own account of my research and contains
as its main content work which has not previously been submitted for a
degree at any tertiary education institution.

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Abstract

Fewer than 200 fish species are found in freshwater habitats in Australia, of which 144 are confined exclusively to freshwater. At least 22 species of exotic freshwater fish have been introduced into Australia, and 19 of these have established self-sustaining populations. However, the parasite fauna of both native and exotic freshwater fishes in Australia is poorly known. This is particularly the case in the south-west of Western Australia, where there have been no previous comprehensive studies of the parasites of 14 native species and nine or more exotic species of fish found in freshwater habitats.

This study represents a survey of the parasites of freshwater fishes in the South West Coast Drainage Division and reports 44 putative species of parasites in 1429 individual fishes of 18 different species (12 native and six exotic) from 29 locations. Parasites were found in 327 (22.88%) fishes, and of the infected fishes, 200 (61.16%) were infected with only one species of parasite and 127 (38.84%) were infected with two or more species of parasites. For helminth and arthropod parasites, which were more comprehensively surveyed than protozoan and myxozoans, I found 37 species compared to 77 species found in a recent study of fishes from the East Coast Drainage Division.

The present study demonstrated that parasitic infection was significantly more common in native fish species (mean prevalence of infection with any species of parasite = 0.36 ± 0.09) than in exotic fish species (0.01 ± 0.12). Parasites were found in all native fish species, but in only two exotic fish species that were examined.

Parasite regional and component community diversity were estimated by species richness (the number of species, S) and by an index of taxonomic diversity (H_T). Both parasite species richness and parasite taxonomic diversity were significantly greater in native fish species (mean $S = 10.5 \pm 2.3$ SE; mean $H_T = 1.19 \pm 0.14$ SE) than in exotic fish species (mean $S = 1.6 \pm 3.3$ SE; mean $H_T = 0.27 \pm 0.20$ SE). These relationships were consistent over all geographic locations that were sampled. The reduced parasite load of exotic species compared to native species has been previously reported across a wide range of taxa. It is thought to arise partly because founding populations of hosts have a low probability of harbouring the species' total parasite fauna, and partly because parasites that infect introduced exotic species may not be able to maintain their life cycle in the new environment. It has been suggested that a reduced parasite load increases the competitive ability of exotic species compared to native species (the parasite release hypothesis) and this may partly explain the abundance and apparent competitive success of exotic over native species of freshwater fish in the South West Coast Drainage Division.

For native species of fish, there were major differences among species in both prevalence of parasitic infection and parasite community diversity, but this variation was not related to fish size, whether the fish were primarily freshwater or primarily estuarine, or whether they were primarily demersal or pelagic.

In this study, I report two new parasites in south western Australian waters. Both are copepod parasites; *Lernaea cyprinacea* and a new species of *Dermoergasilus*. The *Dermoergasilus* appears to be native to the south-west of Western Australia and has been described as *Dermoergasilus westernensis*. It differs from previously described

species in the genus principally by the armature of the legs. This new species was found on the gills of freshwater cobbler, *Tandanus bostocki* and western minnow, *Galaxias occidentalis* in two different river systems.

Lernaea cyprinacea is an introduced parasitic copepod found on the skin and gills of freshwater fishes in many areas of the world. The parasite has not previously been reported in Western Australia. We found infestations of *L. cyprinacea* on four native fish species (*G. occidentalis*; *Edelia vittata*; *Bostockia porosa*; *T. bostocki*) and three introduced fish species (*Carassius auratus*; *Gambusia holbrooki*; *Phalloceros caudimaculatus*) at two localities in the Canning River, in the south-west of Western Australia. The parasite has the potential to have serious pathogenic effects on native fish species, although it appears to be currently localised to a small section of the Canning River.

Over all localities from which fishes were sampled in the present study, the proportion of native freshwater fishes with parasitic infections and the component community diversity of the parasite fauna of native fishes were both negatively related to habitat disturbance, in particular to a suite of factors (river regulation, loss of riparian vegetation, eutrophication and presence of exotic fish species) that indicate increased human usage of the river and surrounding environment. The reduced parasite load and diversity in native fishes from south-west rivers with greater human usage was due principally to the loss of a number of species of trematode, cestode and nematode endoparasites which use fishes as intermediate hosts. Other studies have also found that endoparasites with complex life cycles are most likely to be adversely affected by environmental changes, presumably because

any environmental changes which impact on either free-living parasite stages or on any of the hosts in the complex train of parasite transmission will reduce parasite population size and may cause local extinction of the parasite species.

The most heavily infected species of native freshwater fish in the South West Coast Drainage Division was *T. bostocki* with 96% of all individuals containing at least one species of parasite. As with most freshwater fishes of south-west Australia, *T. bostocki* is limited in its distribution to waterways with relatively low salinity. The degree of parasitism and histopathology of internal and external organs in *T. bostocki* from the Blackwood River was examined over a period of rapid, seasonal changes in water salinity. As salinity increased, the infracommunity richness and prevalence of ectoparasites on the skin of fishes decreased, while the infracommunity richness and prevalence of endoparasites increased. This was associated with a decrease in histopathological lesion scores in the skin and an increase in histopathological lesion scores in internal organs, particularly the intestine. I hypothesise that the seasonal spike in salinity had two contrasting effects on parasitic infections of *T. bostocki*. Firstly, it increased the mortality rate of parasites directly exposed to water, leading to a decrease in ectoparasitic infection and associated pathology. Secondly, it suppressed immune function in fish, leading to a decreased mortality rate of parasites not directly exposed to water and a more severe pathological response to endoparasitism.

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Publications

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CHAPTER 1

General introduction

1.1 Drainage systems in the south-west of Western Australia

The South West Coast Drainage Division (S.W.C.D.D.) includes streams flowing into the Indian Ocean and Southern Ocean between Dongara and Esperance in Western Australia (Figure 1.1). The major rivers in south-western Australia are; Moore, Swan-Avon, Serpentine, Murray, Collie, Margaret, Harvey, Gardner, Bremer, Capel, Blackwood, Donnelly, Warren, Shannon, Frankland, Kent, Kalgan and Pallinup (Figure 1.1). The climate is temperate Mediterranean with warm, dry summers and cool, wet winters (Allen *et al.* 2002). The rivers of the south-west have changed greatly in the 160 years since European settlement, partly because of direct changes to water flow through damming and water extraction, but more often because of the indirect impacts of changes in land use through agriculture, industry, forestry, mining and recreation (Olsen & Skidmore 1991).

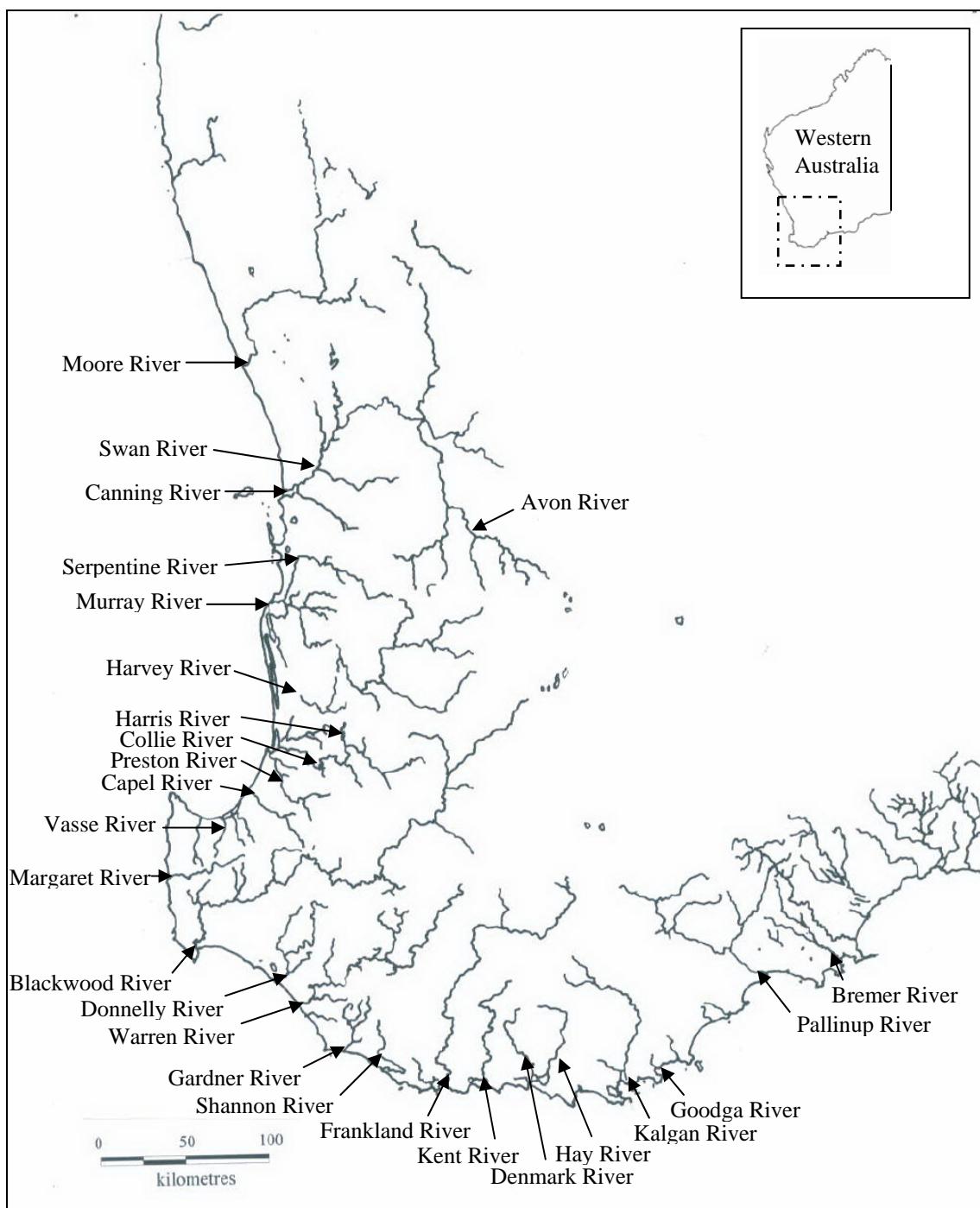


Figure 1.1 The South West Coast Drainage Division of Australia, showing major rivers.

1.2 Native freshwater fishes

Approximately 200 species of native fish from 59 families are found in freshwater habitats in Australia (Allen *et al.* 2002). In the south-west of Western Australia only 14 native species are found. Of these, 10 species are confined exclusively to freshwater and eight species are endemic to the region (Morgan *et al.* 1998). The eight fish species endemic to the S.W.C.D.D. are freshwater cobbler (*Tandanus bostocki*), salamanderfish (*Lepidogalaxias salamandroides*), western minnow (*Galaxias occidentalis*), black-stripe minnow (*Galaxiella nigrostriata*), mud minnow (*Galaxiella munda*), nightfish (*Bostockia porosa*), western pygmy perch (*Edelia vittata*) and Balston's pygmy perch (*Nannatherina balstoni*). The other two freshwater species found in this region are the trout minnow (*Galaxias truttaceus*) and common jollytail (*Galaxias maculatus*) (Morgan *et al.* 1998, 2003). The other fishes which are commonly found in freshwater in the south-west are the anadromous lamprey (*Geotria australis*), and the estuarine western hardyhead (*Leptatherina wallacei*), Swan River goby (*Pseudogobius olorum*) and south-west goby (*Afurcagobius suppositus*) (Morgan *et al.* 1998).

Most Australian freshwater fishes are of recent marine origin and many are dependent on accessing tidal waters during some stage of their life cycle (Russell *et al.* 2003). The diversity of freshwater fishes found in a region depends on factors such as water quality, habitat quality and diversity, and trophic dynamics, all of which may be influenced by anthropogenic processes (Pusey & Arthington 2003). Australian freshwater fish fauna are under pressure from a range of influences including man-made environmental changes (Pollino *et al.* 2004), translocation of

some native fishes (Russell *et al.* 2003) and the introduction of exotic species (Howe *et al.* 1997; Morgan *et al.* 2003; Pollino *et al.* 2004).

1.3 Exotic freshwater fishes

Exotic fishes were first brought to Australia by European settlers in the late 1800s. The fishes were widely released with the intention of providing for recreational angling and an additional food source (Allen *et al.* 2002). Since then, many other fishes have been introduced to Australia from many parts of the world for the purposes of aquaculture, biological control and as aquarium pets (Howe *et al.* 1997; Molony *et al.* 2004). Although many species of introduced fish were unable to adapt to the Australian environment, particularly to the seasonal nature of stream flow (Allen *et al.* 2002), others have been able to establish self-sustaining populations. Many factors have aided the establishment of introduced fishes, such as fish behavior (Molony *et al.* 2004), minimal resource competition (Russell *et al.* 2003), suitable water temperatures (Russell *et al.* 2003; Pusey & Arthington 2003), changes in river flow (Pollino *et al.* 2004), suitable habitat for spawning (Pollino *et al.* 2004) and abundant food supply (Morgan *et al.* 2002; Pusey & Arthington 2003).

A total of 22 exotic fishes are currently known from Australian fresh waters (Allen *et al.* 2002). In south-western Australia, the major introduced fish species are rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), eastern mosquitofish (*Gambusia holbrooki*), redfin perch (*Perca fluviatilis*), koi carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), one-spot livebearer (*Phalloceros caudimaculatus*) and swordtail (*Xiphophorus helleri*) (Allen *et al.* 2002; Morgan *et al.* 2004).

Rainbow trout and brown trout were introduced into Western Australia in the early 1900s. Rainbow trout are native to the Pacific coast of North America, from Alaska to Mexico, while brown trout are native to Europe, from Iceland and Scandinavia southward to Spain and North Africa and eastward to the Black and Caspian Seas (Cadwallader 1996). Both species were introduced for recreational fishing purposes as in south-western Australia there are no native freshwater fishes of interest for anglers except freshwater cobbler (Molony 2001).

Redfin perch, native to western and northern Europe and across Siberia, were also released as a sport fish into many of the river systems of S.W.C.D.D. (Morgan *et al.* 2002). Although some self-sustaining populations have established (e.g. Tay *et al.* 2007), the distribution of the species is limited because of its' inability to survive high summer temperatures and increasing salinities in the region (Morgan *et al.* 2002).

Mosquitofish were introduced to Australia during the 1920s, firstly as aquarium pets, but were soon released widely into natural waters to control mosquito populations (Allen *et al.* 2002) and have since become widely established. They are native to North and Central American rivers draining into the Gulf of Mexico.

In recent years, ornamental fish keeping has become a popular hobby in many countries (Moravec 1999) including Australia (Mouton *et al.* 2001). The worldwide trade in aquarium fishes has led to the introduction of many new fish species into Australia, some of which, including koi carp, goldfish, one-spot livebearer and swordtail, have adapted to their new environment and quickly formed self-sustaining

populations in the wild (Allen *et al.* 2002; Morgan *et al.* 2004). Goldfish and koi carp, in particular, can survive well in temperate regions and are now widespread in both Australia and South Africa (King *et al.* 1997; Mouton *et al.* 2001).

1.4 Effects of exotic fishes

Introduced fishes may have a number of detrimental effects on native fish species, including degradation of habitat and water quality, predation, aggressive interactions such as fin nipping, suppression of reproductive activity, competition for food and other resources and introduction of exotic fish diseases and parasites (Arthington & McKenzie 1997; Howe *et al.* 1997; Dove & Ernst 1998; Morgan *et al.* 2004).

Carp are now widespread in eastern Australia and their high standing stocks have created major concerns about their effects on water quality in Australian freshwater systems, by causing and increasing the frequency of algal blooms (King *et al.* 1997).

Mosquitofish have been shown to impact on small endemic fish species in the USA (Moyle *et al.* 1986; Douglas *et al.* 1994), and in Australia they have been associated with damaged caudal fins of native fishes, predation on juvenile native fishes and competition with native fishes for food, leading to retarded growth and suppression of reproductive activity (Howe *et al.* 1997; Gill *et al.* 1999; Morgan *et al.* 2004). The few studies undertaken in Western Australia all suggest that redfin perch may be a major factor contributing to the loss or reduction of some populations of native fishes and decapod crustaceans through predation (Pen & Potter 1992; Beatty 2000; Morgan *et al.* 2002, 2004). Trout have been implicated in the decline of native freshwater fishes and amphibians in eastern Australia through predation and competition for food and space (Crowl *et al.* 1992; Arthington & McKenzie 1997;

Gillespie 2001), and have also been shown to predate heavily on the endemic S.W.C.D.D. freshwater crayfish, marron (*Cherax cainii*) (Tay *et al.* 2007).

1.5 Parasites of freshwater fishes

The parasite fauna of Australian freshwater fishes is poorly known relative to that of other continents (Dove & Ernst 1998) and in Western Australia, there have been no comprehensive surveys of freshwater fish parasites. Limited information on parasite populations in freshwater fishes in eastern Australia has been published by Dove *et al.* (1997), Dove & Ernst (1998) and Dove (2000). Dove (2000), for example, found 109 species of parasites in 18 fish species in south-eastern Queensland, but much more work is needed to explore the field.

Parasites found in freshwater fishes are primarily protozoans, myxozoans, helminths (platyhelminths, nematodes and acanthocephalans), hirudineans and crustaceans (Tonguthai 1997). Although both ectoparasites and endoparasites are common in fishes, Tonguthai (1997) observed that internal parasites are able to cause much greater damage to their hosts than external parasites.

1.5.1 Helminth parasites

Helminths may have direct or indirect life cycles. Direct life cycles involve a single host, with sexual reproduction occurring on or in the host and eggs or larvae usually leaving the host and spending some time free-living in the environment before infecting the same species of host as the one from which it was discharged, without the obligatory involvement of any other host species.

Many parasites have more complex or indirect life cycles which involve two or more hosts. Larval stages occur in one or more intermediate hosts, followed by sexual reproduction of mature adults in the definitive host. Intermediate hosts are essential for the completion of larval development and the parasite sometimes undergoes a period of asexual multiplication within them. Paratenic hosts are additional, non-obligatory hosts in the life cycle, which larval parasites may infect but in which they do not undergo further development before infecting the next intermediate or definitive host. Intermediate and paratenic hosts are often adversely affected by the presence of the parasite, making them more susceptible to capture by predatory definitive hosts and thereby permitting completion of the parasites life cycle (Crompton & Joyner 1980). Many helminth parasites use fish as either intermediate or as paratenic hosts, with the life cycle being completed when fish are ingested by piscivorous fish, bird or mammal definitive hosts.

The damage caused by helminths to their host is generally related to the intensity of infection (the number of parasites within the infected host) and the depth of parasite penetration within the host tissue. Most intestinal helminths, such as trematodes, cestodes and nematodes, do not induce severe damage to the vertebrate gasterointestinal tract (Dezfuli *et al.* 2003), unless they are present in large numbers, when they may induce growth retardation (Tonguthai 1997). However, some acanthocephalan genera, such as *Acanthocephalus* (Taraschewski 2000), *Pomphorhynchus* (Dezfuli *et al.* 2002) and *Southwellina* (Dezfuli *et al.* 1998), penetrate through the intestinal wall and provoke extensive damage to the gasterointestinal tract. The larval stages (metacercariae) of some trematodes infect

fish gills and may disrupt the respiratory system leading to high mortality (Tonguthai 1997).

1.5.1.1 Monogeneans

Monogeneans are mostly ectoparasitic platyhelminths with direct life cycles. They typically parasitise the gills and external body surfaces of fishes and are generally host specific (Buchmann & Bresciani 2006). The most recognisable morphological character of the group is a posterior adhesive apparatus called an opisthaptor.

Monogeneans are usually divided into two main lineages (monopisthocotyleans and polyopisthocotyleans), which differ markedly in morphological, physiological and reproductive features (Buchmann & Bresciani 2006). Monopisthocotyleans are epithelial browsers and polyopisthocotyleans are blood feeders.

Fletcher & Whittington (1998) estimate that there may be 500 species of monogeneans on freshwater fishes in Australia, although only about 5% of these have been described. They recorded 26 species of monogeneans from 17 species of freshwater fish in Australia, including *Gyrodactylus kobayashii* from an exotic fish, *C. auratus*. According to Fletcher & Whittington (1998), another 14 species have been identified only to the generic level, including two *Anchylodiscus* sp., four *Dactylogyrus* sp. and five *Gyrodactylus* sp.

Two of the most common genera of monogeneans on fishes throughout the world are the monopisthocotyleans *Dactylogyrus* (family Dactylogyridae) and *Gyrodactylus* (family Gyrodactylidae). Dactylogyrids are oviparous and mainly gill parasites, while gyrodactylids are viviparous and live mainly on the skin, fins and gills (Dove

& Ernst 1998; Matejusova *et al.* 2001). Dove & Ernst (1998) reported for the first time four species of monogeneans of the genera *Dactylogyrus* or *Gyrodactylus* from introduced freshwater fishes in Australia; they were *Gyrodactylus bullatarudis* from *Poecilia reticulata* and *Xiphophorus helleri*, *Gyrodactylus macracanthus* from *Misgurnus anguillicaudatus*, *Dactylogyrus extensus* from *C. carpio*, and *Dactylogyrus anchoratus* from *C. auratus*.

1.5.1.2 Trematodes

Digenean trematodes are endoparasitic platyhelminths with indirect life cycles involving one or more intermediate hosts (the first of which is almost always a mollusc), in which occur successive larval forms undergoing asexual multiplication (Berra & Au 1978). Adult trematodes are normally endoparasitic in the gasterointestinal tract, blood vessels or other natural body cavities of vertebrates.

Fishes may act as either definitive intermediate or paratenic hosts in trematode life cycles. Most adult-stage trematodes in fishes are parasites of the gasterointestinal tract. Adult trematodes in fishes are usually host specific and rarely cause significant harm to their hosts, although there are some notable exceptions (Paperna & Dzikowski 2006). The main pathogenic effects from trematode infections in fish hosts occur from larval metacercariae. Metacercariae tend to be less host specific than adult trematodes and may affect fish growth and survival, as well as being a source of infection to definitive hosts and other piscivorous vertebrates, including people (Paperna & Dzikowski 2006). Many larval trematodes enter the fish host by active invasion of the cercarial stage (Jhansilakshmibai & Madhavi 1997; Poulin *et al.* 1999) and transmission success depends on the behavior of the cercariae

(Jhansilakshmibai and Madhavi 1997). Following cercarial invasion, metacercariae often encyst and almost always produce an inflammatory response in the host, with enclosure of the parasite in a fibrous capsule (Paperna & Dzikowski 2006). Encapsulated metacercariae may infect various organs, including the gills, skin, eyes and viscera. The distribution of metacercariae within the host may be affected by parasite behavior as well as host behavior and habitat (Ondrackova *et al.* 2002). Cysts formed around metacercariae in the skin of a number of trematode genera, including *Uvulifer*, *Crassophiala* and *Ornithodiplostomum*, may contain cells heavily loaded with melanin, forming pinhead-sized black spots (black spot disease) (Lane & Morris 2000; Paperna & Dzikowski 2006). White spot disease or white grub is found in visceral organs such as the kidneys, liver and heart, and is commonly caused by the metacercariae of *Posthodiplostomum minimum* (Lane & Morris 2000). Yellow grubs or spots are metacercariae which are embedded intermuscularly or subcutaneously in fish and commonly caused by clinostomiids such as *Clinostomum complanatum* (Lane & Morris 2000; Paperna & Dzikowski 2006).

According to Cribb (1998), the trematode fauna of Australian fishes is particularly poorly studied. Cribb (1998) observed that 289 trematode species have been reported from nearly 300 species of marine teleosts, which represent less than 10% of the 3300 teleost species found in Australian coastal waters. The trematode parasites of freshwater fishes in Australia are almost completely undescribed.

1.5.1.3 Cestodes

Cestodes (tapeworms) are endoparasitic platyhelminths with indirect life cycles involving one or more intermediate hosts. Fishes may act as either definitive,

paratenic or intermediate hosts. Adult cestodes are typically found in the gasterointestinal tract, while larval stages may be found in a variety of organs; adults are generally more host-specific than larval cestodes (Dick *et al.* 2006). Adult cestodes in the gasterointestinal tract may cause mechanical damage or reduced nutrient absorption, but most serious pathology in fish hosts is caused by migrating larvae (Dick *et al.* 2006). Loot *et al.* (2001) pointed out that host-parasite interactions between cestodes and fishes have rarely been investigated in natural environments.

Beveridge and Jones (2002) reported 14 species of adult cestode in teleosts in Australia; according to them, cestodes of Australian teleosts have been poorly studied and most that have been found have been in marine fishes in Australian coastal waters. Of the few cestodes that have been described from freshwater fishes in Australia, most have been introduced, either with exotic fish species or migrating avian hosts (Pollard 1974).

The Asian fish tapeworm *Bothriocephalus acheilognathi* is parasitic as an adult in the gasterointestinal tract of fishes. It was described originally from cyprinid fishes in China, although it now has a much wider distribution because of the introduction of its cyprinid definitive hosts, particularly carp (*C. carpio*) and grass carp (*Ctenopharyngodon idella*), to many other countries for aquaculture and the control of aquatic vegetation (Dove *et al.* 1997; Dove & Fletcher 2000; Perez-Ponce de Leon *et al.* 2000; Nie *et al.* 2000; Salgado-Maldonado & Pineda-Lopez 2003).

Bothriocephalus acheilognathi was first reported in Australia by Dove *et al.* (1997) in *C. carpio*, eastern mosquitofish (*G. holbrooki*) and western carp gudgeon (*Hypseleotris klunzingeri*) in the Australian Capital Territory and New South Wales.

Dove and Fletcher (2000) believed that infections with *B. acheilognathi* may play a significant role in the interaction between native and exotic fishes in Australia, but there have been no comprehensive ecological studies of the parasite in the Australian environment.

Ligula intestinalis is a Northern Hemisphere cestode, first reported in Western Australia by Morgan (2003). It has a complex life-cycle that involves the adults parasitising fish-eating birds, the eggs being released from the birds, hatching and entering copepods, which are subsequently consumed by planktivorous fishes, especially cyprinids (Pollard 1974; Weekes & Penlington 1986; Loot *et al.* 2001). The plerocercoid larvae are always found free in the body cavity of the fish host (Pollard 1974; Morgan 2003), where they cause severe disfigurement, reproductive retardation (Loot *et al.* 2002; Morgan 2003) and behavioural changes (Loot *et al.* 2001; Brown *et al.* 2002), making the fish more susceptible to avian predation. A number of birds have been reported as definitive hosts of *L. intestinalis*, including blue heron (*Ardea herodias*), herring gull (*Larus argentatus*), common loon (*Gavia immer*), American merganser (*Mergus merganser*), belted kingfisher (*Ceryle alcyon*) and American osprey (*Pandion cavolinensis*) (Black & Fraser 1984).

1.5.1.4 Nematodes

Nematodes (roundworms) are endoparasites that may have direct life cycles, such as *Camallanus cotti* (Levsen 2001), although most species that infect fishes have indirect life cycles, with a single intermediate host and one or more paratenic hosts. Fishes may be either definitive, intermediate or paratenic hosts for nematodes (Stromberg & Crites 1974; Bhaibulaya *et. al.* 1979; Coyner *et. al.* 2001; Akther *et al.*

2004), with adult parasites usually found in the gasterointestinal tract and larvae in a variety of organs, including the gasterointestinal tract, skin, body cavity and viscera (Molnár *et al.* 2006). When fishes are intermediate hosts, definitive hosts are typically fish-eating birds (Bhaibulaya *et al.* 1979; Bhaibulaya & Indra-Ngarm 1979; Spalding & Forrester 1993; Perez-Ponce de Leon *et al.* 2000; Coyner *et al.* 2001), fish-eating mammals (Bhaibulaya *et al.* 1979), carnivorous reptiles (Jackson & Tinsley 1998; Perez-Ponce de Leon *et al.* 2000; Akther *et al.* 2004) or amphibians (Jackson & Tinsley 1998; Perez-Ponce de Leon *et al.* 2000).

Although nematodes may be important pathogens of fishes, of greater concern is usually their role in transmitting parasites to humans, who become accidental hosts. The most common of these fish-borne zoonotic diseases is anisakiasis, caused by larval anisakid nematodes, usually of the species *Anisakis simplex* or *Pseudoterranova decipiens*. Anisakiasis can take a number of different clinical forms, depending on the location and histopathological lesions caused by the larvae. The most important of these clinical forms is invasive gastrointestinal anisakiasis, where the live larva penetrates the gastric or intestinal mucosa, producing either acute or chronic symptoms (Lymbery & Cheah 2007). Other zoonotic diseases caused by nematodes transmitted to people from fish include capillariasis (caused by *Capillaria philippinensis*), marked by diffuse abdominal pain and intermittent chronic diarrhea, and gnathostomiasis (caused by *Gnathostoma* sp.), due to visceral larval migration (Bhaibulaya *et al.* 1979; Suankratay *et al.* 2001; Ko 2006).

There have been some studies of nematodes, particularly anisakids, in marine fishes in Australia (Cannon 1977; Lymbery *et al.* 2002; Doupe *et al.* 2003), but little is

known of nematodes in Australian freshwater fishes. Pollard (1974) reported worms of *Eustrongylides* (possibly *E. gadopsis*) in the common jollytail (*G. maculatus*) in Victoria and Chapman *et al.* (2006) found both *Eustrongylides* sp. and *Contracaecum* sp. in *G. maculatus* in Western Australia. Both parasite species use fishes as intermediate and paratenic hosts, with piscivorous birds as definitive hosts. Coyner *et al.* (2002) observed that fish infected with larval *Eustrongylides* have behaviour modifications that make them more susceptible to predators, which may play an important role in parasite transmission in natural populations.

1.5.1.5 Acanthocephalans

Acanthocephalans are endoparasitic helminths with complex, indirect life cycles involving arthropod intermediate hosts and vertebrate final hosts; vertebrate paratenic hosts are also common. Fishes may act as definitive hosts, in which the adult parasite is found in the gasterointestinal tract, or as paratenic hosts, in which the larva (cystacanth) occurs in a variety of extra-intestinal sites (Hine & Kennedy 1974; Lyndon & Kennedy 2001; Fielding *et al.* 2003). Some acanthocephalan species have developed the ability to modify the behaviour of their intermediate hosts to enhance the probability of ingestion by the definitive host (Fielding *et al.* 2003; Perrot-Minnot 2004; Bauer *et al.* 2005). Successful transmission of acanthocephalans is also assisted by characteristics of their eggs such as structure and chemical composition (Shin 1986).

Acanthocephalans have a world-wide distribution in both marine and freshwater fishes and have been reported in many countries (Hine & Kennedy 1974; Evans *et al.* 2001; Lyndon & Kennedy 2001; Nickol 2006). *Pomphorhynchus laevis* is one of the

most widely distributed species and is very common in both salmonid and cyprinid fishes in Europe (Hine & Kennedy 1974; Evans *et al.* 2001). Little is known, however, about the occurrence of acanthocephalans in freshwater fishes in Australia.

1.5.2 Crustacean parasites

Parasitic crustaceans, found in the classes Copepoda, Branchiura and Malacostraca, are invariably ectoparasitic on fishes and have a direct life cycle, often with a considerable period of time spent off the host. Parasitic stages are usually blood feeders on the gills, fins or skin of the host and in large numbers can have serious pathogenic effects (Lester & Hayward 2006).

Most of the ca 2,000 species of parasitic crustaceans that have been described are copepods, and the majority of copepods that infest freshwater fishes come from the families Lernaeidae and Ergasilidae (Ho 1998; Lester & Hayward 2006). Lernaeids or anchor worms have a wide geographic distribution, although the great majorities are from Asia and Africa. According to Ho (1998), 113 species of lernaeid copepods have been reported from 322 species of freshwater fishes belonging to 161 genera in 41 families. There are over 40 species in the genus *Lernaea*. The most well studied, and probably the most widespread species is *Lernaea cyprinacea* (Al-Hamed & Hermiz 1973; Sanderson 1974; Bullow *et al.* 1979; Timmon & Hemstreet 1980; Marcogliese 1991; Medeiros & Maltchik 1999; Toro *et al.* 2003; Bond 2004; Perez-Bote 2005). Other species include *Lernaea cruciata* (Joy 1973; Lewis *et al.* 1984), *Lernaea minuta* (Kularatne *et al.* 1994) and *Lernaea piscinae* (Shariff 1981).

Larval stages (copepodids) of *Lernaea* infest mostly the gills the host, but the main pathogenic effects occur from adult females, which use a holdfast ('anchor') to lodge in the body musculature, often at the base of the fins (Shariff 1981; Ho 1998). This produces lesions leading to such effect as mild oedema, infiltration of neutrophils, infiltration of macrophages, proliferation of fibroblast and chronic granulation tissue, and destruction of scales (Joy & Jones 1973). Although species of *Lernaea* are not native to Australia, they have been recorded from a number of native and cultured fish species in eastern Australia (Ashburner 1978; Hall 1983; Callinan 1988; Rowland & Ingram 1991; Dove 2000; Bond 2004).

The Ergasilidae is one of the major families of fish-parasitising poecilostome copepods, in which only female adults are parasitic, with larval stages and male adults being planktonic (Ho *et al.* 1992). The females usually find and infest their hosts after mating and undergo a metamorphosis in which the adults change their body shape and increase in size before beginning egg production (Amado & Rocha 2001). Most ergasilids infest the gills of their host and may cause extensive gill damage and severe haemorrhage, with inflammation and epithelial hyperplasia associated with the attachment and feeding of the parasite (Lester & Hayward 2006).

The genus *Ergasilus* is the largest genus of ergasilids, comprising nearly two thirds of the species currently included in the family (El-Rashidy & Boxshall 2002). About 140 species are currently known and most of them are found in freshwater habitats (Ho *et al.* 1992). Twelve species of ergasilids are currently known from Australia, with six species in *Ergasilus* (*E. orientalis*, *E. australiensis*, *E. lizae*, *E. intermedius*, *E. spinilaminatus* and *E. ogawai*), three in *Dermoergasilus* (*D. semicoleus*, *D.*

acanthopagri and *D. amplexens*), one in *Paraergasilus* (*P. acanthopagri*) and one in *Neoergasilus* (*N. spinipes*) (Byrnes 1986; Kabata 1992).

1.5.3 Protozoan parasites

Over 31 000 extant species have been described in the Kingdom Protozoa, with roughly two-thirds of these being free-living species and the remainder parasites, commensals and symbionts (Adlard & O'Donoghue 1998). Protozoan parasites from at least seven different phyla, encompassing both endoparasites and ectoparasites with a wide range of life cycles and pathologies, have been described from fishes. Although the majority of these species have been described from marine fishes, a number of species of dinoflagellates, kinetoplastids, ciliophorans, apicomplexans and microsporidians have been found to cause disease in freshwater fishes (see Woo 2006). Adlard & O'Donoghue (1998) recorded 56 protozoan species known from Australian fishes. Although more species have been added since that time (Dove 2000) there is still very little known about protozoans in freshwater fishes in Australia.

Among the most important ectoparasites of freshwater fishes are species of ciliated protozoans (Phylum Ciliophora), and the most pathogenic of these is *Ichthyophthirius multifiliis*, causing “white spot disease” (Buchmann *et al.* 2001). *I. multifiliis* has a worldwide distribution and appears to parasitise all species of scaleless, freshwater fish at all growth stages, from juvenile to adult (Sao Clemente *et al.* 2000; Xu *et al.* 2005; Dickerson 2006). The life cycle of the parasite is divided into three stages; trophont, tomont (which divides into tomites) and theront (Buchmann *et al.* 2001). The trophont is the parasitic stage, residing and feeding in

the epidermis of a fish host where it can attain a diameter of up to 1 mm (Buchmann *et al.* 2001). The other stages are free-living and undergo a cycle of asexual reproduction in the environment before theronts infest a new host (Dickerson & Clark 1998). The most obvious clinical signs of infestation with *I. multifiliis* are disseminated white surface lesions on the body and gills; these may become ulcerated and secondarily infected by bacteria, with extensive inflammatory reactions (Dickerson 2006). Fish often become lethargic and cease feeding (Sao Clemente *et al.* 2000).

Trichodinids are ciliated protozoans which are commonly found on freshwater fishes throughout the world. They generally have a low host specificity (Basson & Van As 2006). Many species are commensals which cause little or no harm to their fish hosts, but a number are important ectoparasites, which in large numbers can cause serious epithelial damage to the skin and gills (Basson & Van As 2006). Dove & O'Donoghue (2005) found 21 trichodinid species, both exotic and (presumably) native, on 33 species of freshwater fish throughout eastern Australia. They speculated that the Australian trichodinid fauna may include up to 150 as yet undescribed species and represents a major source of unexplored biodiversity from native and introduced freshwater fishes.

1.5.4 Myxozoan parasites

Myxozoans are metazoan parasites that are commonly found in both marine and freshwater fishes throughout the world. Some species may have a direct life cycle, but many have an indirect life cycle, with both fish and invertebrate (often oligochaete) hosts. Myxozoans may infect a wide range of organs, including the gills,

skin, muscles, digestive tract, central nervous system and viscera, with varying degrees of pathology (Feist & Longshaw 2006). Taxonomy within the phylum is still uncertain, and species are identified primarily on the basis of their spore morphology (Lom & Arthur 1989). Some of the most important myxozoan pathogens in freshwater fishes are from the genus *Myxobolus*, one of the largest genera in the family Myxobolidae (Landsberg & Lom 1991; Lom & Dykova 1992; Kent *et al.* 2001).

In Australia, the prevalence and geographic distribution of myxozoan infections in freshwater fishes is largely unknown (Boreham *et al.* 1998). Dove (2000) reported five putative species of *Myxobolus* and a species of *Myxidium* in freshwater fishes in eastern Australia. Spores of *Myxobolus plectroplites*, a previously described pathogen from the freshwater fish *Plectroplites ambiguous*, were detected in human fecal samples from patients showing signs of abdominal pain or diarrhea (Boreham *et al.* 1998).

1.6 Environmental factors influencing parasitism

Parasites are pervasive and important components of ecosystems through their diverse effects on host population dynamics, community interactions and habitat structure (Marcogliese 2004; Thomas *et al.* 2005). Parasites, because of their need to locate and infect a host in at least one stage in their life cycle, are also vulnerable to natural and anthropogenic environmental changes that occur within ecosystems (Tinsley 2005). Changes in parasite population sizes and community organisation, therefore, may act as both an indicator of environmental changes and a driver of further changes in ecosystem structure and function.

1.6.1 Natural environmental changes

Noble (1960) pointed out that migratory fishes often change the nature of their parasites as they move from one habitat to another, suggesting that factors such as temperature, salinity, water quality, mechanical barriers and food supply continuously restrict or encourage the growth, development and transmission of parasites. Successful transmission of parasites from host to host requires reproduction (usually within the host), release of infective stages from the host, survival of infective stages within the environment, location of a new host and entry into the host, and is therefore dependent on both host behaviour and parasite behavior, as well as external environmental conditions (Crompton & Joyner 1980; Krause *et al.* 1999; Pietrock & Marcogliese 2003).

Water temperature is often a critical environmental parameter for parasites of fishes, with effects upon parasite survival, growth, timing of transmission, distribution and host hormone cycles (Stromberg & Crites 1975; Jackson *et al.* 2001; Tubbs *et al.* 2005; Hakalahti *et al.* 2006). The influence of temperature is often reflected by seasonal differences in parasite prevalence and intensity. Ogut and Palm (2005), for example, found that protozoan infections in fishes were greatest during late autumn, winter and early spring. In the nematode *Camallanus oxycephalus*, growth, maturation and egg production are greatest during the warm seasons and cool temperatures cause delayed transmission of the parasite (Stromberg & Crites 1975). The monogeneans *Gyrodactylus rugiensoides* and *Gyrodactylus rugiensis* were found to be different sizes between spring and summer on their host *Pomatoschistus pictus* (Huyse & Volckaert 2002). Jackson *et al.* (2001) observed differences in the embryonic developmental pattern of the monogeneans *Protopolyystoma orientalis* and

Protopolyystoma xenopodis in relation to water temperature. Barker and Cone (2000) found a significant loss from fishes of the monogenean *Pseudodactylogyrus anguillae* with water temperatures above 22°C. Water temperature also affects parasite reproduction rates. Tubbs *et al.* (2005), for example, found that at lower temperatures, the monogenean *Zeuxapta seriolae* had markedly reduced reproductive potential, with development to maturity occurring more slowly and fewer eggs being produced than at higher temperatures compared with *Benedenia seriolae*. Reproduction rate is also influenced by water temperature in copepods such as *Ergasilus* sp. and *Lernaea* sp. (Bulow *et al.* 1979; Timmons & Hemstreet 1980; Marcogliese 1991; Barker & Cone 2000; Saarinen & Taskinen 2004; Perez-Bote 2005).

Chemical characteristics of the water may also have important effects on parasites of fishes. Saarinen and Taskinen (2004), for example, found decreased prevalence of the copepod *Paraergasilus rylovi* in water with pH values below 5.6. Moller (1978) observed that parasite species richness was less in fishes in brackish water (such as in estuaries) than in marine and freshwater ecosystems, because of the effects of changing salinity on ectoparasites and on exposed stages (such as eggs and free-swimming larvae). Jackson *et al.* (2001) observed that changes in water chemistry may affect the transmission of parasites by disturbing larval hatching and development (Jackson *et al.* 2001). Trematode cercariae, for example, may fail to achieve a full multi-layered protective cyst when water chemistry parameters change (Morley *et al.* 2003). Special adaptations, such as the thick-shelled eggs of acanthocephalans and the spores of myxozoans, may allow parasite stages which are

exposed to adverse environmental conditions to survive until conditions improve (Shin 1986; Hedrick *et al.* 1998).

The prevalence and intensity of many parasites of fishes is affected by water velocity. Barker and Cone (2000), for example, found that increased stream velocity decreased the prevalence and abundance of ergasilid copepods on fishes and Saarinen and Taskinen (2004) observed that *Paraergasilus rylovi* larvae are flushed away from potential new hosts before they become infective. According to Berra and Au (1978) and Poulin *et al.* (1999), trematode cercariae are also continuously swept away in running water and have less chance to penetrate a host.

1.6.2 Anthropogenic environmental changes

Human impacts on the aquatic environment may also affect the parasites of freshwater fishes. Anthropogenic changes that may affect parasite communities include pollution, biodiversity loss, climate change, habitat alteration and the introduction of exotic species (Lafferty & Kuris 2005). The effects of pollution have been most intensively studied. Parasites can interact with environmental pollution in different ways, depending on the species and life cycle stage, leading to either increases or decreases of parasitism in polluted environments (Sures 2004). Pietrock *et al.* (2001), for example, observed that trematode species diversity in fish was decreased in polluted environments, presumably because pollutants impaired the life span of the parasites' free-living transmission stages. On the other hand, the prevalence and intensity of parasitic gill ciliates and monogeneans has been found to increase in polluted environments, apparently because of an increase in host susceptibility (Galli *et al.* 2001; Lafferty & Kuris 2005).

Whereas parasitism is always expected to decrease with the extinction of free-living hosts, climate change and habitat alteration may, like pollution, have complex effects on parasites, making it difficult to generalise. The most clear prediction of anthropogenic climate change is a global increase in average temperatures, which will affect different parasite/host relationships in different ways (Marcogliese 2001; Lafferty & Kuris 2005). Likewise, habitat alteration may increase parasitism, as for example when soil excavation exposes the eggs of the nematode *Eustrongylides ignotus* to potential intermediate hosts (Coyner *et al.* 2002) or decrease parasitism, as has occurred for a variety of vector-borne parasitic diseases when wetlands have been drained or converted to other land uses (Lafferty & Kuris 2005). Perhaps most complex of all, however, is the relationship between parasitism and introduced exotic species, which is exemplified in the role of parasites in freshwater fish invasions throughout the world.

1.7 The role of parasites in freshwater fish invasions

The invasion of freshwater ecosystems by exotic fish species is an accelerating phenomenon throughout the world, leading to the global homogenisation of freshwater fish faunas (Rahel 2002). Invasive exotic species, including freshwater fishes, are economically costly, may cause extensive ecological damage and are a leading threat to global biodiversity (Kolar 2003). In Australia, human assisted movement of freshwater fishes has occurred since the earliest days of European settlement (Lintermans 2003) and approximately 22 species of exotic freshwater fishes have now been introduced (Arthington & McKenzie 1997; Allen *et al.* 2002). The role of parasites in freshwater fish invasions may be complex, with parasites having both direct effects on exotic and native fish species and indirect effects by

mediating the interactions between exotic and native fishes (Prenter *et al.* 2004).

Three major patterns of host/parasite associations can be distinguished

(Taraschewski 2006): (1) exotic fish species may be introduced without the parasites of their source area; (2) parasites may be introduced with exotic species and then transfer to native species; (3) parasites may transfer from native to exotic species.

1.7.1 Parasite loss in exotic fishes

A number of studies across a wide range of taxa have found that invading exotic species harbour fewer parasites than native species (Torchin *et al.* 2002, 2003; Mitchell & Power 2003). Averaging across taxa, exotic animals leave approximately 84% of their parasite species behind (Lafferty & Kuris 2005). Invading exotic species typically start with small founder populations which, by chance, may not have the full parasite complement of their source area. Furthermore, those parasite species which are introduced with their host species may be unable to maintain their life cycle in the new environment, particularly if environmental conditions are different or if they require other hosts which are not present in the area. For example, the naturalisation of the trematode species *Amurotrema dombrovskajae* in Hungary has been prevented by the lack of a suitable molluscan intermediate host (Molnar 1984).

Even when parasites from the source area manage to invade the new environment, there is often a delay of one to several decades. The acanthocephalan *Paratenuisentis ambiguus*, for example, was first recorded in the Weser River in Germany about 25 years after the introduction of its intermediate host *Gammarus tigrinus* (Taraschewski 2006). It has been suggested that the loss of parasites by an invading

exotic species may lead to greater demographic success, providing them with a competitive advantage over native species, but there are few empirical data to support this hypothesis (Prenter *et al.* 2004; Taraschewski 2006).

1.7.2 Transmission of parasites from exotic to native fishes

Although invading exotic species often have fewer parasites than either native species or populations of the exotic species in its natural range, some parasites may still be introduced along with the exotic host. Parasites with a high prevalence in the source population of hosts and with either a simple, direct life cycle or vertical transmission, are more likely to be introduced and to persist on exotic fish in the new environment (Molnar 1984; Bauer 1991; Hayward 1997; Baldwin & Goater 2003; Prenter *et al.* 2004). Exotic ornamental fishes, in particular, have often been implicated in the introduction of new parasite species (Moravec *et al.* 1999; Sao Clemente *et al.* 2000; Levsen 2001; Mouton *et al.* 2001). For example, Sao Clemente *et al.* (2000) described how *Ichthyophthirius multifiliis* infecting ornamental fishes from Brazil were exported to Europe (Germany and England) and Asia (Japan).

Introduced parasites may subsequently transfer from exotic to native fish species. The risk of such transfer is greater when the parasite has low host specificity and when native and exotic fish species are closely related (Bauer 1991). In this situation, the parasite is often more damaging to the new host because the host and parasite have not coevolved and the host may therefore lack defenses against the parasite (Dove & Ernst 1998; Prenter *et al.* 2004). For example, *Myxobolus cerebralis*, which causes whirling disease in European trout, has spread from introduced, cultured trout to native trout in North America, with severe consequences for native populations

(Lafferty & Kuris 2005). In addition, even parasites which are normally nonpathogenic may become important in native hosts if the native populations are malnourished or stressed from competitive interactions with exotic fishes or from other environmental pressures (Scott 1988).

1.7.3 Transmission of parasites from native to exotic fishes

Once an exotic fish species has colonised a new area, native parasites may transfer to it from their original hosts. Jimenez-Garcia *et al.* (2001), for example, found that native North American monogenean parasites were infecting introduced African cichlids, presumably assisted by their direct life cycle and the close phylogenetic relationship between native and exotic hosts. Empirical studies have found that, on average, about four species of native parasites occur in invading exotic animals (Torchin *et al.* 2003). While this is not enough to compensate for the release from their natural enemies that exotic species experience, it may be important in helping to maintain transmission of native parasites. By gaining a wider host base, native parasites could increase in prevalence, intensity and geographic range (Lafferty & Kuris 2005).

1.8 Thesis objectives and structure

The broad aim of this study was to investigate the parasite fauna of native and exotic freshwater fishes in the south-west of Western Australia. More specifically, my research objectives were to:

- 1) identify the range of parasite species found in native and exotic freshwater fish species;

- 2) compare parasite prevalences and parasite community structure between native and exotic species;
- 3) where possible, distinguish introduced from native parasite species and investigate the likely impacts of introduced parasites on native fish species;
- 4) determine the effect of anthropogenic environmental changes on parasitism in native fish species;
- 5) describe the morphology of the new species of parasite found;
- 6) investigate the pathology of infection caused by parasites and water quality in the native species *Tandanus bostocki*.

This thesis contains five research chapters. Chapter 2 is a general survey of the parasites found in native and exotic freshwater fish species in the south-west of Western Australia. In Chapter 3, I report the first record of an introduced copepod parasite, *Lernaea cyprinacea* in Western Australia and describe it's infestation of native fish species. Chapter 4 is a description of a new, native copepod species found on the endemic freshwater cobbler, *Tandanus bostocki*. In Chapter 5, I examine the relationship between anthropogenic processes which impact freshwater ecosystems and the parasite fauna of native fish species found in those ecosystems, while Chapter 6 is a more intensive study of the effects of one of these impacts (stream salinisation) on parasitism in a population of freshwater cobbler, *Tandanus bostocki*. Chapter 7 is a general discussion, putting my research in the context of the role of parasites in the invasion of exotic freshwater fish in the streams and rivers of the south-west of Western Australia.

CHAPTER 2

Parasites of native and exotic freshwater fishes in the south-west of Western Australia

2.1 Introduction

Compared to other areas of the world of similar size, Australia has a relatively depauperate, although highly endemic, freshwater fish fauna (Allen *et al.* 2002). Fewer than 200 species of fish are found in freshwater habitats in Australia, of which 144 are confined exclusively to freshwater. In the extreme south-west of Australia, which is isolated from the rest of the country by extensive arid zones, only 10 species of freshwater fish are found and eight of these are endemic to the region. This is the highest proportion of endemism among freshwater fishes in all of the 11 major geographic drainage divisions in Australia (Morgan *et al.* 1998).

The endemic species of freshwater fish in the South West Coast Drainage Division (S.W.C.D.D) are salamanderfish (*Lepidogalaxias salamandroides*) (the sole member of the family Lepidogalaxiidae), freshwater cobbler (*Tanadus bostocki*) (Plotosidae), nightfish (*Bostockia porosa*) (Percichthyidae), western pygmy perch (*Edelia vittata*), Balston's pygmy perch (*Nannatherina balstoni*) (both in the family Nannopercidae), western minnow (*Galaxias occidentalis*), black stripe minnow (*Galaxiella nigrostriata*) and mud minnow (*Galaxiella munda*) (all in the family Galaxiidae).

The two remaining species, common jollytail *Galaxias maculatus* and trout minnow *Galaxias truttaceus* (Galaxiidae) are widely distributed throughout southern Australia. In addition to these 10 freshwater species, the anadromous lamprey (*Geotria australis*) (Geotriidae) and the estuarine western hardyhead (*Leptatherina*

wallacei) (Atherinidae), Swan River goby (*Pseudogobius olorum*) and south-west goby (*Afurcagobius suppositus*) (Gobiidae) are also found in freshwater systems in the south-west.

The freshwater fishes of the south-west, like those in other parts of Australia, are threatened by a range of anthropogenic processes. The most important of these are river regulation, the extraction of water for irrigation, salinisation and sedimentation as a result of land clearing, eutrophication from agricultural and urban wastes, and the accidental or deliberate introduction of exotic animals and plants (Howe *et al.* 1997; Pen 1999; Morgan *et al.* 2003; Pollino *et al.* 2004).

At least 22 species of exotic freshwater fish have been introduced into Australia, and 19 of these have established self-sustaining, feral populations (Arthington & McKenzie 1997; Allen *et al.* 2002). In the S.W.C.D.D., the most important of these are rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) (Salmonidae), redfin perch (*Perca fluviatilis*) (Percidae), mosquitofish (*Gambusia holbrooki*), one spot livebearer (*Phalloceros caudimaculatus*) (Poeciliidae), pearl cichlid (*Geophagus brasiliensis*) (Cichlidae) and goldfish (*Carassius auratus*) (Cyprinidae). Exotic fishes may impact upon native species through predation, competition or the introduction of exotic pathogens and parasites (Arthington & McKenzie 1997; Howe *et al.* 1997; Dove 2000).

The parasite fauna of both native and exotic freshwater fishes in Australia is very poorly known. There have been a limited number of studies in eastern Australia (e.g. Callinan 1988; Rowland & Ingram 1991; Dove & Ernst 1998; Dove 2000; Dove &

O'Donoghue 2005), but apart from two reports of helminth infections in native galaxids (Morgan 2003; Chapman *et al.* 2006), almost nothing is known of the parasites of freshwater fishes in Western Australia. This lack of knowledge is both surprising and unfortunate. Parasites are often important regulators of host population sizes through their direct effects on host morbidity and mortality (Anderson & May 1992; Hudson *et al.* 1998), and they can represent significant threats when populations are already declining due to other causes (McCallum & Dobson 1995; Holmes 1996). In particular, the introduction of new parasites in exotic hosts can be a major source of disease emergence (Freeland 1993; Prenter *et al.* 2004; Torchin *et al.* 2005; Taraschewski 2006). Parasites may also affect host population sizes indirectly, by mediating competitive and predatory interactions, and this can be an important factor in determining the outcome of invasions by exotic species (Prenter *et al.* 2004; Torchin *et al.* 2005; Taraschewski 2006).

In this chapter, I provide the first comprehensive report of the parasites of freshwater fishes in south-western Australia. My aims were to survey the parasite fauna, particularly helminths and copepods, of as many native and exotic fish species as possible over the range of the S.W.C.D.D. and to compare the prevalence of parasitic infection among fish species and geographic localities.

2.2 Materials and methods

2.2.1 Sampling sites

Fishes were collected from 29 localities in 12 separate rivers, covering the range of the South West Coast Drainage Division of Western Australia (Figure 2.1). To minimise unnecessary sampling of fishes, I utilised, where possible, samples

collected for ongoing studies of fish biology. Thus, my sampling effort, while comprehensive in terms of geographic range, was not equally distributed over this range. Some rivers, particularly the Blackwood and Canning Rivers, were sampled at multiple localities, while most other rivers were sampled at only one or two localities (see Figure 2.1). Both lentic and lotic systems were sampled, including permanent rivers and streams, permanent and ephemeral freshwater pools and lakes, and water storage areas (see Morgan & Gill 2000). At each locality, latitude and longitude were recorded using GPS.

2.2.2 Fish sampling

Fishes were sampled at each locality using either 24 or 12 volt backpack electro-fishers, or from fyke nets (11.2 m wide with two 5 m wings and a 1.2 m wide mouth fishing to a depth of 0.8 m, and a 5 m pocket with two funnels) and seine nets (5, 10 and 15 m); all nets being of 2-3 mm woven mesh. Collected fishes were identified to species in the field and dead fishes were immediately preserved in 70 % ethanol. Live fishes were maintained in aerated containers and transported to the laboratory where they were kept about 2 to 3 days in site-specific aquaria until euthanised for examination. Prior to dissection, all fishes were weighed and measured for total length (TL) (i.e. body length including the caudal fin). For all fish species which were captured, I recorded their origin (native or exotic), primary habitat (freshwater or estuarine), secondary habitat (demersal or pelagic) and maximum size (mm), based on information in Morgan *et al.* (1998).

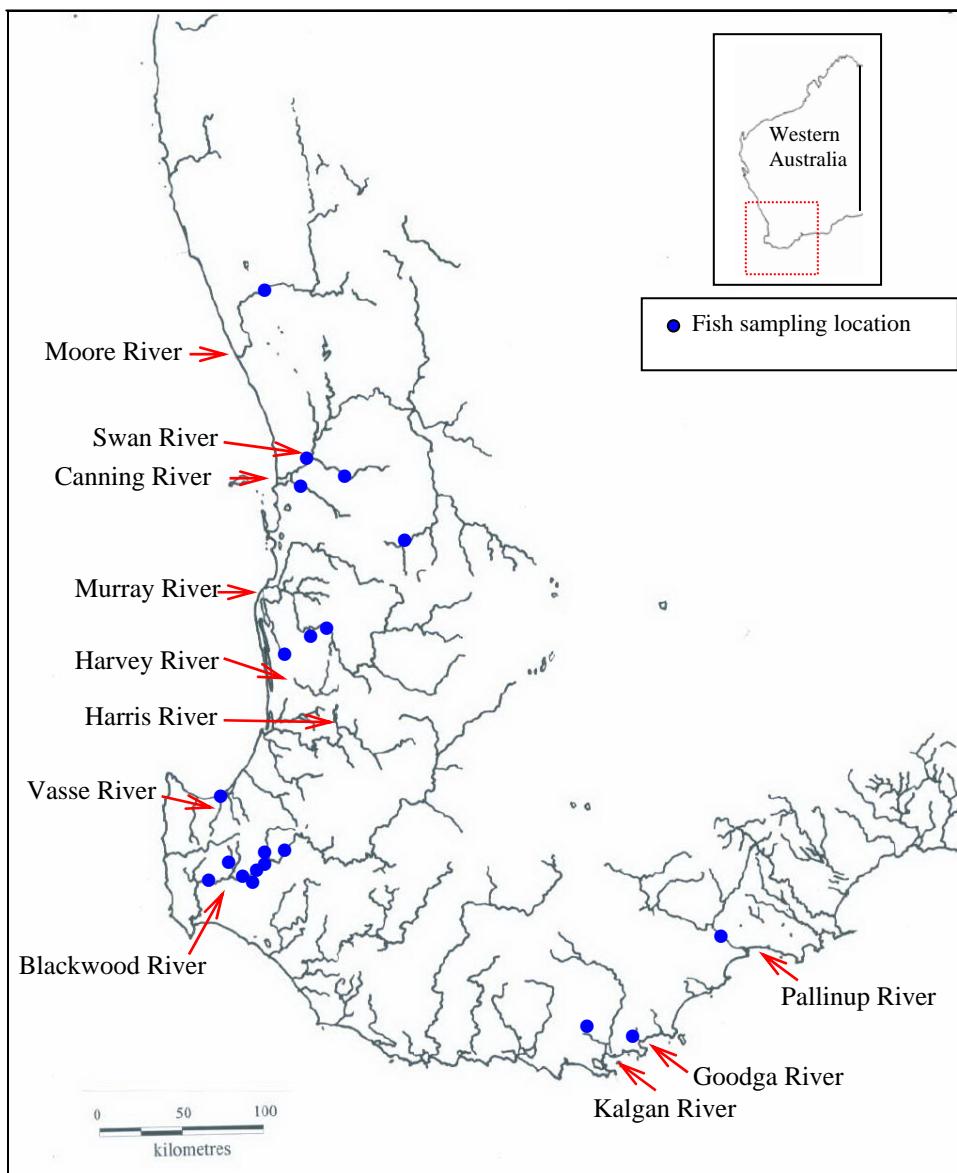


Figure 2.1 Localities from which fishes were sampled in the South West Coast Drainage Division.

2.2.3 *Parasite sampling*

Fishes were dissected using the general methods of Dove (2000) and Berland (2005).

Briefly, each fish was treated with physiological saline to reduce desiccation, and then systematically examined for parasites. Firstly the skin, gills and fins were examined using a combination of eye and dissecting microscope. Then the skin was scraped with a scalpel and the scrapings examined using both dissecting and

compound microscopes. Gills were removed, examined individually under a dissecting microscope and then scraped as for the skin. Eyes, muscles and visceral organs (liver, kidney, spleen, gall bladder, gonads and gastrointestinal tract) were dissected, teased apart and examined for parasites using a dissecting microscope, and squash preparations were taken and examined under a compound microscope. Histological sections were not routinely taken.

All detected parasites were removed and treated with physiological saline to retain morphological definition (Berland 2005). Where possible, photographs were taken of live specimens and they were then fixed and preserved in either 70% ethanol (crustaceans, nematodes, cestodes) or 5-10% formalin (trematodes, protozoans, myxozoans). If necessary, specimens were cleared in lactophenol (nematodes and crustaceans) and prepared as whole mounts for microscopic identification. Protozoan and myxozoan cysts were dissected prior to preparation; trematode metacercaiae were not excysted. Parasite specimens were identified to the lowest taxon possible using the taxonomic keys of Schmidt (1970), Kabata (1979), Anderson *et al.* (1980) and Lom & Dykova (1992). Identification to species level was rarely possible and specimens were assigned to morphospecies based on consistent differences in morphology and site of infection.

Two samples of a cestode, identified morphologically as *Ligula* sp., were sent to Dr Géraldine Loot, Laboratoire Evolution et Diversité Biologique, Université Paul Sabatier, for molecular analyses. Three DNA regions were sequenced, the ITS2 region of rDNA and two mitochondrial genes (cytochrome oxidase subunit I and

cytochrome B), and the sequences compared with other isolates of *Ligula* from around the world, as described by Bouzid *et al.* (2008).

2.2.4 Data analysis

Parasitic infections at both local (i.e. within rivers) and regional scales (i.e. within the entire S.W.C.D.D) were examined. Measures of parasitism are therefore described at both of these scales. For each fish species, the overall prevalence of parasitic infection (i.e. the proportion of fish infected with any species of parasite) as well as prevalence of each parasite species was recorded. Parasite regional and component community (following Bush *et al.* 1997) diversity were estimated by species richness (the number of species, S) and by a taxonomic diversity (H_T) index, which is the Shannon-Wiener diversity index (Shannon 1948) calculated from the number of species in each major taxonomic grouping of parasites: protozoans/myxozoans; monogeneans; trematodes; cestodes; nematodes and copepod crustaceans.

Differences in parasite prevalence or diversity among fish species and localities were investigated using Chi-square tests or analyses of variance, respectively. Differences in these parameters among groups of fish species (e.g. native or introduced, freshwater or estuarine, benthic or pelagic) were compared by analyses of variance of species values. Relationships with continuous variables, such as fish total length, were investigated with linear regression analyses. Prior to undertaking any analyses which assumed normality, all variables were tested for normality and transformed if necessary.

Measures of parasite prevalence are relatively independent of host sample size, but diversity measures are much more affected, especially for small sample sizes (<40 hosts) (Lopez 2005; Jovani & Tella 2006; Marques & Cabral 2007). When comparing these parameters among fish species, species groups and localities, I attempted to correct for differences in sample size in a number of ways. Firstly, any samples with less than 10 host individuals were excluded from analyses. Secondly, measures of prevalence, intensity and diversity were regressed against host sample size and, if a significant relationship was found, host sample size was included as a covariate in the analysis. Thirdly, expected (smoothed or rarefaction) parasite species accumulation curves were calculated for each host species to gauge the adequacy of species richness estimates. Expected species richness and 95% confidence intervals were calculated cumulatively for each host sample size using the analytical formulae of Colwell *et al.* (2004), implemented with the software EstimateS 7.5 (Colwell 2005).

Similarities in parasite species composition among different fish species, species groups or localities were estimated from parasite presence/absence data using the Bray-Curtis coefficient (Bray & Curtis 1957). Patterns of similarity were assessed with non-metric multidimensional scaling (Kruskal & Wish 1978) of pairwise similarity coefficients. Stress values, or the distortion between the original similarity rankings and similarity rankings in the ordination plot, were compared at different dimensionalities of ordination to determine the most suitable plot dimensions. A stress value of less than 0.2 was taken to indicate a reasonable representation of rank similarities by the ordination plot (Clarke 1993). The significance of differences in parasite species composition among fish species, species groups or river systems was

tested by a permutation procedure applied to the pairwise similarity matrix (ANOSIM, implemented using the computer package PRIMER 5.0; Clarke & Gorley 2001).

2.3 Results

2.3.1 Extent of parasitic infection

A total of 1429 individual fishes of 18 different species (12 native and six exotic) from the 29 sampling localities were examined for parasites. Three hundred and twenty seven of these fishes (0.23) were infected with parasites and of the infected fishes, 200 (0.61) were infected with only one species of parasite, while 127 (0.39) were infected with two or more species of parasite.

The number of fishes captured per river ranged from four to 462. Table 2.1 shows the prevalence of parasitic infection and the parasite diversity for all rivers in which 10 or more fishes were sampled. Prevalence of infection was not related to the number of fishes sampled per river, but parasite diversity increased significantly as fish sample size increased (for S, $r^2 = 0.45$, $P < 0.05$; for H_T , $r^2 = 0.43$, $P < 0.05$). There was a trend for prevalence of infection and parasite diversity to increase from north to south, although there were no significant relationships between latitude and either prevalence ($r^2 = 0.38$, $P = 0.06$), parasite species richness ($r^2 = 0.24$, $P = 0.16$) or parasite taxonomic diversity ($r^2 = 0.22$, $P = 0.17$).

Table 2.1 Rivers which were sampled (arranged from north to south), total number of fishes collected (N), overall proportion of infected fishes (prevalence), total number of parasite species found per river (S) and parasite taxonomic diversity per river (H_T).

River	N	Prevalence	S	H_T
Moore River	59	0	0	0
Canning River	441	0.12	8	1.04
Murray River	42	0.02	1	0
Harvey River	113	0	0	0
Harris River	20	0	0	0
Vasse River	46	0.02	1	0
Blackwood River	462	0.28	32	1.52
Kalgan River	139	0.67	17	1.67
Goodga River	76	0.64	17	1.37
Pallinup River	20	0	0	0

2.3.2 *Parasite fauna*

Forty four putative species of parasites were found; six species of protozoans, one myxozoan, two monogeneans, 11 digeneans, nine cestodes, 12 nematodes and three copepod crustaceans (Table 2.2). The parasite sampling procedures which were used were designed principally to detect helminth and arthropod parasites, so the number of protozoan and myxozoan species which were found is almost certainly an underestimate. Although only very few parasites could be identified to species level, morphological differences between all the species listed in Table 2.2 were generally marked and consistent. Most of these putative parasite species were not strictly host

specific (infecting a mean of 2.6 ± 0.2 host species), but were relatively restricted in geographic distribution (found in a mean of 1.7 ± 0.1 rivers).

The 44 species of parasites found in south-western Australia included representatives of all major parasite groups which infect fish, except acanthocephalans. Of the protozoans, the two species of flagellates and the ciliates *Trichodina* sp., *Chilodenella* sp. and *Ichthyophthirius* sp. were all ectoparasitic, principally on the gills and less commonly on the skin, while the microsporidian species was an intracellular parasite of the intestinal wall, kidney and gonads. The ectoparasitic myxozoan species, found in the gills, was tentatively assigned to the genus *Myxobolus*, but preliminary taxonomic studies have suggested that it is quite distinct and may be a new species (F. Ivan & P. O'Donoghue, pers. comm.).

The monogeneans *Dactylogyrus* and *Gyrodactylus* were ectoparasitic on gills. Both are likely to be introduced parasites, but confirmation of this awaits species identification. They were found only on native fish species; *Dactylogyrus* sp. on *T. bostocki* and *N. balstoni*, and *Gyrodactylus* sp. on *G. occidentalis*.

Two species of adult trematodes were found, both in the gastrointestinal tract of their hosts. One of these species has a spiny tegument and may be a lepocrediid or plagiochid (T. Cribb, pers. comm.), but both are likely to be new species and further taxonomic work is proceeding. Nine different metacercarial forms were found, in a much wider range of hosts than the adult species and in a range of sites, including the skin, gills, muscle, gastrointestinal tract, liver, kidneys, spleen, gall bladder and gonads. Metacercarial cysts associated with dark, raised lesions on the skin ('black

spot disease') were identified as *Diplostomum* (probably *D. galaxiae*; Chapman *et al.* 2006). Further taxonomic identification of the other metacercariae will require molecular studies and/or infection experiments to produce adult worms.

Three types of adult cestodes were found in the gastrointestinal tract; one is likely to be a new species of caryophyllidean, but the other two were identified only from fragments of gravid proglottids found in two separate host fishes and further taxonomic placement has not been possible. The other six species of cestodes were all at the larval stage. *Ligula* is thought to have a Northern Hemisphere distribution and has presumably been introduced into Australia. It was found in the abdominal cavity of six native fish species (*G. occidentalis*, *G. maculatus*, *G. truttaceus*, *E. vittata*, *P. olorum* and *A. suppositus*). Isolates from *G. maculatus* and *G. truttaceus* were sequenced for the rDNA ITS2 region, mtDNA cytochrome oxidase subunit I gene and mtDNA cytochrome B gene. The sequences were very similar (although not identical for the COI gene) and most closely related to Chinese isolates of *Ligula*, within a clade of diverse and widely distributed haplotypes (clade B of Bouzid *et al.* 2008). At present it is not clear if this clade is conspecific with the European species *Ligula intestinalis* (Bouzid *et al.* 2008). The other five species of larval cestodes, which have not been identified to date, were found in a variety of sites in the viscera, including the liver, gallbladder, heart and gastrointestinal tract.

Two adult nematode species, *Spirocammallanus* sp. and *Pseudocapillaria* sp., were found in the intestine of fishes. *Spirocammallanus* has not previously been recorded in Australia and may have been introduced; it was found in the exotic poeciliid *G. holbrooki* and one native fish species (*G. occidentalis*) in the Canning River, near

Perth. The *Pseudocapillaria* specimen appears to be a new species and a descriptive taxonomic study is currently underway. The other 10 putative nematode species were all larval stages and found principally in the abdominal cavity, although some were also recovered from the liver, kidney, gonads and gastrointestinal tract. All were anisakids, except one species of *Eustrongylides*, morphologically similar to *E. gadopsis* described by Johnston and Mawson (1940).

Three species of adult copepod crustaceans were found on the skin and gills of fishes. *Lernaea cyprinacea* has been introduced to Australia, presumably on cyprinid hosts (see Chapter 3). It was found only on fishes from the Canning River. Four native fish species (*G. occidentalis*, *E. vittata*, *B. porosa* and *T. bostocki*) and three introduced fish species (*C. auratus*, *G. holbrooki* and *P. caudimaculatus*) were infested. The other two species are most likely native ergasilids. One of these is known from only two specimens and taxonomic description is awaiting further samples, while the other appears to be a new species of *Dermoergasilus* (see Chapter 4).

Table 2.2 All parasite species found in fishes from the South West Coast Drainage Division during this study, number of fish species infected and number of rivers in which the parasite species was found. * Indicates likely exotic origin.

Parasite species	Number of fish species infected	Number of rivers infected
Protozoa		
<i>Trichodina</i> sp.	3	2
<i>Chilodenella</i> sp.	3	1
<i>Ichthyophthirius</i> sp.	1	1
Microsporidian sp.	5	3
Flagellates A	3	2
Flagellates B	4	3
Myxozoa		
<i>Myxobolus</i> sp.	1	1
Monogenea		
* <i>Dactylogyrus</i> sp.	2	1
* <i>Gyrodactylus</i> sp.	1	1
Trematoda		
Metacercaria A	3	3
Metacercaria B	4	3
<i>Diplostomum</i> sp.	3	2
Metacercaria C	6	3
Metacercaria D	4	2
Metacercaria E	1	1
Clinostomidae sp.	1	1
Metacercaria F	2	1
Metacercaria G	1	1
Digenean adult A	2	1
Digenean adult B	1	1
Cestoda		
* <i>Ligula</i> sp.	5	3
Gryporhynchidae A	2	2
Gryporhynchidae B	2	2
Gryporhynchidae C	8	3
Gryporhynchidae D	2	2
Caryophyllaeidae sp.	1	1
Cestode A	3	2
Cestode adult B	1	1
Cestode adult C	1	1
Nematoda		
<i>Eustrongylides</i> sp.	4	4
<i>Contracaecum</i> sp. A	6	2
<i>Contracaecum</i> sp. B	3	1
<i>Contracaecum</i> sp. C	1	1
<i>Anisakis</i> sp.	1	1
* <i>Spirocammallanus</i> sp.	2	1
<i>Pseudocapillaria</i> sp.	1	1
Anisakidae A	4	3
Anisakidae B	4	3
Anisakidae C	1	1
Anisakidae D	1	1
Anisakidae E	1	1
Crustacea		
* <i>Lernea cyprinacea</i>	4	1
Ergasilidae sp.	2	1
<i>Dermoergasilus</i> sp.	2	2

2.3.3 Pathology of infection

Gross pathological effects of infection were seen only in a minority of cases.

Myxobolus infections were associated with gill damage and increased mucus production. All hosts infected with *Ligula* sp. had abdominal distension, with displacement of internal organs, and appeared to be in poor condition. Dilepidid cestode larvae caused severe lesions in the liver when in large numbers.

Eustrongylides were mainly encapsulated in the abdominal cavity, but were occasionally associated with visceral organs, where they caused discolouration and haemorrhage, and in one case in the skin, causing a deep lesion with secondary infection. *Lernaea* infestations were often associated with haemorrhage and skin damage at the site of attachment, and native fishes appeared to be more severely affected than exotic fishes (Chapter 3).

2.3.4 Differences in parasitism among fish species

The number of fish captured per species ranged from two to 398, a reflection of both relative abundances and differences in distribution of different fish species. Table 2.3 shows the prevalence of parasitic infection and the parasite diversity for all fish species with a sample size greater than 10 individuals. Prevalence of infection was not related to the number of fish sampled, but there was a trend for a greater parasite diversity as the fish sample size increased (for S, $r^2 = 0.23$, P = 0.07; for H_T, $r^2 = 0.26$, P = 0.05). Regional species richness curves for each fish species with a sample size greater than 10 individuals are shown in Appendix I. Examination of these curves indicates that an asymptote was approached only for *A. suppositus*, *E. vittata*, *G. maculatus*, *G. occidentalis*, *P. olorum* and *T. bostocki*, suggesting that species richness was underestimated for *B. porosa*, *G. holbrooki*, *G. truttaceus*, *N. balstoni*,

L. wallacei and *P. caudimaculatus*. On the basis of these results, host sample size was used as a covariate in all comparisons of parasite species richness or taxonomic diversity among fish species.

Table 2.3 Fish species and total numbers sampled (N), overall proportion of infected fish (prevalence), total number of parasite species found per fish species (S) and parasite taxonomic diversity per fish species (H_T).

Fish species	Common name	N	Prevalence	S	H_T
Native					
<i>Tandanus bostocki</i>	Freshwater cobbler	72	0.96	22	1.58
<i>Galaxias maculatus</i>	Common jollytail	40	0.90	13	1.36
<i>Pseudogobius olorum</i>	Swan River goby	87	0.56	20	1.51
<i>Nannatherina balstoni</i>	Balston's pygmy perch	12	0.33	4	1.04
<i>Galaxias occidentalis</i>	Western minnow	398	0.28	25	1.66
<i>Galaxias truttaceus</i>	Trout minnow	27	0.15	7	1.35
<i>Afurcagobius suppositus</i>	South-west goby	33	0.12	2	0.69
<i>Edelia vittata</i>	Western pygmy perch	220	0.10	3	1.10
<i>Leptatherina wallacei</i>	Western hardyhead	86	0.09	8	0.97
<i>Bostockia porosa</i>	Nightfish	56	0.07	2	0.69
Exotic					
<i>Gambusia holbrooki</i>	Mosquitofish	268	0.05	7	1.35
<i>Phalloceros caudimaculatus</i>	One spot livebearer	47	0.02	1	0
<i>Carassius auratus</i>	Goldfish	40	0	0	0
<i>Geophagus brasiliensis</i>	Pearl cichlid	16	0	0	0
<i>Perca fluviatilis</i>	Redfin perch	14	0	0	0

Parasitic infection was significantly more common in native fish species (mean prevalence = 0.36 ± 0.09) than in exotic fish species (0.01 ± 0.12) (single factor ANOVA, $F = 8.95$, $P < 0.01$). Parasites were found in all native fish species, but in only two of the exotic fish species that were examined. Similarly, both parasite species richness and parasite taxonomic diversity were significantly greater in native fish species (mean $S = 10.5 \pm 2.3$; mean $H_T = 1.19 \pm 0.14$) than in exotic fish species (mean $S = 1.6 \pm 3.3$; mean $H_T = 0.27 \pm 0.20$) (single factor ANOVAs with fish sample size as a covariate: for S , $F = 13.33$, $P < 0.01$; for H_T , $F = 18.91$, $P < 0.001$). These relationships were consistent over all geographic locations that were sampled.

Despite these differences in parasite prevalence and diversity, there were no significant differences in species composition between native and exotic fish species (Figure 2.2; ANOSIM, $R = 0.18$, $P = 0.18$), indicating that no particular suite of parasites were found in either natives or exotics.

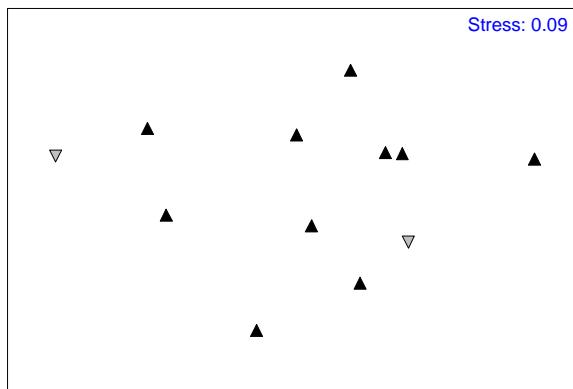


Figure 2.2 Multidimensional scaling plot of pairwise similarity coefficients in species composition between native (black symbols) and exotic (grey symbols) fish species.

Considering only native fish species, there were major differences among species in both prevalence of parasitic infection and parasite species diversity, even when allowing for differences in geographic distribution and sample size. For example, among six native species where more than 30 individuals were sampled from the Blackwood River, prevalence ranged from 0.03 to 0.96, parasite species richness ranged from 1 to 22 and parasite taxonomic diversity ranged from 0 to 1.58.

This variation could not be accounted for by obvious differences in habitat or size. No significant differences were found among fish species which were classed as primarily freshwater or primarily estuarine in either prevalence of infection (single factor ANOVA; $F = 0.40$, $P = 0.54$), parasite species richness (single factor ANOVA with fish sample size as a covariate; $F = 0.16$, $P = 0.70$) or parasite taxonomic diversity (single factor ANOVA with fish sample size as a covariate; $F = 0.63$, $P = 0.45$). Similarly, there were no significant differences among fish species which were primarily demersal or primarily pelagic in prevalence (single factor ANOVA; $F = 0.01$, $P = 0.98$), parasite species richness (single factor ANOVA with fish sample size as a covariate; $F = 0.22$, $P = 0.65$) or parasite taxonomic diversity (single factor ANOVA with fish sample size as a covariate; $F = 0.48$, $P = 0.51$). There was no significant relationship between maximum length of native fish species and parasite species richness ($r^2 = 0.19$, $P = 0.21$) or taxonomic diversity ($r^2 = 0.20$, $P = 0.20$), but prevalence of infection did increase with maximum length ($r^2 = 0.46$, $P < 0.05$). This was almost entirely due, however, to the relatively high infection rate in *T. bostocki* (freshwater cobbler), which are by far the largest native species; when *T. bostocki* was removed from the analysis, there was no longer a significant relationship between prevalence and maximum length ($r^2 = 0.06$, $P = 0.52$).

2.4 Discussion

2.4.1 Parasite fauna of freshwater fishes in south western Australia

The South West Coast Drainage Division contains less than half as many species of freshwater fish as the other major drainage systems of eastern and northern Australia (Morgan *et al.* 1998). The relative paucity of fish species in this region is also reflected in a depauperate parasite fauna. I found 44 putative species of parasites in 1,429 individual fish of 18 different species throughout the region. Dove (2000), by contrast, recorded 109 different parasite species from only 330 individual fish of 18 different species in south eastern Queensland, in the East Coast Drainage Division. It is very likely that my study severely underestimated the number of protozoan and myxozoan species in the south-west, but even when only helminths and arthropods are considered, I found 37 species compared to 77 species in Dove's (2000) study. Parasite diversity is, of course, expected to be influenced by host diversity and studies in other parts of the world with depauperate freshwater fish fauna have also recorded few species of parasites. In Hawaii, for example, Font and Tate (1994) found nine species of helminths in 220 fishes of seven different species, while Choudhury *et al.* (2004) found 17 parasite species in 1,435 fishes belonging to 11 species in the Little Colorado River, Arizona.

The prevalence of parasitic infection and parasite diversity differed markedly among different rivers and different fish species over the South West Coast Drainage Division. Geographically, there was a (non-significant) trend for rates of parasitic infection to increase with increasing latitude; this relationship between geographical locality and parasitism is addressed in Chapter 5. Differences in prevalence of parasitism or parasite diversity among fish species could not be accounted for by

obvious differences in habitat or size, as have been found to affect parasite infection rates in other studies of marine and freshwater fishes (Luque & Poulin 2008). The one factor that overwhelmingly accounted for differences in parasitism in the present study was the origin of the fish species, whether native or exotic.

2.4.2 Parasites of native and exotic fishes

Invasive exotic species constitute a major threat to aquatic biodiversity throughout the world (Arthington & McKenzie 1997; Rahel 2000, 2002; Ricciardi 2004). In the South West Coast Drainage Division of Western Australia, at least nine exotic fish species have been introduced and established self-sustaining populations. Some of these introductions have been officially sanctioned, with release of fishes for angling (e.g. *Oncorhynchus mykiss*, *Salmo trutta*, *Perca fluviatilis*) or mosquito control (*Gambusia holbrooki*), while others have involved accidental or deliberate releases of ornamental fishes (e.g. *Carassius auratus*, *Phalloceros caudimaculatus*). Morgan *et al.* (2004) documented the potential impacts of exotic fishes on native species from predation and competition for food and habitat, but until now there has been no consideration of the role of parasites in this process.

Parasites may influence the success or failure of exotic animal invasions in a number of ways (Prenter *et al.* 2004). Firstly, parasites may mediate competitive interactions between exotic and native species. The parasite release hypothesis proposes that introduced species often escape the parasites that infect them in their native range, leading to increased body sizes, greater population densities and enhanced competitive ability (Keane & Crawley 2002; Torchin *et al.* 2002, 2003; Mitchell & Power 2003). In the present study, I found that exotic freshwater fish species in the

south-west of Western Australia were much less likely to be parasitised than native species, and had significantly reduced regional parasite diversity. This reduced parasite load of exotic species compared to native species was also found by Dove (2000), when comparing the parasites of native freshwater fishes and exotic poeciliids in eastern Australia, and has been reported across a wide range of taxa throughout the world (Torchin *et al.* 2002; 2003; Mitchell & Power 2003). It is thought to arise partly because over-dispersion of parasites among individual hosts means that founding populations of hosts have a low probability of harbouring the species' total parasite fauna, and partly because parasites which are introduced may not be able to maintain their life cycle in the new environment (Bauer 1991; Dove 2000; Torchin *et al.* 2003; Prenter *et al.* 2004).

Despite the number of studies that have found reduced parasitism in exotic invasive species, there are few empirical data to support the other elements of the competitive release hypothesis, namely that reduced parasite loads improve demographic performance and competitive ability of exotics. Torchin *et al.* (2001) showed that introduced populations of the European shore crab (*Carcinus maenas*), were not infected by parasitic castrators, and had greater body size and biomass than their source populations, but to my knowledge there is no direct evidence that loss of parasites improves the competitive ability of exotic species. This is an important area for further research before we can attribute the abundance and apparent competitive success of exotic freshwater fishes, such as *G. holbrooki*, over native species in the South West Coast Drainage Division (Morgan *et al.* 2004).

The other ways in which parasites may influence the impact of exotic invasive species on native species is through parasite transfer, either the transfer of exotic parasites to native fishes or the transfer of native parasites to exotic fishes. Despite the lower prevalence of parasitic infection and reduced diversity of parasites in exotic fishes, compared to native fishes, there were no obvious differences in species composition between exotics and natives. This suggests that transfer of parasites has been occurring.

2.4.3 Transfer of parasites from exotic to native fishes

Despite the typically low parasite load of exotic species, some novel parasites may be introduced and then be transmitted to native species. The infection of naive native hosts by an introduced parasite may have severe consequences on host population size. For example, the monogenean *Nitzchia sturionis* was introduced to the Aral Sea with the Caspian Sea sturgeon *Huso huso*; the parasite then infected the native Aral Sea sturgeon *Acipenser nudiventris*, increasing rapidly in numbers and causing extensive mortality (Dogiel & Lutta 1937, cited in Bauer 1991). Similarly, the fungal parasite *Aphanomyces astaci* was introduced to Europe with the North American crayfish *Austropotamobius pallipes*, causing local extinction of the native European crayfish *Pascifastacus leniusculus* (Holdich & Reeve 1991).

Only five of the 44 parasite species in the present study appear, from previous distribution records, to be exotic in origin; *Gyrodactylus* sp., *Dactylogyrus* sp., *Spirocammallanus* sp., *Ligula* sp. and *Lernaea cyprinacea*. All of these are generalist parasites and were found on native fishes. *Gyrodactylus*, *Dactylogyrus* and *Spirocammallanus* were quite restricted in their distribution, being found in only one

river and on one or two host species. *Lernaea cyprinacea* was also restricted to one river, although it was found on a wider range of hosts; the distribution and prevalence of *L. cyprinacea* infections are considered more fully in Chapter 4. The most likely route of introduction of all these species is with cyprinid aquarium fish.

Ligula sp. had the widest geographic distribution (three different rivers) and host range (five different native fish species) of all the exotic parasite species that were found in this study. It is also potentially the most pathogenic. This tapeworm has a complex life cycle, with adults found in the intestine of piscivorous birds. Eggs are voided with faeces into the water, where they are ingested by the first intermediate host, copepod crustaceans. Copepods are then eaten by fish, which form the second intermediate host. Within the fish host the parasite develops into a plerocercoid larva, which may live for 12-14 months and grow up to 200 mm in length. Heavily infected fish have grossly distended abdomens, markedly reduced gonad mass, and are weak and slow-moving, making them an easy target for predators. Chapman *et al.* (2006) reported significant reductions in body weight and gonad weight of *Galaxias maculatus* infected with *Ligula* in Moates Lake, a permanent freshwater lake into which the Goodga River drains on the south coast of Western Australia. When infected fish are eaten by birds, the ingested parasites develop to the adult stage and produce eggs within two days.

Ligula is more likely to have been introduced with fish hosts than with migratory birds, because the adult stage of the parasite is short-lived (< 5 days). Pollard (1974) suggested that *Ligula* in eastern Australia could have been introduced with *Salmo trutta*, *Oncorhynchus mykiss* or *Perca fluviatilis*, all of which have been recorded as

hosts in the northern hemisphere. Once the parasite was introduced into the south-west of Western Australia, numerous piscivorous birds could act as definitive hosts. White-faced herons, *Egretta novaehollandiae*, little pied cormorants, *Phalacrocorax melanoleucos*, and hoary-headed grebes, *Poliocephalus poliocephalus*, are all abundant in the localities where *Ligula* specimens were found and are all known predators of native freshwater fish (Barker & Vestjens 1989; Chapman *et al.* 2006)

2.4.4 Transfer of parasites from native to exotic fishes

Although many endemic native parasites may not be able to transfer to exotic species, those that do may increase their biotic potential through an expanded host range. The exotic hosts may then act as reservoirs, allowing the parasite to maintain high population densities even as native host populations decline. Of the eight parasite species found on exotic hosts in this study, six were presumably native parasites which had transferred to new host species. *Gambusia holbrooki*, the most abundant and widespread exotic fish species, harboured six species of parasite, all of which were native. Although the potential for *Gambusia* to negatively impact on native freshwater fish populations through resource competition, aggressive interference competition and predation on eggs and juveniles has been noted by Lloyd (1990), Gill *et al.* (1999), Arthington and MacKenzie (1997) and Morgan *et al.* (2004), there has as yet been no consideration of the dangers posed by this species acting as a reservoir for naturally occurring parasitic infections of native fish.

CHAPTER 3

An introduced parasite, *Lernaea cyprinacea* L., found on native freshwater fishes in the south-west of Western Australia

3.1 Introduction

The South West Coast Drainage Division (S.W.C.D.D) contains a depauparate, but highly endemic freshwater fish fauna, with eight of the 10 native species found in the region being endemic (Morgan *et al.* 1998; see Chapter 2). A number of exotic fish species have also been introduced into south west rivers, either deliberately or as escapees from aquaculture or the aquarium trade (Morgan *et al.* 2004; see Chapter 2). Morgan *et al.* (2004) documented a number of potentially deleterious impacts of introduced fishes on native species in the south west, including predation, competition and habitat alteration. Introduced fishes may also transfer exotic diseases to native species, and this has been an increasing cause of concern for the health of freshwater environments throughout the world (Bauer 1991; Kennedy 1993; Arthington & McKenzie 1997; Levy 2004). In Chapter 2, I documented five species of parasites that appear to be exotic, on native fish species in the south-west of Western Australia.

Lernaeosis is a disease of freshwater fishes caused by parasitic copepods of the family Lernaeidae (anchor worms). About 110 species of lernaeids have been described in 14 different genera (Ho 1998). The most common species is *Lernaea cyprinacea* L., which has been widely translocated with cultured fish species and is now found throughout North America, Europe, Asia, southern Africa and eastern Australia (Hoffman 1970; Lester & Hayward 2006). *Lernaea cyprinacea* has a very

wide host range and has been found on more than 45 species of cyprinids, as well as fish belonging to many other orders and occasionally on tadpoles (Tidd & Shields 1963; Lester & Hayward 2006).

Although *L. cyprinacea* is not native to Australia, the parasite has been recorded from a number of native and cultured fish species in New South Wales and Victoria, in south-eastern Australia (Ashburner 1978; Hall 1983; Callinan 1988; Rowland & Ingram 1991; Dove 2000; Bond 2004). My study provides the first report of *L. cyprinacea* in Western Australia, and in this chapter I report the geographic distribution, host range and pathogenic effects of the parasite.

3.2 Materials and methods

As part of a larger study on the parasite fauna of freshwater fishes in Western Australia (see Chapter 2), 1429 fishes of 18 different species from 29 localities in 12 rivers, spanning the extent of the South West Coast Drainage Division were sampled for parasites (Figure 3.1). After *Lernaea* sp. infestations were found at one locality in the Canning River, a further five localities in the Swan/Canning system were sampled (Figure 3.1). Adult and juvenile fishes were captured during summer and autumn, 2005-2007, using a combination of seine nets (3 mm mesh), fyke nets (2 mm mesh) and electrofishers. Water temperature and conductivity were recorded for each locality on each sampling occasion. Fishes were returned to the laboratory, weighed and measured for total length (TL), and the skin and gills examined externally for *Lernaea* infestation using a dissecting microscope. *Lernaea* specimens were removed and preserved in 70% ethanol. After clearing in lactophenol, they were mounted whole for identification using a compound microscope. Parasite data

were expressed as prevalences (proportion of infested hosts) and intensities of infestation (number of parasites per infested host). Ninety five percent confidence intervals were calculated for prevalences, assuming a binomial distribution, and intensities, from 2,000 bootstrap replications, using the software Quantitative Parasitology 3.0 (Rózsa *et al.* 2000). Differences in size between infested and non-infested hosts were tested by analysis of variance.

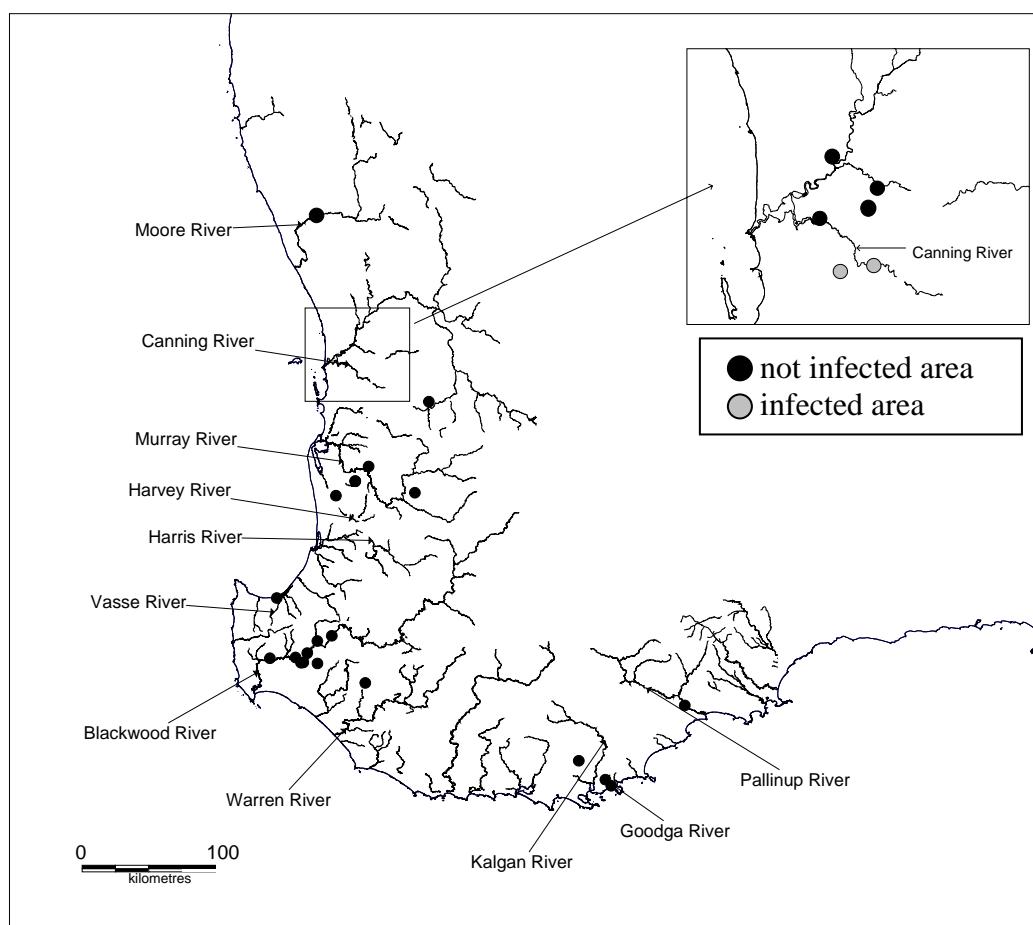


Figure 3.1. South West Coast Drainage Division, showing sampling locations. Black circles indicate that no fishes from that location were infested with *Lernaea cyprinacea*, grey circles indicate that *Lernaea cyprinacea* were found on some fishes.

3.3 Results

Of the 12 different rivers sampled, *L. cyprinacea* were found only on fishes from the Swan/Canning system. Infested fish were found at two of six localities in this system (Figure 3.1). Water temperatures at these localities over the sampling period ranged from 17-30°C and salinities from 2,000-4,000 mgL⁻¹. Overall prevalences (i.e. prevalences of infestation for all fish species) at the two localities were 0.06 (n = 63 fishes) at Soldier Crossing and 0.18 (n = 231 fishes) at Southern River.

Four native fish species (*Galaxias occidentalis*, *Edelia vittata*, *Bostockia porosa*, *Tandanus bostocki*) and three introduced fish species (*Carassius auratus*, *Gambusia holbrooki* and *Phalloceros caudimaculatus*) were infested at the two localities. Prevalences differed between fish species, with the native species *G. occidentalis* and *E. vittata* being most heavily infested (Table 3.1). For these two species, infested fish tended to be larger, although this difference was significant only for *E. vittata* (mean TL of infested fish = 38.5 mm, mean TL of non-infested fish = 32.2 mm, F = 6.28, P < 0.01). For the introduced *C. auratus*, infested fish were significantly smaller, although the sample size of infested fish was low (mean TL of infested fish = 75.5 mm, mean TL of non-infested fish = 104.2 mm; F = 4.04, P < 0.05). Most infested fishes contained a single parasite, with a mean intensity over all fish species of 1.29 (95% confidence interval = 1.12-1.45; range = 1-3). Of the 45 *Lernaea* that were found, 96% were adults and 72% were attached to the base of the dorsal fin of their host, with the remainder attached to either the base of the caudal, pectoral or pelvic fins or to the general body surface.

Table 3.1 Prevalence (with 95% confidence interval in parentheses) of *L.cyprinacea* infestation in seven species of fish captured at two localities in the Canning River, Western Australia.

Species	Location	N	Prevalence
<i>Galaxias occidentalis</i>	Southern River	40	0.40 (0.25-0.60)
	Soldier Crossing	20	0.00
<i>Edelia vittata</i>	Southern River	76	0.20 (0.12-0.30)
	Soldier Crossing	13	0.23 (0.07-0.52)
<i>Bostockia porosa</i>	Southern River	10	0.10 (0.00-0.48)
	Soldier Crossing	10	0.10 (0.00-0.48)
<i>Tandanus bostocki</i>	Southern River	1	1.00
<i>Carassius auratus</i>	Southern River	39	0.10 (0.04-0.24)
<i>Gambusia holbrooki</i>	Southern River	39	0.02 (0.00-0.14)
	Soldier Crossing	20	0.00
<i>Phalloceros caudimaculatus</i>	Southern River	27	0.04 (0.00-0.18)

The morphology of the copepodid larva and the attachment organ of the adult female are shown in Figure 3.2, 3.3 and 3.4. All parasites were morphologically similar to *Lernaea cyprinacea* as described by Kabata (1979).



Figure 3.2 *Lernaea cyprinacea* larva found on infested fish.

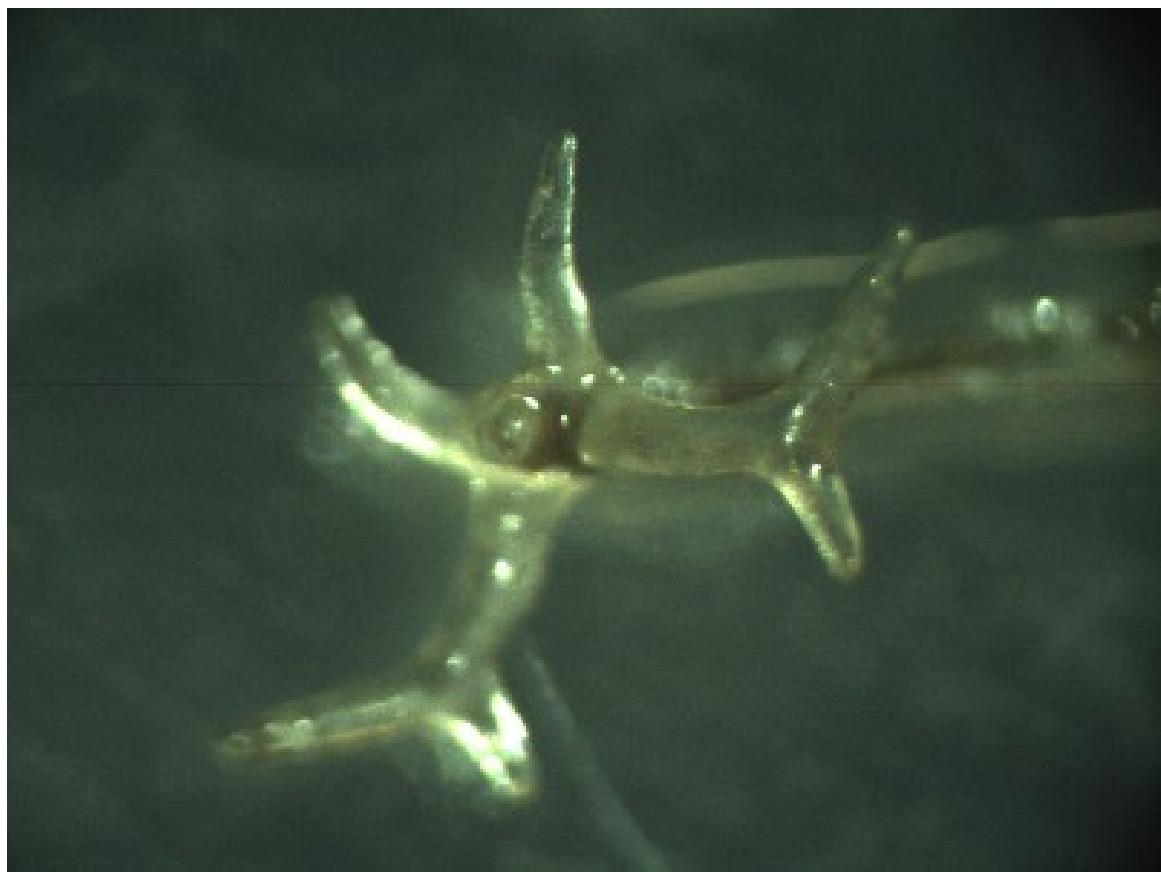


Figure 3.3 Attachment organ of adult female *Lernaea cyprinacea*

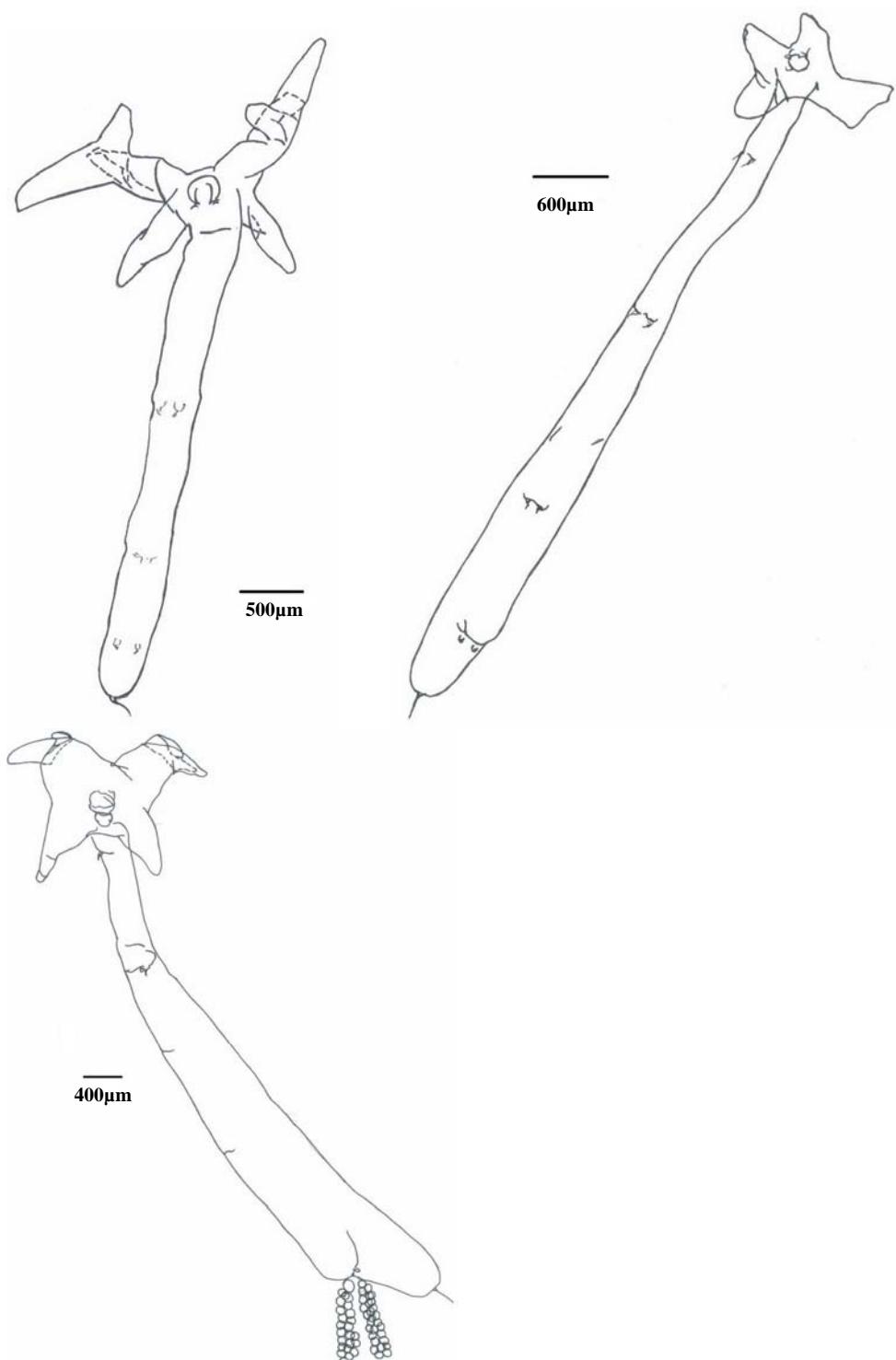


Figure 3.4 Adult female *Lernaea cyprinacea* found on the infested fish from this study, showing different morphological forms of the attachment organ.

I found extensive hemorrhages and ulceration at attachment sites in infested native fish (Figure 3.5) and also captured several fish without *L. cyprinacea*, but with large scars at the base of their dorsal fins, which are likely to have been caused by previous infestations.

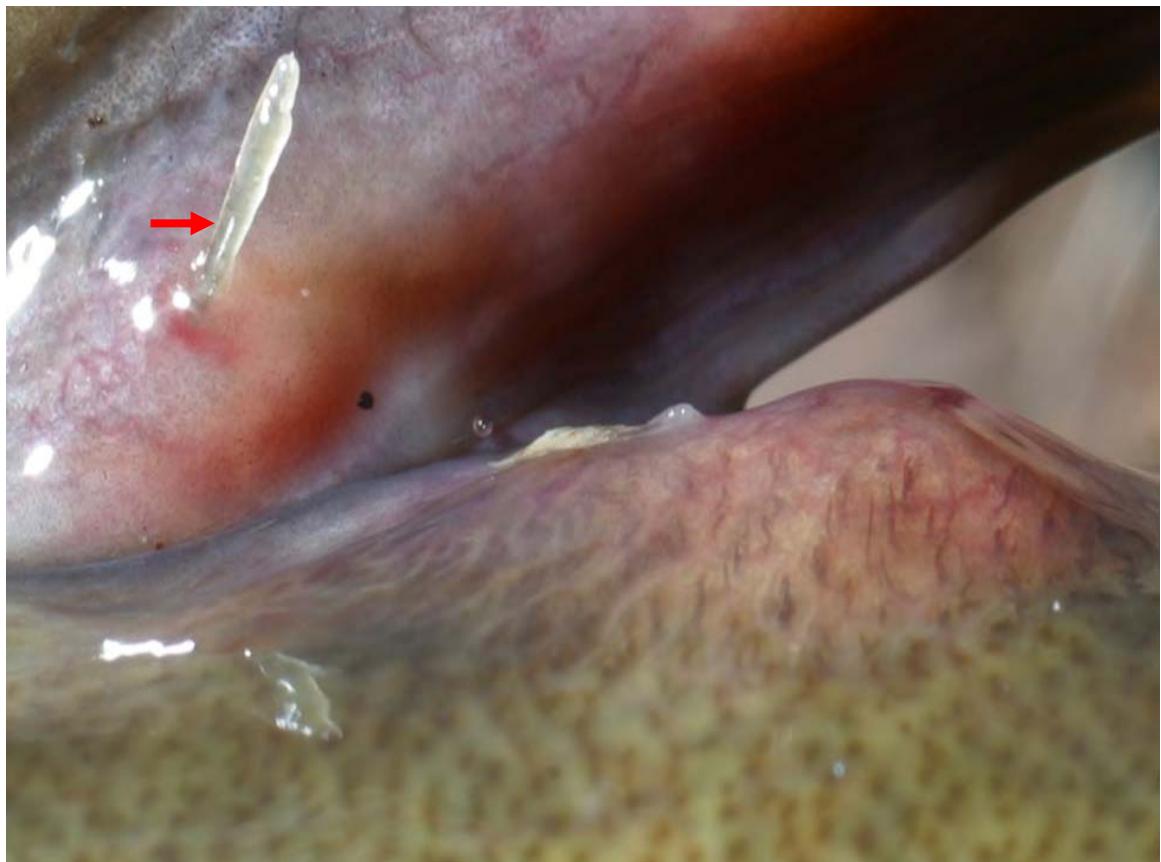


Figure 3.5 Ulcerated lesions at the site of attachment of female *Lernaea cyprinacea* (arrow) to a freshwater cobbler (*Tandanus bostocki*) host.

3.4 Discussion

This is the first account of *L. cyprinacea* infestation on fish in Western Australia. It appears that the species in Western Australia is most likely to be *L. cyprinacea*, although the considerable morphological plasticity of species of *Lernaea* complicates

morphological identification (Kabata 1979; Lester & Hayward 2006), and definitive confirmation of species identity will require molecular genetic studies.

Lernaea cyprinacea has been widely introduced throughout the world, presumably through the translocation of cyprinid hosts such as *C. auratus* and European carp, *Cyprinus carpio*. This is also the likely origin of the parasite in Western Australia, possibly through the release or escape of infested aquarium fish into natural waterways. Morgan *et al.* (2004) have reported *C. auratus* and *C. carpio* from many streams, irrigation drains and lakes in the vicinity of Perth, and also from a number of other natural waterways between the Moore and Vasse Rivers on the Swan Coastal Plain.

I found *L. cyprinacea* infestations on seven different species of fish, with the greatest prevalence on the native species *G. occidentalis* and *E. vittata*, rather than the natural cyprinid hosts of the parasite. Differences in infestation levels among different host species have also been reported in many other studies (e.g. Demaree 1967; Shariff & Kabata 1986; Marcogliese 1991; Bond 2004) and may result from different encounter frequencies between host and parasite, from differences in the rate of attachment of the parasite to different host species or from differences in the immune response of different host species to the parasite. At this stage, I have no information on the reason for the greater rate of infestation of *G. occidentalis* and *E. vittata*, but may lie in morphology (e.g. *G. occidentalis* has no scales) or migratory behavior, nothing that host species undertake considerable migration that may expose them to parasites.

The results suggest that *L. cyprinacea* is currently confined to a relatively small section of the Canning River, although future surveys around the Perth metropolitan area are necessary to confirm this. The spread from this population is likely to be slow, because of the life cycle of the parasite and the physical characteristics of the river, which is short and separated from other river systems by an extensive estuary. Female *Lernaea*, attached to the body of their host, produce eggs which hatch into free-living naupliar larvae. After about four days, the naupliar larvae moult to infective copepodid larvae, which attach, usually to the gills of a host fish. Copepodids moult to adults after a week or more, depending on the temperature, with optimal development occurring at 28-36°C and little development below 20°C (Shields & Tidd 1968; Marcogliese 1991; Lester & Hayward 2006). Adult males die within 24 hours and fertilised females either attach to the same host or swim to another host. Distribution and migration of the parasite in the south-west is likely to be restricted by its direct life cycle, temperature-dependent development, low salinity tolerance and reduced survival in fast-flowing water (Bulow *et al.* 1979; Medeiros & Maltchik 1999; Lester & Hayward 2006).

Lernaea infestations often have serious pathogenic effects on their fish hosts. Copepodites may cause disruption and necrosis of gill epithelium, while attachment of adult females usually causes hemorrhages, muscle necrosis and an intense inflammatory response, sometimes associated with secondary bacterial infections (Khalifa & Post 1976; Berry *et al.* 1991; Lester & Hayward 2006). Bond (2004) demonstrated high mortality rates and reduced swimming ability, which might predispose to greater predation rates, in two species of native eastern Australian freshwater fish (*Galaxias olidus* and *Nannoperca australis*) infested with *Lernaea*.

There is evidence that the pathological effects of *Lernaea* infestations are greater on smaller fish because the attachment organ of the parasite penetrates more deeply into the body of the fish, often causing damage to internal organs (Khalifa & Post 1976; Lester & Hayward 2006). Most native freshwater fishes in the south-west of Western Australian are much smaller than typical cyprinid hosts, and the greater prevalence of infestation on larger native fish in this study may result from an increased mortality rate of infested small native fish, although this is speculative and remains to be tested experimentally.

Elimination of *L. cyprinacea* from the Canning River is not likely to be achieved. Although the parasite appears to be relatively confined, it has spread to a number of different fish species, both native and introduced. A number of chemical treatments are effective against copepodids (although less effective against embedded adults or nauplii), and in a closed culture system these can be applied over a number of weeks to break the life cycle of the parasite (Lester & Hayward 2006). In an open, natural river system this is not a feasible proposition. The best prospect for containing the spread of the parasite is to prevent future releases of infested hosts into other river systems. This will require an extensive education campaign to alert the public to the threat posed by this, and other exotic diseases, which may be associated with aquarium fish.

CHAPTER 4

A new species of *Dermoergasilus westernensis* n. sp. (Copepoda: Ergasilidae) from freshwater fishes in the south-west of Western Australia

4.1 Introduction

The family Ergasilidae comprises 25 genera and more than 260 species of ectoparasitic copepods, found principally on freshwater, brackish and marine teleosts. Although a number of species of ergasilids have been described from Australian fishes, there are likely to be many more undescribed species (Byrnes 1986; Kabata 1992). This is particularly the case in freshwater environments, as the parasitic fauna of Australian freshwater fishes has been very poorly studied.

As part of a parasitological survey of freshwater fishes in the South West Coast Drainage Division (S.W.C.D.D) of Western Australia (Chapter 2), I found a new ergasilid species, belonging to the genus *Dermoergasilus* Ho & Do 1982, on two species of hosts. In this chapter, I describe the new species and compared it with related species in the genus.

4.2 Materials and methods

Specimens of an unidentified copepods were found on the gills of freshwater cobbler, *Tandanus bostocki*, caught in the Blackwood River (34° 07'S 115° 29'E) and on the gills of western minnow, *Galaxias occidentalis*, caught in the Swan River (31° 56'S, 115° 54' E), both sites occur within the S.W.C.D.D. of Western Australia. All specimens were preserved in 70% ethanol, stained with Chlorazol black E, dissected and mounted as temporary preparations in lactophenol. Mounted specimens were

observed with a Motic BA200 microscope, and measurements and illustrations were made using the software Motic Images Plus 2.0. All measurements are presented in micrometres as the mean, with range in parentheses.

Descriptions are based on four specimens from the Blackwood River and four specimens from the Swan River. These differed in size, so measurements are presented separately for each population, but did not differ in any details of body shape or the structure of appendages. Body terminology follows Kabata (1992), El-Rashidy & Boxshall (2001) and Montu & Boxshall (2002). All descriptions refer only to female copepods, as males and larval stages are free living and their morphology is not known.

4.3 Results

4.3.1 *Dermoergasilus westernensis* n. sp.

Description

All measurements are shown in Table 4.1. Body gradually narrowed posteriorly (Figure 4.1A). Cephalothorax oblong, with anterior margin rounded and posterior transversely rounded, transverse suture-like division extending across posterior half of dorsal surface and partly over lateral walls. Anterior half of dorsal surface markings shaped like inverted ‘T’ (Figure 4.1A). Antennule and antenna visible from dorsal view (Figure 4.1A). First pedigerous somite incorporated into cephalothorax. Free pedigerous somites decreasing in width posteriorly (Figure 4.1A). Fifth pediger fused with genital double-somite. Genital double-somite wider at anterior and gradually decreasing posteriorly (Figure 4.1B). Ventral surface of genital double-somite with coarse spinules and one straight row of spinules at the anterior margin.

Table 4.1 Measurements of body characters in specimens of *Dermoergasilus westernensis* n. sp. from two different localities and hosts. All measurements in micrometres as the mean, with range in parentheses. For each population, n = 4.

Character	Locality (host species)	
	Blackwood River (<i>Tandanus bostocki</i>)	Swan River (<i>Galaxias occidentalis</i>)
Total length (excluding antennae and setae of caudal rami)	1118.6 (1032.8-1212.4)	929.8 (752.5-1113.4)
Cephalothorax		
length	649.6 (598.4-708.0)	499.9 (335.5-640.1)
Width	246.6 (216.2-294.9)	238.7 (166.3-306.4)
Free pedigerous somite length	69.1 (51.2-81.6)	49.2 (31.8-60.8)
Genital double somite		
length	116.5 (109.2-127.3)	115.6 (83.8-143.7)
Width	88.2 (84.7-91.1)	84.3 (74.2-95.3)
Abdominal somite I		
length	78.0 (72.7-82.4)	63.3 (32.3-81.5)
Width	65.6 (60.7-68.9)	59.7 (50.6-69.9)
Abdominal somite II		
length	21.7 (20.1-23.5)	17.8 (14.2-23.6)
Width	52.2 (38.4-57.9)	49.2 (39.0-58.5)
Abdominal somite III		
length	23.7 (22.1-24.7)	20.2 (14.7-26.0)
Width	44.1 (33.0-50.1)	22.5 (17.7-29.5)
Caudal rami		
length	21.8 (21.0-23.2)	13.4 (10.3-19.6)
Width	18.2 (17.8-18.9)	16.8 (14.5-18.9)

Abdomen with 3 free somites. Ventral surface of abdominal somite with single row of spinules at the anterior, whereas second abdominal somite with single row of spinules along the posterior margin (Figure 4.1B). Caudal rami armed with a digital process and three setae; one long seta medially, two small setae from lateral margin (Figure 4.1C).

First antenna 6-segmented, tapering; first segment bearing 3 setae; second, 8; third, 4; fourth, 4; fifth, 4; sixth, 6 (Figure 4.2A). Second antenna 4-segmented, comprising coxobasis and 3-segmented endopod (Figure 4.2B). Coxobasis short; second segment nearly 1.5 times longer than coxobasis. Third segment curved and about half length of second segment. Terminal claw strongly recurved and about two-thirds length of third segment. Second antenna, except terminal claw, covered with inflated transparent membrane.

Mouthparts consisting of mandible, maxillule and maxilla (Figure 4.2C). Mandible with two blades anteriorly and one blade posteriorly. Larger anterior blade bearing row of curved strong teeth along posterior edge. Other two blades with row of thin spinules on the anterior margin. Maxillule with two setae, one slightly shorter. Maxilla 2-segmented, proximal segment larger, distal segment small and spinulose. Interpodal sternites wide and ornamented, with a few rows of spinules at posterior margin (Figure 4.2D).

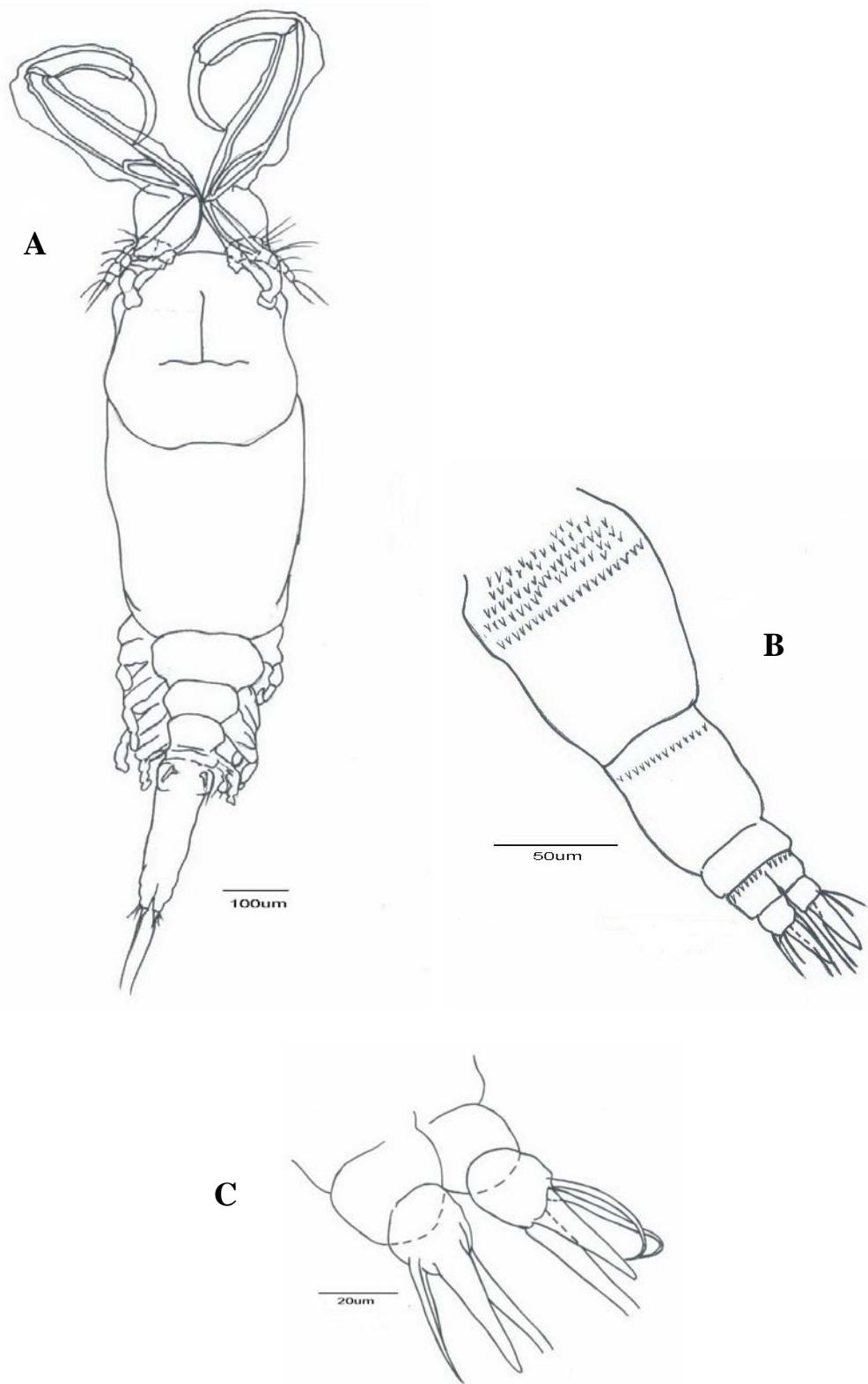


Fig. 4.1 *Dermoergasilus westernensis* n. sp., adult female. (A) Dorsal view. (B) Genital complex to distal end, dorsal view. (C) Anal somite and caudal rami, dorsal view.

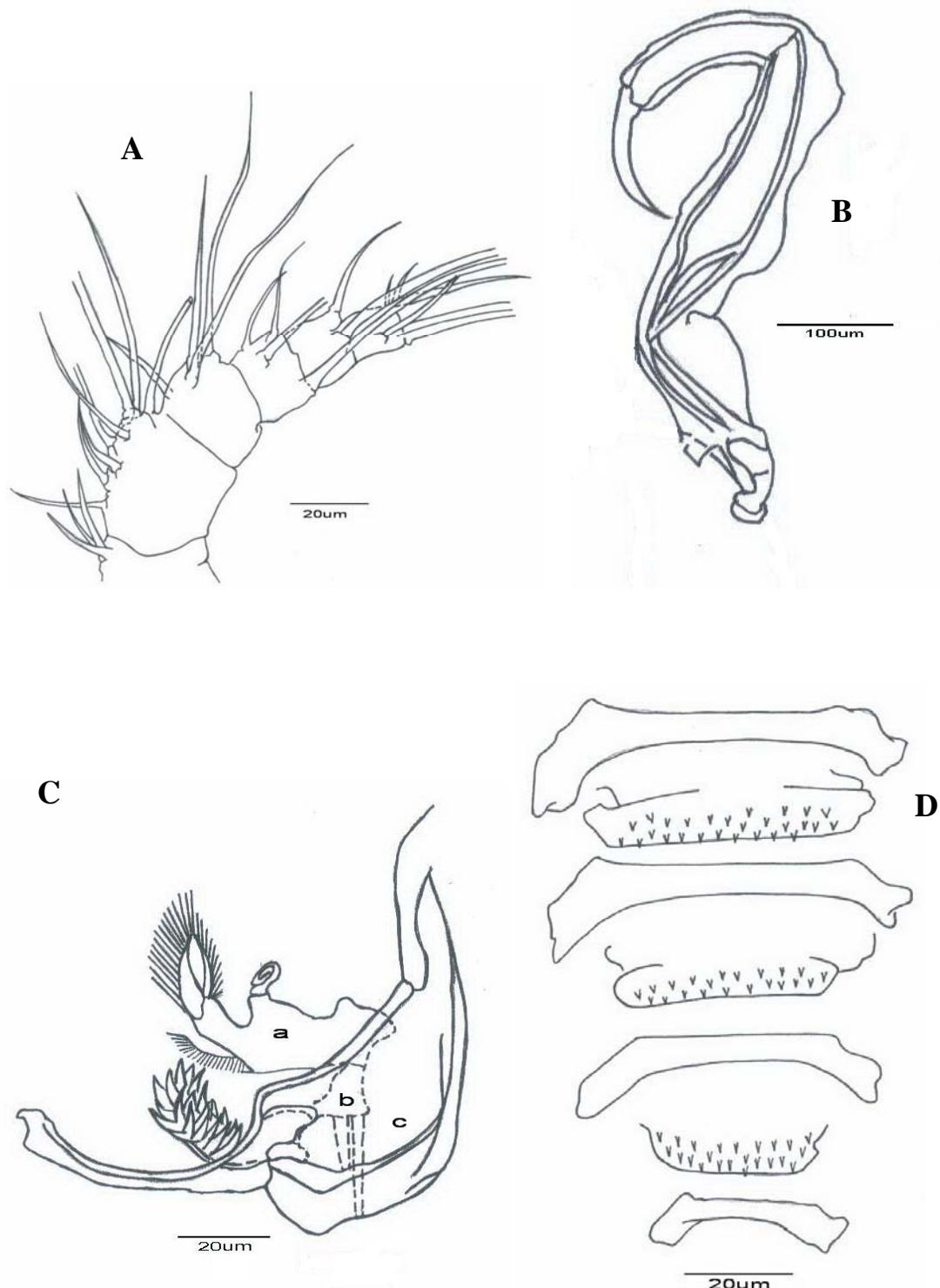


Fig. 4.2 *Dermoergasilus westernensis* n. sp., adult female. (A) Antenna. (B) Antennule. (C) Mouth parts: a- mandible, b-maxillule, c-maxilla. (D) Interpodal sternites.

Swimming legs 1-4 with rami 3-segmented, except 2-segmented exopod of leg 4.

Leg armature as follows (spines - roman numerals; setae - Arabic numerals):

	Coxa	Basis	Endopod	Exopod
Leg 1	0-0	1-0	0-1; 0-1; II,4	I-0; 0-1; II,5
Leg 2	0-0	1-0	0-1; 0-1; I, 4	I-0; 0-1; 6
Leg 3	0-0	1-0	0-1; 0-1; I,4	I-0; 0-1; 6
Leg 4	0-0	1-0	0-1; 0-2; I,3	I-0; 5; -

Leg 1 (Figure 4.3A) with outer seta on posterior surface of basis and patch of spinules at inner distal surface. Both rami segments spinulate laterally; first exopodal segment pinnulate medially; outer apical spines on both rami spinulate along lateral margins. Leg 2 (Figure 4.3B) with outer seta on posterior surface of basis and row of spinules on posterior margin; both rami segments spinulate laterally; first exopodal segment pinnulate medially; outer apical spine on endopodal segment spinulate along lateral margin. Leg 3 (Figure 4.3C) with outer seta on posterior surface of basis and row of spinules on posterior margin; both rami segments spinulate laterally; first exopodal segment pinnulate medially; outer apical spine on endopodal segment spinulate along lateral margin. Leg 4 (Figure 4.4A) with outer seta on posterior surface of basis and row of spinules on posterior margin; 2-segmented exopod; both rami segments spinulate laterally; first exopodal segment pinnulate medially; outer apical spine on endopodal segment spinulate along lateral margin. Leg 5 (Figure 4.4B) with two segments; first segment short, with seta; second segment with two terminal setae of unequal length.

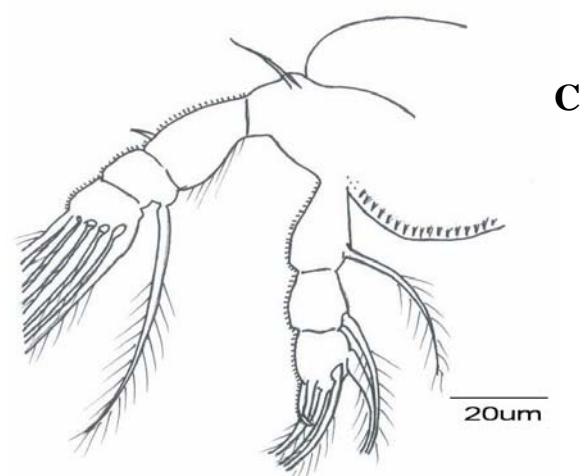
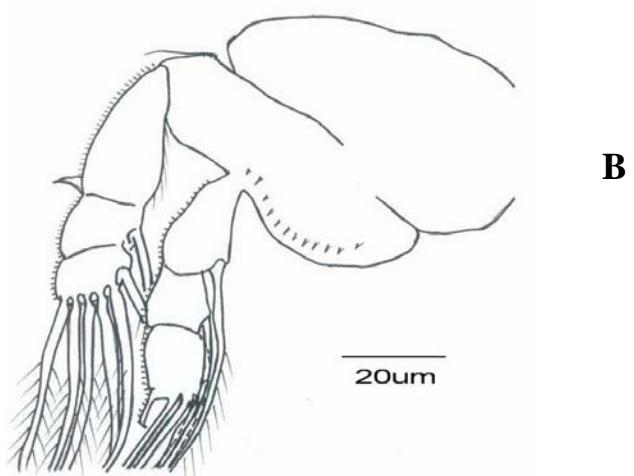
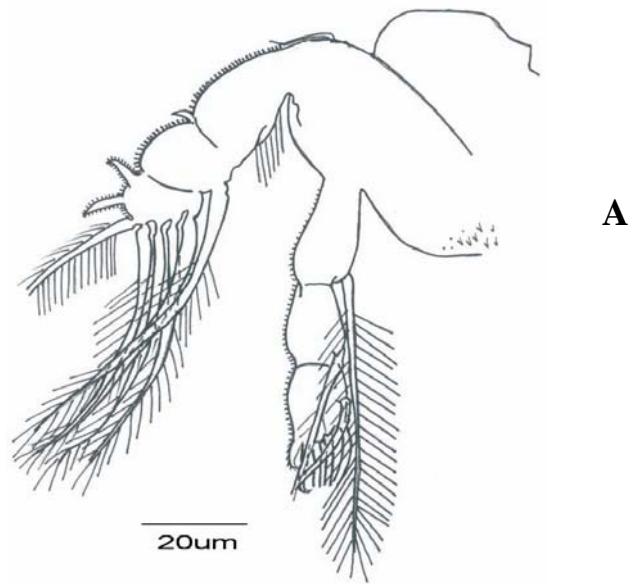


Fig. 4.3 *Dermoergasilus westernensis* n. sp., adult female. (A) First swimming leg, anterior. (B) Second swimming leg, anterior. (C) Third swimming leg, anterior.

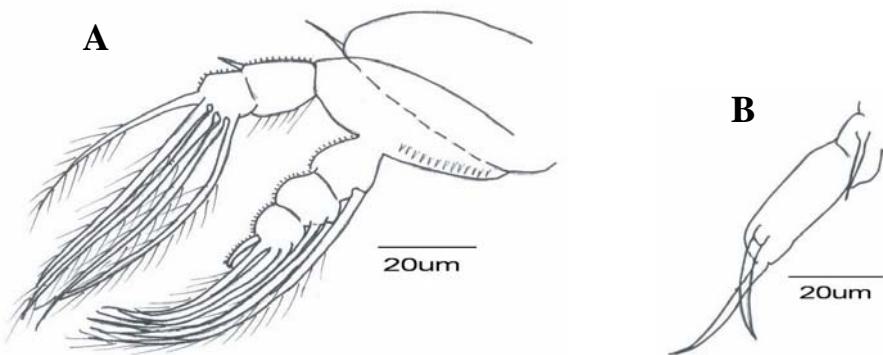


Fig. 4.4 *Dermoergasilus westernensis* n. sp., adult female. (A) Fourth swimming leg, anterior. (B) Fifth leg.

Type material: Holotype (WAM C40042) and two paratypes (WAM C40043, WAM C40044) deposited in the Western Australian Museum (WAM).

Type-host: *Tandanus bostocki* Whitley, 1944 (Plotosidae).

Type-locality: Jalbarrangup, Blackwood River, Western Australia ($34^{\circ} 07'S$ $115^{\circ} 29'E$).

Site: Gills

Etymology: The specific name indicates the Western Australian origin of this new species.

4.4 Discussion

The genus *Dermoergasilus* was proposed by Ho & Do (1982) and, although it is regarded as of uncertain validity, is currently retained pending a generic level revision of the family Ergasilidae (Kabata 1992; El-Rashidy & Boxshall 2001).

Species of *Dermoergasilus* are found in the Indian and Indo-West Pacific regions.

Ten species have been previously described, from a range of host groups (Table 4.2). Four of these species have been found in Australia, all from marine or estuarine hosts

in coastal areas (Byrnes 1986; Kabata 1992). The only previous record of *Dermoergasilus* in Western Australia is *D. acanthopagri* Byrnes 1986, from the sparid *Acanthopagrus butcheri* (Byrnes 1986).

Table 4.2 Species of *Dermoergasilus* previously described (based on data in El-Rashidy & Boxshall 2001).

Species	Geographic range	Host range (family)
<i>D. acanthopagri</i>	Australia	Sparidae
<i>D. amplectens</i>	Australia, India, Japan, South Africa, Russia	Mugilidae, Cichlidae, Gerreidae, Hemirhamphidae, Megalopidae, Chanidae
<i>D. intermedius</i>	Australia	Clupeidae, Plotosidae, Percichthyidae
<i>D. semicoleus</i>	Australia	Belonidae
<i>D. coleus</i>	Borneo, India, Philippines, South Africa	Belonidae, Sparidae
<i>D. curtus</i>	India	Mugilidae
<i>D. longiabdominalis</i>	India, Philippines, Madagascar	Mugilidae
<i>D. mugilis</i>	South Africa	Mugilidae
<i>D. semiamplectens</i>	Burma, China, India	Mugilidae
<i>D. varicoleus</i>	India, Iraq	Mugilidae

The new species of *Dermoergasilus* described in this chapter is the first record in Australia of this genus on purely freshwater host species. Specimens from the Blackwood River, found on *T. bostocki*, were larger than specimens from the Swan River, found on *G. occidentalis*, although they were identical in body shape and structure of the appendages. The size difference may indicate genetically differentiated populations or may be a phenotypic response to different host species or environmental conditions.

The new species is morphologically distinct from all previously described species of *Dermoergasilus*. The armature of the exopods of legs 1-4 differentiates it from *D. amplexens* (Dogiel & Akhmerov 1952), *D. coleus* (Cressey 1970), *D. mugilis* Oldewage & van As 1988 and *D. semicoleus* (Cressey 1970); and the armature of the endopod differentiates it from *D. acanthopagri* Byrnes 1986 (see Ho *et al.* 1992). In leg armature, the new species is most similar to a group formed of *D. varicoleus* Ho, Jayarajan & Radhakrishnan 1992, *D. longiabdominalis* El-Rashidy & Boxshall 2001, *D. semiamplexans* El-Rashidy & Boxshall 2001 and *D. curtis* El-Rashidy & Boxshall 2001, all of which are parasites of mugilids. The new species can be distinguished from *D. varicoleus* in the armature of the first antenna, the relative proportions of the second antenna and the absence of the minute terminal spine on the digital process of the caudal ramus; from *D. longiabdominalis*, which has a long caudal ramus seta ornamented by fine spinules, rather like a rasp; from *D. semiamplexans* in the armature of the first antenna, the relative proportions of the second antenna and the relative proportions of the genital double somite and the three abdominal segments; and from *D. curtis*, which has a hyaline inflated cuticle only around the first endopod segment of the antenna (El-Rashidy & Boxshall 2001).

CHAPTER 5

Environmental factors affecting parasitism of native freshwater fishes in the south-west of Western Australia

5.1 Introduction

In recent years, there has been a growing recognition of the crucial roles played by parasites in determining the flow of energy and nutrients through an ecosystem (Poulin 1999; Marcogliese 2004; Hudson *et al.* 2006). Parasites, by their nature, are embedded in the food webs of the communities in which they occur (Marcogliese & Cone 1997; Lafferty *et al.* 2006) and may have important impacts on ecosystem function through their regulation of host population size (Hudson *et al.* 1998), mediation of competition between hosts (Poulin 1999; Thomas *et al.* 2000) and roles as ecosystem engineers (Thomas *et al.* 1999). Environmental changes which affect the abundance and diversity of parasites, therefore, are likely to impact on ecosystems at many different trophic levels.

This suggests that parasites may be useful indicators of environmental stress on ecosystems and a number of studies have found that anthropogenic impacts may lead to changes in parasite fauna (MacKenzie *et al.* 1995; Lafferty 1997; Landsberg *et al.* 1998; Galli *et al.* 2001; Sures 2004; Marcogliese 2005). An important limitation of parasites as ecosystem bioindicators, however, is the difficulty in establishing causal pathways between environmental changes and changes in parasitism. Rates of parasite infection may increase if environmental changes enhance the survival of infective parasite stages, increase the contact between infective stages and their hosts, or increase the susceptibility of the host to infective stages of the parasite. It is

also possible, however, for rates of parasite infection to decrease if environmental changes reduce the survival of infective parasite stages or limit the contact between infective stages and their hosts. Furthermore, parasites as a group are taxonomically diverse and have a bewildering array of life cycles, and the same environmental change is likely to affect different parasite species in very different ways (Lafferty 1997). Understanding parasite biology, therefore, is essential to understanding how environmental changes will change parasite abundance and diversity (Marcogliese 2004).

Freshwater ecosystems in the south-west of Western Australia have a depauperate, but highly endemic freshwater fish fauna, which is under threat from a range of anthropogenic processes, including river regulation, the extraction of water for irrigation, salinisation and sedimentation as a result of land clearing, eutrophication from agricultural and urban wastes, and the accidental or deliberate introduction of exotic animals and plants (Howe *et al.* 1997; Pen 1999; Morgan *et al.* 1998, 2003; Pollino *et al.* 2004). Forty four different species of parasites have been found in freshwater fishes in the South West Coast Drainage Division; six species of protozoans, one myxozoan, two monogeneans, 11 digeneans, nine cestodes, 12 nematodes and three copepod crustaceans (see Chapter 2). In this chapter, I examine the geographic distribution of these parasite species in relation to environmental factors potentially impacting on the aquatic habitats in which they occur. My aims were to determine the correlations between habitat disturbance and both the prevalence of parasitic infection and the diversity of parasite taxa in native freshwater fishes.

5.2 Materials and methods

5.2.1 Fish sampling

Fishes were collected from 24 localities in nine separate rivers in the South West Coast Drainage Division of Western Australia. At each locality, the latitude and longitude was recorded using GPS, and scored against five measures of habitat disturbance (river regulation, loss of riparian vegetation, salinisation, eutrophication and presence of exotic fish species) on a semi-quantitative scale from 1 (pristine) to 5 (heavily disturbed). Assessments were made by one person using site inspections, on-site measurements of conductivity (YSI 30-25 FT conductivity meter), laboratory analysis of total nitrogen and total phosphorous concentrations and records from previous studies of environmental conditions and fish distributions (Morgan *et al.* 1998).

Between 11 and 157 fishes from 10 different species were sampled at each locality using either 24 or 12 volt backpack electro-fishers, fyke nets or seine nets. Collected fishes were identified to species in the field and dead fishes were immediately preserved in 70 % ethanol. Live fishes were maintained in aerated containers and transported to the laboratory where they were kept about 2 to 3 days in site-specific aquaria until euthanised for examination.

5.2.2 Parasite sampling

Fishes were dissected using the general methods of Dove (2000) and Berland (2005), as described in Chapter 2. Briefly, each fish was treated with physiological saline to reduce desiccation, and then systematically searched for parasites. First the skin, gills and fins were examined using a combination of eye and dissecting microscope. Then

the skin was scraped with a scalpel and the scrapings examined using both dissecting and compound microscopes. Gills were removed, examined individually under a dissecting microscope and then scraped as for the skin. Eyes, muscles and visceral organs (liver, kidney, spleen, gall bladder, gonads and gastrointestinal tract) were dissected, teased apart and examined for parasites using a dissecting microscope, and squash preparations were taken and examined under a compound microscope.

Histological sections were not routinely taken.

All detected parasites were removed and treated with physiological saline to retain morphological definition (Berland 2005). Where possible, photographs were taken of live specimens and they were then killed, fixed and preserved in either 70% ethanol (crustaceans, nematodes, cestodes) or 5-10% formalin (trematodes, protozoans, myxozoans). If necessary, specimens were cleared in lactophenol (nematodes and crustaceans) and prepared as whole mounts for microscopic identification. Protozoan and myxozoan cysts were dissected prior to preparation; trematode metacercaiae were not excysted. Parasite specimens were identified to the lowest taxon possible using the taxonomic keys of Anderson *et al.* (1980), Schmidt (1970), Kabata (1979) and Lom & Dykova (1992). Identification to species level was rarely possible and specimens were assigned to morphospecies based on consistent differences in morphology and site of infection. Following identification, parasites were classed as exotic or native in origin, on the basis of previous distribution records (see Chapter 2), and as ectoparasites or endoparasites, on the basis of site of infection and life history of the parasite taxon.

5.2.3 Data analysis

For each locality, I recorded the overall prevalence of parasitic infection (i.e. the proportion of all native fishes of any species infected with any species of parasite). Parasite component community (following Bush *et al.* 1997) diversity was estimated by species richness (the number of species, S) and by an index referred to a taxonomic diversity (H_T), which is the Shannon-Wiener diversity index (Shannon 1948) calculated from the number of species in each major taxonomic grouping of parasites: protozoans; myxozoans; monogeneans; trematodes; cestodes; nematodes and copepod crustaceans.

Relationships among habitat disturbance variables measured at all sampling localities were investigated using Spearman's rho, followed by principal components analysis. Relationships between habitat variables and parasite prevalence or diversity among localities were investigated with forward stepwise multiple regression, with host sample size and number of host species collected at a locality included in the model (see Chapter 2). Prior to undertaking any analyses which assumed normality, all variables were tested for normality and transformed if necessary.

Similarities in parasite species composition among different localities were estimated from parasite presence/absence data using the Bray-Curtis coefficient (Bray & Curtis 1957). The significance of differences in parasite species composition among localities with different measures of habitat disturbance was tested by binning localities into disturbance groups (see Results) and then applying a permutation procedure to the pairwise similarity matrix (ANOSIM, implemented using the computer package PRIMER 5.0; Clarke & Gorley 2001). The contribution of

individual parasite species to dissimilarities between disturbance groups was assessed by averaging the Bray-Curtis similarity term for each species over all pairwise locality combinations, using the SIMPER procedure in PRIMER 5.0.

5.3 Results

5.3.1 Habitat disturbance

There were significant correlations among all habitat disturbance variables which were measured at the 24 different sampling localities, except for salinisation, which was correlated only with the presence of exotic fish species (Table 5.1). Principal components analysis of the correlation matrix extracted two factors explaining 82% of the variance among localities; the first factor had significant loadings for all variables except salinisation, while the second factor had significant loadings only for salinisation and the presence of exotic fish species. Following varimax rotation, factor scores were used to create two new habitat disturbance variables, which I call human usage (factor 1) and salinisation (factor 2).

Table 5.1 Matrix of Spearman's rho correlations between five measures of habitat disturbance scored at 29 sampling localities; river regulation, loss of riparian vegetation, salinisation, eutrophication and presence of exotic fish species.

Significance of correlations indicated by * P < 0.05, ** P < 0.01, *** P < 0.001.

	Regulation loss	Vegetation loss	Salinisation	Eutrophication
Vegetation loss	0.72***			
Salinisation	-0.11	0.04		
Eutrophication	0.55**	0.66***	0.30	
Exotic fish	0.51**	0.52**	0.49*	0.61**

5.3.2 *The effect of habitat disturbance on parasite prevalence and diversity*

Differences in parasitism of native fish species among sampling localities were partially explained by differences in habitat disturbance, with fewer parasites in more disturbed localities. Multiple regression analyses found significant effects of both human usage and total number of native fishes sampled, but not of salinisation or number of species of fish sampled, on prevalence of parasitic infection ($r^2 = 0.42$; for human usage $F = 6.67$: $P < 0.05$; for number of fishes $F = 5.82$, $P < 0.05$), number of parasite species ($r^2 = 0.48$: for human usage $F = 8.92$; $P < 0.01$; for number of fishes $F = 12.22$, $P < 0.01$) and taxonomic diversity of parasites ($r^2 = 0.41$: for human usage $F = 4.22$; $P = 0.05$; for number of fishes $F = 8.28$, $P < 0.01$).

These analyses were based on parasites from all native species of fish that were sampled and therefore confound differences in fish fauna and differences in parasite fauna among localities. Unfortunately, species of fish were not distributed widely

enough over the different localities to allow separate analyses, but three lines of evidence suggest that the results were not due to the effect of habitat disturbance on fish fauna. First, the number of native fish species found at a locality did not have a significant effect in any of the multiple regression analyses. Second, there was little change in the multiple regression results when the two most heavily infected native fish species (*Tandanus bostocki* and *Galaxias maculatus*), each with a very limited sampling distribution (one and three localities, respectively) were excluded from the analyses. Third, there was no significant relationship between the similarity of localities in their composition of fish species and their similarities in composition of parasite species ($\text{Rho} = -0.12$, $P = 0.78$).

The major contributors to the negative relationship between human usage of localities and the prevalence and diversity of parasitism in those localities were endoparasites (mostly trematodes, cestodes and nematodes) rather than ectoparasites (mostly protozoans, monogeneans and copepod crustaceans). When endoparasites and ectoparasites were analysed separately using linear regression, only endoparasites showed significant associations with human usage scores (Figure 5.1; for prevalence, $r^2 = 0.29$, $P < 0.01$; for S , $r^2 = 0.18$, $P < 0.05$; for H_T , $r^2 = 0.18$, $P < 0.05$). (S = number of species; H_T = taxonomic diversity).

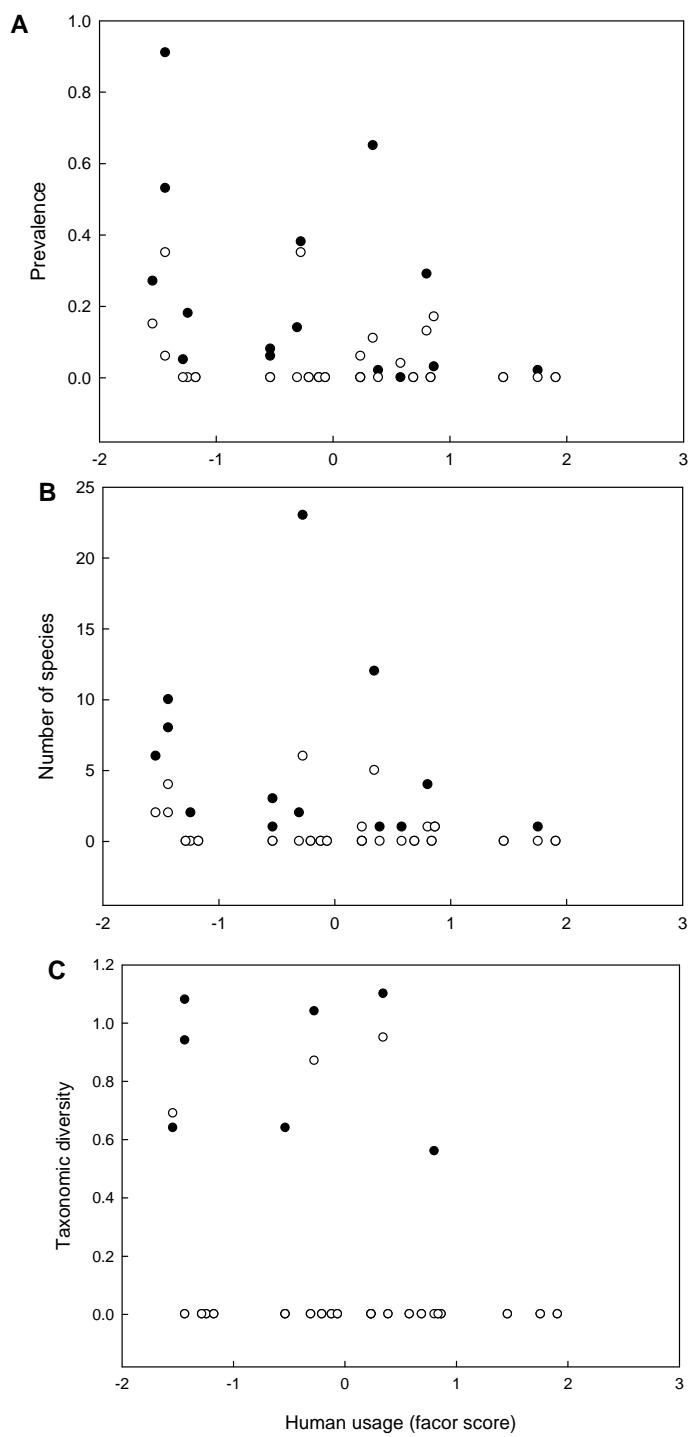


Figure 5.1 Relationships between human usage (measured as a factor score) and (A) prevalence of parasitic infection, (B) number of parasite species and (C) taxonomic diversity of parasites for ectoparasites (white circles) and endoparasites (black circles).

5.3.3 The effect of habitat disturbance on parasite species composition

When localities were grouped into ‘pristine’ (factor 1 score < -0.30) and ‘degraded’ (factor 1 score > 0.30) sites, there was a significant difference between the two groups in parasite species composition (ANOSIM; $R = 0.18$, $P < 0.05$). SIMPER analysis found that over 40% of this difference was due to the relatively greater abundance in pristine localities of the larvae of two species of anisakid nematodes, a species of trematode and the introduced cestode *Ligula* sp., and the relatively greater abundance in disturbed localities of larvae of the nematode *Eustrongylides* sp. and adults of the introduced copepod *Lernaea cyprinacea*.

5.4 Discussion

Ecological indicators are measurable characteristics of an ecosystem, which are usually employed to monitor the effects of specified or unspecified environmental impacts, or to monitor the success of ecosystem restoration projects (Carignan & Villard 2002; Niemi & McDonald 2004). A primary distinction can be made between pressure indicators, which are direct measures of the environmental impacts and response or state indicators, which are measures of ecosystem structure (including biotic composition and diversity) or ecosystem function. In freshwater ecosystems, pressure indicators are typically chemical measures of water quality, such as temperature, pH, nutrient content or salinity, while response indicators are measures of biological entities or processes.

The value of parasites as ecological response indicators in ecosystems is twofold. First, there is increasing evidence that the diversity of parasites in an ecosystem has an important influence on ecosystem function (Hudson *et al.* 2006). Second, because

parasites encompass a wide taxonomic range and a great diversity of life cycles, it is likely that at least some parasite species in an ecosystem will be affected by any particular environmental impact, either directly or through effects on their host species (Marcogliese 2004). Environmental impacts, therefore, will often change the composition of the parasite community in an ecosystem, which may in turn lead to further changes in the composition of the free living hosts of the parasites.

I found that the proportion of native freshwater fishes with parasitic infections and the component community diversity of the parasite fauna of fishes in the south-west of Western Australia were both negatively related to habitat disturbance, in particular to a suite of factors (river regulation, loss of riparian vegetation, eutrophication and presence of exotic fish species) that indicate increased human usage of the river and surrounding environment. These data are consistent with a number of other studies from Australia and around the world, which have found that anthropogenic changes to the environment often decrease the abundance and/or diversity of parasites, especially in aquatic hosts (Khan & Thulin 1991; MacKenzie *et al.* 1995; Landsberg *et al.* 1998; Dove 2000). The reduced parasite load and diversity in native fish from south-west rivers with greater human usage was due principally to the loss of a number of species of trematode, cestode and nematode endoparasites which use fishes as intermediate hosts. Other studies have also found that endoparasites with complex life cycles involving transmission between a number of different host species are most likely to be adversely affected by environmental changes; this is presumably because any environmental changes which impact on either free-living parasite stages or on any of the hosts in the complex train of parasite transmission

will reduce parasite population size and may cause local extinction of the parasite species (Lafferty 1997; Galli *et al.* 2001; Marcogliese 2004).

Despite the overall reduction in parasitic infections and parasite diversity in localities with greater human usage, some parasite species were found more frequently in these localities, in particular the introduced copepod crustacean *Lernaea cyprinacea* and the nematode *Eustrongylides*. *Lernaea* is likely to have been recently introduced with aquarium fish and its direct life cycle and limited potential for rapid dispersal (see Chapter 3) means that it is still confined to rivers close to urban centres. In addition, there is some evidence that ectoparasites of fish, in contrast to endoparasites, often increase in abundance and diversity in response to anthropogenic changes to the environment, perhaps because of a reduced host immune response (Lafferty 1997; MacKenzie 1999; Marcogliese 2004). *Eustrongylides* is a native species (Chapter 2) and has an indirect life cycle, with two intermediate hosts (oligochaete worms and fish) and a piscivorous bird definitive host. Unlike other endoparasites of fish, however, prevalence of infection with *Eustrongylides* has been found to increase in response to anthropogenic impacts on rivers, presumably because oligochaete abundance often increases with organic enrichment of the sediment (Coyner *et al.* 2003).

The associations found between the extent of human usage of rivers and the degree of parasitic infection and diversity of parasites found in native freshwater fishes, represent a starting point for further study. My data did not allow me to examine the effect of environmental factors on individual parasite species/host species relationships, which will be an essential next step before we can confidently ascribe

causal processes to differences in parasite prevalence and diversity in different localities. Nevertheless, the study has suggested that human impacts on freshwater environments may not only reduce overall parasite diversity, but may also change the composition of parasite communities, favouring some species of parasites at the expense of others. It has also indicated which species of parasites and which species of fish may be suitable for a more comprehensive study of the effects of environmental impacts on the parasites of freshwater fishes in the south-west of Western Australia.

CHAPTER 6

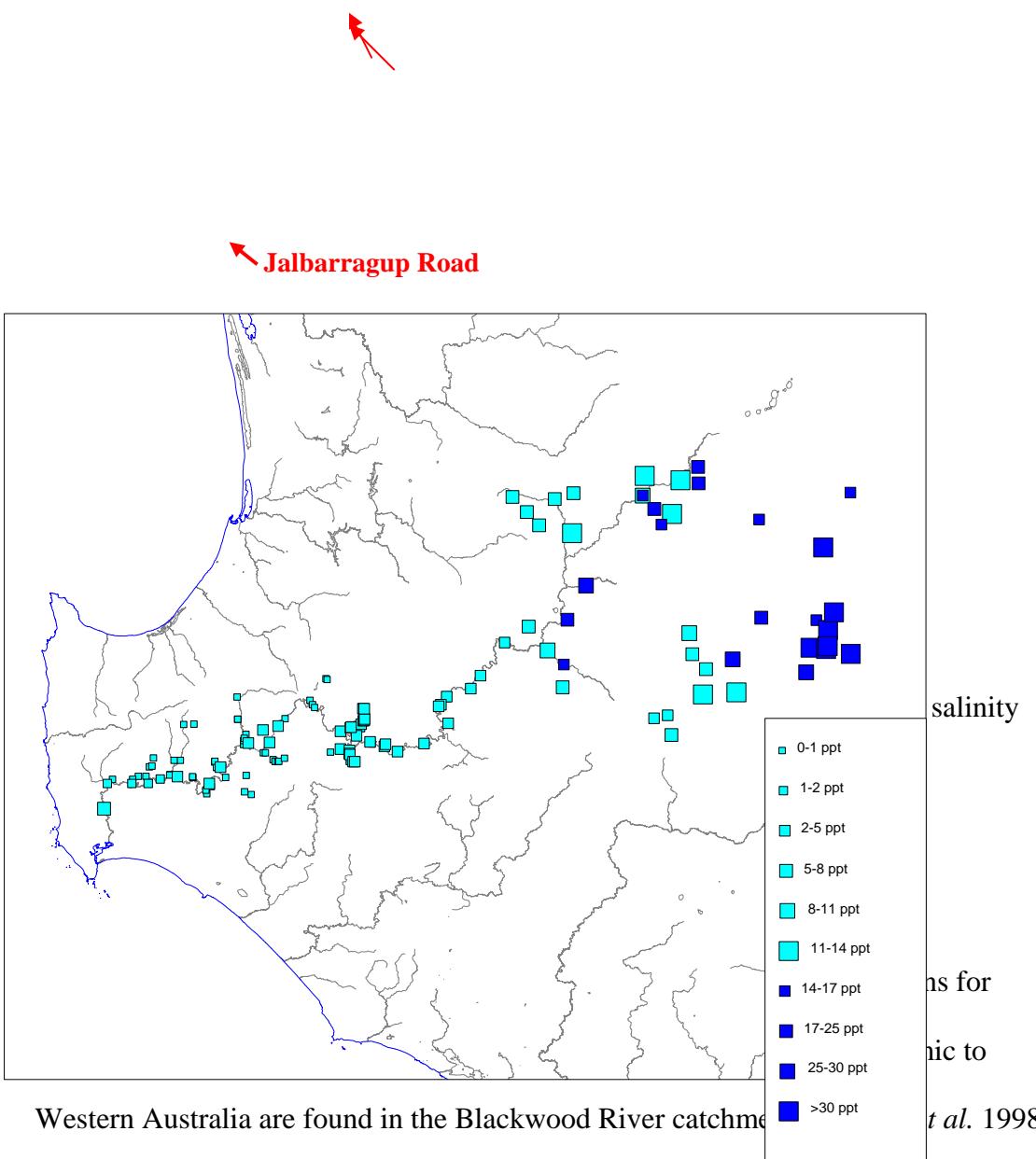
Salinisation and parasitic infection in freshwater cobbler (*Tandanus bostocki*) from the Blackwood River

6.1 Introduction

One of the major threats to freshwater ecosystems in the south-west of Western Australia is salinisation, caused by land clearing for agriculture throughout much of the region. The replacement of deep-rooted, perennial, native vegetation with annual crops and pastures has led to rising groundwater in agricultural areas, mobilising salts stored in the soil profile (Beresford *et al.* 2001). The salinisation of streams and rivers occurs from the direct seepage of saline groundwater into the river system, from salt deposited on the surface of the land being washed into drainage lines, and by the accidental or deliberate discharge of saline groundwater from drainage schemes used to repair salinised land (Lymbery *et al.* 2003).

One of the largest and most heavily salinised rivers in the south-west is the Blackwood River (Morgan *et al.* 2003). Salinity varies from as high as $21,600 \text{ mgL}^{-1}$ in the upper catchment to approximately $2,000 \text{ mgL}^{-1}$ in the lower reaches of the main channel (Mayer *et al.* 2005; Figure 6.1). Over the last 60 years, salinities in the main channel of the lower Blackwood River have been increasing at a mean annual rate of about 25 mgL^{-1} (Schofield & Ruprecht 1989; Mayer *et al.* 2005). The seasonal variation in salinity in the main channel of the lower Blackwood River does not follow the usual pattern for salinised rivers in Australia of rising salinity over summer, peaking with the first rains and then decreasing with winter flows. Instead, salinity falls over summer because an increasing proportion of water flow is

contributed by discharge from fresh groundwater aquifers in the lower, forested parts of the catchment and then, as the major rains begin in winter, salinity rises sharply due to water flow from the upper, saline parts of the catchment (Mayer *et al.* 2005; Morgan *et al.* 2003).



halotolerant fishes and many are now restricted to the lower reaches (Morgan *et al.* 2003, Beatty *et al.* 2006). One such species is the freshwater cobbler, *Tandanus bostocki*.

In a survey of parasites of native and exotic freshwater fishes in the South West Coast Drainage Division, *T. bostocki* was the most heavily infected species, with 96% of all individuals containing at least one species of parasite (Chapter 2). *Tandanus bostocki* is often more difficult to catch with standard sampling procedures than other species of freshwater fish in the south-west due to nocturnal behavior. All *T. bostocki* in the survey were from the lower reaches of the Blackwood River, where a major monitoring study was underway, using fyke nets that were set overnight to capture this nocturnal species (Beatty *et al.* 2006). Anecdotal evidence obtained during sampling suggested that seasonal changes in salinity in the river may be influencing parasite loads and tissue pathology in captured fish.

Relationships between water quality, parasitism and pathological responses may be complex. The environmental factors are often themselves correlated, leading to a series of direct and indirect effects on the fish. Some organs may respond more specifically than others to these effects and could be used as indicators for pollutants (Burkhardt-Holm *et al.* 1997; Arellano *et al.* 2004).

In this chapter, I examine the levels of parasitism and histopathology of internal and external organs in *T. bostocki* sampled from the Blackwood River over a period of rapid changes in water salinity. My aims were to compare the parasite diversity and severity of histopathological lesions in different organs at different levels of salinity.

6.2 Materials and methods

6.2.1 Fish sampling

Tandanus bostocki were sampled from Jalbarragup Road crossing (34.0421°S, 115.6025°E), within the main channel of the Blackwood River, just upstream of the input from freshwater aquifers into the river (Figure 6.1). As part of an ongoing monitoring study, water temperature, conductivity, pH and dissolved oxygen have been measured regularly at this site since 2005. Conductivity shows regular seasonal fluctuations associated with saline water moving down the river from the upper catchments with the first heavy rains of the winter (Figure 6.2).

Fish were sampled by fyke nets (11.2 m wide with two 5 m wings and a 1.2 m wide mouth fishing to a depth of 0.8 m, and a 5 m pocket with two funnels all of 2 mm stretched mesh) on five separate occasions; three times before and twice immediately after the spike in conductivity in early July 2007. Because relatively small numbers of fish were captured on each sampling occasion, individual captures were pooled into low salinity or high salinity categories.

Live fish were maintained in aerated containers and transferred to the laboratory where they were kept in aquaria for a maximum of two days until euthanised for parasite examination. Prior to dissection, all fish were weighed to the nearest 0.01 g and measured for total length (TL) (i.e. body length including the caudal fin) to the nearest 1 mm.

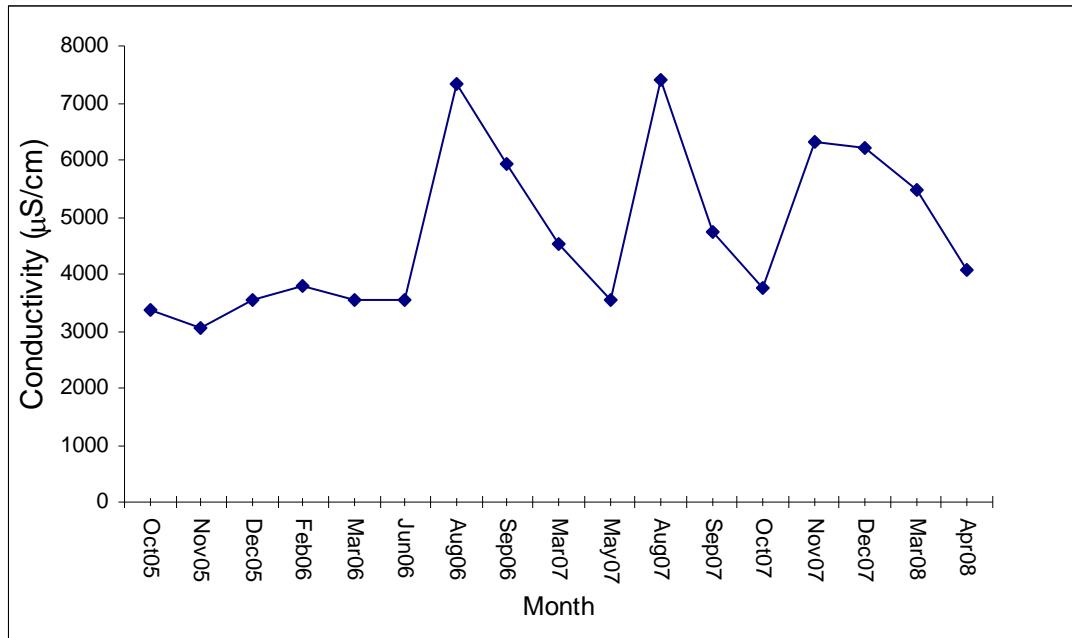


Figure 6.2 Seasonal changes in water conductivity ($\mu\text{S}/\text{cm}$) at Jalbarragup Road crossing in the Blackwood River.

6.2.2 *Parasite sampling*

Fish were dissected using the general methods of Dove (2000) and Berland (2005), as described in Chapter 2. Briefly, each fish was treated with physiological saline and then systematically searched for parasites. First the skin, gills and fins were examined using a combination of eye and dissecting microscope. The skin was scraped with a scalpel and the scrapings examined using both dissecting and compound microscopes. Gills were removed, examined individually under a dissecting microscope and then scraped as for the skin. Eyes, muscles and visceral organs (liver, kidney, spleen, gall bladder, gonads and gastrointestinal tract) were dissected, teased apart and examined for parasites using a dissecting microscope, and squash preparations were prepared and examined under a compound microscope. All detected parasites were removed and treated with physiological saline to retain morphological definition (Berland 2005). Where possible, photographs were taken of

live specimens and they were then killed, fixed and preserved in 70% ethanol. If necessary, specimens were cleared in lactophenol (nematodes and crustaceans) and prepared as whole mounts for microscopic identification. Protozoan and myxozoan cysts were dissected prior to preparation; trematode metacercaiae were not excysted. Parasite specimens were identified to the lowest taxon possible using the taxonomic keys of Schmidt (1970), Kabata (1979), Anderson *et al.* (1980) and Lom & Dykova (1992). Identification to species level was rarely possible and specimens were assigned to morphospecies as described in Chapter 2. Following identification, parasites were classed as ectoparasites or endoparasites, on the basis of site of infection and life history of the parasite taxon.

6.2.3 Histological examination

After examination for parasites, the skin, gills, liver, kidney and intestine were removed from each fish. Tissues were fixed in 10% formalin, then processed for paraffin sections and stained with haematoxylin and eosin (H & E). Prepared sections were examined under a light microscope and histopathological alterations were evaluated semi-quantitatively using methods modified from Schwaiger (2001). The severity of tissue lesions were ranked on a scale from 1 to 3, where grade 1 = no pathological/ parasitological alterations, grade 2 = focal mild to moderate changes, grade 3 = extended severe pathological alterations/extensive parasite infection. Histopathological images were captured using the software Motic Images Plus 2.0.

6.2.4 Data analysis

6.2.4.1 Parasitological data

For all fish sampled and for fish from each salinity grouping (low or high), the prevalence (proportion of fish infected) of each parasite species was recorded. Confidence intervals (95%) were calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa *et al.* 2000). Differences in parasite prevalence between groups of fish from different salinities were investigated using a Fisher exact test, with a Bonferroni correction to maintain an experiment-wide error rate of 0.05.

Parasite infracommunity (following Bush *et al.* 1997) diversity was estimated for each fish by species richness (the number of species, S). Differences in species richness between groups of fish from different salinities were investigated using analysis of variance, following tests for normality and homogeneity of variances. Relationships with continuous variables, such as fish weight or length, were investigated with linear regression analysis.

Similarities in parasite infracommunity species composition between individual fish were estimated from parasite presence/absence data using the Bray-Curtis coefficient (Bray & Curtis 1957). The significance of differences in parasite species composition between groups of fish from different salinities was tested by a permutation procedure applied to the pairwise similarity matrix (ANOSIM, implemented using the computer package PRIMER 5.0; Clarke & Gorley 2001). The contribution of individual parasite species to dissimilarities between groups was assessed by averaging the Bray-Curtis similarity term for each species over all pairwise

combinations of fish using the SIMPER procedure in PRIMER 5.0 (Clarke & Gorley 2001).

6.2.4.2 Histopathological data

For each fish, severity of lesions were recorded for each organ and for all external (skin and gills) and internal organs (liver, kidney and intestine) by summing histopathological scores. Mean scores were compared between groups using analysis of variance or, if the data were not normally distributed, a nonparametric Wilcoxon rank-sum test.

6.3 Results

6.3.1 Parasitology

Forty eight *T. bostocki* were sampled from Jalbarragup Road crossing in the Blackwood River; 34 before and 14 immediately after the spike in conductivity which occurred in July, 2007. All fish were infected with at least one species of parasite, with the mean parasite infracommunity species richness being 4.7 ± 0.3 . Species richness was significantly greater in larger fish (with fish weight, $r^2 = 0.41$, $P < 0.0001$; with fish length $r^2 = 0.35$, $P < 0.0001$). Nineteen putative species of parasites were found; two species of protozoans, one myxozoan, one monogenean, seven trematodes, two cestodes, five nematodes and one copepod crustacean. All of these parasite species have been previously described in Chapter 2. Over all fish that were sampled, prevalences for each parasite species varied from 0.021 to 1, with a mean of 0.246 ± 0.070 (Table 6.1).

Table 6.1 Parasite species found in *Tandanus bostocki* sampled from the Blackwood River, and prevalence (with 95% confidence intervals in parentheses) of each parasite species over all 48 fish; 34 fish sampled in low salinity conditions and 14 fish sampled in high salinity conditions. * indicates a significant difference in prevalence between salinities at P < 0.05, with the Bonferroni.

Parasite species	Prevalence (all fish)	Prevalence (low salinity)	Prevalence (high salinity)
<i>Ichthyophthirius</i> sp.	0.125 (0.056-0.248)	0.147 (0.060-0.362)	0.071 (0.004-0.317)
Microsporidian sp.	0.375 (0.248-0.521)	0.235 (0.114-0.410)*	0.714 (0.426-0.896)*
<i>Myxobolus</i> sp.	1 (0.922-1)	1 (0.902-1)	1 (0.762-1)
<i>Dactylogyrus</i> sp.	0.333 (0.211-0.479)	0.441 (0.276-0.619)	0.071 (0.004-0.317)
Metacercaria B	0.313 (0.196-0.458)	0.176 (0.080-0.337)*	0.643 (0.371-0.847)*
Metacercaria D	0.021 (0.001-0.111)	0.029 (0.002-0.156)	0 (0-0.238)
Metacercaria E	0.125 (0.056-0.248)	0.176 (0.080-0.337)	0 (0-0.238)
Metacercaria F	0.521 (0.374-0.657)	0.353 (0.201-0.530)*	0.929 (0.683-0.996)*
Clinostomidae sp.	0.021 (0.001-0.111)	0.029 (0.002-0.156)	0 (0-0.238)
Digenean C	0.021 (0.001-0.111)	0.029 (0.002-0.156)	0 (0-0.238)
Digenean D	0.354 (0.227-0.500)	0.206 (0.098-0.381)*	0.714 (0.426-0.896)*
Dilepididae B	0.021 (0.001-0.111)	0 (0-0.098)	0.071 (0.004-0.317)
Caryophyllaeidae sp.	0.125 (0.056-0.248)	0.088 (0.024-0.232)	0.214 (0.061-0.500)
<i>Anisakis</i> sp.	0.021 (0.001-0.111)	0.029 (0.002-0.156)	0 (0-0.238)
<i>Pseudocapillaria</i> sp.	0.063 (0.017-0.174)	0.059 (0.016-0.176)	0.071 (0.004-0.317)
Anisakidae A	0.083 (0.029-0.196)	0.118 (0.041-0.276)	0 (0-0.238)
Anisakidae B	0.021 (0.001-0.111)	0.029 (0.002-0.156)	0 (0-0.238)
Anisakidae E	0.125 (0.056-0.248)	0.088 (0.024-0.232)	0.214 (0.061-0.500)
<i>Dermoergasilus</i> sp.	1.00 (0.922-1.00)	1 (0.902-1)	1 (0.762-1)

Eighteen species of parasites were found amongst 34 fish sampled before the spike in salinity and 12 species of parasites were found amongst 14 fish sampled after the salinity spike. When corrections were made for multiple testing, significant differences in prevalence among fish from different salinities were found only for a species of microsporidian, a larval trematode species and an adult trematode species (Table 6.1).

Parasite infracommunity richness was significantly greater in fish from the high salinity group (5.7 ± 0.5 species per fish) than in fish from the low salinity group (4.3 ± 0.3 species per fish; $F = 6.01$, $P < 0.05$). This was not related to size of the fish sampled, which did not differ significantly between the two groups. When parasite species were divided into endoparasites and ectoparasites, fish from the high salinity group had significantly more endoparasite species (3.6 ± 0.5 compared to 1.6 ± 0.3 species per fish; $F = 10.52$, $P < 0.01$), but significantly fewer ectoparasite species (2.1 ± 0.2 compared to 2.6 ± 0.1 species per fish; $F = 4.66$, $P < 0.05$) than fish from the low salinity group.

There were significant differences in parasite species composition between fish in the high and low salinity groups (ANOSIM, $R = 0.20$, $P < 0.01$). SIMPER analysis showed that more than 70% of this difference was due to the relatively greater occurrence at high salinities of three species of endoparasitic trematodes (digenean D, metacercaria F and metacercaria B) and an endoparasitic microsporidian, and the relatively greater occurrence at low salinities of the ectoparasitic monogenean *Dactylogyrus* sp. and the ectoparasitic protozoan *Ichthyophthirius* sp.

6.3.2 Histopathology

A variety of histopathological lesions were observed in all organs examined. Using the semi-quantitative scoring system, lesions were most severe in the gills, with all fish showing grade 3 lesions; other organs showed less severe lesions and were more variable in lesion scores among fish (Table 6.2).

Table 6.2 Mean and coefficient of variation (CV) in histopathological lesion scores for five different tissues in 48 *Tandanus bostocki* sampled from the Blackwood River, Western Australia.

	Organ				
	Gill	Skin	Liver	Kidney	Intestine
Mean score	3	2.2	1.9	2.7	2.0
CV score	0	0.4	0.2	0.1	0.4

Gill lesions consisted of severe inflammatory reactions, with general fusion of lamellae and no interlamellar spaces. These changes were always more severe at the site of parasitic infection, particularly with *Myxobolus*, *Dermoergasilus*, *Dactylogyirus*, *Ichthyophthirius* and occasionally encysted trematode metacercariae (Plates 6.1, 6.2, 6.3). Skin lesions were principally associated with infections of *Ichthyophthirius*, which led to goblet cell activity and sloughing of the epidermal layer (Plate 6.4) and encysted trematode metacercariae, which were surrounded by host fibroblast tissue (Plate 6.5).

In the liver, lesions consisted of necrotic cells, cloudy degeneration of hepatocytes, lack of zymogen granules in the exocrine cells (Plate 6.6) and inclusion bodies in the

hepatocytes (Plate 6.6A). One fish had a cyst on the liver, surrounded by thin cells with spindle-shaped nuclei (Plate 6.6B). Histological changes in the kidney were vacuolation and hypertrophy of tubular epithelial cells, which caused a narrowing of the tubular lumen. These lesions were often accompanied by cloudy swelling and fine eosinophilic granules, and sometimes by hyaline droplets which caused the nuclei to be displaced to the basal side of cells (Plate 6.7A). These lesions were sometimes, but not always, accompanied by infections with a species of microsporidian in the tubular lumen, glomeruli or hematopoietic tissue (Plate 6.7 B, C). Intestinal lesions, particularly sloughing, breakage and loss of the epithelium were usually associated with infections of nematodes or adult trematodes (Plate 6.8).

Total lesion scores (i.e. over all tissues) were positively related to the number of different parasite species (parasite infracommunity species richness) present in an individual fish ($r^2 = 0.17$, $P < 0.01$) and were significantly greater in fish from the high salinity group than in fish from the low salinity group (ANOVA, $F = 19.08$, $P < 0.001$), but the pattern of the differences varied among organs. Skin lesions were more severe at low salinities, although the difference between the groups was not quite significant (Wilcoxon test, $P = 0.08$), whereas lesions in the kidney, liver and intestine were more severe at high salinities, although differences were significant only in the intestine (Wilcoxon test, $P < 0.01$; Figure 6.3).

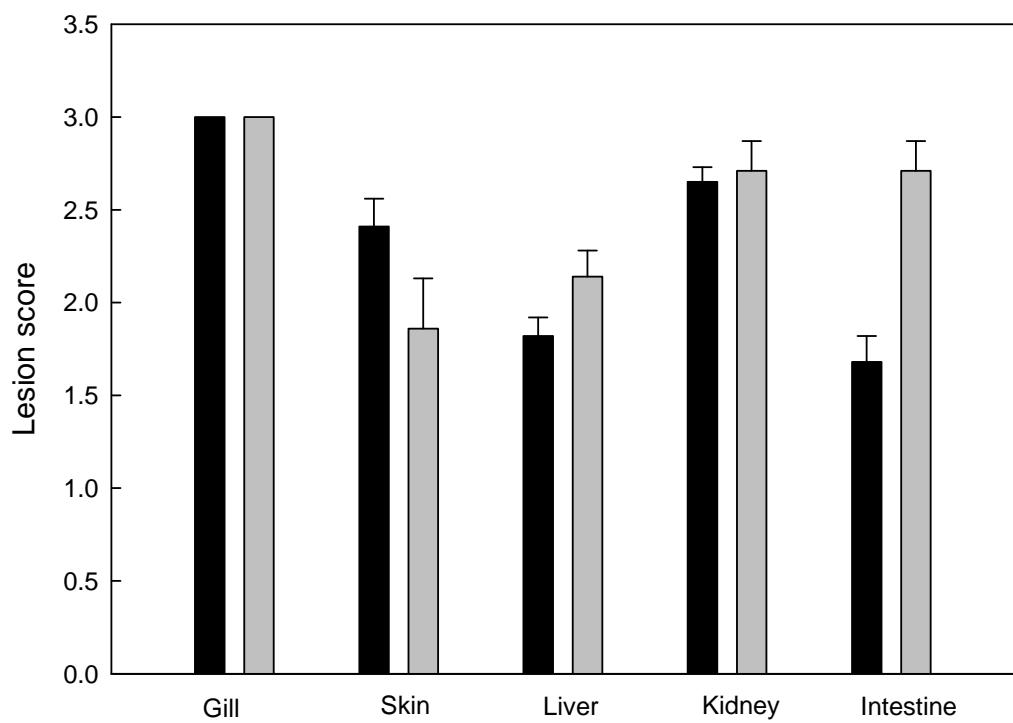


Figure 6.3 Mean lesion scores (with standard error bars) for gill, skin, liver, kidney and intestinal tissue in *Tandanus bostocki* sampled from the Blackwood River at low salinities (black bars) and high salinities (grey bars).

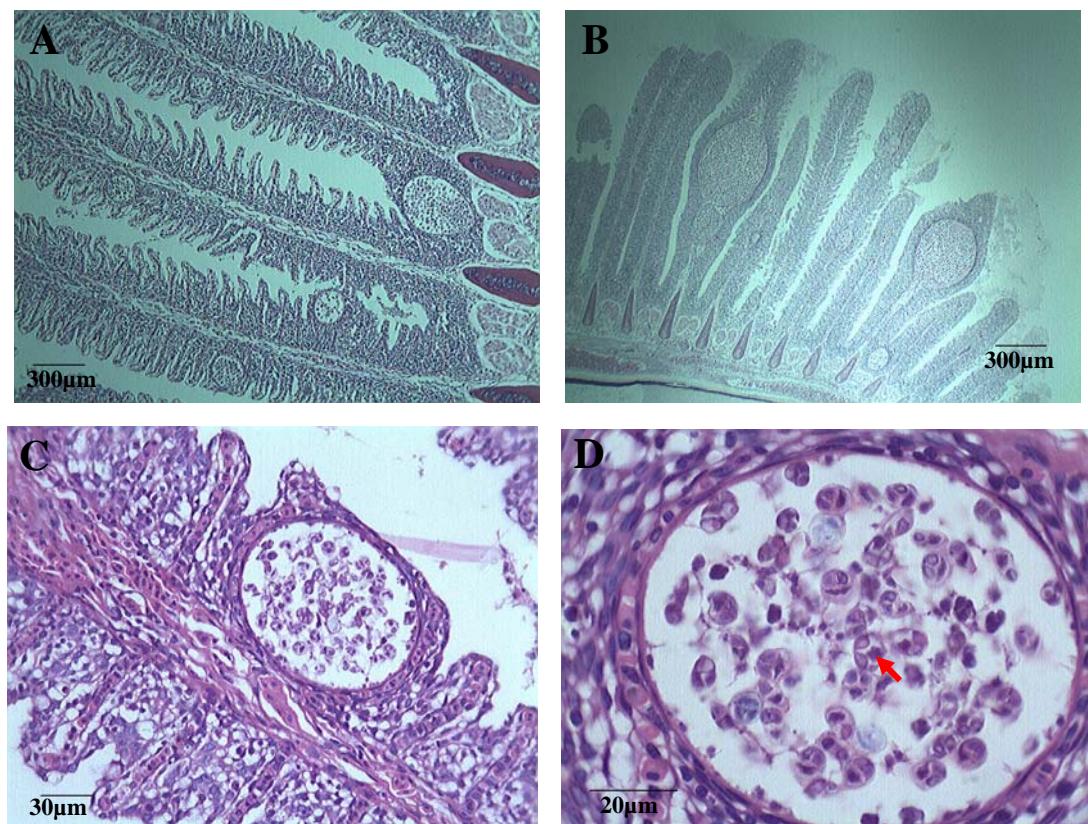


Plate 6.1 (A) (B) Heavy infection of the gills of *Tandanus bostocki* by *Myxobolus* sp. Note the greater severity of inflammatory reactions at the sites of infection, compared to non-infected sites. (C) (D) Cyst of *Myxobolus* sp., with surrounding fibroblast cells and spores (arrow).

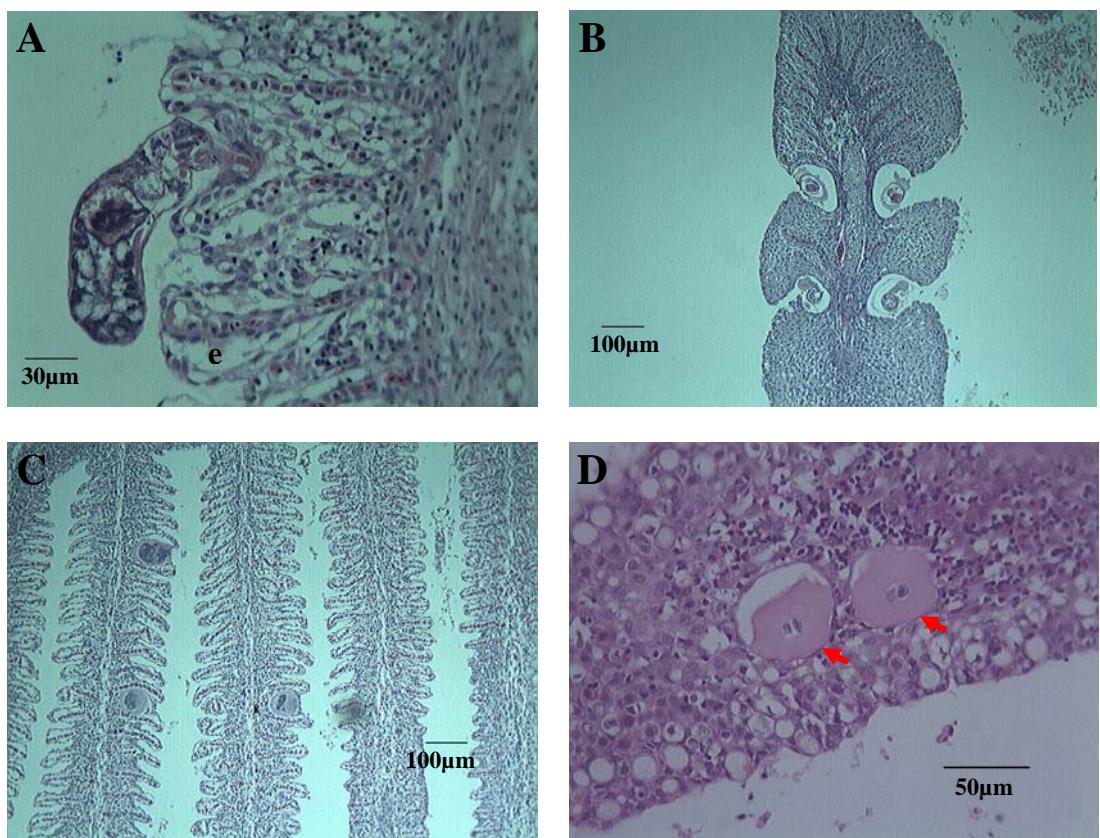


Plate 6.2 Infestation on the gills of *Tandanus bostocki* with (A) *Dactylogyrus* sp., (B) *Dermoergasilus* sp. and (C) *Ichthyophthirius* sp. Note the epithelial hyperplasia, hypertrophy, oedema (labelled e) and fusion of secondary lamellae associated with parasitic infestation. (D) The large unknown cell (possibly a parasitic stage) with two nuclei on the gills (arrow).

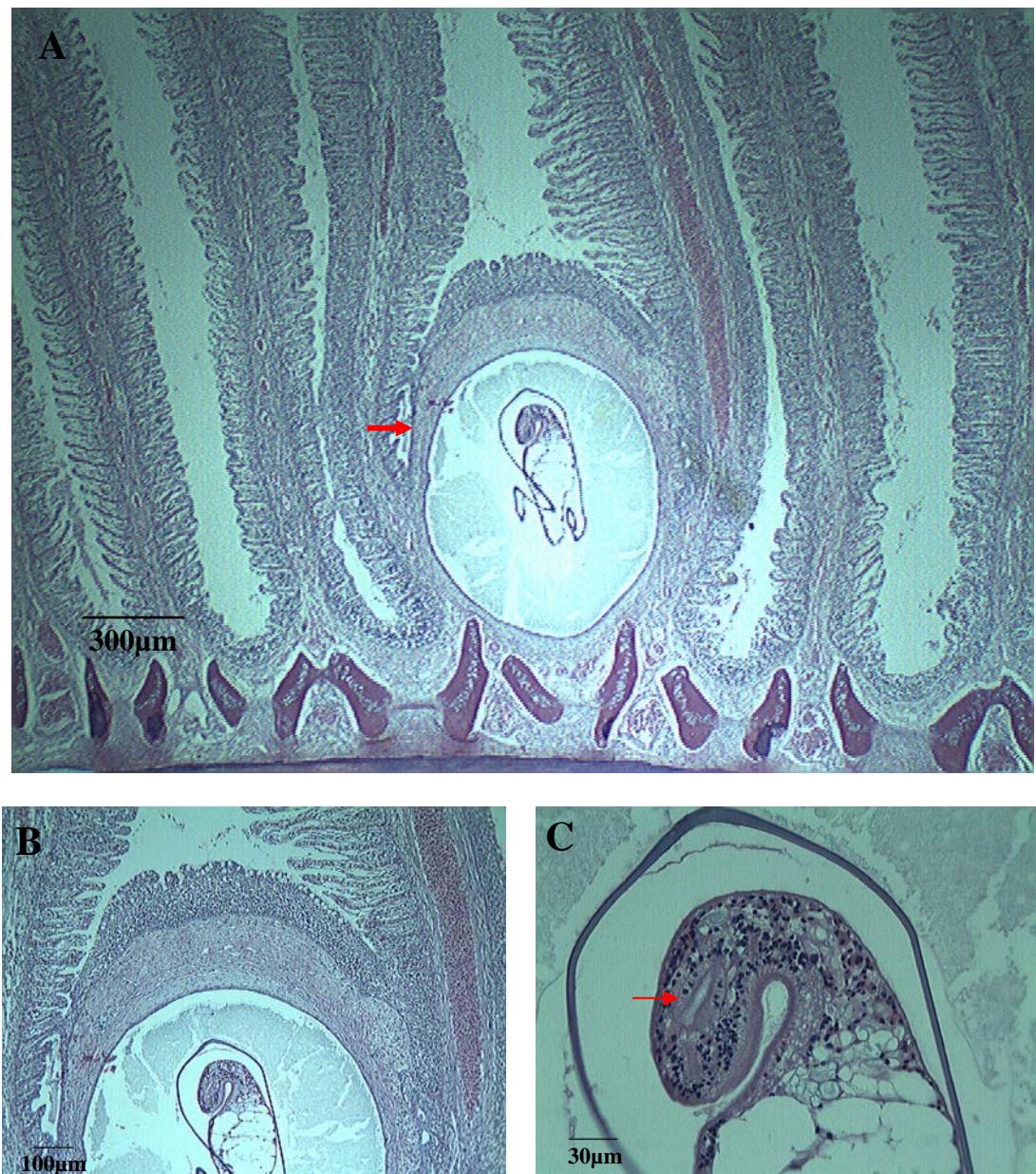


Plate 6.3 (A) Encysted trematode metacercaria (arrow) at the junction of the gill filaments of *Tandanus bostocki*. (B) Thick host fibroblast tissue and inflammatory cell reactions at the area of infections. (C) The organism within the cyst with a sucker (arrow).

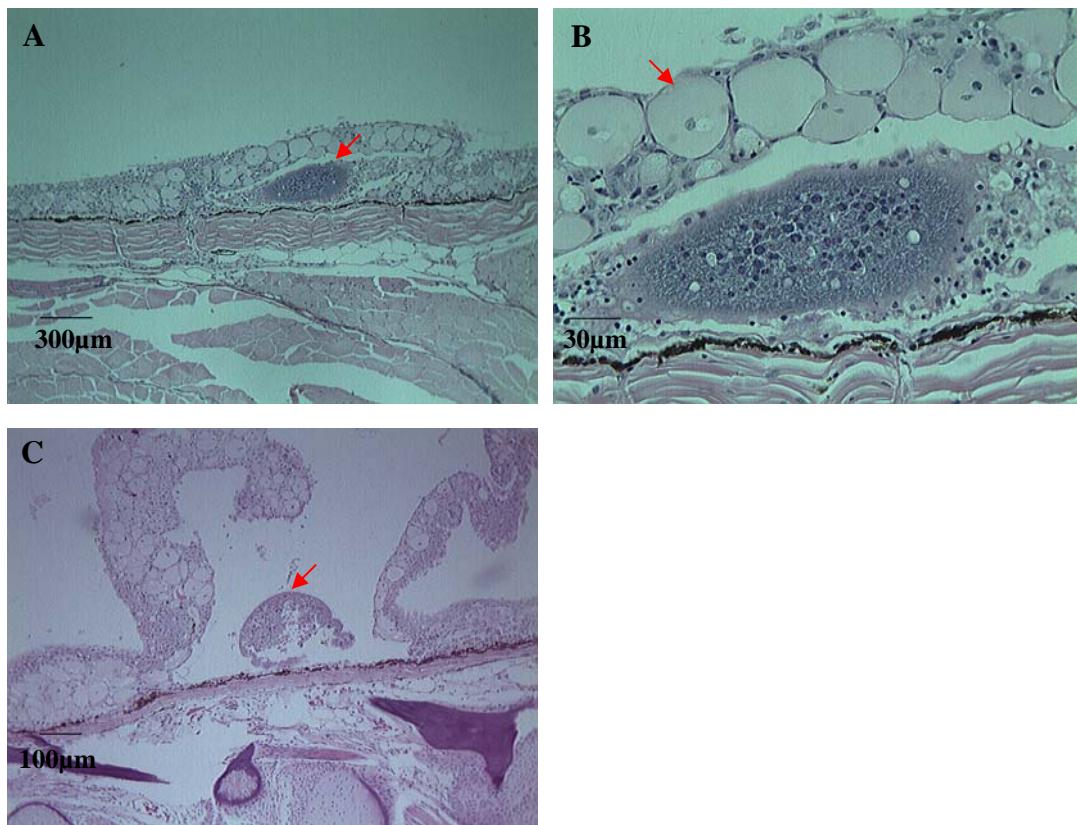


Plate 6.4 (A) *Ichthyophthirius* sp. (arrow) beneath the epidermis of *Tandanus bostocki*. (B) Active goblet cells (arrow) at the site of infection. (C) Sloughing of the epidermis over the organism (arrow).



Plate 6.5 Encysted trematode metacercaria under the skin of *Tandanus bostocki*. The cyst has broken the skin layers (arrow). Note the organism debris (A) in the middle of the cyst and surrounded by an eosinophilic hyaline capsule, and the thick host fibroblast tissue surrounding the cyst.



Plate 6.6 (A) Lack of zymogen granules in the exocrine cells (a). Blood vessel (bv). (B) A cyst in the liver (a). The cyst was surrounded by thin cells with spindle shaped nuclei (arrow).

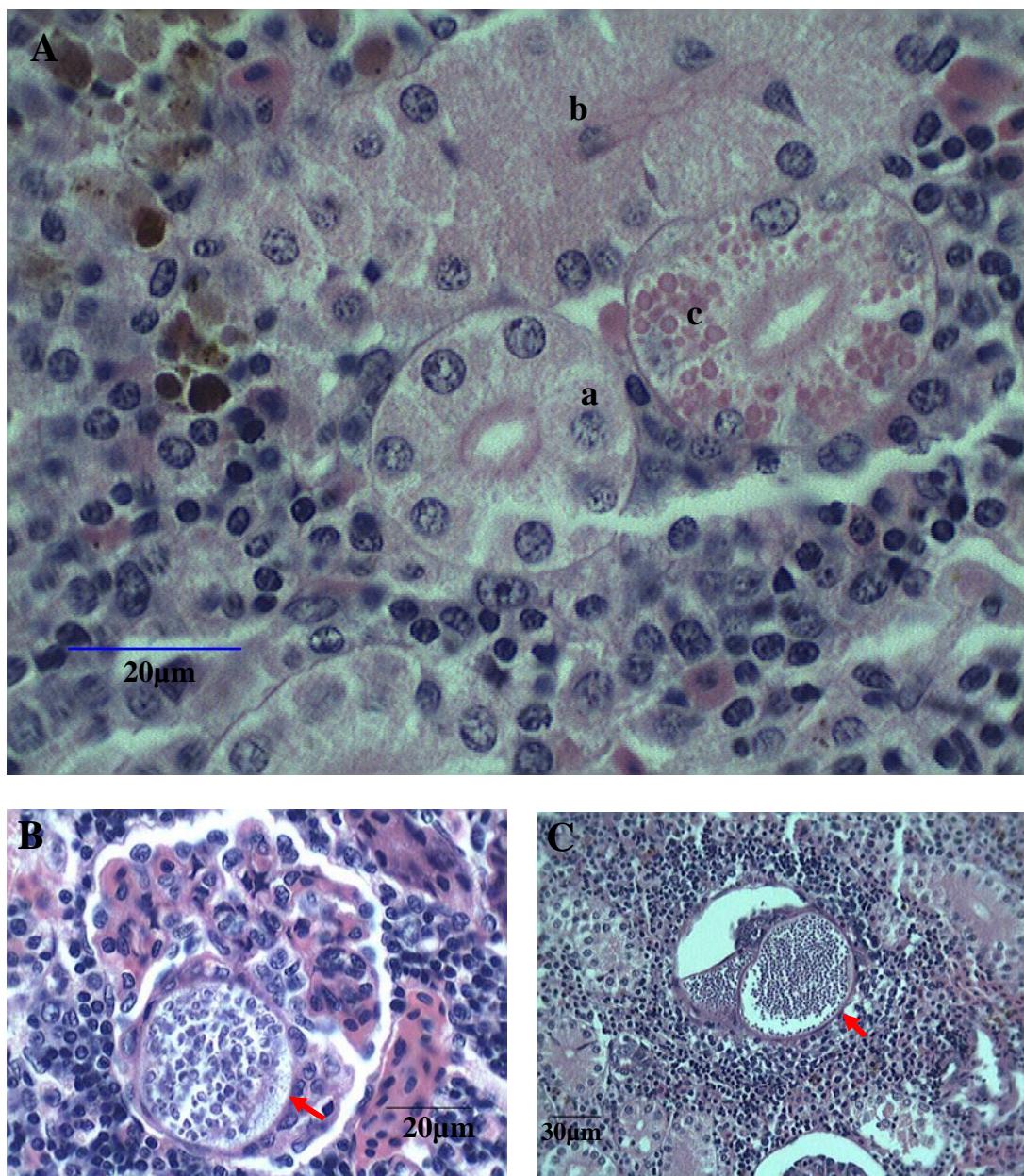


Plate 6.7 (A) Histological lesions in the kidney of *Tandanus bostocki*: (a) degeneration of epithelial cells of renal tubule (a), hypertrophied cells and narrowing of tubular lumen (b) and hyaline droplet degeneration in the epithelium of renal tubule. (B) Microsporidia cyst (arrow) in glomerulus. (C) Microsporidia cyst (arrowed) in hematopoietic tissue. Note host fibroblast tissue surrounding the cyst.

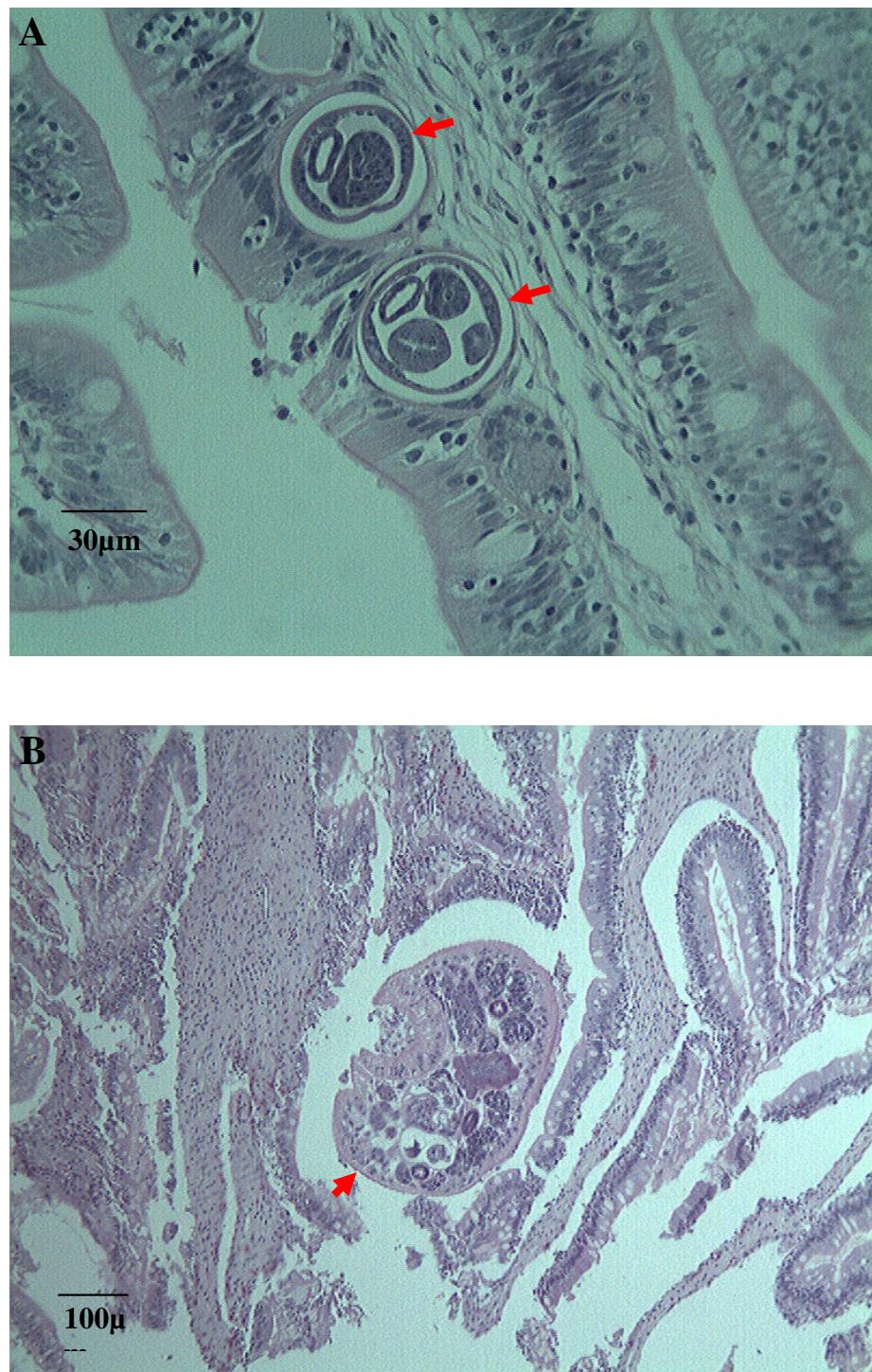


Plate 6.8. Transverse sections of (A) nematode and (B) trematode infections (arrow) in the intestine of *Tandanus bostocki*. Note the sloughing, breakage and loss of intestinal epithelium.

6.4 Discussion

6.4.1 Parasitic infections in T. bostocki

A general survey of parasites of freshwater fishes in the S.W.C.D.D. (Chapter 2) found that freshwater cobbler, *T. bostocki*, was the most heavily infected species. This has been confirmed in the present study; all fish which were sampled were infected, with a mean parasite infracommunity richness of 4.7 (± 0.3) species and a mean prevalence per parasite species of 0.246 (± 0.070). This level of parasitism is much greater than that found in most other native species of freshwater fish in the south-west of Western Australia (see Chapter 2), although it is similar to levels in six species of native freshwater fish in eastern Australia, studied by Dove (2000).

The most prevalent parasites were a species of myxozoan, tentatively assigned to the genus *Myxobolus* and a copepod crustacean, which appears to be a new species of *Dermoergasilus* (see Chapter 4). Both of these species were found in all fish that were examined. Other relatively common parasites were a microsporidian, adult and larval (metacercaria) trematodes and a species of the monogenean *Dactylogyrus*, which is likely to have been introduced (see Chapter 2).

6.4.2 Parasitic infections and histopathology

Histopathological changes, including inflammation, eosinophilia and cellular hypertrophy were found in gill, skin, liver, kidney and intestinal tissue. Often, these were generalised reactions, but in many cases they were closely associated with, and likely to be a response to, parasitic infection. This is suggested not only from the histological findings of parasites surrounded by pathological changes in host tissue (Plates 6.1 – 6.8), but also by a positive relationship between overall

histopathological response and the number of different parasite species infecting individual fish. Tissue changes in response to parasites have been studied only rarely in fishes. The other pathological study showed closely related changes of tissues with my findings such as hyperplasia and lamellae fusion of the gill filaments caused by *Paramoeba* sp. (Zilberg & Munday, 2000) and monogeneas (members of the Diplectanidae) (Nowak *et al.* 2004). In grouper (*Epinephelus tauvina*), severe gill necrosis was associated with infection by *Benedenia epinepheli* (Monogenea) (Jithendran *et al.* 2005).

6.4.3 Relationship between salinity, parasitism and histopathology

A sharp increase in salinity, resulting from the flow of salinised water from the upper catchment of the Blackwood River, was associated with an increase in parasitism, as measured by parasite infracommunity richness, and a greater histopathological response in *T. bostocki*. Differences in water quality have been linked to pathological changes in fish in previous studies (e.g. Schwaiger *et al.* 1997; Schwaiger 2001; Velmurugan *et al.* 2007) and have been ascribed to both direct toxic effects of contaminants in the water and indirect effects from parasites and pathogens, which are more prevalent or more virulent in contaminated water. Disentangling these different causal pathways is very difficult in field studies and usually requires controlled laboratory experiments (Schwaiger 2001).

In the present study, a comparison of the effects of increased salinity on different species of parasites and on the histopathology of different organs suggests that the pathological effects of salinity are mostly indirect, via its effect on parasite infections. Firstly, although the overall number of parasite species per fish was

greater in fish sampled after an increase in salinity, there was a clear difference between ectoparasite and endoparasite species; ectoparasite species richness was greater in fish at low salinities, while endoparasite species richness was greater in fish at high salinities. Second, although the overall histopathological lesion score was greater in fish sampled after the increase in salinity, different organs responded differently; lesion scores were greater in skin tissue at low salinities and greater in liver, kidney and (especially) intestinal tissue at high salinities.

Explanation of these results may be that the sharp spike in salinity experienced by fish in the Blackwood River has two, contrasting effects on their parasitic infections. Increasing salinity may subsequently increase the mortality rate of those parasites directly exposed to water, leading to a decrease in ectoparasitic infection and associated pathology. Previous studies have found that some ectoparasitic copepods (Felley *et al.* 1987) and protozoans (Finley 1930; Chu *et al.* 2001) are sensitive to increases in salinity. In the present study, a marked decrease in the prevalence of the ectoparasite *Ichthyophthirius* on the skin of *T. bostocki* was found at higher water salinities, which may have been a result of parasite mortality as salinity increased. Although histopathological lesion scores in the skin of *T. bostocki* decreased at higher salinities, in line with the decrease in ectoparasitic infections, those in the gills did not. This may be partly because the major gill ectoparasites, *Myxobolus* and *Dermoergasilus*, were not adversely affected by increasing salinity and partly because gill tissue is itself very sensitive to environmental contamination (Cengiz 2006), offsetting the effects of reduced pathology from parasites.

In addition to directly affecting ectoparasites and therefore reducing parasitic infection, however, increasing salinity may also suppress immune function in fish, as has been found previously by Landsberg *et al.* (1998) and in response to other environmental stressors such as temperature (Dykova & Lom 1978; Lamkova *et al.* 2007), dissolved oxygen (Landsberg *et al.* 1998) and pollution (Schwaiger *et al.* 1997; Schwaiger 2001; Valtonen *et al.* 2003). This may lead in turn to an increase in parasite infections, an increase in secondary infections from viral, bacterial and fungal pathogens and a more severe pathological response to parasitism (Vethaak 1992; Lafferty & Kuris 1999).

6.4.4 Implications for fish and parasite populations

The winter spike in salinity observed in the Blackwood River in this study is a regular, seasonal occurrence (Mayer *et al.* 2005; see Figure 6.2). This transient increase in salinity is unlikely to have long-term effects on parasite population sizes, even for those ectoparasites which appear to have been reduced in abundance on *T. bostocki*, as most will have at least one stage which is resistant to environmental conditions (Moller 1978). It also clearly not affecting intermediate hosts of those parasites, such as trematodes, with complex, indirect life cycles.

The effects on fish populations of increased parasitism and greater pathological response as a result of the annual salinity spike are more difficult to judge. Although there are no experimental data on the salinity tolerance of *T. bostocki*, it is restricted in its current geographic distribution to areas of water bodies that are not severely salinised, such as the lower catchment of the Blackwood River (Morgan *et al.* 1998, 2003). Although the population in the lower Blackwood River is apparently coping

with regular, seasonal increases in salinity, these are occurring at a critical time, just prior to spring spawning (Beatty *et al.* 2006). The increase in parasitism and parasite-related pathological changes at this time may impact reproductive success, although there is currently no evidence that this is the case. Nevertheless, with a predicted increase in stream salinisation due to increasing temperatures and decreased rainfall in the south-west of Western Australia (Hughes 2003), any processes that place additional stress upon native freshwater fishes in the region deserve further study.

CHAPTER 7

General conclusions

7.1 Freshwater fishes of the South West Coast Drainage Division

In the south-west of Western Australia only 14 native species of fish are found in freshwater habitats. Ten of these species are confined exclusively to freshwater and eight are endemic to the region (Morgan *et al.* 1998). According to Morgan *et al.* (1998), many of the native freshwater fish species found in south-western Australia occur in very specific habitat types and currently have very restricted distributions. This is likely due to the many anthropogenic changes that have occurred to Western Australia's rivers and streams since European settlement. These changes are particularly evident in the South West Coast Drainage Division (S.W.C.D.D), where only one of 19 river basins is not substantially degraded by the construction of dams, extraction of water for irrigation, salinisation and sedimentation as a result of land clearing, agricultural runoff, mine wastes, and the introduction of exotic animals and plants (Olsen & Skitmore 1991; Pen 1999).

At least nine, and possibly more species of exotic fish have been introduced into the S.W.C.D.D. and established self-sustaining feral populations (Morgan *et al.* 2004). The establishment of exotic fishes is often enhanced by their ability to tolerate degraded habitats and also to thrive in foreign environments.

In the current study, I sampled 1429 individual fishes of 18 different species (12 native and 6 exotic) from 29 locations throughout the S.W.C.D.D.

7.2 Parasites of freshwater fishes in the South West Coast Drainage Division

This is the first comprehensive study on the parasites of native and exotic fishes in the inland waters of south-west Western Australia. Given the known threats to the native freshwater fish fauna of the region, the lack of data on parasitic infections is both surprising and unfortunate, because parasites are very important mediators of ecosystem function and therefore of how invading species may affect ecosystem function (Prenter *et al.* 2004; Thomas *et al.* 2005; Torchin *et al.* 2005; Taraschewski 2006).

In this study, I found 44 putative species of parasites in the 18 different species of fish that were sampled from the S.W.C.D.D. This regional parasite diversity is much lower than that found by Dove (2000) in the East Coast Drainage Division, south eastern Queensland, where 109 different parasite species were recorded from only 330 individual fishes of 18 different species. My study undoubtedly underestimated the number of protozoan and myxozoan species present, but even when only helminths and arthropods are considered, I found 37 species compared to 77 species in Dove's (2000) study. The depauperate parasite fauna in the south-west is probably a reflection of the depauperate fish fauna; the region contains less than half as many species of freshwater fish as the other major drainage systems of eastern and northern Australia (Morgan *et al.* 1998).

The majority of the parasites found in this study appeared to be indigenous, with previous distribution records suggesting that only five of the 44 putative species were exotic in origin. As far as it was possible to ascertain, most of the native species have not previously been described. All of the specimens from this study are held in a

reference collection at the Fish Health Unit, Murdoch University, and taxonomic work has been commenced on a number of the species. In this study, I have described, based on morphological characteristics, a new copepod species from the genus *Dermoergasilus*, but much further work remains to be done to document the parasite biodiversity of native freshwater fishes in the south-west of Western Australia.

7.3 Reduced parasitism of exotic fishes

The results showed that exotic freshwater fish species in the south-west of Western Australia were much less likely to be parasitised than native species, and had significantly reduced regional parasite diversity. Despite the lower prevalence of parasitic infection and reduced diversity of parasites in exotic fishes, compared to native fishes, there were no obvious differences in species composition between exotics and natives. This reduced parasite load of exotic species compared to native species was also found by Dove (2000) in his study of freshwater fishes in eastern Australia. According to Torchin *et al.* (2003) and Prenter *et al.* (2004), the reduced parasitism of introduced exotic species has several causes, including a low probability of the introduction of parasites with founding populations, absence of other required hosts in the new environment and the host-specific limitations of native parasites adapting to new hosts.

The parasite release hypothesis proposes that the reduction in parasitism of introduced exotic species leads to increased body sizes, greater population densities and enhanced competitive ability when compared to native species with which they interact (Keane & Crawley 2002; Torchin *et al.* 2002; 2003; Mitchell & Power

2003). There is, however, little empirical evidence for these effects and this is a very important topic for further research as we try to understand and manage the spread of exotic fishes at the expense of native fishes throughout the S.W.C.D.D. and, indeed, throughout almost all river systems in Australia.

7.4 Transmission of parasites from exotic to native fishes

When exotic parasites are introduced to a new area with invading hosts, they may transfer to native hosts. According to McIntyre (1996), introduced parasite species might be competitively superior to native parasite species in the new environment and thus might colonise new hosts relatively quickly. All of the five presumed introduced parasites species that were found in this study (*Gyrodactylus* sp., *Dactylogyrus* sp., *Spirocamballanus* sp., *Ligula* sp. and *Lernaea cyprinacea*) are generalist parasites and were found on native fishes. *Gyrodactylus*, *Dactylogyrus* and *Spirocamballanus* were quite restricted in their distribution, being found in only one river and on one or two host species. *Lernaea cyprinacea* was also restricted to one river, although it was found on a wider range of hosts. *Ligula* sp. is probably the most pathogenic and had the widest geographic distribution (three different rivers) and host range (five different native fish species) of all the exotic parasite species that were found in this study. The most likely route of introduction of all these exotic parasite species is with cyprinid aquarium fish.

It has been suggested that introduced parasites are often more pathogenic to new, native hosts than to their original host, because the new hosts may lack evolved defenses against the parasite (Dove & Ernst 1998; Prenter *et al.* 2004). There is some evidence from the present study that *Lernaea* infections may occur more readily, and

have more serious pathogenic consequences, for native fishes in the S.W.C.D.D. Experimental work in a laboratory situation is required, however, to confirm this and to determine whether it is due to differences in behaviour and/or to differences in immune response between native and exotic fishes. I have some anecdotal evidence that exotic cyprinid and native galaxiid hosts are equally susceptible to infection by *Lernaea* copepodids, but that cyprinids are better able to tolerate or to resist sustained infestation by adult *Lernaea*.

7.5 Transmission of parasites from native to exotic fishes

I found eight parasite species on exotic fish hosts in the S.W.C.D.D. and six of these were presumably native parasites which had transferred to new host species. Exotic hosts may act as reservoirs, allowing native parasites to maintain high population densities even as native host populations decline and this could be important in the conservation of native fish species. At present, we have little information on the factors favouring the transmission of native parasites to exotic hosts or on the relative pathogenicity of native parasites in native or exotic hosts; this is an area where much more work is needed.

7.6 The effect of habitat disturbance on parasitism in native fishes

In this study, the proportion of native freshwater fishes with parasitic infections and the component community diversity of the parasite fauna of fishes in the south-west of Western Australia were both negatively related to habitat disturbance, in particular to a suite of factors (river regulation, loss of riparian vegetation, eutrophication and presence of exotic fish species) that indicate increased human usage of the river and surrounding environment. The reduced parasitism of fishes in disturbed habitats was

primarily due to a loss of endoparasitic helminths with complex, indirect life cycles, a phenomenon that has also been found in a number of other studies (Lafferty 1997; Galli *et al.* 2001; Marcogliese 2004). Despite the overall reduction in parasitic infections and parasite diversity in localities with greater human usage, some parasite species were found more frequently in these localities, possibly because of a reduced host immune response or a positive effect of disturbance on intermediate hosts. This illustrates the importance, when considering the value of parasites as indicators of environmental quality, of understanding parasite biology and considering different host/parasite associations on a case by case basis (Marcogliese 2004).

7.7 The effect of salinity on parasitism in freshwater cobble, *Tandanus bostocki*

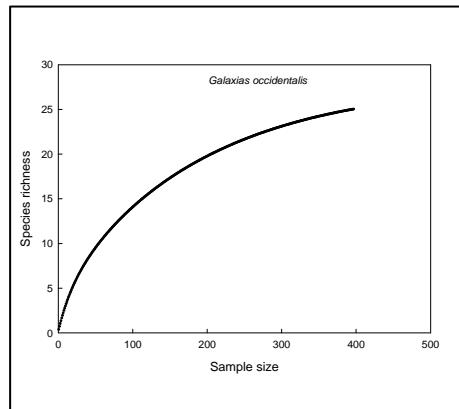
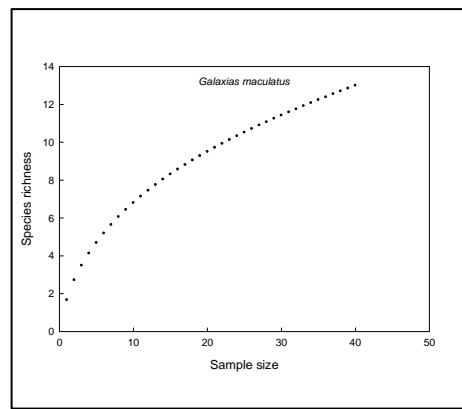
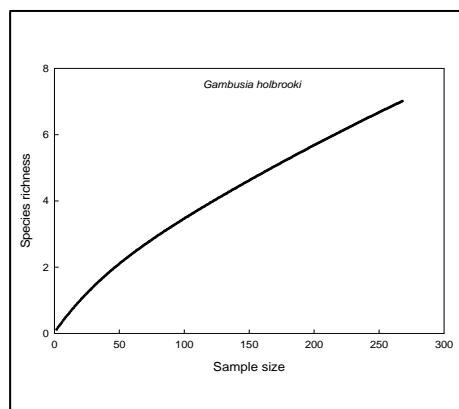
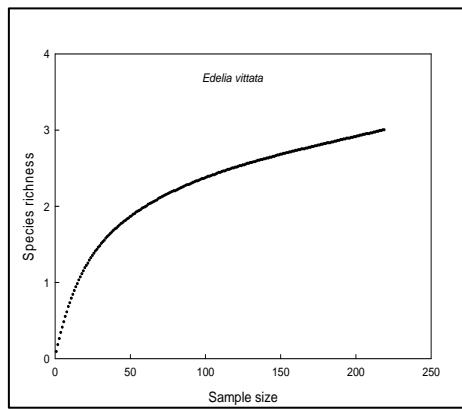
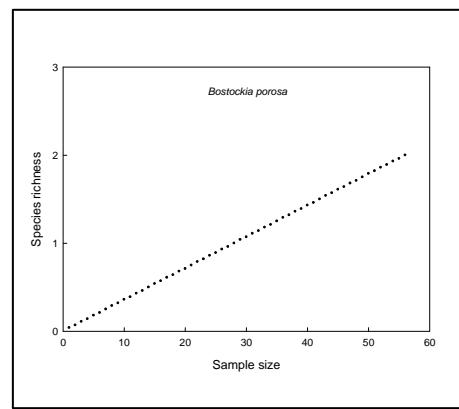
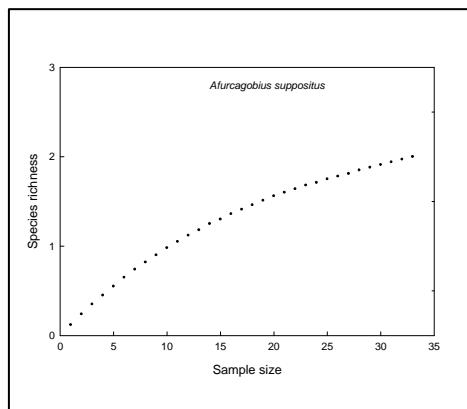
The overwhelming importance of host and parasite biology in trying to unravel environmental effects on parasitism was further underscored in a study of the effects of regular, seasonal changes in salinity on parasitism of *T. bostocki* in the Blackwood River. In this case, spikes of increased salinity occur every year as saline water is washed down the river from the upper catchments; these salinity spikes appear to produce contrasting effects on the diversity and pathology of endoparasitic and ectoparasitic infections.

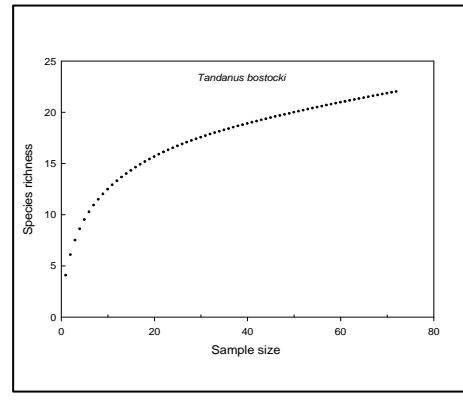
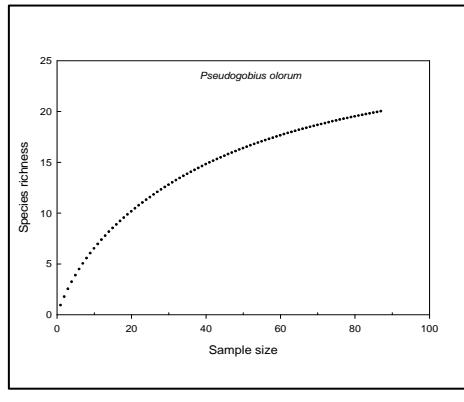
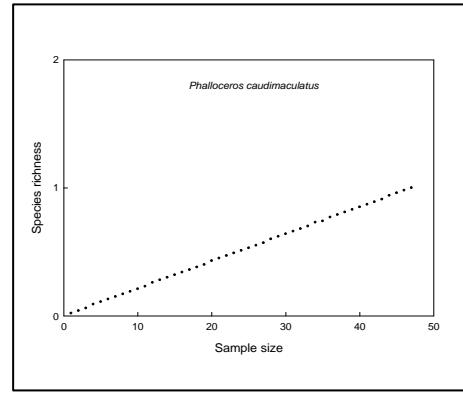
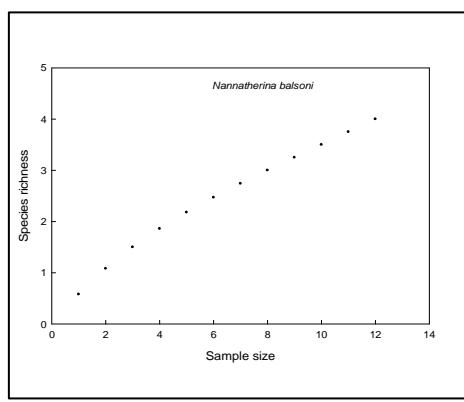
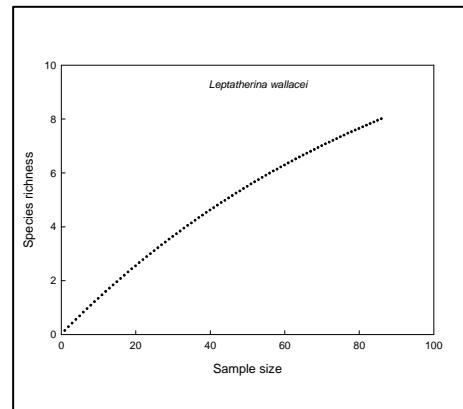
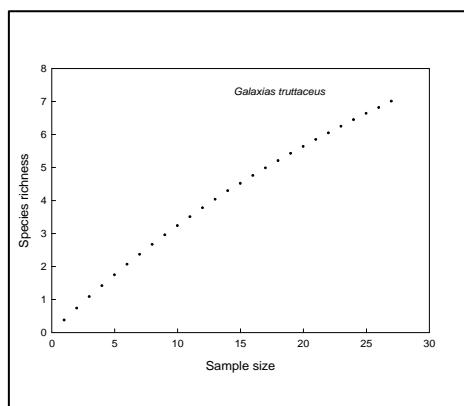
Ectoparasite species diversity decreased and parasitic skin lesions became less severe as salinity increased. This presumably occurred because ectoparasites were directly exposed, and adversely affected by, the abrupt salinity change, something that has been found in previous studies (Moller 1978; Garcia & Williams 1985). By contrast, endoparasite species diversity and the severity of parasitic intestinal lesions increased, possibly because endoparasites are largely protected from osmotic damage

in changing salinities, but the immune system of the fish host may be compromised (Landsberg *et al.* 1998; Lafferty & Kuris 1999).

At this stage, we do not understand the long-term implications of these regular changes in salinity, and the associated changes in parasite fauna and pathological effects, on the population of *T. bostocki* in the Blackwood River. Further studies are required to confirm my hypotheses on the contrasting effects of salinity spikes on endoparasitic and ectoparasitic infections. I suggest that more field observations over a number of seasons, and experimental studies on the relationship between salinity and parasite pathology are required. If more information is established, pathological lesions as a result of parasitic infections of fish may be found to be a useful indicator of the effects of salinisation on the ecosystem health of rivers in the south-west of Western Australia.

APPENDIX 1 – SPECIES ACCUMULATION CURVES





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