

**Can constructed wetlands act as
effective surrogate breeding habitats for
amphibian communities in areas where
few natural wetlands remain?**

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This thesis is presented for the degree of Doctor of Philosophy of the University of
Western Australia, School of Animal Biology.

February 2006

ABSTRACT

Amphibians are now considered more threatened and are declining at a more rapid rate than either birds or mammals. Habitat loss and degradation is considered the most threatening force in amphibian decline, yet very little research has explored the impacts that habitat modification may be having on amphibian populations in Australia.

Constructed wetlands, may potentially play a vital role in mediating the impacts of habitat loss in areas where many of the natural wetlands have been removed. However, the role that constructed wetlands can play in the conservation of amphibian species is far from clear. This study attempts to fill in some of the information gaps in Western Australia by investigating the capacity of constructed wetlands to support healthy frog communities. Specifically, I attempted to determine if the species composition and density of tadpoles and calling male amphibians differed significantly between constructed and natural wetlands? If so, 1) were there habitat differences between natural and constructed wetlands which strongly influence the amphibian community composition? 2) is predation likely to be an important factor and is its effect mediated by habitat complexity?

The study showed that the species richness of calling males did not differ greatly between constructed and natural wetlands in the south-west of Australia, but tadpole species richness did. Community composition of both calling males and tadpoles varied substantially between the two wetland types. The results suggest that the amount of native vegetation in the terrestrial landscape and riparian zone and wetland water regime all strongly influenced the ability of a wetland to support species rich tadpole communities. The abundance of most species (adults and tadpoles) correlated positively with the amount of native vegetation in the landscape and negatively with increasing wetland water permanence. Predator trials revealed that the reproductive output of amphibians in constructed ponds is likely to be detrimentally impacted by the presence of the introduced fish predator, *Gambusia.holbrooki*, but these predator impacts may be mediated by habitat complexity in the form of water colour and structural diversity.

Results of this study have several implications for conservation and wetland management and for the design and development of constructed wetlands. Wetlands should not be considered in isolation from the surrounding landscape. While at the pond level, water regime, morphology of the banks, presence of predators, the density

of riparian vegetation and structure of aquatic habitats all appear to influence the communities of amphibian that actively call and reproduce there. This study emphasises that constructed wetlands should be designed with a diversity of habitats. All constructed wetland and restoration projects should aim to mimic the hydrology, morphology and habitat types of the original natural wetlands of the region.

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1.0 Introduction

According to the first global assessment of amphibian populations, amphibians are now considered more threatened and are declining at a more rapid rate than either birds or mammals (Houlahan et al. 2000; Stuart et al. 2004). In 2004, 1856 species (32.5%) of amphibians were considered threatened under the IUCN red list categories of vulnerable, endangered or critically endangered. Trends illustrate that the status of amphibians has deteriorated substantially from the 1980's when 31% of species were considered globally threatened and of these approximately 4% were considered critically endangered (CE). Today the number of amphibians in the CE category has almost doubled and the World Conservation Union Global Amphibian Assessment (GAA) estimates that between 9 and 122 amphibian species have become extinct since 1980 (Stuart et al. 2004). Of the 435 species that qualified for listing in IUCN categories of higher threat since 1980, 183 species (42%; 20 endemic to Australia), are suffering significant losses due to habitat destruction and modification (Stuart et al. 2004).

In 2004, the GAA identified that habitat loss and degradation are by far the greatest threat to amphibians, affecting nearly 4,000 species (IUCN Conservation International and NatureServe. 2004). The number of species impacted by habitat loss and degradation is almost four times greater than the next most common threat, pollution (IUCN Conservation International and NatureServe. 2004) and is considered a far greater threat than disease, or overexploitation. However, despite this apparent global threat and the continued loss of frog species to habitat destruction and alteration (IUCN Conservation International and NatureServe. 2004), very little research, has explored the impacts that habitat modification may be having on amphibian populations in Australia (Hazell 2003). In 1981, Rawlinson (1981) noted that Australian amphibian research had primarily focussed on taxonomy, evolution and ecology, with very little attention paid to the effects of human impacts, despite the ecological significance of these processes. Rawlinson (1981) emphasised the need for researchers and wildlife authorities to focus attention on the impacts of human activities on the Australian frog fauna. Yet in 2005, over 20 years later, when less than 7% of Australia's total landmass is reserved for land conservation (Australian Bureau of Statistics 2001) this situation has not altered greatly (Hazell 2003).

In a recent review of frog ecology studies in modified landscapes of Australia, Hazell (2003) noted that while many threats associated with frog habitat loss and change have been identified there is little quantitative information on frog-habitat relationships in modified landscapes, the role that habitat fragmentation is having on frog populations or investigations on the connectivity required between terrestrial and aquatic frog habitat. Consequently, frogs have largely been ignored in efforts to revegetate and manage for the conservation of Australian biota outside reserves and they are often overlooked in the compilation of conservation plans (Hazell 2003). Hazell (2003) concluded that frog conservation research in Australia must focus on the impacts of modified landscapes to avoid land-management decisions and conservation strategies based on inappropriate assumptions of how biota respond to landscape change.

Since 1788 forest cover across Australia has been reduced by an estimated 38% (National Forest Inventory Australia 1998) and over half of Australia's wetlands have been destroyed (Environment Australia, 1997). In some regions this destruction has been even more wide spread. For example, on the Swan Coastal Plain of Western Australia, 75% of the original wetlands have now been filled or drained and in the south-east of South Australia 89% of the natural wetlands have now been destroyed (Environment Australia 1997). Of those wetlands still remaining in Australia, many suffer adverse affects from anthropogenic activities including altered hydroperiod, pollution and the impacts of exotic invasive species (Environment Australia 1997; Lake 1995). It is therefore no surprise that post-European land clearing is considered to be the major factor affecting the distribution of many frog species in south-eastern Australia today (Gillespie & Hines 1999). The impact of habitat changes are clearly illustrated by the 34 species of Australian frogs or 62% of the current listing of Australian species on the IUCN list of vulnerable, endangered, critically endangered or near threatened list that are considered to be declining as a consequence of habitat loss, habitat fragmentation and declining habitat quality (IUCN Conservation International and NatureServe 2005). As highlighted by Hazell (2003) few studies in Australia have examined how frogs respond to habitat loss and its associated impacts and although the creation of wetlands has been considered advantageous for some frogs (e.g. Tyler & Watson 1999) knowledge of how frogs use these habitats is limited.

1.1 Use of habitats by amphibians in wetlands in modified landscapes

Globally, research on amphibian habitat use has received some attention. For example, in the Eastern Palouse (Northern Idaho, USA), where over 97% of natural wetlands have now been removed (Black et al. 1999), artificial ponds constitute the majority of the wetland areas available for amphibian use (Monello & Wright 1999). Recent investigations have shown that the artificial ponds in this region support several amphibian species, but at least two species have not effectively exploited these ponds for reproduction or dispersal (Monello & Wright 1999). Monello & Wright (1999) discovered that non-native fish were the most significant factor influencing the occurrence and reproduction of amphibian populations. In ponds with no fish, the age of the pond, the amount of emergent vegetation and the distance from agricultural, forest and/or grassland areas appeared to be the strongest factors influencing the occurrence of frog species. They concluded that although constructed wetlands in Eastern Palouse are playing a role in the conservation of some frog species, their value as conservation refuges are limited by their anthropogenic setting and the presence of exotic fish. They suggested it would be more suitable if conservation wetlands were created in areas of limited human disturbance where exotic fish are absent (Monello & Wright, 1999).

Similarly, Knutson et al (2004) studied small constructed agricultural ponds in southeastern Minnesota (USA), to assess their value as amphibian breeding sites. They found that many amphibian species in the area were effectively utilising the ponds for reproduction and noted that reproductive success was closely associated with pond characteristics such as water quality, vegetation and predators. They also identified that there was consistently low reproductive success in ponds in landscapes which were grazed and attributed this to poor water quality and the disturbance within the wetlands associated with grazing. The researchers concluded that small agricultural ponds, which are properly managed, may help sustain amphibian populations in landscapes where natural wetland habitat is rare (Knutson et al. 2004).

Similar studies in Italy (Ildos & Ancona 1994; Pavignano et al. 1990), the Netherlands (Laan & Verboom 1990; Stumpel & van der Voet 1998), Laos (Stuart & Davidson 1999), Brazil (Eterovick 1999), the United Kingdom (Baker & Halliday 1999; Beebee 1985; 1997) and the USA (Babbitt & Tanner 2000; Quinn et al. 2001) have

identified that frogs can and will utilise constructed ponds, provided suitable biotic and abiotic components are met. They have also identified that a species habitat requirements in a single region are often broad and ponds with a range of habitats are more likely to support a larger species pool, than ponds with limited habitat complexity.

Given Australia's large amphibian diversity (215 species IUCN Conservation International and NatureServe 2005) and the proportion of endemic species declining as a result of habitat modification, research in Australia on the role that artificial habitats can play in amphibian conservation has been relatively limited. Only ten published Australian studies, known to me, have focussed on the role constructed or highly modified wetlands can play in providing viable habitat to amphibian species (Hazell et al. 2001; Hazell et al. 2004; Healy et al. 1997; Humphries 1979; Pyke & White 1996; Tyler & Watson 1999; Watson et al. 1995; Williamson & Bull 1994; 1996; 1999). Four of these studies focussed on a single species (*Crinia signifera*, Williamson & Bull 1994; 1996; 1999; *Litoria aurea*, Pyke & White 1996) limiting the overall application of the studies to wider conservation systems. Two other studies (Hazell et al. 2001; Hazell et al. 2004) did not investigate the reproductive success of frogs in the wetlands studied making it difficult to determine the ecological significance of these habitats for the persistence of amphibian populations. Of the remaining studies, only one, Watson et al (1995) identified successful reproductive output in artificial wetlands. But, Watson et al. (1995) failed to compare breeding success in constructed wetlands with nearby natural wetlands and therefore the study provided only limited information on the value of these areas in a wider context.

1.2 *Factors influencing the persistence of amphibian species in modified landscapes*

1.2.1 *Introduced aquatic predators in modified landscapes*

Predation is considered to be one of the major ecological factors influencing the survival of amphibian larvae and hence the overall structure of amphibian communities (Alford 1989; Fauth 1990; Tejedo 1993). It therefore follows that the modification of wetland predator dynamics, such as the introduction of an exotic predator or a predator formerly excluded, is likely to substantially impact on larval amphibian communities and the subsequent reproductive success of those communities in that wetland. Babbitt

& Tanner (2000) showed that the composition of amphibian larval communities of previously fish free ponds, changed substantially with the introduction of fish predators, while ponds unaffected by the fish maintained tadpole communities commensurate with the previous years census. As identified by Hecnar and M'Closkey (1997) in natural systems, species of tadpole that are vulnerable to predation by fish predators survive in water bodies where predatory fish are absent and those vulnerable to invertebrate predators survive in water bodies with fish that reduce the density of invertebrate predators. However, what is not clear is what happens to amphibians in environments where there is no longer any choice?

In Australia one of the most widespread and common introduced aquatic predators of amphibian larvae, is the mosquitofish *Gambusia holbrooki* (Gillespie & Hero 1999; Pusey et al. 1989). The species is exceptionally tolerant of a broad range of environmental conditions (Pen & Potter 1991b) and will inhabit marshes, lakes, dams, slow-flowing streams and associated billabongs and aqueducts. However, it is generally most abundant in modified habitats near human settlement (Allen 1989; McDowall 1996). Its widespread and invasive nature has meant that *G. holbrooki* has received a great deal of research attention (Gillespie & Hero 1999). Numerous studies have identified that the fish has a negative impact on a variety of amphibian larvae including the vulnerable Australian native frog, *Litoria aurea* (Morgan & Buttemer 1996; White & Ehmann 1997; White & Pyke 1996). However, as noted by Gillespie and Hero (1999) the evidence that *G. holbrooki* has a major impact on the abundance of any Australian amphibian species is unclear. Most studies have only identified that the fish can kill a variety of anuran larvae often under very artificial conditions. Few studies to date have examined the effects that interactions between fish and habitat may have on amphibian persistence and most fail to examine the relative impacts of syntopic native predators and the role that habitat complexity may play in mediating the impacts of the fish (see Gillespie & Hero 1999).

1.2.2 *The availability of habitat*

Most pond-breeding amphibians rely primarily on two types of habitat: wetlands for breeding and terrestrial habitats for feeding, shelter and dispersal (Guerry & Hunter 2002). Therefore, the availability of habitat in both the aquatic and terrestrial

environments is likely to play a key role in the ability of a species to utilise and persist in a specific patch. One of the most significant and obvious impacts of habitat loss and modification is the fragmentation of habitat that once was relatively continuous. This disconnection of required habitat patches could have severe implications for the persistence of amphibians in a region. Laan & Verboom (1990) found that amphibian species richness in artificial pools in the Netherlands was strongly related to the distance to the nearest woodland, identifying a relationship between isolation and the probability of potential colonists arriving at a patch. The researchers concluded that the probability of occurrence of a species in suitable patches increases with source proximity and an increasing connectivity of the landscape (i.e. the presence of woodlands nearby; Laan & Verboom, 1990). Similarly, Hazell et al., (2001) discovered a positive relationship between the extent of native canopy cover in the surrounding landscape and frog species richness. Terrestrial habitat have been described as important amphibian habitats which increase habitat connectivity and provide suitable foraging and shelter environments during periods when the frogs are not breeding particularly in highly modified environments (Hazell et al. 2001).

The habitat within (e.g. water quality, hydroperiod, aquatic vegetation, etc) and immediately in association with a wetland can also influence amphibian assemblages (e.g. Hazell et al. 2001; Hazell et al. 2004; Ildos & Ancona 1994; Jansen & Healey 2003; Knutson et al. 2004; Pavignano et al. 1990; Pyke & White 1996; Stumpel & van der Voet 1998). A number of studies have shown that aquatic vegetation type and its density were extremely important in determining the local distribution of amphibians in wetlands. However, the response of species within a community is not always consistent, with some species likely to have a close affinity with relatively dense vegetation, whilst others demonstrate a clear preference for more open water habitats and relatively sparse riparian vegetation (Hazell et al. 2001; Ildos & Ancona 1994; Stumpel & van der Voet 1998). Most researchers linked this variation in habitat density association to the different life history traits of the studied species and concluded that wetlands that contain a large variety of habitats are most likely to support a rich and varied amphibian community (Hazell et al. 2001; Ildos & Ancona 1994; Stumpel & van der Voet 1998).

Unfortunately constructed wetlands are not known for their habitat complexity. Typically, artificial wetlands are constructed to create a water body of sufficient size to serve a specific purpose, e.g. water holding for the lowest capital outlay (Hammer

1992). As noted by Broome and Jarman (1983) constructed wetlands are often structurally and biologically less complex and diverse than natural wetlands in the same environment. As a consequence, artificial wetlands tend to be poor mimics of natural wetlands and have limited habitat availability in both the aquatic and terrestrial zones. Brock et al. (1999) identified that the artificial selection for deep steep sided water supply reservoirs and small dams has replaced a mosaic of heterogenous wetland types in the landscape by a relatively less diverse system across most of rural Australia.

The variation in response of amphibian species identified by Hazell (2001) and others highlights the importance of understanding the ecological requirements of all frog species in a community and how each will respond to habitat change. To date most studies in Australia that have investigated amphibian utilisation of highly modified environments have focussed on eastern Australian communities or species. This skewed focus is most likely associated with the large number of declining species that are primarily found on the eastern seaboard of Australia (IUCN Conservation International and NatureServe 2005). This region of Australia has had a long and relatively intense history of habitat loss and fragmentation and has been the primary area of focus for amphibian researchers (Roberts, J. D., pers. com.). In comparison, habitat clearance and modification in Western Australia has been relatively recent (Burbidge et al. 2004; Sauders & Curry 1990). A detail clearly illustrated by the fact that to date only three anuran species endemic to Western Australia have been recommended for inclusion on the IUCN listing as a consequence of some form of habitat loss or degradation (IUCN Conservation International and NatureServe. 2004). However, the high level of endemism (49%; Tyler et al. 2000) in Western Australia's amphibian fauna and the increasing level of habitat removal emphasise the need to establish long term studies on the impacts of habitat modification before large numbers of amphibian species start to decline.

The need for proactive measures to prevent a negative amphibian response to habitat modification in the future is further emphasised by the potentially extended time lags between habitat loss and species response. Recent research has identified that the impacts of population losses may not be immediately evident and substantial time lags can occur. Hitchings & Beebee (1998) identified that the impacts of population isolation that occurred in England approximately 60 years prior is only just being realised. Habitat fragmentation resulting from urban development has influenced the

genetic diversity and fitness of the relatively common species *Bufo bufo*, placing in doubt the long-term viability of urban populations (Hitchings & Beebee 1998).

1.2.3 *The role of constructed wetlands*

As identified, the role that constructed wetlands can play in the conservation of amphibian species is far from clear (Quinn et al. 2001). In Australia, research has been patchy and mostly qualitative, restricting the ability of land managers to develop conservation plans that address even some of the most basic requirements of Australian amphibian species. Understanding the role that constructed wetlands play in the conservation of Australian amphibian may be vital to ensure the long-term persistence of many native species. Effective conservation decisions and long-term management cannot be made without thorough knowledge of the processes that affect the persistence of species in modified landscapes. Steps must therefore be taken to reduce these information gaps and to broaden our understanding of the impacts of habitat loss and modification on our amphibian fauna.

This study attempts to fill in some of those information gaps in Western Australia by investigating the capacity of constructed wetlands to support healthy frog communities. Specifically, I compared natural and constructed wetlands and determined whether amphibian communities in constructed wetland mimic those found in natural wetlands in the same geographical location. This was achieved by answering the following questions:

- 1) Do the species composition and density of tadpoles and calling male amphibians differ significantly between constructed and natural wetlands? If so,
 - i) are there habitat differences between natural and constructed wetlands which strongly influence the amphibian community composition?
 - ii) is predation likely to be an important factor and is its effect mediated by habitat complexity?

Answering these questions will improve our understanding of the physical characteristics that influence the suitability of artificial wetlands to act as successful breeding habitat for Western Australian frog species. This information is vital in

developing an understanding of the conservation value of artificial wetlands and to enable effective management decisions in the future. Developing knowledge of the importance of various habitat attributes will also encourage better wetland design and restoration for the enhancement of habitat and enable the development of multifunction wetlands.

2.0 Variation in species richness and the relative abundances of calling males in anuran communities in constructed and natural wetlands.

2.1 Introduction

Recent quantitative research has identified that amphibian populations are declining on a world-wide scale (Houlahan et al. 2000) and to date 34 species have been listed as extinct, while 427 species have been listed as Critically Endangered (the category of highest threat) under the IUCN classification (Stuart et al. 2004). Forty eight percent of the rapidly declining species are threatened by unidentified processes and the remaining 52% are declining as a result of over-exploitation or significant loss of habitat (Stuart et al. 2004). In Australia where over half of the natural wetlands have been destroyed since European settlement (Environment Australia 1997), habitat destruction has been identified as one of the major causes of amphibian declines (Gillespie & Hollis 1996; Hazell 2003; Wardell-Johnson & Roberts 1991). Habitat degradation processes such as pollution/climate change (Bertram & Berrill 1997; Bidwell & Gorrie 1995; Bishop 1992), the introduction of exotic predators (Gillespie & Hero 1999; Gillespie & Hollis 1996; Hines et al. 1999; Laurance et al. 1996; McDonald & Alford 1999), disease (Berger et al. 1999; Bradford 1991) and changes to wetland hydrology (Babbitt & Tanner 2000) have also been identified as posing considerable threat to the persistence of many amphibian species in Australia.

However, not all human activity is necessarily deleterious. A large number of new water bodies have been created as a consequence of development and these water bodies may be used by a variety of fauna. The types of created water bodies include farm dams, water reservoirs, pits created from mining activities, effluent treatment wetlands, evaporative salt works, etc. By 1974 over 100 000 farm dams had been constructed in the south-west of Western Australia (McComb & Lake 1988b) and by 1988 over 400 000 farm dams had been recorded as constructed across the nation (McComb & Lake 1988a). In addition, thousands of reservoirs and numerous mining pits and other man-made wetlands (intentional and unintentional) have been created throughout Australia. These waterbodies represent a substantial area of potential habitat

for flora & fauna that rely upon fresh water to complete their life cycle. Records of Australian fauna utilising these artificial “wetlands” abound. For example in April of 1983 it was estimated that of 120 000 migratory waders surveyed in the Broome/Port Hedland region of Western Australia, over 25% were feeding in constructed ponds at the Leslie Salt Works. A similar survey in Port Phillip Bay, Victoria showed that over 75% of the waders recorded were found on constructed ponds of three salt works and a large constructed wetland in treatment sewage works near Werribee (McComb & Lake 1988a).

Use of man-made ponds by animal groups such as birds (e.g. McComb & Lake 1988a; Worrall et al. 1997) and invertebrates (e.g. McComb & Lake 1988a; Sanders 2000; Worrall et al. 1997) have been well documented. In contrast, although a number of studies have focused on frog populations in constructed wetlands in Australia, few have shown that man-made ponds can substitute for natural wetlands as alternative habitat for frog communities. In a number of studies (e.g. Hazell et al. 2001; Hazell et al. 2004; e.g. Ildos & Ancona 1994; Monello & Wright 1999; Stuart & Davidson 1999) frog species have been shown to utilise man-made ponds. However, far fewer studies have compared the presence and abundance of species in constructed wetlands to the densities and species composition commonly found in natural wetlands in the same region. Hazell et al. (2004) showed that frog community composition differed in natural and constructed ponds, even though frog species richness was similar in both systems. Hazell et al (2004) also showed that chorus size can be significantly higher in natural than constructed wetlands for a single species (*Limnodynastes tasmaniensis*) but their results indicated that this relationship was mediated by the presence of fish. Understanding, whether amphibian communities that use artificial ponds are comparable to those in nearby natural wetlands is important because it will provide information about the ability of constructed wetlands to act as surrogates for natural wetlands in areas where these habitats have been removed. Further, we may gain insight into the reasons why man-made wetlands do or do not perform this function with the obvious implications for the construction and management of artificial wetlands.

The aim of this study was to determine whether the species composition and relative abundances of chorusing amphibians differed significantly between man-made ponds and natural wetlands in the south-west of Australia. A second aim was to determine whether species composition and relative abundances of calling amphibians

was related to any particular habitat differences that occurred between constructed and natural wetlands.

2.2 Methods

2.2.1 The study area

The Capel region (33.33°S and 115.34°E) is located on the Swan Coastal Plain of the southwest of Australia, approximately 200km south of Perth (Figure 2.1). The area originally supported mixed woodlands of tuart (*Eucalyptus gomphocephala*) and *Banksia* spp., interspersed with swamps and wetlands (Davies 2002). However, in the late nineteenth century the area grew as a centre for farming and much of the swamp habitat was cleared and exploited to provide good fertile soil for horticulture (Davies 2002), while woodland areas were cleared to make way for agriculture and mining development.

Estimates indicate that by 1992, 67% of the southwest region of Western Australia, had been cleared for agriculture, urban development and mining (Environment Western Australia, 2006). Clearing continues to this day with monitoring showing that between 1996 and 2004 there has been a net decrease in the vegetation cover in 64% of the bioregions of this corner of Western Australia (Environment Western Australia, 2006). Of the 48 940ha of land that comprises the landcare district of Capel only 37.6% remains vegetated (Shepherd et al, 2002)

Today the Capel region's major industries are mining, agriculture and forestry (Environment Australia, 2006). Over 90% of the mineral production in Australia occurs in the southwest of Western Australia with the majority of the industry focussed in the Capel, Busselton and Jangardup districts (South West Area Consultative Committee, 2006). This high intensity mining (Figure 2.2) in combination with other industry based activities (i.e. agricultural development, forestry etc) and the high population growth in the region has resulted in a highly modified landscape with few natural wetlands remaining. Estimates indicate that up to 75% of the natural wetlands on the Swan Coastal Plain have been removed since European settlement in the district (Chambers & McComb, 1994). Today most of the wetland systems in the southwest region are those that have been created as a consequence of mining excavations which intercepted the water table or other human disturbance.

2.2.2 Study site

I surveyed 16 wetlands in the Capel region (33.33°S and 115.34°E) of southwestern Australia (Figure 2.3) for amphibians from April 2001 to December 2002. Eight of the wetlands were natural, (i.e. they were water bodies that were not constructed, but may have been modified through vegetation clearance or the presence of introduced species) and were subject to seasonal water fluctuations. The remaining 8 were ponds constructed between 1975 and 1990 during the extraction of mineral rich sands (Davies, 2002; Doyle, pers. com.).

Initially the constructed wetlands were used for the disposal and treatment of mine-waste water (Chambers & McComb 1994). Water quality testing in the 1980's indicated that many of the ponds suffered from low pH, had excessively high loads of iron and magnesium and were highly phosphorus deficient (Davies, 2002). However, subsequent works within the wetlands to create more natural wetland systems and the upgrading of the wastewater treatment facilities in the processing plant, has amended most of these issues. Subsequent tests have indicated that all of the study wetland's consistently recorded neutral pH and iron and magnesium concentrations have dropped to acceptable levels (Chambers & McComb, 1994; Doyle, pers. com; Meney, pers. com.). Water levels in the constructed wetlands ranged from permanently wet ponds to seasonally fluctuating waterbodies. ranged in size from 2.5 to 3.1ha when full. Wetlands ranged in size from 2.5 to 3.1ha and their depth ranged from 0.5 to 3m deep at maximum capacity.

Wetland were chosen based on their size known water quality and accessibility. Some mine-constructed wetlands were not accessible due to the quick sand like nature of their sediment and strict site protocols limiting site access. All wetlands need to be access both during daylight and nocturnal hours and therefore sampled wetlands were restricted to those where this was deemed acceptable by the mining companies. Wetlands also need to be in relatively close proximity to allow consecutive sampling in a single night. Of those that were deemed accessible, wetlands with very poor water quality (i.e. low pH, high metal content, etc) were excluded. Given the constraints a total of eight constructed wetlands were available for the 2 year project.

It was assumed that all amphibians that bred in the region could colonise all the wetlands sampled. Previous research at a number of the constructed wetlands (Davies,

2002) indicated that all species of frog common in the region were present at the site. Monitoring of amphibian movement indicated that most of the individuals were colonising the sites from nearby natural wetlands (Davies 2002). The close proximity of natural wetlands to all of the constructed wetlands was likely to provide ideal source populations of all available amphibian species to each of the constructed wetlands.

2.2.3 *Calling activity*

Density estimates for the frog communities were made using nocturnal audio counts along two 100m transects at each of the 16 wetlands. This is considered an effective way to inventory species composition and the relative abundances of calling amphibian males that exploits the species specific calling behaviour of breeding males. All call counts were undertaken by a single surveyor who was skilled in the audio differentiation of species in the region. This ensured all calling species were quickly and accurately identified during all surveys providing consistent recording between study sites. Approximately one hour after sunset and before 01:00 I walked along each transect and stopped every 10m and listened for 30 seconds. All frogs heard calling within 5m radius of my observation point during each 30 second period were counted. These data provided a density measure of the calling males of each species for each transect in each wetland. This sampling method does not account for female, juvenile or non-breeding male abundance and therefore it does not provide a measure of the total abundance of each species. The measure does however, provide a good indication of the relative size of the calling male population between wetlands and can be a very effective way to inventory species composition (Zimmerman 1994). Driscoll (1998) identified that this method of measuring calling male abundance was effective at measuring between 76 and 96% of the calling males of *Geocrinia alba* and *G. vitellina*, respectively. Similarly, Stevens and Paskowski (2004) noted that calls surveys of *Rana sylvatica* provided a good indicator of the relative number of egg masses of this species in a pond. Counts of adult calling frogs were therefore assumed to provide a good representation of the number of breeding adult males present at a wetland.

Density and species composition measures were made every four to six weeks between March and November 2001 and 2002 providing a total of 16 measures for each wetland. Repeated monitoring over the two, 9 month periods, encompassed the breeding season of the entire amphibian community for the area, and provided seasonal

density estimates in the wetlands and the identification of any inter-site variability in the peak of the calling season for each species. As no explosive breeders occur in this region it was assumed that the repeated measures over the known breeding season of all potential amphibian species would ensure that all frog species utilising the ponds would be recorded.

Transects were identified after an initial pilot study to determine frog calling habitats. Transects were positioned to enable sampling of all potential habitat types that frogs were heard calling in. The habitat sampled included wetland margins (areas that only flooded during high rainfall periods), deep pools and shorelines. The section of the transects that ran along shorelines moved as water levels rose and fell. This ensured that species that called in the aquatic zone were consistently sampled throughout their breeding seasons. The start time for each transect was randomised between one hour after sunset and 01:00 to ensure audio surveys were not always conducted at the same time.

2.2.4 *The presence of Gambusia holbrooki*

Each wetland was surveyed for the presence of the introduced fish *Gambusia holbrooki* in each sample period. Their presence or absence was determined through the use of visual surveys, followed by sweep net sampling. This fish species is commonly noted to aggregate along the warm shallow margins of wetlands during daylight hours (Krumholz 1948, Winkler 1979). This behaviour enabled effective visual identification of the presence of this species in wetland habitat. However, all wetlands were also rigorously sampled for fish and other predators using sweep nets as part of the sampling protocol outlined in chapter 3.

2.2.5 *Environmental parameters*

Vegetation in each wetland was divided into 2 categories, aquatic (vegetation below the winter high-water mark) and riparian (habitat above the winter high-water mark). The percentage of the water surface containing submerged, floating, or emergent vegetation was estimated (recorded as AV, aquatic vegetation) within 10m of each audio transect. Along the sections of the transect that sampled the shoreline and consequently moved with the fluctuating water levels, the measured vegetation was based on an average across the areas which were sampled. The percentage cover that riparian (reeds, sedges, etc) or canopy vegetation provided within 10m above the high water mark on each transect was also estimated (recorded as RV, riparian vegetation). Aerial photographs (1:25 000) were examined to estimate the percentage of native vegetation in and surrounding each wetland at 500m (FV, 500m vegetation) and 1000m (OV, 1000m vegetation) radius. Water permanence (WP) was estimated during the survey period to provide a proportion of months with surface water for each wetland.

2.2.6 *Data analysis*

I analysed total species richness and maximum abundance data for each calling frog species in each wetland over the two year field study and related calling frog abundance and species richness to habitat variables (AV, RV, FV, OV & WP). As the vegetation in the surrounding landscape (FV and OV) and the permanence of the water (WP) were strongly correlated and the overall sample size of the test was small relative to the number of variables a multiple linear regression using all independent variables was likely to lack power. Therefore, I ran a Principle Components Analysis (PCA) using a correlation matrix and varimax rotation on the arcsine (\sqrt{x}) transformed habitat variables (AV, RV, FV, OV & WP) prior to the multiple linear regression (SPSS 9). This reduced the number of variables used in the regression and provided a more powerful analysis while removing the influence of collinearity. The model included an entry analysis using the components from the PCA to determine which components most strongly contributed to the model. In instances where one component was identified to not strongly influence the model, the component was removed from the multiple linear regression and the model re-run with the excluded component.

Several species were also tested to determine whether there was a relationship between occurrence of a species and the presence of the introduced, predatory fish *Gambusia holbrooki*. The presence/absence of a species in relation to the presence/absence of the fish, *G. holbrooki*, was tested using a Chi-squared test on a contingency table. Wetlands in each year (i.e. 2001 and 2002) were treated as separate measures as fish presence and absence changed between the two seasons as did the presence of calling activity for a number of species. This provided a total sample size of 32 for the test and prevented the occurrence of low frequencies (<5) in the contingency table which can influence the strength of the chi-squared tests (Quinn & Keogh 2002).

Differences in the abundances of a species between each wetland type were tested using a repeated-measures analysis of variance (ANOVA). The test revealed whether the calling males of a particular species were more commonly found in a particular wetland type (constructed or natural). The peak calling month was determined by identifying the single sample which recorded the maximum average

abundance across the two transects for the focal species in each wetland type in each year. Data from this sample were subsequently log (number calling males +1) transformed to normalise data before analysis. The peak abundance data of each species of calling male on each transect in each wetland were then analysed in a repeated-measures ANOVA. Individual wetlands were treated as randomly chosen experimental units and were nested within wetland type (fixed, $n=2$). Year (fixed, $n=2$) was the repeated measure. These analyses revealed whether the abundances of calling males were significantly different between wetland types (constructed vs natural wetlands).

Differences in the species richness between each wetland type were tested using a repeated-measures ANOVA. Species richness was identified by counting the number of species recorded along each transect, in each wetland in each year, giving 2 replicates per wetland, repeatedly sampled over two years (2001 and 2002). Individual wetlands were treated as randomly chosen experimental units and were nested within wetland type (fixed). Year (fixed) was the repeated measure. This test identified whether richness differed markedly between each wetland type.

2.3 Results

2.3.1 Environmental parameters

Below average rainfall in 2001 (Figure 2.4) resulted in unusually low water levels in several wetlands. One constructed wetland dried for the first time since its construction, eliminating a large population of the introduced predatory fish *G. holbrooki* from the wetland in early 2002. Several natural wetlands that had not dried for approximately 10-15 years were completely dry for much of 2002. While, two strongly ephemeral natural wetlands (i.e. on average held water for less than 7 months of the year) remained almost completely dry and only received surface water for a few weeks in 2002. One natural wetland did not hold any surface water over the study period but this wetland did support a large population of calling males of the burrowing frogs *Heleioporus eyrei* and *P. guentheri*.

Aquatic vegetation (AV) within the wetlands was similar in the two wetland types (mean AV constructed = 40.2 ± 11 s.e., $n = 8$ and natural = 53.42 ± 5.8 s.e., $n=8$). On average, constructed wetlands had less surrounding vegetation than natural wetlands

(Figure 2.5). The only exception was the natural wetland, Lake McCarley, which had only 10% cover of remnant vegetation within 1km of the wetland. This was due to the wetland's location on a dairy farm and its proximity to old sand mining areas. On average constructed wetlands retained water for most of the year with an average of $95\% \pm 3.12$ s.e., $n=16$, of the total survey months having some surface water across the treatment group. In comparison, on average surface water was only noted in natural wetlands in about $51\% \pm 12.28$ s.e., $n=16$, of the survey months.

2.3.2 *Frog communities in constructed and natural wetlands*

A total of 10 species of anuran were recorded in the 16 sampled wetlands (Tables 2.1 and 2.2). Of these, *Crinia glauerti* was the most common and was recorded in 15 wetlands with densities varying from zero to 36 frogs/transect. The least common species were *Heleioporus psammophilus* and *C. georgiana* with only two and five individual males, respectively, recorded calling over the two year study. None of the recorded species was exclusive to constructed wetlands. *Pseudophryne guentheri* and *H. psammophilus* were only recorded in natural wetlands.

On average the total species richness over the two year study (i.e. the total number of species recorded in each wetland over the entire study) in constructed wetlands was 5.125 ± 0.51 s.e., $n = 8$, species of calling male while natural wetlands recorded 6.37 ± 0.40 s.e., $n = 8$, species of calling male. Within year species richness (i.e. the number of species recorded within a single calendar year) differed markedly in natural wetlands between 2002 (Table 2.2) and 2001 (Table 2.1) in natural wetlands (6 species ± 0.53 s.e., $n = 8$ and 4.62 species ± 0.53 s.e., $n = 8$ respectively). In comparison the within year species richness varied little between years in the constructed wetlands; both 2001 and 2002 recording an average of approximately 4 species within each year (± 0.56 s.e., $n = 8$ in 2001 and ± 0.51 s.e., $n = 8$ in 2002: Tables 2.1 and 2.2).

There was no significant difference in the species richness between wetland types over the two year study (Table 2.3). However, a significant difference was identified in the interaction between wetland type and year (Table 2.3). A review of the data revealed that this difference was associated with the increase in the species richness from 2001 to 2002 in natural wetlands and an opposite response in constructed wetlands (Figure 2.6). The interaction with the nested term, year x wetland [wetland type],

indicates that there was also substantial variability between individual wetlands within wetland types between years.

2.3.3 *Habitat characteristics in constructed and natural wetlands and the occurrence of frogs*

The PCA identified two components that had eigenvalues greater than one and explained over 88% of the variance (Table 2.4). The loadings based on the correlation matrix showed that component 1 represented the landscape vegetation (FV and OV) and water permanence (WP), with vegetation within 500m (FV) and 1000m (OV) of a wetland loaded positively onto this component, while water permanence loaded negatively. This indicated that more disturbed landscapes (i.e. cleared of natural vegetation) were characterised by wetlands of increasing water permanence. Component 2 was a contrast between the aquatic vegetation (AV) and the riparian vegetation (RV), with aquatic vegetation loaded positively to the component and riparian vegetation loaded negatively. The contrast showed that wetlands with greater densities of riparian vegetation typically had less vegetation in the aquatic zone.

An ordination plot of the PCA components revealed component 1 (landscape vegetation and water permanence) contributed the most to the differentiation between constructed and natural wetlands (Figure 2.7). The plot also indicated that natural wetlands varied substantially along component 1, showing some variation in the vegetation in the landscape and the permanence of the water between individual natural wetlands. In contrast, constructed wetlands varied more along component 2 (local vegetation) suggesting some variation in the amount of aquatic and riparian vegetation between individual constructed wetlands. Multiple linear regression models based on these two components were fitted to all species recorded except for the calling males of *H. psammophilus* and *C. georgiana*. The low abundances of calling males of both species prevented meaningful analysis. In instances where one component was identified to not strongly influence the model the component was removed from the multiple linear regression and the model re-run with the excluded component.

The abundance of calling males of *C. insiginifera*, *G. leai*, *L. moorei* and *L. dorsalis* showed no relationship with the tested habitat components extracted from the PCA. Initial regression models showed that Component 2 (local vegetation) did not contribute strongly to the model for any of the tested species. This component was therefore removed from the multiple linear regression for all species. Component 1

(Landscape vegetation and water permanence) was positively associated with *Heleioporus eyrei* and *P. guentheri* and explained 63% and 45% of the variation in the abundances of calling males of each species respectively. *Crinia glauerti* and *L. adelaidensis*, responded negatively to component 1 (local vegetation) with 38% and 30% of the variance explained by the model respectively (Table 2.5). Species richness showed no relationship with the tested habitat variables.

Chi-squared tests were conducted on the presence/absence of calling males of each species relative to the presence/absence of the introduced fish *G. holbrooki*. Only one species, *G. leai*, was found to have any significant relationship with the presence of the introduced fish. *Geocrinia leai* was less likely to occur at sites with *G. holbrooki* ($\chi^2 = 9.791$; $df = 1$; $P = 0.002$; $\phi = -0.553$; $P = 0.002$).

2.3.4 Calling males in constructed and natural wetlands

ANOVA tests were used to determine whether calling males of a particular species were more common in constructed or natural wetland. These tests were not conducted for calling males of the species *C. georgiana* due to the low numbers across both wetland types. In addition, both *H. psammophilus* and *P. guentheri* were only noted to be calling in natural wetlands (Table 2.1 and 2.2) and therefore statistical comparisons of variance between wetland types (constructed and natural) could not be conducted for either of these species.

Crinia insignifera and *H. eyrei* both showed variation in the abundances of calling males in individual wetlands between years (Table 2.6). Calling males of *H. eyrei* increased in numbers in 6 of the natural wetlands between 2001 and 2002, while three of the four constructed wetlands in which the species was recorded calling had reduce numbers of calling males of this species in 2002 compared to 2001. Of the 9 wetlands with higher abundances of calling males of *C. insignifera*, four were constructed, while only two natural wetlands had reduced numbers of calling males in 2002 compared to four of the constructed wetlands.

Five of the seven species tested showed significant variation between replicate wetlands within wetland types (wetland [wetland type]; Table 2.6 and 2.7). This indicates that the population abundance of calling males of *C. glauerti*, *G. leai*, *L. adelaidensis*, *L. moorei* and *L. dorsalis*, varied substantially between individual wetlands. However, the response to year x wetland type was consistent for all species,

with none of the tested species showing a significant variation between years between wetland type. Of the 7 species tested both *C. insignifera* and *H. eyrei* were significantly more common in natural ponds, while *C. glauerti* and *L. adelaidensis* were more commonly found calling in constructed wetlands (Table 2.6). Figure 2.8 illustrates that for *C. glauerti* this trend was consistent across both years. In contrast, calling male *L. adelaidensis* were more common in constructed wetlands in 2001 (Figure 2.9) but in 2002 there was no apparent preference for either wetland type. Significant differences in abundances of calling male *L. adelaidensis* during the peak calling month (sample 6) were not detected between years (Table 2.6).

Crinia insignifera were more common in 2002 than in 2001 (Table 2.6, Figure 2.10). There was no interaction between the main terms, year and wetland type, showing that the trend to increased calling in 2002 was consistent in both constructed and natural wetlands. Numbers of calling males of *G. leai*, *L. moorei* and *L. dorsalis*, did not differ significantly between constructed and natural wetlands (Table 2.7).

2.4 Discussion

2.4.1 *Biological attributes influencing frog communities in constructed and natural wetlands*

The aim of this study was to determine whether the community of calling males differed between constructed and natural wetlands within the same geographical location and to highlight whether habitat variables may be related to the presence of calling males at a wetland. This study identified that species richness was not significantly influenced by a wetlands origin (natural or constructed), but species composition varied between wetland types, with a number of individual species influenced by a wetland's origin and habitat variables, such as vegetation density and extent, water permanence and the presence of introduced predatory fish. Two species of frog did not call in constructed wetlands, but no species of frog was exclusive to constructed wetlands. In general burrowing species favoured natural wetlands, while the presence of calling males at a particular wetland type (natural or constructed) for several species appeared to be influenced by sample year. This may reflect the between year variation in hydroperiod experienced at many of the wetlands.

The preference of some species to call at natural wetlands in this study is consistent with the results of Monello & Wright (1999). Monello & Wright (1999) identified that artificial ponds could support several amphibian species (Eastern Palouse, Northern Idaho, USA), but noted that at least two amphibian species had not effectively exploited these ponds for reproduction or dispersal. Their research identified that the absence of some species from constructed ponds was strongly linked to the presence of an introduced, predatory fish. In ponds with no fish, the age of the pond, the amount of emergent vegetation and distance from agricultural, forest and/or grassland areas appeared to be the strongest factors influencing the occurrence of amphibians. Monello & Wright (1999) concluded that although constructed wetlands in their study area were playing a role in the conservation of some frog species, their value as conservation refuges was limited by their anthropogenic setting and the presence of exotic fish that preyed on tadpoles. Likewise, my study showed that habitat variables such as the amount of vegetation in the landscape, the presence of introduced predatory fish and the permanence of the water could influence the likelihood of recording a calling male of a frog species.

The abundance of *Geocrinia leai* was not influenced strongly by the extent of native vegetation or wetland type, but the presence of calling males was negatively correlated with the presence of the introduced, predatory fish, *G. holbrooki*. Studies by Monello & Wright (1999) identified that non-native fish were the most significant factor influencing amphibian species occurrence and reproduction. Similarly, Resetarits & Wilbur (1991) identified that both male and female *Hyla chrysoscelis* would often discriminate between ponds based on the fish species present within that pond. Notably, calling males of *H. chrysoscelis* actively avoided calling in ponds containing the predatory black-banded sunfish (*Enneacanthus chaetodon*). In contrast, Hazel et al. (2001) reported that the presence of fish did not appear to influence frog occurrence in farm dams in southeastern Australia. I have no data indicating whether males of *G. leai* choose calling sites based on the absence of fish or if the tadpoles of the species are vulnerable to *G. holbrooki* and this should be investigated further.

For the most part, association with habitat variables matched what is known about the ecology of each of the studied species. The negative association between water permanence and two species, *H. eyrei* and *P. guentheri*, is consistent with their reproductive strategies. These burrowing species rely on fluctuating water levels in the riparian zones of wetlands as they deposit eggs in burrows in autumn in anticipation of

winter flooding events (Main 1965). In addition, these predominately terrestrial species responded positively to dense native vegetation in surrounding terrestrial zones. The relationship detected here could reflect a dependence on upland vegetation zones, perhaps for feeding and shelter. Bamford (1992) reported that *H.eyrei* were regularly captured 2.5km away from the nearest possible breeding site and noted that consistent recapture of individuals within woodland habitats indicated that the species had a relatively sedentary period in woodland habitats between breeding seasons.

The two species that responded positively to increasing amounts of native vegetation in the landscape (*P. guentheri* and *H. eyrei*) were also positively associated with natural wetlands (*P. guentheri* was only found in natural wetlands). This apparent preference for natural wetlands over constructed wetlands may be a function of the wetland's locality. In this study, all constructed wetlands were set in highly modified and cleared landscapes. In comparison, the majority of natural wetlands were set in landscapes with relatively large amounts of remnant native vegetation. Monello & Wright (1999) indicated that constructed wetlands were limited in their suitability as conservation refuges for frogs primarily because of their location in a highly disturbed setting. It is therefore probable that the juxtaposition of the constructed wetlands in a predominately cleared landscape and their mostly permanent hydroperiod was the driving force behind wetland choice for breeding males of these species. The relationship between the extent of native canopy cover and these two frog species is consistent with other studies throughout the world. Hazell et al (2001) and others (Laan & Verboom 1990; Vos & Stumpel 1995), have typically shown strong positive relationships between increasing amounts of native vegetation and the number of amphibian species. Alternatively, altered soil conditions or constructed wetland bathymetry could potentially influence the occurrence of burrowing species. Porej and Hetherington (2005) discovered that there was strong evidence for a positive association between amphibian species presence and the presence of shallow littoral zones in constructed ponds in the USA. They identified that the presence of wetland shallows increased amphibian diversity even in the presence of introduced predators. These factors were not investigated as part of this study and should be researched further in the Australian context.

Two frog species, *C. glauerti* and *L. adalaidensis*, responded negatively to habitat variables loaded strongly on component 1 of the PCA. Component 1 was represented by a negative correlation between landscape vegetation density (OV, RV & FV) and

water permanence (WP) and contributed most strongly to the significant difference between constructed and natural wetlands in relation to habitat variables (Figure 2.7). This result may reflect a preference by these species for permanent water. Alternatively, it may highlight the importance of open areas as habitat for these species. *Crinia glauerti*, has been identified as an opportunistic breeder (Tyler et al. 2000), and *L. adelaidensis* has been known to call for extended periods when surface water is available (Main et al. 1959). It is therefore probable that permanent water may provide a chance for an extended breeding season for these species. The results presented here do, however, suggest that constructed wetlands in highly modified environments provide suitable calling habitat for these species if they have permanent water.

2.4.2 *Between year variation in amphibian calling male communities in natural and constructed wetlands*

The very low rainfall received in the study area in 2001 resulted in many of the wetlands having below average water levels and consequently reduced hydroperiods. This appears to have strongly influenced the calling male communities utilising the wetlands. For example, calling males of *C. insignifera* were far more abundant in wetlands in 2002 than in 2001 (Figure 2.10). Similarly, in the natural wetlands, species richness was significantly higher in 2002 than in 2001. Babbitt and Tanner (2000) identified that annual variation in rainfall resulted in significant changes in species composition in modified ponds in south-eastern Florida. In drought conditions it is likely the opportunity for calling activity in natural wetlands is reduced in response to the limited hydroperiod. In such instances permanent constructed wetlands may provide refuge habitat for a number of species. However, for other species, constructed wetlands in an anthropogenic setting with introduced predators are unlikely to provide a complete surrogate system.

2.5 Conclusion

Results from this study demonstrate that landscape habitat variables are likely to strongly influence the occurrence of frog species. Therefore, when planning constructed wetlands wetland managers should focus attention on the availability of nearby terrestrial habitats, and patterns of wetland drying and filling if they intend to provide breeding habitat for the full suite of amphibian species. Care should also be taken to avoid introducing exotic fish, e.g. *G. holbrooki*. This study has highlighted that

constructed wetlands do provide calling sites for a number of frog species and these wetlands may provide habitat for calling activity when natural habitats are dry. Later chapters (e.g. Chapter 3) will examine whether constructed wetlands can act as suitable surrogate habitats for successful reproduction of amphibian species.

ACKNOWLEDGEMENTS

I would like to thank Frank Doyle, and Ann Bentley of the Capel Wetlands Centre for assistance with the field work. Thanks to the Jeff and Marjah Hardwick, Wayne Hastie and Mrs Hatfield for allowing me access to their properties to monitor their frog populations. Thanks to Dr R Black for statistical assistance and to Dr. M. Smith and Dr J.D. Roberts for assistance with the manuscript. I would also like to thank the Capel Wetlands Centre for assisting with the funding for this project and for the numerous volunteers, including Dan Edwards, Michael Smith, Dean Paini, Frank Doyle, Phil Harden, Ann, Tracey and others (too many to name) who assisted me with the field work. This research was undertaken as part of a PhD at the University of Western Australia with the assistance of an Australian Postgraduate Award. All work conducted was done with approval from the University of Western Australia Animal Ethics Committee (AEC approval # 00/100/064) and the Department of Conservation and Land Management, Western Australia (Permit # SF SF003880).



Figure 2.1: Location of study area

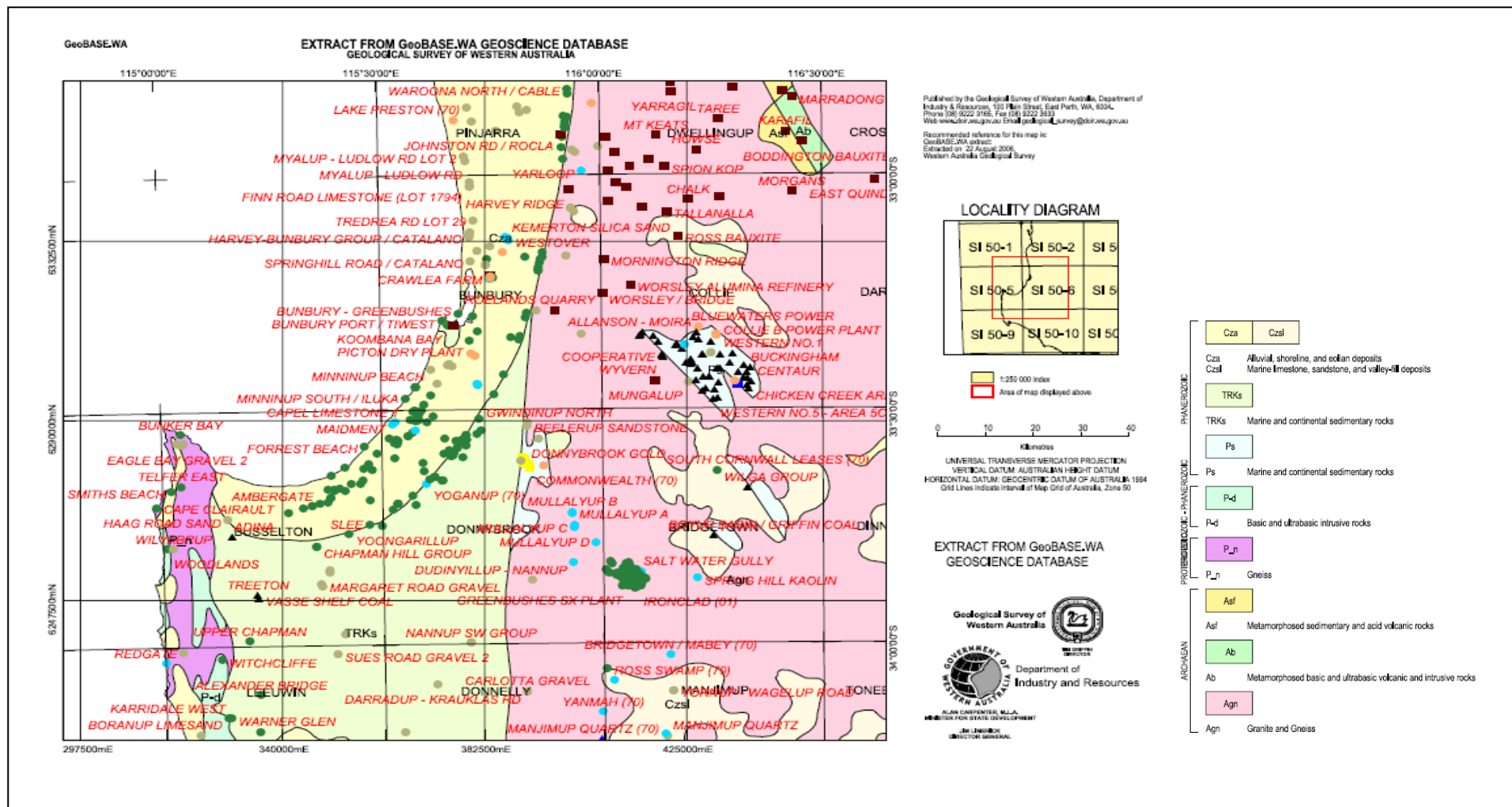


Figure 2.2: Map indicating the number of mines in the Capel district of the south west of Western Australia

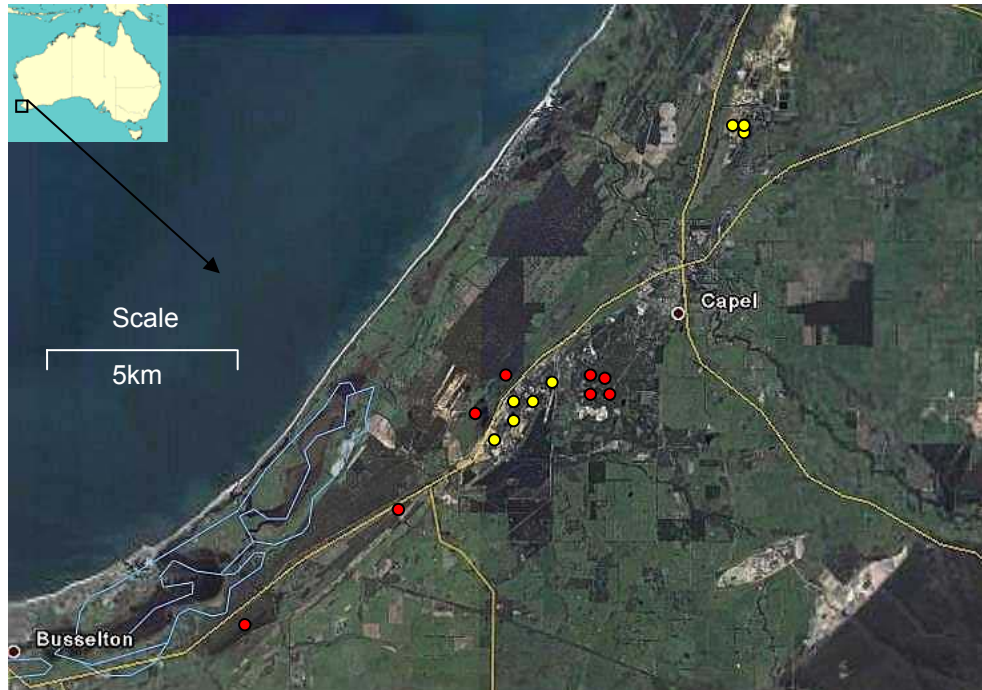


Figure 2.3: Location of wetlands in the study area. Red dots indicate the location of natural wetlands. The yellow dots represent constructed wetlands.

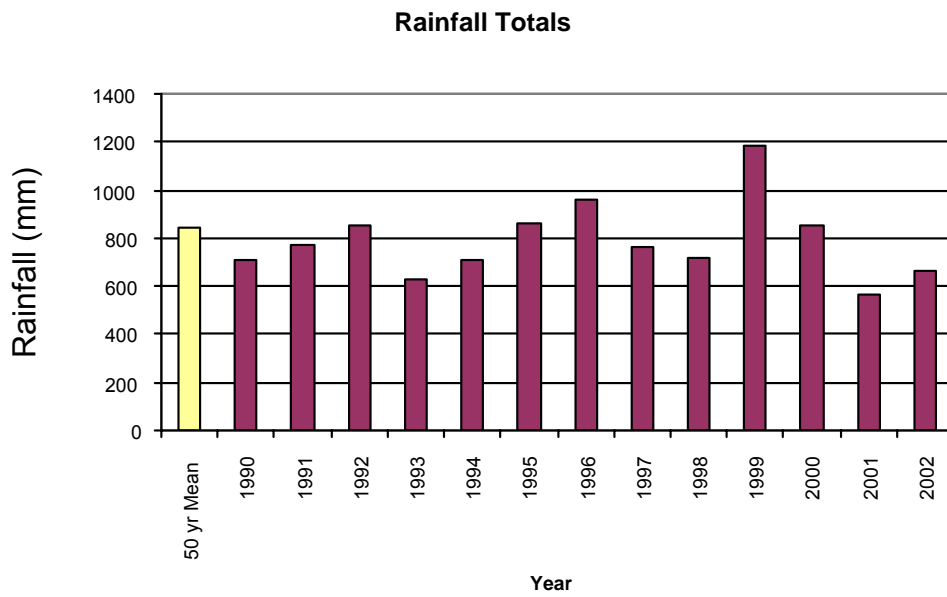


Figure 2.4: Total annual rainfall from 1990 to 2002. The 50 year mean for the study area is also reported.

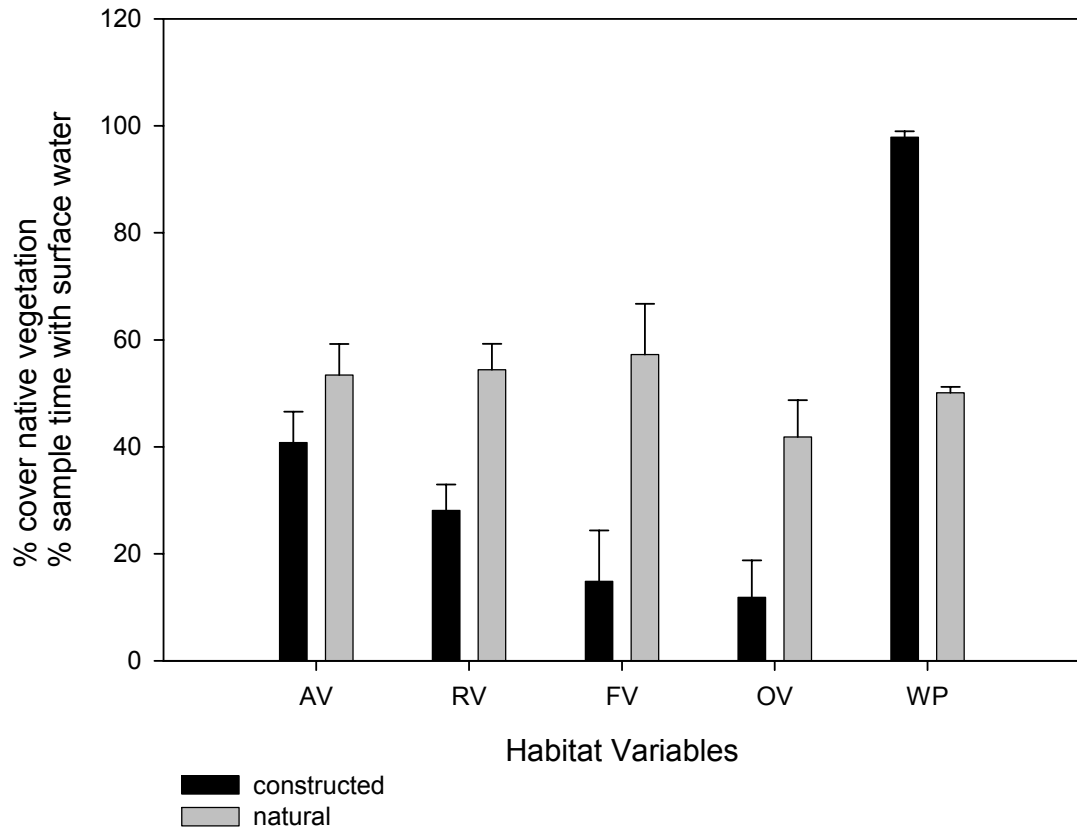


Figure 2.5: Mean percentage cover of vegetation in each zone (n = 8 wetlands/wetland type) and mean percentage of sample period (years) with surface water (n = 2) in constructed and natural wetlands.

AV= % cover of aquatic vegetation, RV= % cover of riparian vegetation within 10m of the transect, FV= % cover of remanent vegetation within 500m of the wetland, OV= % cover of remanent vegetation within 1km of the wetland, and WP = water permanence as % of sample period with surface water. Error Bars = standard error.

Table 2.1: The presence of calling males of each species in each wetland in 2001.

A “P” indicates calling males of that species was present at that wetland in 2001, while a blank space indicates that that species was not recorded in that wetland.

Wetland	Treatment	<i>C. georgina</i>	<i>C. glauerti</i>	<i>C. insignifera</i>	<i>G. leai</i>	<i>H. eyrei</i>	<i>H. psammophilus</i>	<i>L. adalaidensis</i>	<i>L. moorei</i>	<i>L. dorsalis</i>	<i>P. guentheri</i>
Paperbark	constructed		P	P	P			P			
Peninsula	constructed	P	P	P		P		P			
SR2	constructed		P					P	P		
SR3	constructed		P	P				P	P		
SR4	constructed		P	P	P			P			
Swampphen	constructed		P	P	P	P		P	P	P	
Tiger Snake	constructed		P					P			
Wetwoodland	constructed		P	P	P	P		P			
Hardwicks	natural		P	P		P		P			
Hastie A	natural				P	P					P
Hastie B	natural		P	P	P	P					P
Hatfield	natural		P	P	P	P		P	P		
Ludlow 1	natural		P	P	P	P		P			
Ludlow-Hithergreen	natural			P	P	P					
McCarley	natural		P	P		P		P			
Sues Rd	natural			P	P	P		P	P	P	P

Table 2.2: The presence of calling males of each species in each wetland in 2002.

A “P” indicates calling males of that species was present at that wetland in 2002, while a blank space indicates that that species was not recorded in that wetland.

Wetland	Treatment	<i>C. georgina</i>	<i>C. glauerti</i>	<i>C. insignifera</i>	<i>G. leai</i>	<i>H. eyrei</i>	<i>H. psammophilus</i>	<i>L. adalaidensis</i>	<i>L. moorei</i>	<i>L. dorsalis</i>	<i>P. guentheri</i>
Paperbark	constructed		P	P	P			P			
Peninsula	constructed		P	P				P		P	
SR2	constructed		P	P				P	P		
SR3	constructed		P					P			
SR4	constructed		P		P			P		P	
Swampphen	constructed	P	P	P	P	P		P		P	
Tiger Snake	constructed		P	P		P		P			
Wetwoodland	constructed		P	P		P		P			
Hardwicks	natural		P	P		P		P	P	P	P
Hastie A	natural			P	P	P	P	P			P
Hastie B	natural			P	P	P					P
Hatfield	natural		P	P	P	P		P	P		P
Ludlow 1	natural		P	P	P	P		P	P	P	P
Ludlow-Hithergreen	natural		P	P		P		P			
McCarley	natural	P	P	P		P		P		P	
Sues Rd	natural		P	P	P	P		P		P	

Table 2.3: Repeated measures ANOVA of species richness.

Species richness was identified by counting the number of species recorded along each transect in each wetland in each year, giving 2 replicates per wetland, repeatedly sampled over two years (2001 and 2002). Analysis was performed on the statistical package JMP 4.0. Also see Figure 2.3.

<i>Richness</i>				
Source	df	MS	F-ratio	P
Wetland type	1	5.062	1.777	0.296
Wetland[Wetland type] (random)*	14	4.299	2.775	0.033
Year	1	2.250	1.452	0.248
Year x Wetland type*	1	7.562	4.882	0.044
Year x Wetland[Wetland type] (random)*	14	1.549	2.155	0.036
Residual	32	0.719		
Total	63			

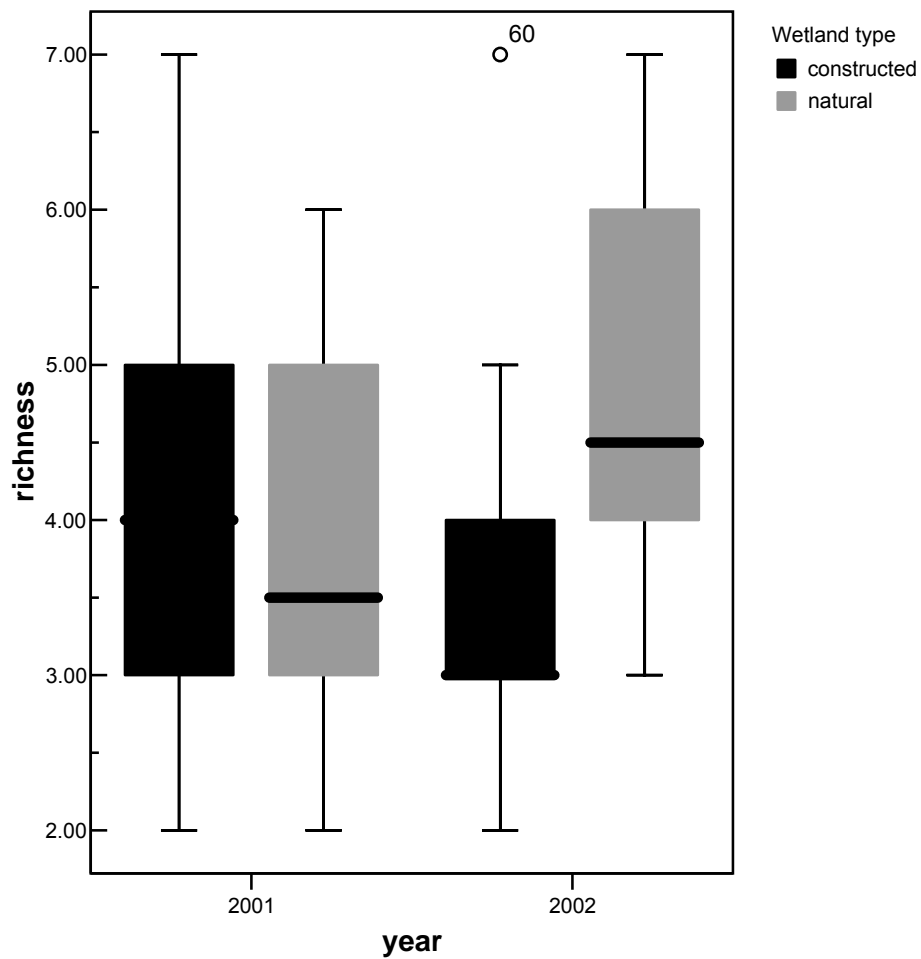


Figure 2.6: Box plots of species richness between wetland types in 2001 and 2002. The centre line represents the sample median. The box border is the interquartile range and the whiskers = range of the data. o = outliers 1.5 to 3 times the box lengths from the upper edge of the box. Also see table 2.1.

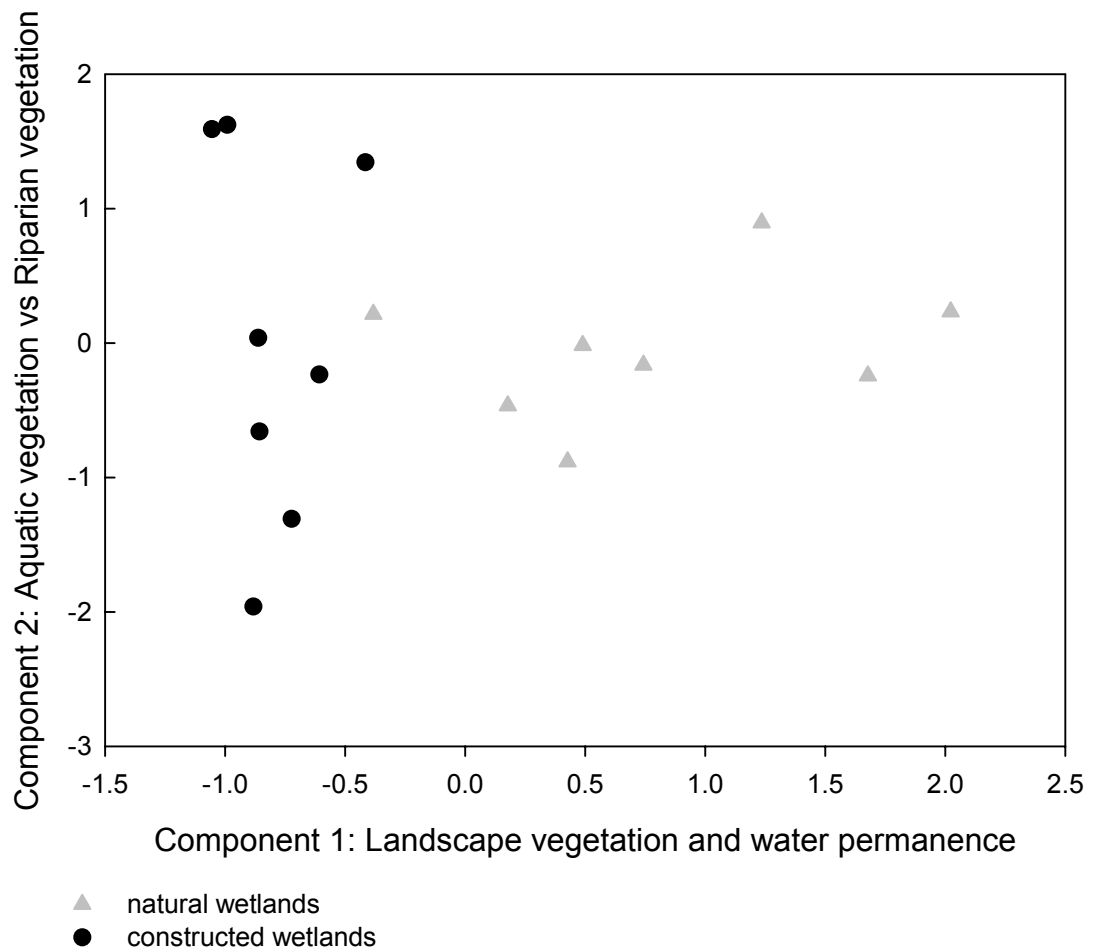


Figure 2.7: Ordination plot for each wetland type (constructed or natural).

The ordination was based on the habitat variables extracted from the PCA. Landscape vegetation and water permanence contributed the most to the differentiation between constructed and natural wetlands. The plot also indicates that natural wetlands varied substantially along component 1, showing some variation in the amount of vegetation in the landscape and the permanence of the water between individual natural wetlands. In contrast, constructed wetlands varied more along component 2 (local vegetation) suggesting substantial variation in the amount of aquatic and riparian vegetation between individual constructed wetlands.

Table 2.4: Results of the PCA with varimax rotation using arcsine (\sqrt{x}) transformed environmental parameter data.

AV= % cover of aquatic vegetation, RV= % cover of riparian vegetation within 10m of the transect, FV= % cover of remanent vegetation within 500m of the wetland, OV= % cover of remanent vegetation within 1km of the wetland, and WP = water permanence as % of sample period with surface water.

Component	Correlation		
	Eigenvalue	Percentage variance	Cumulative variance
1	3.147	62.935	62.935
2	1.259	25.16	88.111
3			

Variable	Component (pattern matrix)	
	1	2
Arcsine (FV) ^{-0.5}	0.936	0.226
Arcsine (OV) ^{-0.5}	0.949	0.107
Arcsine (AV) ^{-0.5}	0.358	0.856
Arcsine (RV) ^{-0.5}	0.616	-0.686
Arcsine (WP) ^{-0.5}	-0.928	0.055

Table 2.5: Multiple linear regression results based on the identified PCA components (1 and 2) for habitat variables relative to maximum abundance of each species measured at each wetland.

In instances where one component was identified to not strongly influence the model the component was removed from the multiple linear regression and the model re-run with the excluded component. Analysis were conducted on the statistical package SPSS 12.0

Species	Model	Unstandardised co-efficients		Standardised B(std)	t	Sig
		B	S.E.			
<i>C. glauerti</i> F _{1,14} =8.753, P=0.01, r ² =0.385	Constant	11.500	1.740		6.610	0.000
	Component 1	-5.316	1.797	-0.620	-2.959	0.010
<i>H. eyrei</i> F _{1,14} =24.349, P=0.00, r ² =0.635	Constant	9.688	1.668		5.807	0.000
	Component 1	8.501	1.723	0.797	4.935	0.000
<i>L. adelaidensis</i> F _{1,14} =6.226, P=0.03, r ² =0.308	Constant	10.125	1.225		8.264	0.000
	Component 1	-3.157	1.265	-0.555	-2.495	0.026
<i>P. guentheri</i> F _{1,14} =11.341, P=0.005, r ² =0.448	Constant	2.063	1.153		1.789	0.095
	Component 1	4.010	1.191	0.669	3.268	0.005

1 **Table 2.6: Results of repeated-measures ANOVA for maximum calling abundance**
 2 **of, *C. insignifera*, *C. glauerti* and *H. eyrei*, *L. adelaidensis***

3 The peak calling month (detailed in parenthesis for each species) was determined by
 4 identifying the single sample which recorded the maximum average abundance across
 5 the two transects for the focal species in each wetland type in each year. Analysis was
 6 completed using the statistical package: JMP version 4.

7

Crinia glauerti, (sample 5 in 2001 & 2002), see figure 2.5

Source	df	MS	F-ratio	P
Wetland type*	1	2.5014	6.5753	0.022
Wetland[Wetland type] (random)*	14	0.3804	24.1092	<0.000
Year	1	0.0014	0.0865	0.773
Year x Wetland type	1	0.0202	1.2774	0.277
Year x Wetland[Wetland type] (random)	14	0.0158	0.1084	1.000
Residual	32	0.1455		
Total	63			

Crinia insignifera,
 (constructed= s3 in 2001, s5 in 2002; natural= s5 in 2001, s4 in 2002), see figure 2.7

Source	df	MS	F-ratio	P
Wetland type*	1	5.0736	11.9664	0.003
Wetland[Wetland type] (random)	14	0.7410	0.9557	0.534
Year	1	4.6712	10.939	0.004
Year x Wetland type	1	1.5192	3.5578	0.075
Year x Wetland[Wetland type](random)*	14	0.7750	8.8605	<0.000
Residual	32	0.0875		
Total	63			

Heleioporus eyrei, (sample 1 in 2001 & 2002)

Source	df	MS	F-ratio	P
Wetland type*	1	4.9550	15.7127	0.0014
Wetland[Wetland type] (random)	14	0.3153	1.4838	0.2349
Year	1	0.3239	1.5240	0.2373
Year x Wetland type	1	0.3347	1.5750	0.2300
Year x Wetland[Wetland type] (random)*	14	0.2125	2.4863	0.0163
Residual	32	0.0854		
Total	63			

Litoria adelaidensis, (sample 6 in 2001 & 2002), see figure 2.6

Source	df	MS	F-ratio	P
Wetland type*	1	1.3502	5.2375	0.038
Wetland[Wetland type] (random)*	14	0.2578	2.5438	0.046
Year	1	0.0152	0.1505	0.704
Year x Wetland type	1	0.1063	1.0490	0.321
Year x Wetland[Wetland type] (random)	14	0.1013	0.9917	0.483
Residual	32	0.1022		
Total	63			

8

9 * indicates significant effect

10

11 **Table 2.7: Results of repeated measures ANOVA for maximum calling abundance**
 12 **of *G. leai*, *L. moorei* and *L. dorsalis*.**

13

The peak calling month (detailed in parenthesis for each species) was determined by
 14 identifying the single sample which recorded the maximum average abundance across
 15 the two transects for the focal species in each wetland type in each year. Analysis was
 16 completed using the statistical package: JMP version 4.

17

<i>Geocrinia leai</i> , (sample 3 in 2001 & 2002)				
Source	df	MS	F-ratio	P
Wetland type	1	0.7127	1.0214	0.3293
Wetland[Wetland type] (random)*	14	0.6977	15.4789	<0.0001
Year	1	0.0943	2.0916	0.1701
Year x Wetland type	1	0.0097	0.2158	0.6494
Year x Wetland[Wetland type] (random)	14	0.0451	0.5271	0.8985
Residual	32	0.0855		
Total	63			
<i>Litoria moorei</i> , (sample 7 in 2001, s6 & s5 in 2002)				
Source	df	MS	F-ratio	P
Wetland type	1	0.15864	1.2672	0.2792
Wetland[Wetland type] (random)*	14	0.12519	2.739	0.0347
Year	1	0.04152	0.9084	0.3567
Year x Wetland type	1	0.06879	1.5051	0.2401
Year x Wetland[Wetland type] (random)	14	0.04571	0.7784	0.6835
Residual	32	0.05872		
Total	63			
<i>Limnodynastes dorsalis</i> , (sample 5 in 2001 & 2002)				
Source	df	MS	F-ratio	P
Wetland type	1	0.0657	0.4612	0.5082
Wetland[Wetland type] (random)*	14	0.1425	2.5279	0.0469
Year	1	0.2376	4.2138	0.0593
Year x Wetland type	1	0.1099	1.9505	0.1843
Year x Wetland[Wetland type] (random)	14	0.0564	0.7988	0.6638
Residual	32	0.0701		
Total	63			

18

* indicates significant effect

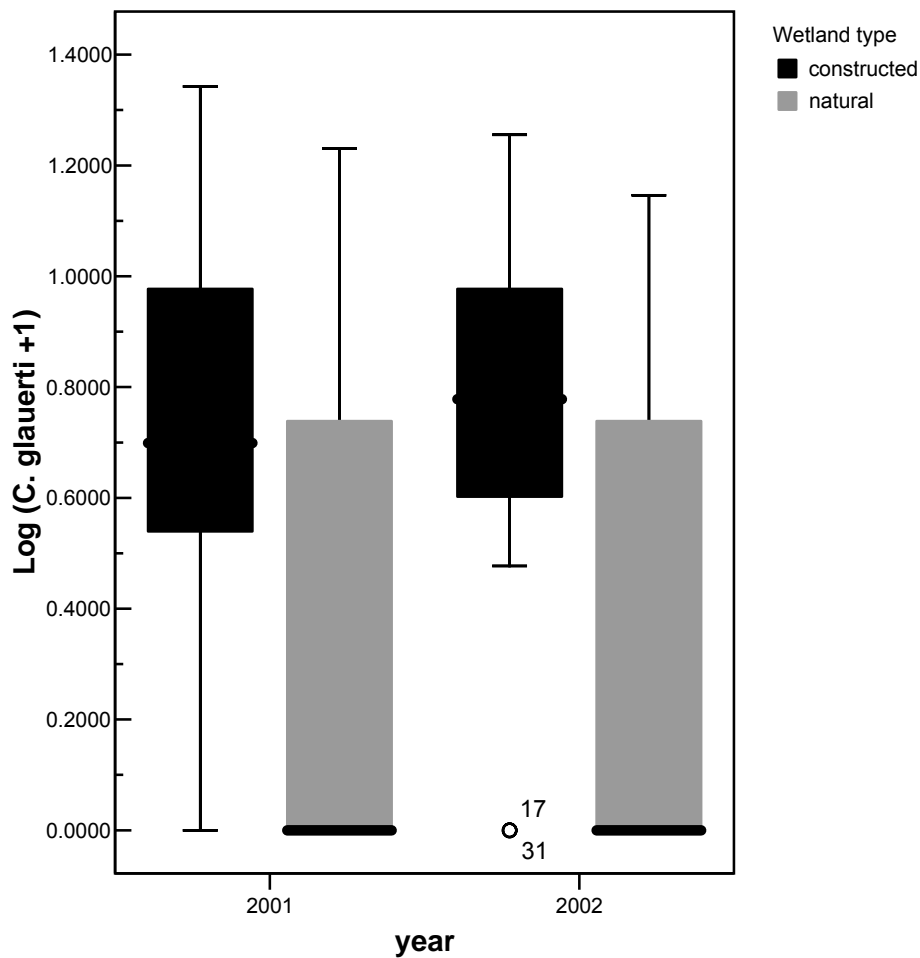


Figure 2.8: Box plots of the number of calling males of *C. glauerti* between wetland types in 2001 and 2002.

Y-axis represents the log (number of calling males of *C. glauerti* + 1). The bold line represents the sample median. The box border is the interquartile range and the whiskers represent the range of the data.

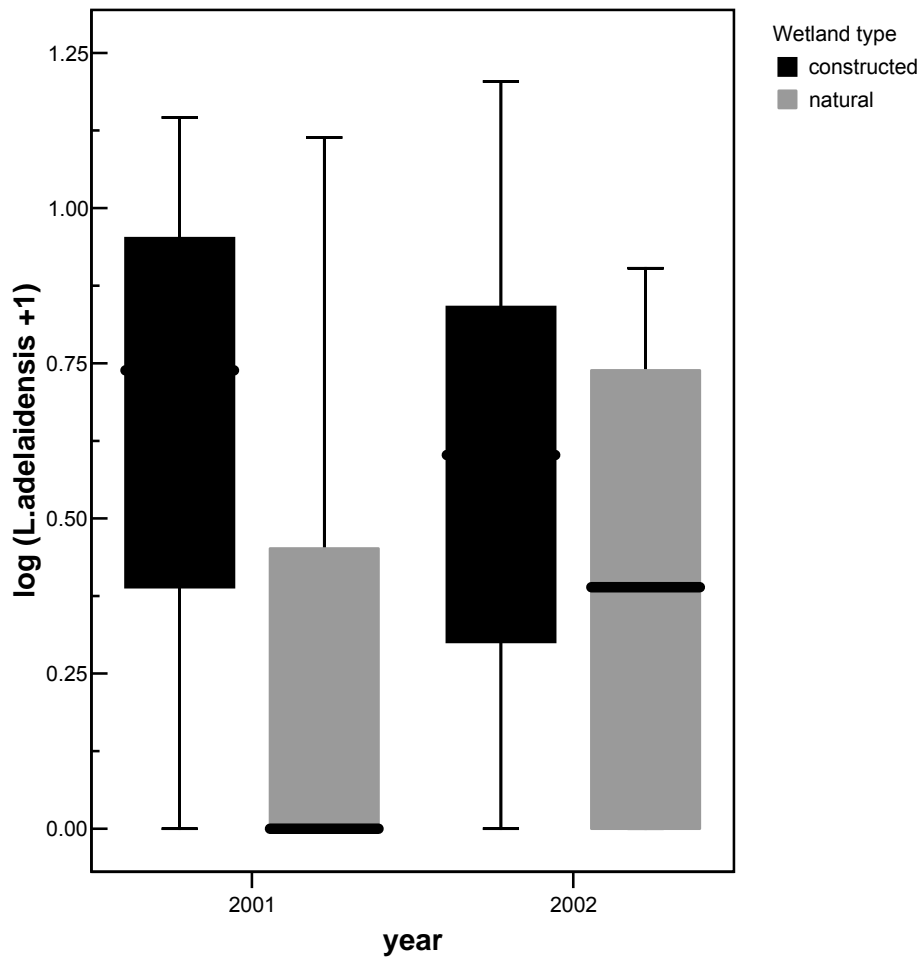


Figure 2.9: Box plots of the number of calling males of *L. adelaidensis* between wetland types in 2001 and 2002.

Y-axis represents the log (number of calling males of *L. adelaidensis* + 1). The bold line represents the sample median. The box border is the interquartile range and the whiskers represent the range of the data.

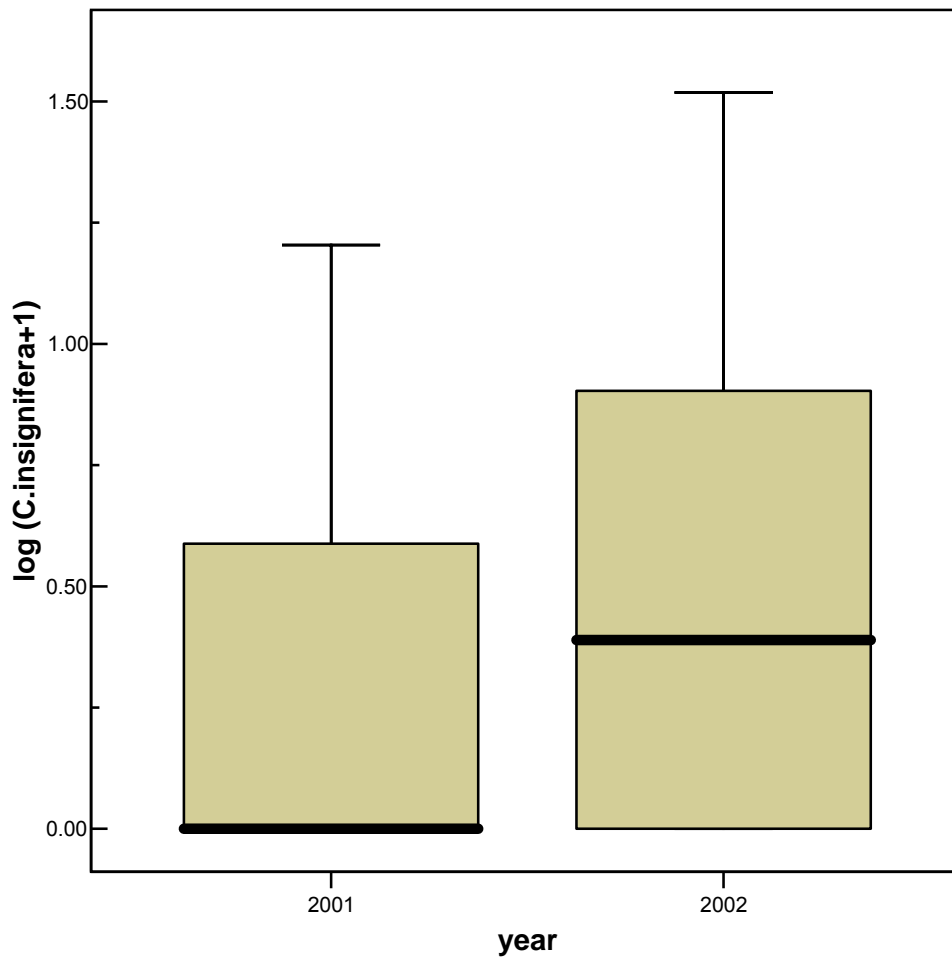


Figure 2.10: Box plots of the number of calling males of *C. insignifera* between years.

Y-axis represents the $\log(\text{number of calling males of } C. insignifera + 1)$. The bold line represents the sample median. The box border is the interquartile range and the whiskers represent the range of the data.

3.0 Variation in species richness and the relative abundances of tadpoles in constructed and natural wetlands.

3.1 Introduction

Frogs in Australia have been noted to exploit a variety of man-made ponds (i.e. ponds that exist as a result of human activities: Hazell et al. 2001; Tyler & Watson 1999; Williamson & Bull 1994; 1996; 1999). However, few studies in Australia have determined whether artificial wetlands can act as successful breeding habitats for anuran communities. A number of studies have focussed on the presence of calling activity in constructed wetlands as this is a quick and reliable method of identifying amphibian species present (Hazell et al. 2001; Hazell et al. 2004) but these studies failed to determine whether calling activity led to reproductive success. Of the Australian studies that examined reproductive output in constructed wetlands several, identified persistent reproductive failure in many of the species calling at the ponds (Healy et al. 1997; Humphries 1979). Only a few studies have shown some successful reproductive output from constructed wetlands in Australia (Pyke & White 1996; Watson et al. 1995). Only one Australian published study known to me, compared the reproductive output of constructed wetland with that found in a natural wetland (van de Mortel & Goldingay 1998). But although the study compared the abundances of calling males and metamorphlings at the two wetland types, quantitative estimates of metamorphlings numbers were only provided for the natural wetland site. As measures of reproduction, (e.g. presence of eggs, tadpoles, metamorphs, etc), are the most sensitive indicator of habitat quality for wildlife (Van Horne 1983) evidence of successful amphibian breeding is vital in the assessment of the conservation value of man-made wetlands for the persistence of amphibian communities.

The absence of research that examines the successful utilisation of constructed (man-made) wetlands as breeding habitats is surprising given the substantial changes that have occurred to natural environments in Australia since the arrival of Europeans and the large number of constructed wetlands in the landscape. Rawlinson (1981) noted that research on Australian frogs has primarily focussed on taxonomy, evolution and ecology, with very little attention paid to the effect of human impacts, in spite of their ecological significance. Unfortunately, despite Australia's greatly altered landscape,

this situation has not changed significantly and the effects of man's activities on frog community persistence and habitat availability remains largely unknown (Hazell 2003).

This study examines the potential for constructed/man-made wetlands to act as surrogates for natural wetlands as anuran breeding habitats, by examining the abundance and richness of tadpole communities in constructed wetlands relative to their natural wetland counterparts. The aim was to determine the role constructed ponds could play in amphibian conservation at the tadpole phase and to understand some of the mechanisms (e.g. habitat complexity, predator presence and calling male abundance) that may be influencing the habitat value of these areas.

3.2 Methods

3.2.1 Study site

I surveyed 16 wetlands in the Capel region (33.33° S and 115.34° E) of southwestern Australia (Figure 2.3) for amphibians from April 2001 to December 2002. Eight of the wetlands were natural, (i.e. they were water bodies that were not constructed, but may have been modified through vegetation clearance or the presence of introduced species) and were subject to seasonal water fluctuations. The remaining 8 were ponds constructed between 1975 and 1990 during the extraction of mineral rich sands (Davies, 2002; Doyle, pers. com.). The constructed wetlands were used for the disposal and treatment of mine-waste water (Chambers & McComb 1994).

Water quality testing in the 1980's indicated that many of the ponds suffered from low pH, had excessively high loads of iron and magnesium and were highly phosphorus deficient (Davies, 2002). However, subsequent works within the wetlands and upgrading of the wastewater treatment facilities in the processing plant, has amended most of these issues. Subsequent tests have indicated that all of the study wetland's consistently recorded neutral pH and iron and magnesium concentrations have dropped to acceptable levels (Chambers & McComb, 1994; Doyle, pers. com.; Meney, pers. com.). Water levels in the constructed wetlands ranged from permanently wet ponds to seasonally fluctuating waterbodies.

3.2.2 Tadpole richness and species relative abundances

The relative abundances of tadpoles were estimated for each species using a combination of sweep netting and tadpole traps. Water in each wetland was swept haphazardly close to the audio transects described in chapter 2. I used a 340x220mm, 0.5mm (mesh size) sweep net, and swept each wetland five times within each visit. All tadpoles captured were identified to species before being released. The majority of captured tadpole species captured during the study were easily discernible in the field. However, hatchlings of the closely related *Crinia insignifera* and *Crinia glauerti* were often difficult to differentiate. Where tadpoles could not be identified in the field they were simply noted as *Crinia* spp. hatchlings before release and these data were excluded from abundance analysis. All potential predators captured within the sweep were also identified to species (vertebrates) or family (invertebrates) to determine the density of predators relative to tadpole density.

Ten unbaited tadpole funnel traps (Lauk 2004) were placed in each wetland to capture any tadpole species which may have been able to avoid capture in sweeps. Tadpoles traps were cylindrical traps made from 1.25L plastic drink bottles, 260mm long x 90mm diameter, with a funnel (the top of another bottle) extending inward at one end. The traps were installed in all accessible habitat types (e.g. open water, dense vegetation, fringing edges, etc.) in the wetland and were set in each pond for 24hours. These data provided a measure of the relative density of tadpoles of each species in each wetland and maximised the opportunity to record the highest richness of species of tadpole and their potential predators. Shaffer et al. (1994) suggested that no single sampling technique was effective in sampling all habitat types within a waterbody and therefore both techniques were employed to maximise the chance of capturing all species of tadpoles present in the waterbodies. Funnel traps have been identified as an effective way to sample amphibian larvae in structurally complex habitats and they have been noted as being effective at measures of relative differences in tadpoles densities (Lauck, 2004). It was assumed that these measures would capture all species present at least once and provide a good estimate of species richness and the relative abundances of each species.

Abundance and species composition measures were made once every four to six weeks between July and November in both 2001 and 2002 providing a total of 8 measures for each wetland. Repeated monitoring between July and November, 2001 encompassed the tadpole developmental period for all tadpole species known in the

area. Repeating the process in 2002 provided seasonal abundance estimates over two seasons and allowed for the identification of any intra and inter-site variability in the abundance of tadpoles for each species.

3.2.3 *Environmental parameters*

Vegetation in each wetland was divided into 2 categories, aquatic (vegetation below the winter high-water mark) and riparian (habitat above the winter high-water mark). The percentage of the water surface containing submerged, floating, or emergent vegetation was estimated (recorded as Aquatic Vegetation: AV) along each audio transect (see Chapter 2). The percentage cover that riparian (reeds, sedges, etc.) or canopy vegetation provided within 10m above the high water mark, on each transect, was also estimated (recorded as Riparian Vegetation: RV). Aerial photographs (1:25 000) were examined to estimate the percentage of native vegetation surrounding each wetland at 500m (vegetation within five hundred metres: FV) and 1000m (vegetation within one thousand metres: OV) radius. Water permanence (WP) was estimated during the survey period to provide a proportion of months with surface water for each wetland.

3.2.4 *Data analysis*

Differences in the species richness between each wetland type, sampling method and year were tested using a split-plot ANOVA (SPSS 12.0). Species richness was identified by counting the number of species recorded by each capture technique in each wetland in each year. This gave 8 replicates for constructed wetlands in each sample year (2001 and 2002) for each capture technique and 7 each year in natural wetlands for each capture technique, as one of the natural wetlands remained dry for the entire study period preventing all tadpole sampling (see section 3.3.1 for details). Wetland type (fixed, $n = 2$) was the between subject factor in the model, with capture technique (fixed, $n = 2$) and year (fixed, $n = 2$) as the within subject factors. This test identified whether richness differed markedly between each wetland type, each sample year and sampling method.

Differences in the number of tadpoles of each species between each wetland type (constructed vs natural) were tested using a repeated-measures ANOVA for each capture method. The peak abundance in each capture method was determined by identifying the single sample which recorded the maximum abundance for the focal species in each wetland type in each year. Data from this sample were subsequently log transformed ($\log_{10}(\text{number tadpoles of species "a"} + 1)$) to normalise the data before

analysis. Within the ANOVA individual wetlands were treated as randomly chosen experimental units and were nested within wetland type (fixed, $n = 2$). Year (fixed, $n = 2$) was the repeated measure. The tests were run independently for each of the two capture techniques. These analyses revealed whether the abundances of tadpoles were significantly different between wetland types (constructed vs natural wetlands) within each capture technique. Analyses were not conducted for tadpoles of *G. leai*, *L. moorei*, and *L. dorsalis* due to the low numbers of tadpoles of these species captured across both wetland types in both years. In addition, *H. eyrei* was only tested on data collected in 2002 in a nested ANOVA as tadpoles were only recorded in one wetland (natural) in 2001.

A linear regression was conducted on the maximum abundance of calling males and tadpoles in each capture method in each wetland in each year to determine if there was a relationship between male calling activity and tadpole abundance for a single species. Tadpoles were treated as the dependent variable and tests were initially conducted using the entire data set with both wetland types. Subsequent tests were also conducted on calling males and tadpoles within each wetland type (e.g. natural or constructed) to identify if a linear relationship existed between calling and activity and tadpole numbers within natural or constructed wetlands. All data were $\log(x+1)$ transformed prior to the test.

The relationship between wetland habitat variables (AV, RV, FV, OV, & WP), total tadpole species richness (sweep and trap data combined) and the maximum abundance data in each capture method for each species of tadpole in each wetland, over the two year field study, were examined using a multiple linear regression. Habitat variables were $\arcsine(\sqrt{x})$ transformed prior to analyses. As vegetation in the landscape (FV and OV) and the permanence of the water (WP) were strongly correlated and the overall sample size of the test was small relative to the number of variables the multiple linear regression test was likely to lack power. Therefore a PCA using a correlation matrix and varimax rotation was run on all the habitat variables (AV, RV, FV, OV, & WP) prior to running the multiple linear regressions, using the statistical package SPSS 12.0. This reduced the number of variables used in the regression and provided a more powerful analysis while removing the influence of collinearity. The two components from the PCA were entered into the model using the enter method of analysis to determine which components most strongly contributed to the model. In instances where one component did not strongly influence the model, the component was removed from the multiple linear regression and the model re-run with the excluded

component. Where scatter plots revealed that any relationship that was present was non-linear, tadpole data were $\log(x+1)$ transformed.

Tadpoles of several species were tested to determine whether there was a relationship between species abundance and the presence of the introduced predatory fish, *Gambusia holbrooki*. The maximum number of tadpoles recorded within a single sample in each year in each wetland was tested relative to the presence or absence of *G. holbrooki*, using a single factor ANOVA for each capture technique. Wetlands in each year (i.e. 2001 and 2002) were treated as separate measures as fish presence and absence in individual wetlands changed between the two seasons. This provided a total sample size of 30 for the test, with the exception of data on tadpoles of *Heleioporus eyrei*. Data from 2001 were excluded for *H. eyrei* as this species was only recorded in a single wetland during that sample year. All tadpole data were log transformed prior to the analyses.

3.3 Results

3.3.1 Environmental parameters

Below average rainfall in 2001 resulted in unusually low water levels in several wetlands (see Figure 3.1 and chapter 2 for details). One constructed wetland dried for the first time since its construction, eliminating a large population of the introduced predatory fish *G. holbrooki* from the wetland in early 2002. Several natural wetlands that had not dried for approximately 10-15 years were completely dry for much of 2002. While, two strongly ephemeral natural wetlands (i.e. on average held water for less than 7 months of the year) remained almost completely dry and only held surface water for a few weeks in 2002. One natural wetland did not hold any surface water over the study period and therefore was excluded from the tadpole sampling, giving a sample size of 7 for natural wetlands and 8 for constructed wetlands for all tadpole abundance data.

On average constructed wetlands retained water for most of the year with an average of $95\% \pm 3.12$ s.e., $n=8$, of the total survey months having some surface water across the treatment group (see Figure 2.2, chapter 2). In comparison, on average surface water was only noted in natural wetlands in about $51\% \pm 12.28$ s.e., $n=8$, of the survey months.

3.3.2 Tadpole richness and species relative abundances in constructed and natural wetlands

A total of seven species of anuran tadpoles were recorded in the 15 sampled wetlands. Of these, five species were captured in both traps and sweeps, while *Geocrinia leai* and *Limnodynastes dorsalis* were only captured in traps. Abundances of tadpoles varied substantially between years and between sampling method. In 2001 *Crinia insignifera* were the most commonly captured tadpoles in both traps and sweeps with individual wetlands recording abundances of between of up to 24 and 62 tadpoles in each of the capture methods respectively (Figure 3.4). In 2002, *Heleioporus eyrei* was the most commonly captured species of tadpole in traps (a maximum abundance of 35 tadpoles/wetland/sample) while *Crinia glauerti* dominated sweep captures with a

maximum abundance of 138 tadpoles captured in a wetland during a single sample. The least common species were *L. dorsalis*, *G. leai* and *Litoria moorei* with a total of only 2, 44 and 75 individual tadpoles captured, respectively, over the two year study. None of the recorded species was exclusive to constructed wetlands. Tadpoles of *L. moorei* and *L. dorsalis* were only recorded in natural wetlands. No tadpoles of *Pseudophryne guentheri* or *Crinia georgiana* were captured in the entire study, despite the presence of calling males of these species in a number of wetlands (see chapter 2).

Tadpole total species richness varied significantly between wetland types over the two year study (Table 3.1 & Figure 3.2) with an average of 1.87 species \pm 0.16 s.e., n = 8) species recorded in constructed wetlands and 2.96 species \pm 0.12 s.e., n = 7) in natural wetlands. Species richness also differed significantly (Table 3.1 & Figure 3.3) between years with a mean of 3.03 species \pm 0.08 s.e., n = 15) in 2002 compared with only 1.73 species \pm 0.09 s.e., n = 15) in 2001 (Table 3.1). This trend was consistent in both constructed and natural wetlands.

There was a significant variation in the species richness recorded in individual wetlands within wetland type (Table 3.1). The main effect, 'capture technique', did not significantly influence tadpole species richness (Table 3.1). However, a review of capture abundances of species between years revealed that there was a large increase in the abundances of tadpoles captured in traps from 2001 to 2002 (0.54 tadpoles \pm 0.08, s.e., n = 545 and 1.40 tadpoles \pm 0.151 s.e., n = 595 respectively) compared with sweeps (2.39 tadpoles \pm 0.47 s.e., n = 270 in 2001 and 4.23 tadpoles \pm 4.27 s.e., n = 300 in 2002). The increase in the number of tadpole captured in traps was primarily associated with a substantial increase in the number of tadpoles of *H. eyrei*, with total captures increasing from 6 individual tadpoles in 2001 to 455 tadpoles in 2002 (Figure 3.4).

3.3.3 Relative abundances of tadpoles in each wetland type

The abundances of three of the tested tadpole species captured in traps varied significantly within wetlands between years (year x wetland [wetland type]: Table 3.2). Tadpoles of *H. eyrei* varied significantly within wetlands in traps. Of the four tadpole species tested, *H. eyrei* was the only species which was significantly ($P < 0.05$) more common in natural ponds at the 0.05 level (Table 3.4; Figure 3.5). However, *C. insignifera* was more common in natural wetlands at the $P < 0.1$ level for both trap and sweep captured tadpoles (wetland type: Tables 3.2 & 3.3; Figures 3.6 & 3.7). A

significant difference ($P < 0.01$) in the abundances of tadpoles of *C. glauerti* captured in sweeps was found between years, with larger numbers of tadpoles captured in 2002 (Table 3.5, Figure 3.8). Numbers of tadpoles of *C. glauerti*, and *L. adelaidensis* did not differ significantly between constructed and natural wetlands (Tables 3.2 & 3.5)

3.3.4 *Relative abundances of calling males and tadpoles in each wetland type*

Of the four species of tadpole tested only two species revealed a significant relationship between calling male activity and tadpole abundance. The number of calling males of *H. eyrei* was positively related to the number of tadpoles for both trap and sweep data (Table 3.6). The relationship between the numbers of tadpoles and the numbers of calling males of *C. glauerti* differed between constructed and natural wetlands with a strong positive relationship in natural wetlands (sweep data: Table 3.6; Figure 3.9) but a negative relationship in constructed wetlands (trap data: Table 3.6; Figure 3.10). Numbers of tadpoles of *C. insignifera* and *L. adelaidensis*, showed no apparent relationship with the abundance of calling males.

3.3.5 *Habitat characteristics and the occurrence of tadpoles*

The PCA identified two components that had eigenvalues greater than one and explained over 88% of the variance (Table 3.7). The loadings based on the correlation matrix showed that component 1 represented the landscape vegetation (FV and OV) and water permanence (WP), with vegetation within 500m (FV) and 1000m (OV) of a wetland loading positively onto this component while water permanence loaded negatively. This indicated that more disturbed landscapes (i.e. cleared of natural vegetation) were characterised by wetlands of increasing water permanence. Component 2 was a contrast between the aquatic vegetation (AV) and the riparian vegetation (RV), with aquatic vegetation loaded positively to the component and riparian vegetation loaded negatively. The contrast revealed that wetlands with greater densities of riparian vegetation typically had less vegetation in the aquatic zone. An ordination plot of the PCA components revealed component 1 (landscape vegetation) contributed the most to the differentiation between constructed and natural wetlands (Figure 3.11). Multiple linear regression models based on these two components were

fitted to all species of tadpole recorded except for *L. dorsalis* as this species was only identified in a single wetland (natural) over the entire study period.

Four species were identified as being significantly influenced by the tested habitat components extracted from the PCA (Table 3.8). Abundances of tadpoles of *H. eyrei* were positively correlated with component 1 from the PCA (Landscape vegetation and water permanence). This component explained 39% and 48% of the variation in numbers of tadpoles of *H. eyrei* captured in traps and sweeps respectively. In contrast, *L. adelaidensis* and *C. glauerti* showed a negative relationship to component 2 (local vegetation) from the PCA. There was a negative relationship with pond properties, with 30% and 32% of the variance in tadpole abundance of *L. adelaidensis* and *C. glauerti*, respectively, explained by the pond vegetation characteristics. There was also a strong positive relationship between species richness and component 1 from the PCA. Landscape vegetation and water permanence (component 1) explained 66% of the variation in tadpole species richness.

Only one species had the two components from the PCA contribute to the model. Numbers of *Crinia insignifera* tadpoles were positively associated with landscape characteristics (component 1) and negatively associated with local vegetation (component 2). The total model contributed to 52% of the variation in tadpole abundance of *C. insignifera* captured in traps.

3.3.6 *The influence of an introduced predator on tadpole richness and relative abundances*

Gambusia holbrooki was more likely to occur in constructed wetlands ($\chi^2 = 19.286$; $df = 1$; $p < 0.0001$; $r^2 = 0.5369$). Species richness was negatively associated with the presence of *G. holbrooki* in both trap (ANOVA: $F_{1,28} = 6.21$, $p = 0.02$) and sweep (ANOVA: $F_{1,28} = 4.76$, $p = 0.04$) data. Abundance of two of the four species of tadpole tested was affected by the presence of the introduced predatory fish. *Gambusia holbrooki* in a wetland was significantly and negatively related to the chances of recording tadpoles of *H. eyrei* in both traps (ANOVA: $F_{1,13} = 10.87$, $p = 0.006$) and sweeps (ANOVA: $F_{1,13} = 9.97$, $p = 0.008$) and trap captured *C. insignifera* (ANOVA: $F_{1,28} = 4.83$, $p = 0.04$). The test was not conducted on the presence/absence of *L. dorsalis* as tadpoles of this species were only recorded in one wetland.

3.4 Discussion

The aim of this study was to determine whether communities of tadpoles differed between constructed and natural wetlands within the same geographical location and to highlight whether any habitat variables may have influenced the abundance of tadpoles of various species. The study identified that the species richness of anuran tadpoles was significantly higher in natural wetlands and that tadpole community composition differed substantially between the two wetland types. These differences could be driven by a number of factors including the available pool of amphibian colonists, and a myriad of abiotic and biotic parameters, many of which may act synergistically. In this study, I examined the influence of the habitat components; landscape vegetation, water permanence, pond habitat characteristics, predator presence and the reproductive potential (i.e. relative abundance of calling males) of each pond relative to the tadpole abundance and richness.

3.4.1 Wetland origin and tadpole response

The lower tadpole species richness recorded in constructed wetlands compared with natural wetlands is consistent with the results of Monello & Wright (1999). Monello and Wright (1999) found that artificial ponds could support a number of amphibian species, but noted that at least two species had not effectively exploited these ponds for reproduction. They concluded that artificial wetlands were often limited in their suitability as conservation refuges for frogs because of their highly disturbed setting (Monello & Wright 1999). Similarly, Laan & Verboom (1990) identified that species richness was higher in wetlands closer to a wood or forest. They concluded that the occurrence of species in a suitable habitat patch increased with source proximity and the increasing connectivity of the landscape. Most of the constructed wetlands within my study were in relatively disturbed landscapes (see Figure 2.2, chapter 2) with a large proportion of the surrounding landscape cleared for mining and farming. In contrast, most of the natural wetlands occurred in areas with good native vegetation cover in the immediate and nearby surrounds.

Tadpoles of *H. eyrei* and *C. insignifera* were more commonly found in natural ponds. This result was anticipated for both species, as calling activity indicated a strong

preference by the adult males of both species to call in natural ponds (see chapter 2). In addition, the success of tadpoles of *H. eyrei* is strongly related to the fluctuating water levels of a wetland riparian zone (Lee 1967). Males call from constructed burrows where mating and oviposition occurs. The burrows are generally located in the seasonally inundated fringes of a wetland or the centre of strongly ephemeral swamps (Lee, 1967). Winter flooding of the wetlands/swamps inundates the burrows and enables the developed embryos to emerge from the burrows to feed and mature. The success of the clutch is therefore dependent on the fringing zones of a wetland experiencing a drying and flooding regime suited to the frogs breeding period. A summer dry down of a wetland enables the adult frogs to establish the burrows and breed in dry or moist burrows. The subsequent winter flooding of this zone at the completion of embryonic development enables the tadpoles to emerge to grow, feed and metamorphose (Lee 1967).

The majority of the constructed wetlands in this study were permanent, steep sided ponds. These provide very little opportunity for breeding adults to establish burrows, as the males tend to prefer sites with little or no gradient (Lee 1967). Furthermore, steep banks would potentially reduce the inundation “window” of the site, reducing the chances of burrows flooding at the optimum time. Of the constructed wetlands, only one supported a number of *H. eyrei* burrows and this wetland had been modified to provide gradually inclined riparian banks, which subsequently provided a predominantly seasonal water regime to most of the wetland. This highlights the need to design wetlands which more adequately reflect natural systems.

The higher abundance of *C. insignifera* tadpoles in natural wetlands may possibly be associated with wetland vegetation. In natural systems, adult *C. insignifera* are most commonly found calling and breeding in ephemeral swamps (Main 1957). They require fallen vegetation and cracks and holes in the soil to provide shelter during the dry summer months as they are unable to excavate holes themselves (Main 1957). Therefore, *C. insignifera* may require good structural diversity components in the riparian zone of the wetland; observations concurrent with the results of my study. As many of the constructed wetlands had only limited availability of vegetation in the riparian zone (see Figure 2.2, chapter 2) and as many were characterised by a permanent water regime it is probable that these habitat components did not favour the persistence of adults of *C. insignifera*. The lower tadpole numbers in constructed wetlands could therefore simply reflect the lower numbers of adult breeding in these wetland types as noted in chapter 2. However, it is important to note that this species is highly

susceptible to predation by the introduced fish, *G. holbrooki* (see chapter 5 and 6) whose presence was strongly linked with constructed wetlands. Therefore, the apparent affinity of *C. insignifera* with natural wetlands could simply be a reflection of the presence of tadpole predators.

3.4.2 Biological attributes influencing tadpole communities

Individual species

All four species of tadpole tested were either influenced by landscape habitat and water permanence variables (*C. glauerti*, *H. eyrei* and *C. insignifera*) or local vegetation variables (*L. adelaidensis*). For all species except *H. eyrei*, the habitat characteristic to which tadpoles responded contrasted with the response of their calling males. Two tadpole species, *H. eyrei* and *C. insignifera*, were strongly and negatively impacted by the presence of the introduced predatory fish, *G. holbrooki*.

Variation in the importance of habitat variables to calling males and tadpoles of *C. glauerti* and *C. insignifera* could reflect the tadpoles' susceptibility to predators such as *G. holbrooki*, female site preference, or other habitat variables not consistently measured (e.g. water quality) which could influence the survival of eggs or tadpoles. Wetlands with a more permanent water regime in this study were more likely to support large populations of the introduced predatory fish *G. holbrooki* and it is highly probable that amphibian reproductive output (i.e. tadpoles) was severely limited by predation. The negative relationship between occurrence of *G. holbrooki* and the abundance of tadpoles of *C. insignifera* in traps supports this. However, as no response was noted for tadpoles of *C. glauerti* to the presence of *G. holbrooki* and no measure was made of female site choice or activity relative to males it is difficult to ascertain the cause with certainty.

In contrast to other tadpole species, tadpoles of *L. adelaidensis* did not appear to be influenced by landscape habitat variables or water permanence. Tadpoles of this species were negatively associated with aquatic vegetation and positively associated with riparian vegetation. As this frog is the only semi-arboreal species studied in detail in this study, it was anticipated that the species would show different habitat preferences compared with the other predominantly ground dwelling anuran species. As indicated by Main (1968) species of *Litoria* (formerly *Hyla*) become cryptozoic in times of drought and rely on humid conditions beneath logs and debris, etc for survival. The

inability of *L. adalaidensis* to burrow would expose the adult frog to periods of desiccation and therefore during summer I anticipated that the species would require access to above ground shelter near a moisture source. If so, *L. adalaidensis* may have a strong affinity with riparian vegetation and perhaps would be more likely to breed in wetlands with well developed riparian vegetation with a predominantly permanent water regime. Indeed, the positive association of calling males of *L. adalaidensis* with water permanence suggests that the water regime of a wetland would strongly influence the presence of this species. This is further emphasised by the species relatively late breeding season (late spring to early summer) which would prevent the species from breeding successfully in wetlands which did not retain water into late summer (i.e. wetlands with insufficient hydroperiod to enable tadpole development).

The complete absence of tadpoles of *P. guentheri* in all wetlands was, in part, anticipated as the majority of calling males of this species were located in a single strongly ephemeral wetland. During the two years of the study, the wetland never held any surface water, eliminating the chances of *P. guentheri* egg development. Numbers in other wetlands were relatively low and the likelihood of capturing tadpoles in these wetlands was low. Similarly, *G. leai* was most commonly found calling in wetlands that held little or no water and hence tadpole numbers captured over the study were very low and generally restricted to wetlands where surface water flooded emergent vegetation in the winter period.

Species Richness

The strong positive relationship between component 1 of the PCA and species richness indicates that water permanence may play an important role in the richness of amphibian communities. Wilbur (1987) stated that the suitability of a particular pond as a breeding site for amphibians is highly dependent on its hydroperiod, as this sets the length of time available for the larval period of potential colonists and their risks of predation. He noted that it is the species specific tolerances to physical factors that determine the pool of species potentially able to exist in any habitat (Wilbur 1987). Success at one point on the water permanence gradient often entails having a phenotype that may hinder performance at other points on the gradient (Wellborn et al. 1996). In many cases water levels in constructed wetlands are artificially maintained (Nield & Townley 1987; Pornpipat 1992), obscuring the natural filling and drying regime commonly experienced in natural wetlands of a region (Chambers & McComb 1994). As identified in this study, a number of species are influenced by the water permanence

and vegetation components of a wetland. Therefore it is probable that artificially maintained water levels could potentially limit the suitability of a wetland for use by some frog species, e.g. *H. eyrei*, *P. guentheri*, especially in locations where few permanently wet water bodies naturally exist (e.g. Swan Coastal Plain, WA; Chambers & McComb, 1994).

It follows therefore, that as the abiotic component (water permanence) of a wetland may influence the tadpole community it is likely to influence the biotic components such as predators and habitat availability. As such, temporary habitats are less likely to support species which are unable to cope with the physical stress of drying and therefore will have a different suite of predators from permanent habitats (Wellborn et al. 1996). Importantly, this constraint prevents colonisation by many key tadpole predators such as fish. Thus the negative relationship between species richness and the introduced fish, *G. holbrooki* was expected. There is extensive evidence to suggest that a number of amphibian species are highly susceptible to predation by *G. holbrooki* (see Gillespie & Hero 1999 for a review), but the strong relationship between water permanence and the persistence of the fish makes it difficult to determine if water permanence, the presence of the fish or the combination of the two parameters is driving the variance in species richness.

Similarly, species richness may also have been influenced by the density of vegetation in the surrounding terrestrial landscape. A number of similar studies throughout the world have noted that species richness is often strongly driven by the connectivity of the landscape with native vegetation (Hazell 2003; Hazell et al. 2001; Hecnar & M'Closkey 1996; Laan & Verboom 1990). Hazell et al. (2001) identified that anuran adult species richness was positively related to the extent of native canopy cover in the surrounding landscape in south-eastern Australia. Likewise, Hecnar & M'Closkey (1998; in Knutson et al. 2004) found anuran species richness closely linked to vegetation cover in the surrounding landscape. But as most of the constructed wetlands and permanently wet wetlands in my study were in highly modified landscapes with little remaining native vegetation, it is again difficult to identify if landscape habitat complexity was the primary mechanism.

Interestingly, calling male species richness did not show a relationship to any of the tested habitat variables (see chapter 2). This could indicate that the reproductive success of multiple species may be more strongly influenced by habitat variables such as water chemistry, female choice of sites for egg deposition, than the activity of calling males. As water chemistry was not consistently measured across all wetland groups it is

not possible to determine if this was the driving force. Similarly, female site choice or female presence was not monitored but it is probable that habitat components may more strongly influence female site selection and they may be less likely to visit wetlands which do not meet these requirements despite the presence of choruses of calling males (2001).

Alternatively, the contrasting responses of individual species of calling males to different habitat components (e.g. the positive association of calling males of *H. eyrei* with landscape habitat variables contrasted with calling males of *L. adalaidensis* which exhibited a negative response with the same habitat components) may have masked vital habitat attributes at the species richness level. Both Hazell et al. (2001) and Stumpel and van der Voet (1998), noted that the opposing responses of several species to a variety of habitat attributes, in their studies, strongly influence the overall species richness response. Variation in species habitat response was clearly observed in my study, with individual species responding differently to landscape habitat variables. A positive relationship was found between landscape habitat and abundances of two species of calling males but, a negative relationship was identified with the same variable for two other species, potentially and effectively reducing the overall effect of species richness for calling males. In contrast, the relationship of tadpole species richness with landscape habitat as identified in the linear regression, may reflect a variation in the influence of habitat variables between adults and tadpoles. It is probable that tadpoles in general are more likely to be strongly influenced by biotic and abiotic components operating within the wetland components rather than landscape habitat variables.

3.4.3 *Between year variation*

Species richness was higher in 2002 compared with 2001 and this response is most likely associated with the variation in the rainfall between the two years which strongly influenced the availability of surface water in many wetlands. Of note was the almost complete absence of tadpoles of *H. eyrei* in tadpole sampling in 2001, yet in 2002 *H. eyrei* tadpoles were the most common tadpole captured in traps. It is probable, that the limited availability of surface water in a number of natural wetland during 2001 in combination with the species apparent preference for natural waterbodies, would

have prevented successful breeding in 2001. Monthly surface water observations in each wetland revealed that in 2001 surface water levels in many wetlands did not reach average flood lines and as a consequence many calling burrows were never flooded by surface water. As the overall activity of calling males between 2001 and 2002, did not vary substantially, it is probable that the low rainfall in 2001 either depressed female activity or resulted in mass tadpole mortality. However, as I did not investigate if females had attended burrows and as no studies to my knowledge have determined if tadpoles of *H. eyrei* can develop to metamorphoses in the absence of burrow flooding, I cannot determine whether low rainfall levels may have caused an almost complete reproductive failure of *H. eyrei* in 2001.

3.5 Conclusion

The results of this study indicate that habitat variables and wetland origin strongly influenced the species richness and composition of the tadpole community. The study has highlighted that some constructed wetlands can be limited in their suitability as successful breeding sites due to the presence of introduced fish and their biotic and abiotic parameters which fail to mimic natural wetland systems. Wetland managers are advised to develop and manage constructed and rehabilitated wetlands to emulate natural wetland systems and to exclude introduced species, such as *G. holbrooki*. Wetlands which have been designed with gently sloping banks, a mixture of deep permanent pools and highly ephemeral littoral zones, well developed riparian vegetation and that are placed in a terrestrial landscape with natural habitat heterogeneity that reflects the varying life histories of species in amphibian communities are likely to provide suitable habitat for a diverse variety of amphibian species. The study has also highlighted that calling activity has only limited value as a tool for assessing the conservation value of a wetland. Knowledge of reproductive is valuable to assess the success of amphibian breeding. Studies that fail to investigate the reproductive output of wetlands are likely to only have limited value as indicators of the conservation value of the wetlands studied. However, it is important to note that measures of tadpoles only provide an assessment of reproductive success in the early stages of the reproductive cycle. True recruitment can only be measured by examining the abundance of metamorphs that emerge from ponds and return to breed. Such measures were beyond the scope of this stud, but future research should consider the role the broader landscape may play on the long-term amphibian recruitment of such ponds.

ACKNOWLEDGEMENTS

I would like to thank Frank Doyle, and Ann Bentley of the Capel Wetlands Centre for assistance with the field work. Thanks to the Jeff and Marjah Hardwick, Wayne Hastie and Mrs Hatfield for allowing me access to their properties to monitor their frog populations. Thanks to Dr R Black for statistical assistance and to Dr. M. Smith and Dr J.D. Roberts for assistance with the manuscript. I would also like to thank the Capel Wetlands Centre for assisting with the funding for this project and for the numerous volunteers, including Dan Edwards, Michael Smith, Dean Paini, Phil Harden and others, too many to name, who assisted me with the field work. This research was undertaken as part of a PhD at the University of Western Australia with the assistance of Australian Postgraduate Award. All work conducted was done with approval from the University of Western Australia Animal Ethics Committee (AEC approval # 00/100/064) and the Department of Conservation and Land Management, Western Australia (Permit # SF SF003880).

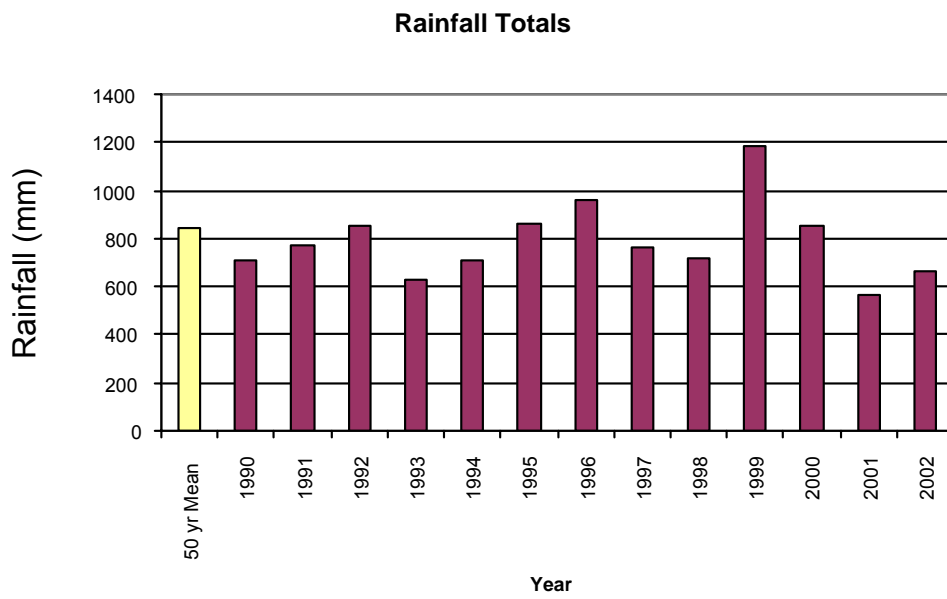


Figure 3.1: Total annual rainfall from 1990 to 2002. The 50 year mean for the study area is also reported.

Table 3.1: Results of the of a split plot ANOVA of tadpole species richness.

Species richness was identified by counting the number of species recorded in each capture technique in each wetland in each year. One natural wetland was excluded from the analysis as the absence of surface water precluded all tadpole trapping. Therefore, only 15 wetlands were included in the analysis; wetland, $n = 15$; constructed: $n = 8$ and natural, $n = 7$. Analysis conducted using statistical package JMP 4.0.

Source	df(num)	MS	F	P
Wetland type*	1	25.3762	6.0136	0.0291
Wetland [wetland type] (random)*	13	54.8571	4.2786	0.0002
Capture technique	1	1.3762	1.3954	0.2447
Capture technique x wetland type	1	0.0429	0.0435	0.8360
Year*	1	18.6012	18.8603	<0.0001
Wetland type x year	1	0.8677	0.8799	0.3540
Capture technique x year	1	1.4583	1.4786	0.2313
Year x wetland type x capture technique	1	0.5250	0.5323	0.4700
Residual	39	0.9863		
Total	59			

** significant at the 0.05 level

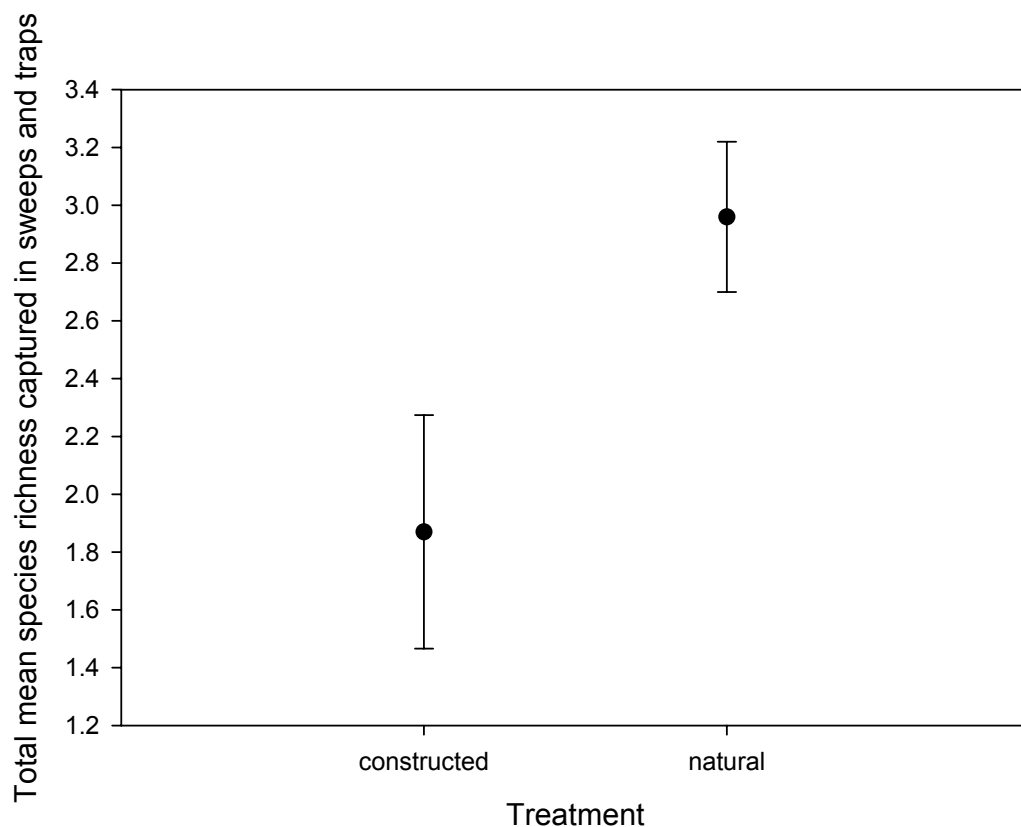


Figure 3.2: Total mean species richness captured (combined across sweeps and traps) in each wetland type.

See Table 3.1 for analysis, $n = 8$, constructed wetlands, $n = 7$ natural wetlands. Error bars = 1 standard error.

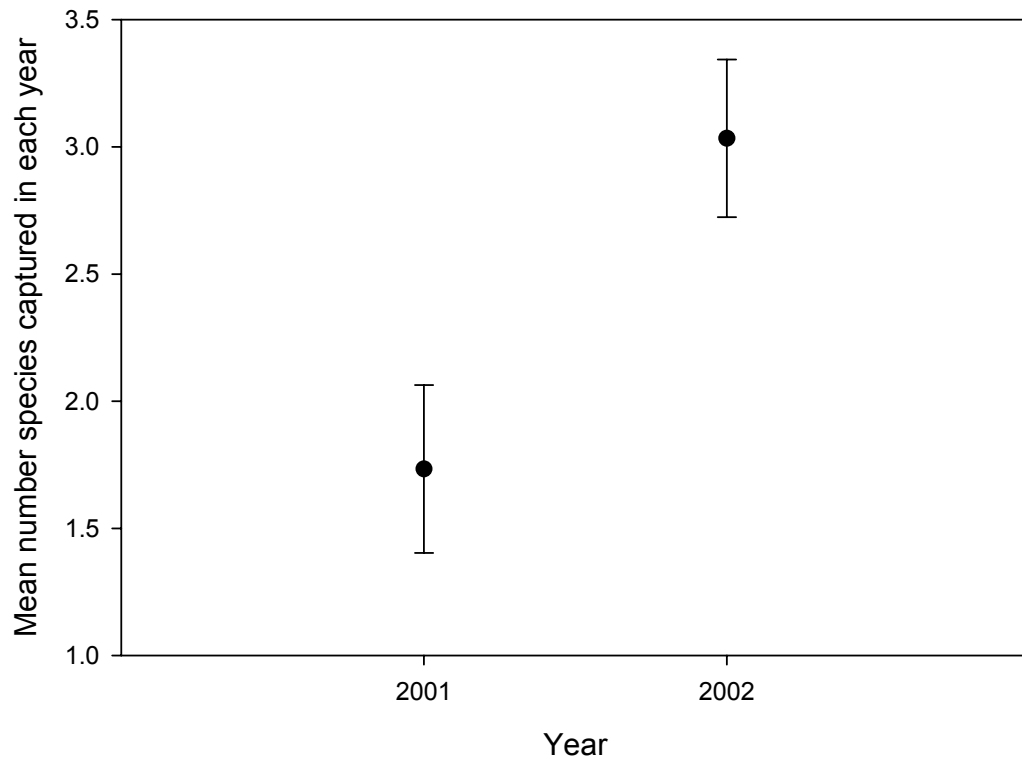


Figure 3.3: Total mean species richness captured (combined across sweeps and traps) in each year (n =15).

See Table 3.1 for analysis. Error bars = 1 standard error

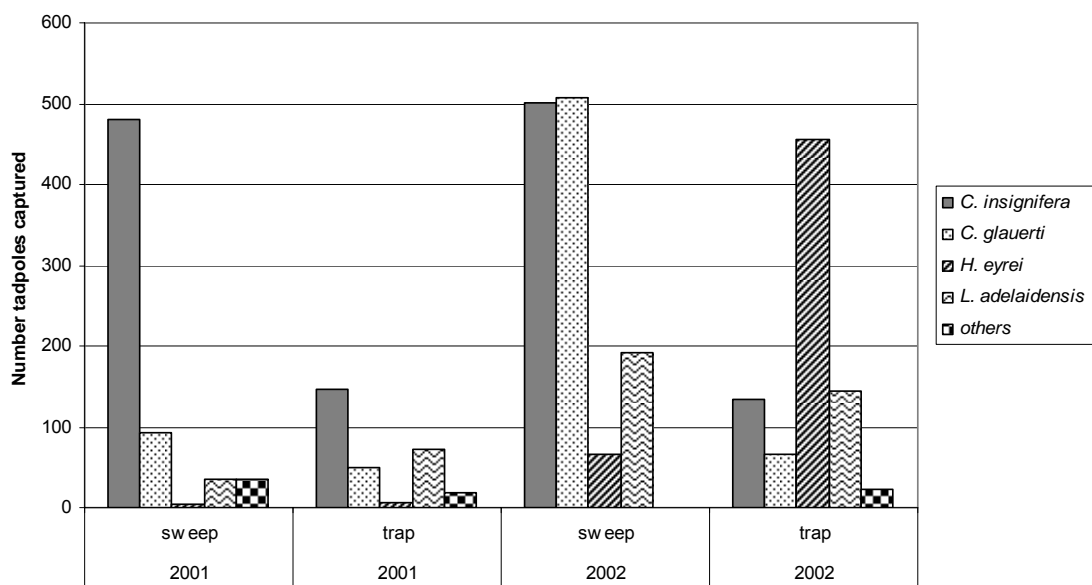


Figure 3.4: Composition of the amphibian tadpole communities captured in each tadpole capture method in each year.

Table 3.2: Results of repeated measures ANOVA for each tadpole species captured in tadpole traps.

(One wetland was excluded from the analysis as the absence of surface water precluded all tadpole trapping. Therefore n=15 for the factor wetland. Tests were undertaken using the statistical program JMP 4.0, using the traditional Expected Mean Square method.)

<i>C. glauerti</i> (sample 6)				
Source	df	MS	F-ratio	P
Wetland type	1	0.0798	1.5319	0.2377
Wetland [wetland type] (random)	13	0.052	0.7731	0.6752
Year	1	0.07	1.0572	0.3226
Wetland type x Year	1	0.050	0.7521	0.4015
Year x wetland [wetland type] (random)**	13	0.067	3.4833	<0.0001
Residual	270	0.0193		
Total	299			
<i>C. insignifera</i> (sample 6)				
Wetland type*	1	2.027	3.2627	0.0941
Wetland [wetland type] (random)	13	0.621	0.8072	0.6474
Year	1	0.276	0.3584	0.5597
Wetland type x Year	1	0.013	0.0169	0.8985
Year x wetland [wetland type] (random)**	13	0.769	4.3492	<0.0001
Residual	270	0.1769		
Total	299			
<i>L. adelaidensis</i> (sample 7)				
Wetland type	1	0.1087	0.1536	0.7015
Wetland [wetland type] (random)	13	0.689	0.9917	0.5059
Year	1	0.730	1.0507	0.3241
Wetland type x Year	1	0.51169	0.7364	0.4064
Year x wetland [wetland type] (random)**	13	0.69483	4.7269	<0.0001
Residual	270			
Total	299			

*significant at the 0.1 level, ** significant at the 0.05 level

Table 3.3: Results of the repeated measures ANOVA (*C. insignifera*) and two-factor ANOVA (*H. eyrei*) for maximum number of tadpoles caught in sweeps only. (One natural wetland was excluded from the analysis as the absence of surface water precluded all tadpole trapping. Therefore n=15 for wetland. Tests were undertaken using the statistical program JMP 4.0, using the Restricted maximum likelihood method (REML).

<i>Crinia insignifera</i> (sample 5, sweeps only) Also see Figure 3.7					
Source	df	Df (den)	MS	F ratio	P
Wetland Type*	1	13	0.427	4.3700	0.0568
Wetland [wetland type] (random)	13	13	18.720	1.1327	0.4128
Year	1	13	0.000	0.0048	0.9460
Wetland type x Year	1	13	0.015	0.1534	0.7016
Year x wetland [wetland type] (random)	13	120	33.280	2.0126	0.2510
Residual	120		0.0978		
Total	149				
<i>H. eyrei</i> (2002 sweep data only, sample 4= constructed, sample 5= natural).					
Wetland type	1	13	0.0473	1.6652	0.2194
Wetland [wetland type] (random)shrunk**	13	60	17.3758	3.6189	0.0003
Residual	60		0.0284		
Total	74				

*significant at the 0.1 level, ** significant at the 0.05 level.

Table 3.4: Results of the nested ANOVA (REML) for maximum number of tadpoles caught in traps. One wetland was excluded from the analysis as the absence of surface water precluded all tadpole trapping (n=15, wetland type). (One natural wetland was excluded from the analysis as the absence of surface water precluded all tadpole trapping. Therefore n=15 for wetland. Data from 5 traps from 1 natural wetland were also excluded as low water levels in one wetland during sample 4 prevented all traps from being deployed. Tests were undertaken using the statistical program JMP 4.0, using the Restricted maximum likelihood method (REML).

<i>H. eyrei</i> (2002 data only, sample 4, traps only) Also see figure 3.5					
Source	df	Df (den)	MS	F ratio	P
Wetland type**	1	13	1.276	4.9153	0.0451
Wetland [wetland type] (random)shrunk**	13	130	404.8070	9.226	0.000
Residual	130		0.2596		
Total	144				

*significant at the 0.1 level, ** significant at the 0.05 level.

Table 3.5: Results of repeated measures ANOVA for each tadpole species captured in sweeps.

One natural wetland was excluded from the analysis as the absence of surface water precluded all tadpole trapping. Therefore n=15 for the factor wetland. Tests were undertaken using the statistical program JMP 4.0, using the traditional Expected Mean Square method.

<i>Crinia glauerti</i> (2001= sample 6, 2002= sample 5)				
Source	df	MS	F-ratio	P
Wetland type	1	0.00192	0.00095	0.9238
Wetland [wetland type] (random)*	13	0.20162	2.4629	0.0584
Year*	1	0.31073	3.7958	0.0733
Wetland type x Year	1	0.05365	0.6554	0.4328
Year x wetland [wetland type]	13	0.0816	0.8034	0.6555
(random)	120	0.1018		
Residual	149	16.297		
Total				
<i>L. adelaidensis</i> (2001: sample 6= constructed, sample 7= natural; 2002: sample 5 = constructed, sample 6=natural.)				
Wetland type	1	0.0195	0.0740	0.7899
Wetland [wetland type] (random)**	13	0.26352	6.8174	0.0007
Year*	1	0.09057	2.343	0.1498
Wetland type x Year	1	0.02253	0.583	0.4588
Year x wetland [wetland type]	13	0.03865	0.811	0.6476
(random)	120	0.0476		
Residual	149			
Total				

*significant at the 0.1 level, ** significant at the 0.05 level.

Table 3.6: The relationship between abundance of calling males† and tadpoles captured in each trap method, based on a linear regression.

Species	Model	Unstandardised co-efficients		Standardised		
		B	Std.E	B(std)	t	Sig.
<i>H.eyrei</i>	Constant	0.037	0.122		0.302	0.767
<i>H.eyrei</i> F _{1,14} =13.232, p=0.003, r ² =0.449	Sweep (all data)	0.283	0.078	0.687	3.638	0.003
<i>H.eyrei</i>	Constant	0.105	0.194		0.543	0.596
<i>H.eyrei</i> F _{1,14} =13.325, p=0.003, r ² =0.451	Trap (all data)	0.449	0.123	0.698	3.650	0.003
<i>C. glauerti</i>	Constant	0.406	0.137		2.966	0.010
<i>C. glauerti</i> F _{1,14} =6.319, p=0.025, r ² =0.262	Sweep (natural only)	0.293	0.116	0.558	2.514	0.025
<i>C. glauerti</i>	Constant	1.165	0.329		3.539	0.003
<i>C. glauerti</i> F _{1,14} =5.555, p=0.034, r ² =0.233	Trap (constructed only)	-0.448	0.190	-0.533	-2.357	0.034

†All wetlands noted to have calling males were utilised in the test under the assumption that if calling males were present, regardless of the presence of surface water, as there was a potential for tadpoles to be present. Therefore, n=16 for the factor wetland.

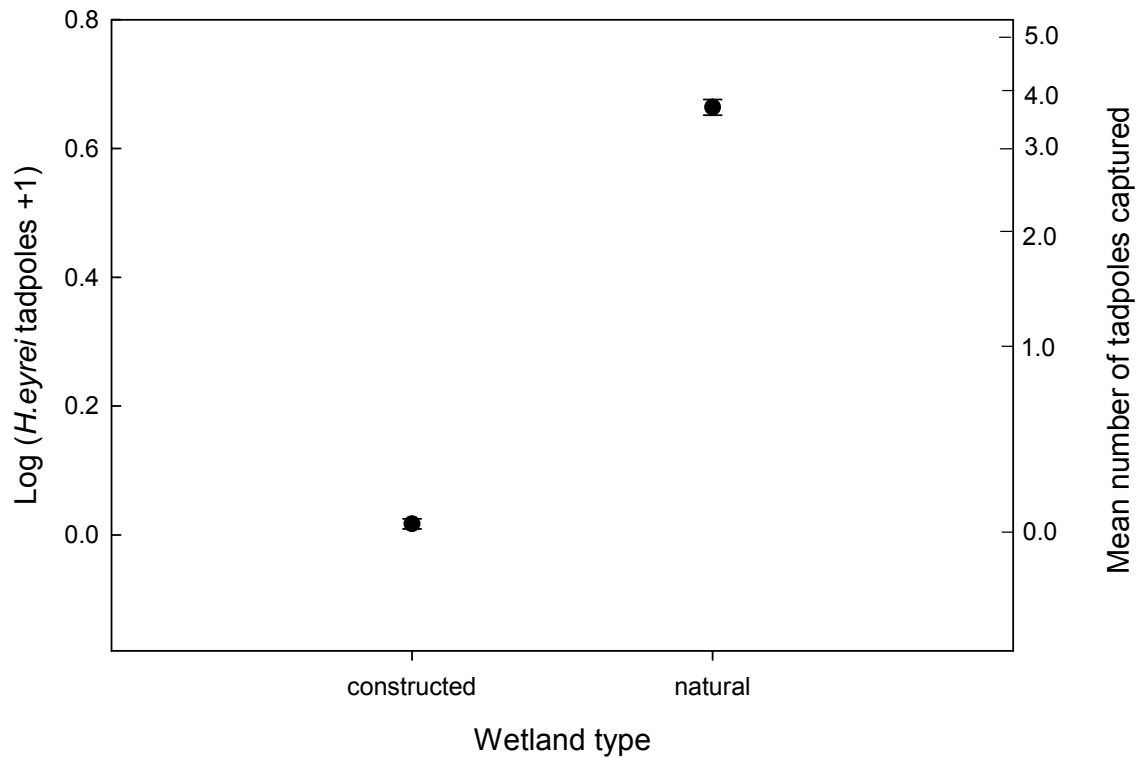


Figure 3.5: Mean number of *H. eyrei* tadpoles captured in traps in constructed and natural wetlands in 2002.

Error bars = 1 standard error. One natural wetland was excluded as it did not hold surface water during the study, in addition several wetlands had reduced surface water in early 2002 reducing the overall trapping effort for some wetlands. Therefore, $n = 320$ constructed wetlands and $n = 275$ for natural wetlands.

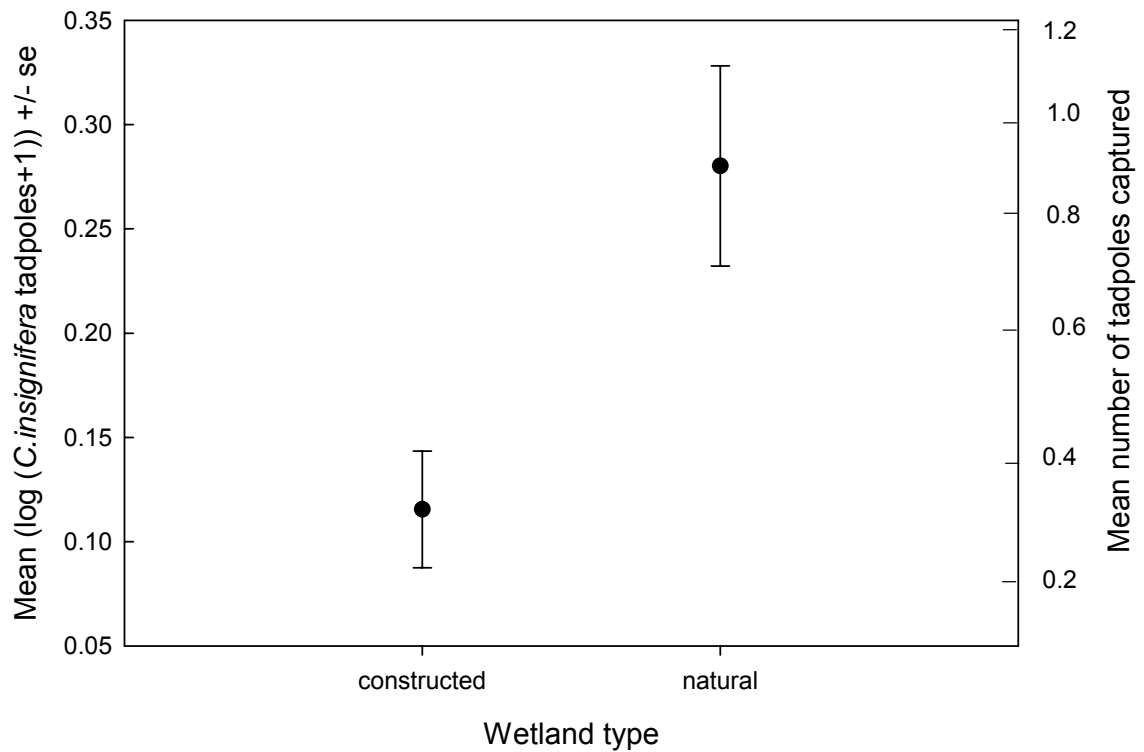


Figure 3.6: Mean number of *C. insignifera* tadpoles captured in traps in constructed and natural wetlands.

Error bars = 1 standard error, n = 530 in natural wetlands and n = 610 in constructed wetlands. One natural wetland was excluded as it did not hold surface water during the study, in addition several wetlands had reduced surface water in early 2001 and 2002 reducing the overall trapping effort for some wetlands.

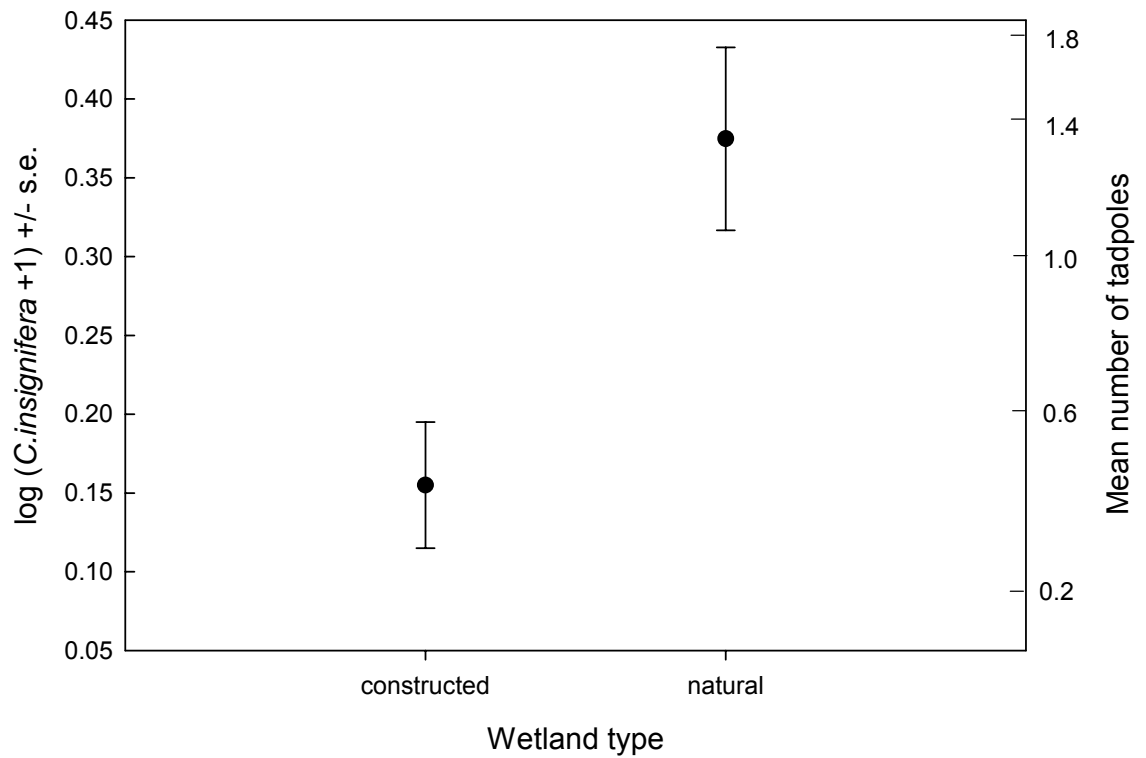


Figure 3.7: Mean number of *C. insignifera* tadpoles captured in sweeps in constructed and natural wetlands.

Error bars = 1 standard error, n = 290 in constructed wetlands, n = 280 in 2 natural wetlands. One natural wetland was excluded as it did not hold surface water during the study, in addition several wetlands had reduced surface water in early 2001 and 2002 reducing the overall trapping effort for some wetlands.

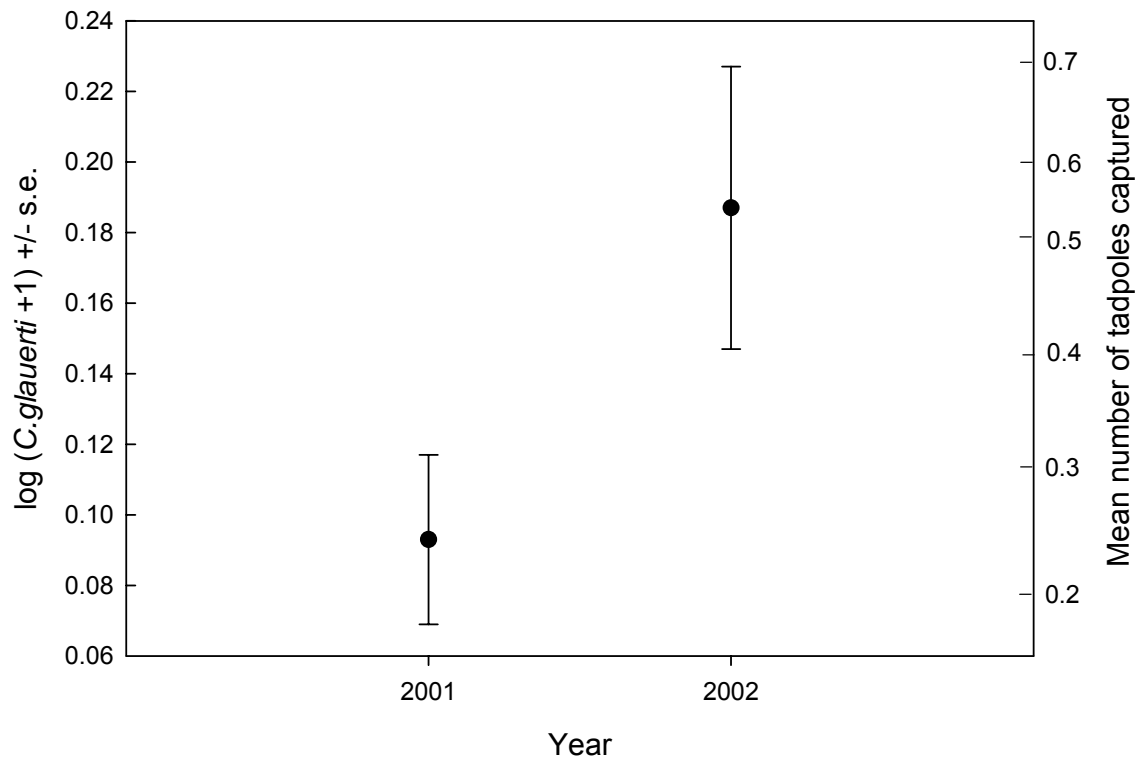


Figure 3.8: Mean number of *C. glauerti* tadpoles captured in sweeps in 2001 and 2002.

Error bars = 1 standard error of the mean, $n = 270$ in 2001, $n = 300$ in 2002. One natural wetland was excluded as it did not hold surface water during the study, in addition several wetlands had reduced surface water in early 2001 and 2002 reducing the overall trapping effort for some wetlands.

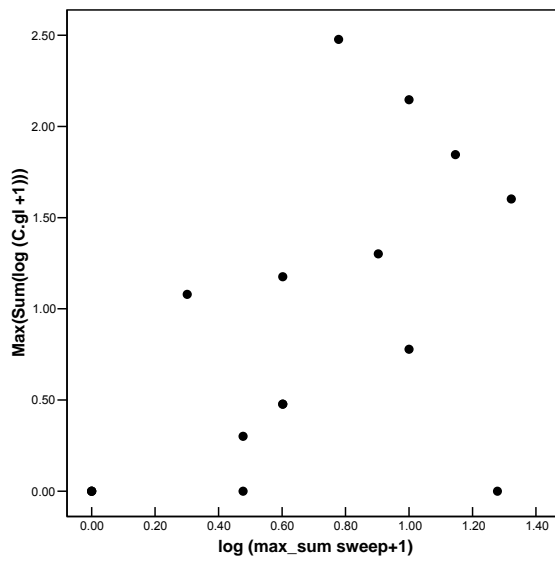


Figure 3.9: Scatter plot of calling males and tadpoles of *C. glauerti* captured in sweeps in natural wetlands

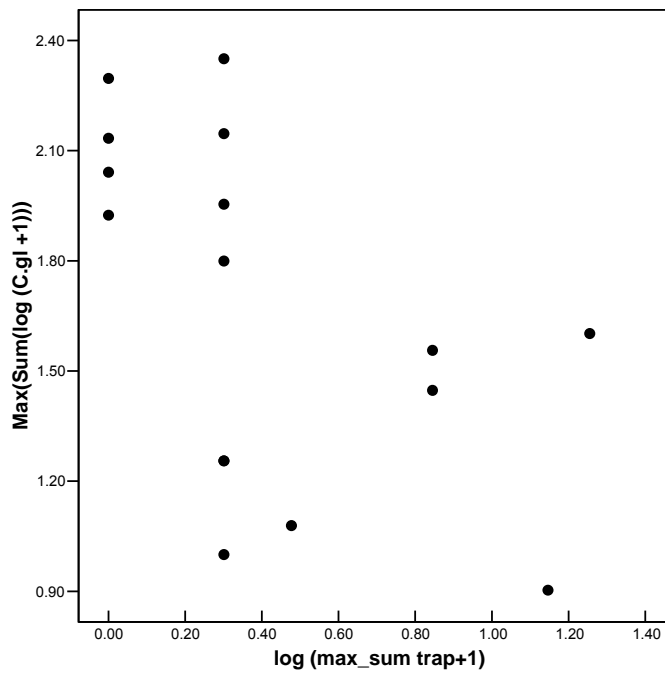


Figure 3.10: Scatter plot of calling males and tadpoles of *C. glauerti* captured in traps in constructed wetlands

Table 3.7: Results of the PCA with varimax rotation using arcsine (x)^{-0.5} transformed environmental parameter data.

Component	Correlation		
	Eigenvalue	Percentage variance	Cumulative variance
1	3.147	62.935	62.935
2	1.259	25.16	88.111
3			

Variable	Component (pattern matrix)	
	1	2
Arcsine (FV) ^{-0.5}	0.936	0.226
Arcsine (OV) ^{-0.5}	0.949	0.107
Arcsine (AV) ^{-0.5}	0.358	0.856
Arcsine (RV) ^{-0.5}	0.616	-0.686
Arcsine (WP) ^{-0.5}	-0.928	0.055

Table 3.8: Multiple linear regression (enter method) results based on the identified PCA components (1 and 2) for habitat variables relative to maximum abundance of each species measured at each wetland.

All statistical analysis were conducted using the statistical package SPSS 12.0.

Source	Model	Unstandardised		Standardised	t	P
		B	SE	B		
Species richness F _{1,14} =25.349, P=0.000, r ² =0.661	Constant	4.165	0.207		20.133	0.000
	Component 1†	1.221	0.243	0.813	5.035	0.000
<i>C. glauerti</i> (sweep) F _{1,14} = 6.174, P =0.027, r ² = 0.322	Constant	21.061	8.811		2.390	0.033
	Component 2†	-21.932	8.827	-0.567	-2.485	0.027
<i>H. eyrei</i> (sweep)* F _{1,14} =12.256, P =0.004, r ² = 0.485	Constant	0.431	0.090		4.808	0.000
	Component 1†	0.368	0.105	0.697	3.501	0.004
<i>H. eyrei</i> (trap)* F _{1,14} =8.253, P =0.013, r ² = 0.388	Constant	0.727	0.155		4.701	0.000
	Component 1†	0.521	0.181	0.623	2.873	0.013
<i>C. insignifera</i> (trap)* F _{1,14} = 6.603, P = 0.012, r ² = 0.524	Constant	0.845	0.094		8.992	0.000
	Component 1	0.307	0.110	0.555	2.785	0.016
<i>L. adelaidensis</i> (trap)* F _{1,14} =5.606, P = 0.034, r ² = 0.301	Component 2	-0.207	0.093	-0.443	-2.222	0.046
	Constant	0.724	0.124		5.855	0.000
	Component 2†	-0.293	0.124	-0.549	-2.368	0.034

* denotes log (x+1) transformed data.

† The two components from the PCA were entered into the model using the enter method of analysis to determine which components most strongly contributed to the model. In instances where one component did not strongly influence the model, the component was removed from the multiple linear regression and the model re-run with the excluded component.

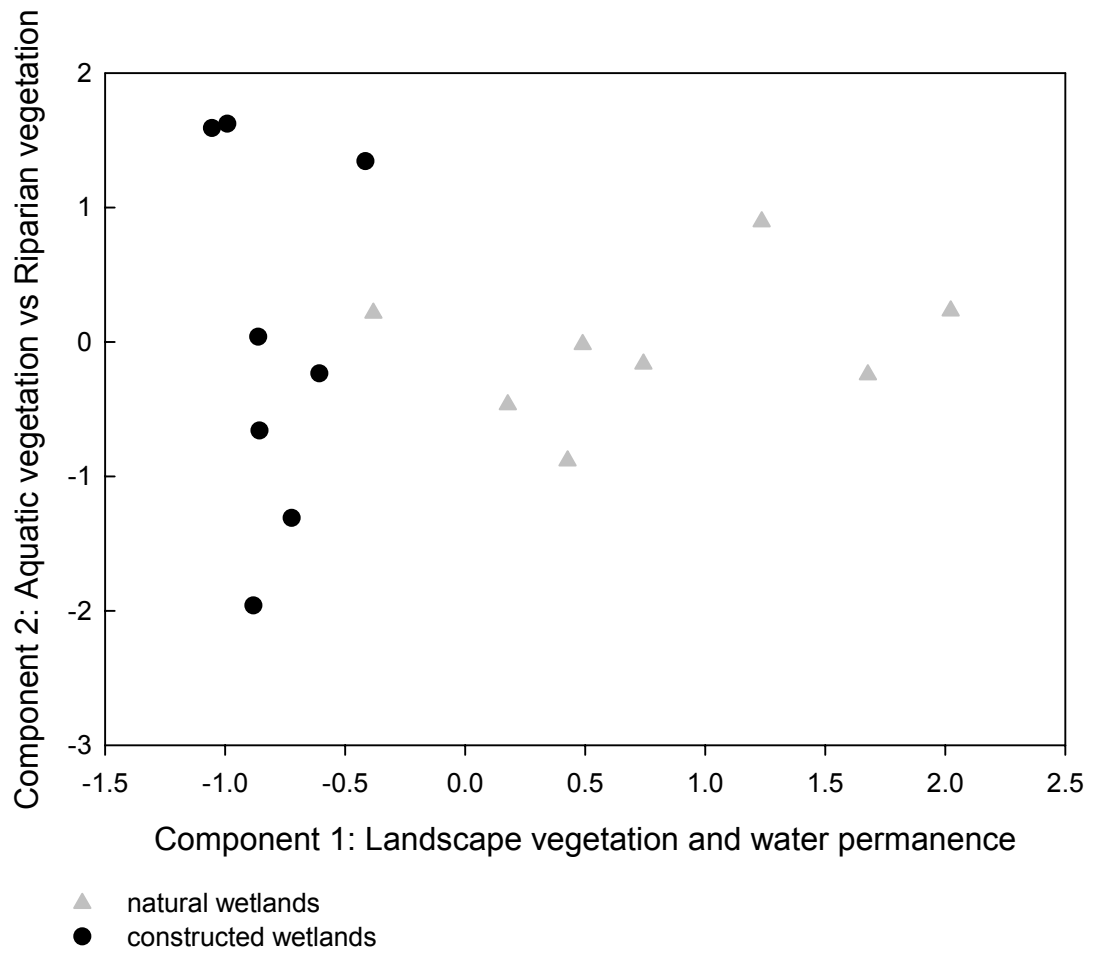


Figure 3.11: Ordination plot for wetland type. The ordination was based on the habitat variables extracted from the PCA.

4.0 Habitat complexity and the survival of tadpoles exposed to two predators: an introduced fish and an odonate larvae.

ABSTRACT

The environmental complexity of a wetland can strongly influence predation success. Tadpoles persisting in wetlands characterised by habitat simplification, such as artificially-constructed wetlands, may experience higher predation rates than tadpoles in more complex environments. A factorial experiment was designed that crossed eight levels of habitat complexity with three predator levels (no predator, introduced fish (*Gambusia holbrooki*) or native, odonate larvae (*Hemicordulia tau*)) to determine the impact of predators on the survival of tadpoles of *Litoria adelaidensis*. Predator type had a large influence on tadpole survival, while habitat complexity in the form of cover and the presence of alternative prey improved tadpole survival in the presence of both tested predators. This study demonstrates that the presence of cover and alternative food sources can play a role in mediating predator-prey interactions in laboratory conditions and highlights the potential impact that the introduced predator *G. holbrooki* may have on native tadpole populations.

4.1 Introduction

The effectiveness of any survival strategy may be strongly influenced by habitat. Habitat complexity in the form of shelter, light availability, abundance and diversity of alternative prey, for example, all potentially modify predator-prey interactions in ecosystems. High levels of physical structure in a system creates more microhabitats, which can provide partial or total refuge for prey and potentially reduce predator capture efficiency (Babbitt & Tanner 1998; Crowder & Cooper 1982; Folsom & Collins 1984). Further, prey density is often positively correlated with habitat structure (Gerking 1957; Macan 1949) as it provides food and substrate to the prey as well as a refuge from predators (Crowder & Cooper 1982). Numerous studies have shown that wetlands with limited habitat complexity have higher predation rates than wetlands with well-developed habitats (Crowder & Cooper 1982; Morgan & Buttemer 1996; Nelson & Bonsdorff 1990). However, most studies only investigate the individual effects of habitat variables such as cover (e.g. Hews 1995), water colour and alternative food on prey survival (e.g. Goodsell & Kats 1999; e.g. Morgan & Buttemer 1996). Complex interactions can occur between two or more variables (Crowder & Cooper 1982; Horat & Semlitsch 1994) and these may be important components to consider when looking at predator-prey interactions (Boone & Semlitsch 2001).

The fish, *Gambusia holbrooki*, has been implicated in the decline of amphibian populations worldwide (Gillespie & Hero 1999) with numerous studies revealing that this fish is an effective predator of many native anuran tadpoles (e.g. Komak & Crossland 2000; e.g. Morgan & Buttemer 1996). Several field studies have identified a negative relationship between fish presence and abundance of various tadpole species (Ildos & Ancona 1994; Pyke & White 1996). However, only a few studies (e.g. Goodsell & Kats 1999; Morgan & Buttemer 1996) have looked at the role habitat complexity may play in mediating the impact of *G. holbrooki*. Understanding the role habitat variables may play in mediating the impact of this introduced predator has become increasingly important as human development continues to reduce habitat availability and quality.

In this study I examined the impact of eight levels of habitat complexity on the survival of the tadpole of a Western Australian anuran, *Litoria adelaidensis*, exposed to *G. holbrooki* or a native predator. *Litoria adelaidensis* is a native anuran species that is common throughout south-western Australia (Tyler et al. 2000) and it typically breeds

in natural wetlands (Cogger 2000) but is now found calling in artificially- constructed wetlands (see chapter 2). Preliminary observations of tadpole abundance in artificially-constructed and natural wetlands in the south-west of Western Australia showed that artificially-constructed wetlands typically supported lower densities of tadpoles of *L. adelaidensis* than nearby natural wetlands (Chapter 3: 2001 data only). However, little information is available on how productive anuran breeding is in these artificial ponds or if these populations rely upon colonisation from more successful nearby breeding habitats (see Marsh & Trenham 2001 for a review of metapopulation studies).

Artificially-constructed wetlands in general are structurally and biologically less complex than natural wetlands (Brock & Jarman 2000; Brock et al. 1999; Hammer 1992). Further, unlike most natural wetlands, which are typically ephemeral in nature, the permanent water regimes of constructed wetlands means they commonly support the introduced fish predator *G. holbrooki* (Gillespie & Hero 1999). Given the simplicity of habitats in constructed wetlands and the presence of the introduced predator *G. holbrooki* the suitability of artificially-constructed wetlands as productive breeding habitats for anurans is questionable. In light of this and the continued destruction of wetlands in Australia, I designed an experiment that would provide the first step in determining if constructed wetlands can play a role in providing adequate, surrogate, breeding habitat for native amphibian species. Using laboratory trials I exposed tadpoles of *L. adelaidensis* to two common predators, the introduced mosquitofish, *Gambusia holbrooki*, and a native odonate, *Hemicordulia tau*, in trials with eight levels of habitat complexity generated by combining three variables: cover, water colour and alternative prey. I evaluated how these components of habitat complexity affect the impact of two common predators on the survival of tadpoles of *L. adelaidensis*.

4.2 Methods

All fish (20-33mm in length) and odonate larvae (25-30mm in total length) were collected from water bodies on the Swan Coastal Plain, Western Australia using a 0.5mm mesh sweep net. Predators were acclimatised to laboratory conditions for a minimum of 24 hours before the trial and were not fed for this period prior to the experiment to standardise hunger levels. Each predator was used only once to prevent acclimatisation to prey and no predator was kept for more than 72hours prior to the trial.

Trials not receiving predators each had a small rock placed in the container to mimic the disturbance created by predator addition.

Tadpoles were raised in the laboratory from egg clutches gathered from 30 amplexing pairs either in the field or the laboratory. Egg clutches were mixed before tadpoles were randomly assigned to each trial to minimise the impact that any genetic or physical attributes, particular to each clutch, may have had on tadpole survival. All tadpoles were between 11 and 13mm in total length: Gosner stages 25 to 26.

The impact of two levels of cover (cover or no cover), two levels of water colour (colour or no colour) and two levels of alternative prey (*Daphnia* or no *Daphnia*) on the survival of tadpoles of *L. adelaidensis* exposed to three predator levels (no predator, fish or odonate), were examined. Between three and five replicates of each of the 24 treatments were run on each of the three trial days, giving a total of 13 replicates of each treatment. This gave a five factor fully orthogonal design (2 x 2 x 2 x 3 x 3).

Experiments were conducted in a controlled temperature room using 8L opaque, polyethylene containers (31 x 28 x 11.5cm) lined with 440ml of a natural wetland sand/mud substrate. Each container was filled with 6L of either tannin stained water ("colour" treatments, secchi depth: 200mm) collected from natural wetlands or transparent water ("no colour" treatments, secchi depth: >1000mm) collected from constructed wetlands, in the Capel region (33.33°S, 115.34°E), south-western Australia. The tannin stained water is likely to be different from the clear water in components other than just colour, such as chemical composition, and therefore the response of tadpoles and/or predators may not necessarily be due to the single component of colour. Water was changed in each container between each trial day and each treatment was randomly allocated to a container. Trials were run for 24 hours commencing at 09:00 on a 12 hour light (20°C): dark (16°C) cycle simulating natural conditions.

Six pieces of shade-cloth (i.e. thickly meshed plastic fabric, 16x13cm) were used to mimic reeds and leaf litter in treatments allocated the "cover treatment". Pilot trials revealed that shade cloth was an effective replacement for natural cover that improved post-experiment processing efficiency. This enabled higher replication of treatments and reduced the risk of contaminating trials with invertebrates that may have been present in natural aquatic vegetation. Three pieces of shade cloth were placed at the base of each tank and held submerged by a small river stone simulating approximately 60% leaf litter cover over the sediment. The remaining three pieces were left unweighted and therefore remained suspended in the water column emulating floating

and emergent aquatics. Approximately 60-80 *Daphnia* were added to trials in addition to the two tadpoles in the "alternative prey" treatments.

Tadpole and predator densities in the trials approximated densities observed in the field (2001 data only). Observations of the abundance of tadpoles of *L. adelaidensis* in constructed wetlands in the field revealed densities of tadpoles between 0.05 and 0.25 tadpoles/L (Figure 4.1). These values translated to 1.5 tadpoles/ treatment, therefore, two tadpoles were allocated to each treatment. *Gambusia holbrooki* densities varied between 0.18 and 1.4 fish/L (Chapter 3: 2001 trap data only). As social species will often behave abnormally in isolation (Casterlin & Reynolds 1977) the trials mimicked the middle of this range with a predator density of 0.83fish/l or 5fish/6L water to two tadpoles of *L. adelaidensis*. In the field, densities of *H. tau* larvae were on average approximately five times lower than *G. holbrooki*; therefore only one odonate/6l tub of water and two tadpoles of *L. adelaidensis* were used for these trials.

4.2.1 Data Analysis

As each replicate had only two tadpoles and the proportion of tadpoles surviving was 0.0, 0.5 or 1.0, variance within treatments was high; I therefore pooled the number surviving in all replicates of each treatment on each of the three days to give a single, average estimate of survival. This simplified design gave three replicates of each of the 24 treatments. The arcsine (square root (mean proportion surviving)) was analysed with day as a blocking factor and the other four fixed factors in a fully orthogonal design (2 x 3 x 2 x 2) using the statistical package JMP 4.0.

4.3 Results

There were no significant interactions between treatments at the 0.05 level (Table 4.1). At the 0.1 level the interaction term Cover x Predator x Water Colour x Alternative prey, was significant. Interpretation of the graphs (Figure 4.2 a-d) show that the result was driven by a variation in the survival rates of tadpoles in treatments with *G. holbrooki* and to a lesser extent *H. tau*. When *G. holbrooki* was present, tadpole survival was highest in treatments with alternative prey, cover and tannin water (Figure 4.2d: 24.2% survival or 0.514 rad. \pm 0.05 s.e., n=3). However, in treatments with cover and clear water tadpole survival was similar regardless if alternative prey was present or not (Figure 4.2c: 2.3% survival or 0.15 rad. \pm 0.15 s.e., n=3). In treatments with no cover and tannin coloured water, alternative prey did not improve the tadpole survival (Figure 4.2b: 0% survival) but in the absence of water colour and cover, alternative prey

increased tadpole survival, with an average survival rate of 1.15% (0.107 ± 0.107 s.e., $n=3$) compared to 0% survival with no alternative prey present. Tadpoles in treatments with *H. tau* had higher survival in all treatments with alternative prey compared to treatments without another food source in all factor combinations except treatments with no cover and clear water. In containers with no cover and clear water, tadpole survival was lower in treatments with alternative prey present (Figure 4.2a: 0.99 ± 0.19 s.e., $n=3$: 70% survival) compared to treatments with no alternative prey (1.04 ± 0.14 s.e., $n=3$, 74.6% survival), although not significantly so. Evaluation of the effect size shows that the influence of these complex interactions on the test was approximately 13 times less than the effect of the main term predator (Table 4.2).

The main factors “Predator”, “Cover” and “Alternative prey” had significant effects on the survival of tadpoles at the 0.05 level (Table 4.1). Survival was highest in treatments with no predator. Tadpole survival was lower in the presence of the odonate predator and lowest with *G. holbrooki* present (Figure 4.3a). The inclusion of cover and alternative prey improved tadpole survival (Figures 4.3b & 4.3c) in the presence of both predators. Survival in the presence of *G. holbrooki* increased from zero radians (0%, $n=6$) in the absence of cover and alternative prey to $0.334 \text{ rad.} \pm 0.108 \text{ s.e.}$, $n=6$ (21.3% survival) in trials with an alternative food source and cover. For tadpoles in treatments with *H. tau*, survival increased from $0.967 \text{ rad.} \pm 0.075 \text{ s.e.}$, $n=6$ (61.6%) in treatments without cover or alternative prey to $1.24 \text{ rad.} \pm 0.105 \text{ s.e.}$, $n=6$ (78.98%) when cover and alternative prey were present. A review of the scaled estimates from the ANOVA which highlights the size of the effect of each treatment level, illustrates that the impact of predators was roughly ten times the influence of both alternative prey and habitat on the survival of tadpoles (Table 4.2).

4.4 Discussion

Under controlled experimental conditions tadpoles of *L. adelaidensis* are highly susceptible to predation by the introduced fish *G. holbrooki*. The impact of odonates (*H. tau*) on tadpole survival was substantially lower than *G. holbrooki* (1.06 radians or 76% and 0.12 radians or 1.21%, respectively; $n=24$). This indicates the potential for wetlands containing *G. holbrooki* to have different mortality regimes for larval *L. adelaidensis* compared with wetlands in which only native invertebrate predators persist. If my results are an indication of how this predator may impact on *L.*

adelaidensis populations in more natural circumstances, *G. holbrooki* may prove to be a highly detrimental species to anuran populations persisting in simplified environments.

4.4.1 *The role of environmental complexity in tadpole survivorship*

This study clearly showed that environmental complexity in the form of habitat/refuges and the availability of alternative food sources increases the survival of tadpoles in simplified, laboratory environments with high predation risk. The influence these factors have on tadpole survival are complex but indicate that alternative prey and habitat complexity in the form of water colour or cover may reduce consumption rates. Both Crowder and Cooper (1982) and Babbitt and Tanner (1998) identified the importance of habitat complexity in the regulation of predation rates: High levels of physical structure in a habitat can reduce predator efficiency by providing complete refuges for the prey, or by providing partial protection for the prey due to reduced predator efficiencies in portions of the habitat. The presence of cover may also alter prey behaviour altering their availability and vulnerability to predators (Crowder & Cooper 1982).

In the case of *H. tau*, a “sit and wait” predator, increased habitat complexity may reduce the incidence of tadpoles encountering the waiting predator. Folsom and Collins (1984) showed that complexity (i.e. sand and substrate with large amounts of aquatic plants) significantly reduced prey capture rates by the odonate larvae *Anax junius*. Crowder and Cooper (1982) showed that at high macrophyte densities, bluegill fish caught fewer prey despite a higher biomass of available prey species. They concluded that reduced encounter rates in structurally complex habitats limited predator capture attempts. Increased structural complexity of habitats increased the surface area in which tadpoles could shelter and therefore predators in complex environments required a longer period to search an area and discover sheltering prey.

The presence of alternative prey in most instances reduced the predator impact on *L. adelaidensis* tadpoles. This suggests that encouraging the presence of alternative prey such as invertebrates may reduce the impact predators have on tadpoles of *L. adelaidensis*. A similar study which compared survival of tadpoles of *Hyla regilla* in the presence of mosquito larvae and the introduced predator *Gambusia affinis* showed that survival was significantly greater with mosquito larvae present than with tadpoles alone (Goodsell & Kats 1999).

4.4.2 *Predatory impact on tadpoles of L. adelaidensis in controlled conditions*

The two predator species tested exhibit very different hunting/foraging techniques. Trials on *H. tau* by Richards and Bull (1990) indicated that hunting in this species is almost exclusively tactile, with trials revealing no detectable influence on the predatory behaviour or efficiency when the eyes of the larvae were covered. Similarly, Folsom and Collins (1984) described most odonate larvae as “sit and wait” predators that rely almost exclusively on tactile cues. Tadpoles would therefore need to come into contact with, or into the close vicinity of an odonate before an attack, making highly active tadpoles more susceptible. Prey activity was identified as one of the primary determinants of the diet of larval odonate *Anax junius* compared with prey exposure (habitat complexity), density or size (Folsom & Collins 1984). Werner and McPeck (1994) identified that the higher predation rates due to a native odonate predator on tadpoles of the bullfrog (*Rana catesbeiana*) compared with green frogs (*R. clamitans*) was primarily due to the higher activity levels of tadpoles of bullfrogs in the presence of odonate predators.

In contrast to odonate predators, *G. holbrooki* has been described as an aggressive feeder that will actively forage for prey (Webb & Joss 1997). This active foraging/hunting would increase the potential for a predator-prey interaction, while prey encounter rates may also be increased if fish movements disturbed resting tadpoles. Pyke and White (2000) and Reynolds (1995) suggested that *G. holbrooki* ignored and rarely attacked stationary tadpoles. However, no quantitative study was conducted on the behavioural patterns of *L. adelaidensis* during this experiment.

4.4.3 *Complex habitat and predator-prey interactions*

The four-way interaction identified in the ANOVA suggests that there may be complex synergistic effects or relationships between habitat and predator-prey interactions. Water colour appeared to play an interesting role, mediating the impacts of *G. holbrooki* with cover present. As *H. tau* is generally considered a tactile predator (Richards & Bull 1990) the predatory impact of this species was considered less likely to be influenced by the availability of light and hence its ability to capture prey should not be influenced substantially by a change in the colour of the water. However, with

the visually stimulated predatory tactics of the fish predator, *G. holbrooki*, it was envisaged that water colour would to some degree mediate its predatory impact (i.e. treatments with cover, tannin water and alternative prey present). Why the reduction in prey impact exposed to *G. holbrooki* only occurred when alternative prey was present and not in treatments with tannin water cover and no alternative prey is unclear. It is probable that chemicals cue in the water may have influence the predatory tactics of this species or alternatively the tadpoles. This may have altered *G. holbrooki*'s prey capture efficiency. How these complex relationships might translate into more natural systems is difficult to determine, but it highlights the importance of undertaking studies which look at the interaction of various habitat components to ensure adequate understanding of processes that may influence predator prey interactions (Boone & Semlitsch 2001).

4.5 Conclusion

The results of this study indicate that the provision of natural/artificial cover in a waterbody might improve survival of *L. adalaidensis* in the presence of introduced predators. Manipulations that increase the abundance of alternative prey, such as provision of cover and/or encouraging algal growth, may also mediate predator impact in constructed wetlands. The results from this experiment should provide a sound basis from which field experiments can be designed to further understand the role habitat complexity plays in mediating the impact of the introduced predator, *G. holbrooki*, on native larval amphibian populations under more natural conditions.

The four way interaction between habitat variables within this study indicates that the influence of habitat variables on predator –prey relations are not independent. Previous studies that have looked at the role of a single habitat variable on predator-prey interactions may be over simplified. Complex interactions such as those shown here and in other studies (e.g. Boone & Semlitsch 2001) indicate that the use of complex designs may prove useful in understanding the ecological implications of simplified habitats such as constructed wetlands.

ACKNOWLEDGEMENTS

I thank Mike Smith for assistance with all facets of experimental setup and the collection of data, Dr. Bob Black and Matt Williams for statistical advice, Frank Doyle, Wayne Hastie and Jeff and Marjah Hardwick for access to wetlands for the collection of water, tadpoles and frogs, the University of Missouri, Department of Biology postgraduate/postdoctoral reading group and Dr. Ray Semlitsch for providing critical assessment of the manuscript. I thank Capel Wetlands Centre for assisting with the funding for this project. All work conducted was done with approval from the University of Western Australia Ethics Committee 00/100/064 and Department of Conservation and Land Management, Western Australia (Permit No.: SF003551).

Table 4.1: Results of the four-factor ANOVA analysis, with date as a blocking factor.

The test was run using pooled data within each treatment on each day giving a single average of survival on each day and a total of three replicates ($n = 3$) for each treatment.

Source	df	MS	F ratio	P
Day	2	0.1408	1.2884	0.2855
Cover*	1	0.2838	10.3858	0.0023
Predator*	2	51.1414	467.8210	<0.0001
Water Colour	1	0.0028	0.1037	0.7489
Alternative prey*	1	0.1916	7.0089	0.0111
Cover x Predator	2	0.1391	1.2727	0.2898
Cover x Water Colour	1	0.0136	0.4974	0.4842
Cover x Alternative prey	1	0.0415	1.5177	0.2242
Predator x Water Colour	2	0.0269	0.2456	0.7832
Predator x Alternative prey	2	0.0849	0.7769	0.4658
Water Colour x Alternative prey	1	0.0132	0.4814	0.4913
Cover x Predator x Water Colour	2	0.0856	0.7833	0.4629
Cover x Predator x Alternative prey	2	0.1370	1.2537	0.2950
Cover x Water Colour x Alternative prey	1	0.0287	1.0511	0.3106
Predator x Water Colour x Alternative prey	2	0.1194	1.0923	0.3440
Cover x Predator x Water Colour x Alternative prey**	2	0.3155	2.8864	0.0659
Error	46	0.0273		
Total	71			

* indicates a significant effect at the 0.05 level, ** indicates a significant effect at the 0.1 level.

Table 4.2: Summary of the estimated size of the effect from the four factor ANOVA for each of the significant terms.

Term	Scaled			
	estimate	Std Error	t ratio	Prob >[t]
Intercept	0.9108	0.0195	46.7501	0.0000
No Cover	-0.0628	0.0195	-3.2227	0.0023
Cover	0.0628	0.0195	3.2227	0.0023
No predator	0.6408	0.0276	23.2563	0.0000
G. holbrooki	-0.7945	0.0276	-28.8354	0.0000
Odonate	0.1537	0.0276	5.5791	0.0000
No Alternative prey	-0.0516	0.0195	-2.6474	0.0111
Daphnia	0.0516	0.0195	2.6474	0.0111
No Cover x No Predator x clear water x no alternative prey	0.0008	0.0276	0.0281	0.9777
No Cover x No Predator x clear water x daphnia	-0.0008	0.0276	-0.0281	0.9777
No Cover x No Predator x tannin water x no alternative prey	-0.0008	0.0276	-0.0281	0.9777
No Cover x No Predator x tannin water x daphnia	0.0008	0.0276	0.0281	0.9777
No Cover x gambusia x clear water x no alternative prey	-0.0577	0.0276	-2.0947	0.0417
No Cover x gambusia x clear water x daphnia	0.0577	0.0276	2.0947	0.0417
No Cover x gambusia x tannins x no alternative prey	0.0577	0.0276	2.0947	0.0417
No Cover x gambusia x tannins x daphnia	-0.0577	0.0276	-2.0947	0.0417
No Cover x odonate x clear water x no alternative prey	0.0569	0.0276	2.0666	0.0444
No Cover x odonate x clear water x daphnia	-0.0569	0.0276	-2.0666	0.0444
No Cover x odonate x tannins x no alternative prey	-0.0569	0.0276	-2.0666	0.0444
No Cover x odonate x tannins x no daphnia	0.0569	0.0276	2.0666	0.0444
Cover x No Predator x clear water x No Alternative prey	-0.0008	0.0276	-0.0281	0.9777
Cover x No Predator x clear water x daphnia	0.0008	0.0276	0.0281	0.9777
Cover x No Predator x tannins x No Alternative prey	0.0008	0.0276	0.0281	0.9777
Cover x No Predator x tannins x daphnia	-0.0008	0.0276	-0.0281	0.9777
Cover x Gambusia x clear water x No Alternative prey	0.0577	0.0276	2.0947	0.0417
Cover x Gambusia x clear water x daphnia	-0.0577	0.0276	-2.0947	0.0417
Cover x Gambusia x tannins x No Alternative prey	-0.0577	0.0276	-2.0947	0.0417
Cover x Gambusia x tannins x daphnia	0.0577	0.0276	2.0947	0.0417
Cover x Odonate x clear water x No Alternative prey	-0.0569	0.0276	-2.0666	0.0444
Cover x Odonate x clear water x daphnia	0.0569	0.0276	2.0666	0.0444
Cover x Odonate x tannins x No alternative prey	0.0569	0.0276	2.0666	0.0444
Cover x Odonate x tannins x daphnia	-0.0569	0.0276	-2.0666	0.0444

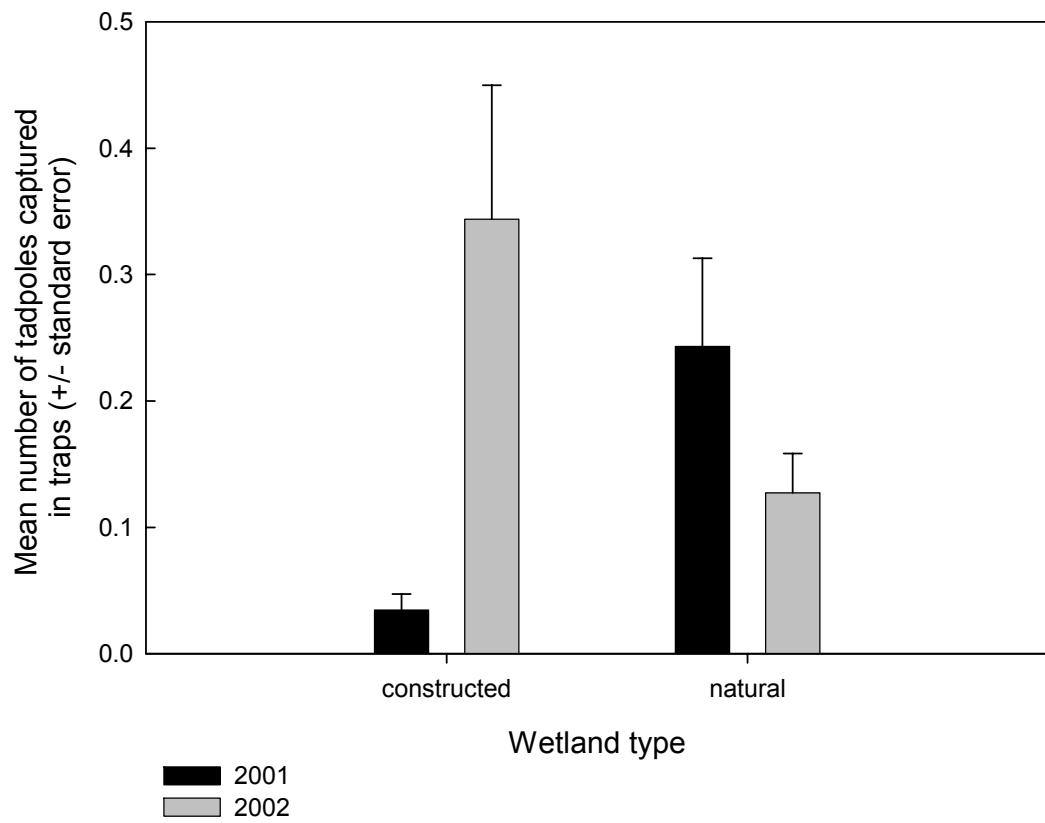
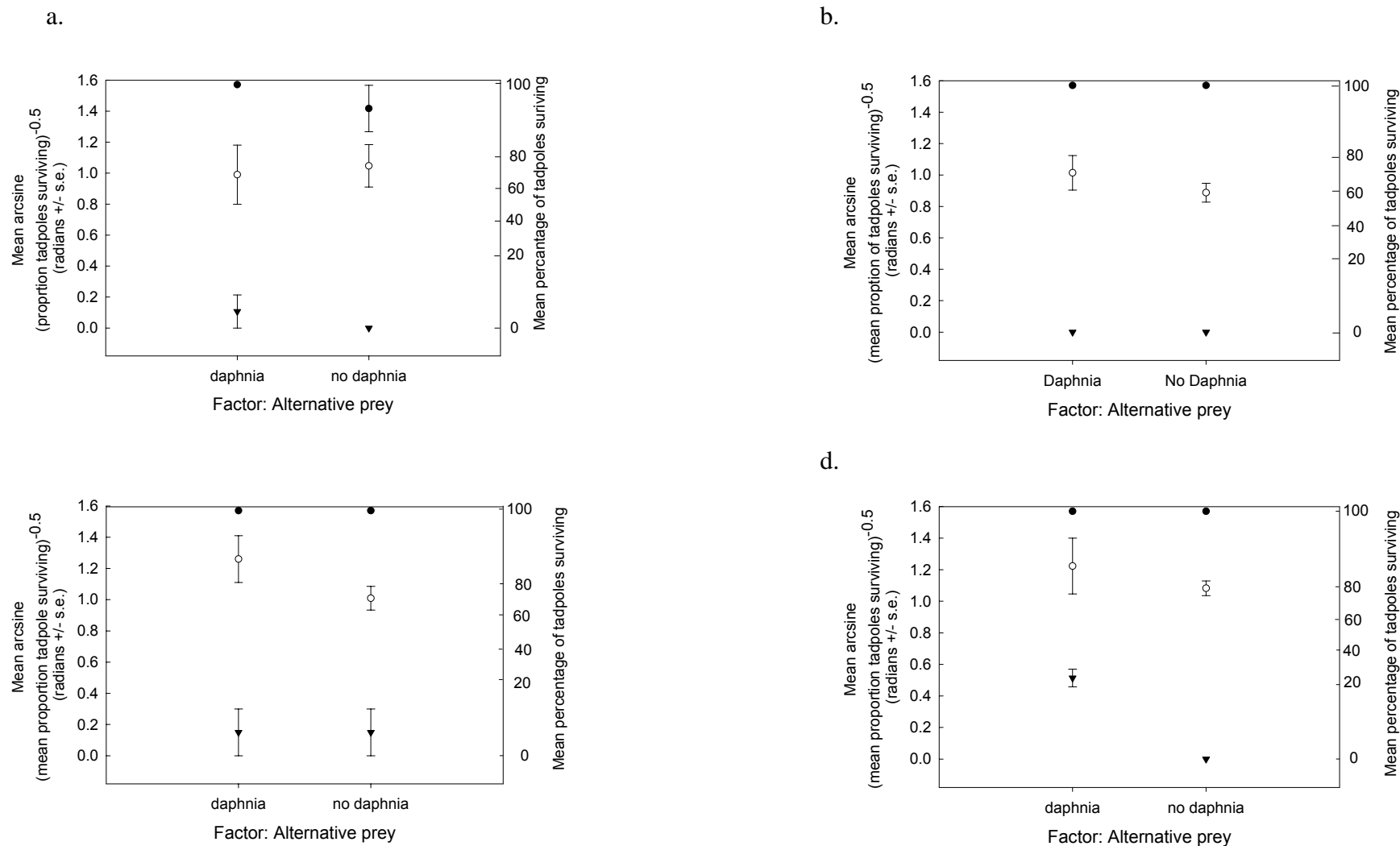


Figure 4.1: The mean number of tadpoles of *L. adelaidensis* captured in traps. Error bars = standard error.



30 **Figure 4.2: Influence of various levels of each factor in the four way interaction of Predator x Cover x Water Colour x Alternative prey (n=3)**
 31 **on the survival of *C. insignifera* in laboratory experiments. A) No cover and clear water, b) No cover and tannin stained water, c) Cover**
 32 **present and clear water and d) Cover present with tannin stained water.**

33 Filled dots (●) represent treatments with no predator, open circles (○) represent treatments with Odonate as a predator
 34 present, while inverted triangles (▼) represent treatments with *G. holbrooki*. Error bars = standard error of the mean.

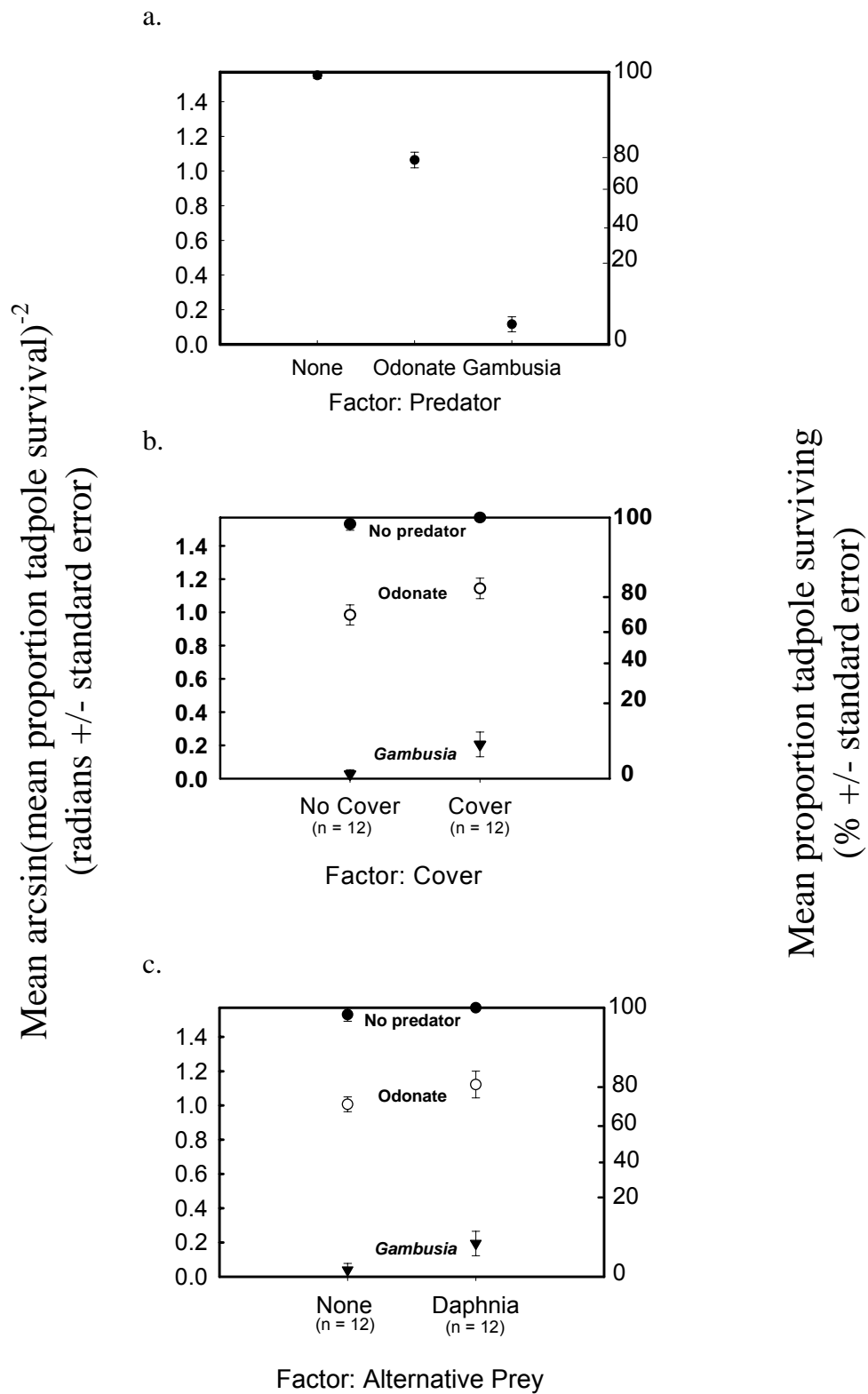


Figure 4.3: Influence of the main factors in the factorial experiment on survival of tadpoles of *L. adelaidensis*: a) predator (n =24), b) cover (n = 12) and c) alternative prey (n=12).

5.0 *Gambusia holbrooki* and survival of *Crinia insignifera* (Anura: Myobatrachidae) in constructed wetlands.

5.1 Introduction

To persist in environments where large proportions of the natural habitat have been removed, native fauna are dependent upon their ability to seek refuge in the remaining natural remnants or to adapt to the newly modified habitats. With only 6.4% of Australia's landmass reserved for conservation (Australian Bureau of Statistics 2001) and with an estimated loss of 50% of wetlands (Environment Australia 1997) and 38% of forested areas across Australia since European settlement (National Forest Inventory Australia 1998) many species have had to adapt to a highly modified environment. For example on the Swan Coastal Plain, south-west of Western Australia, where over 75% of the natural wetlands have been removed, filled or developed (Chambers & McComb 1994; Environment Australia 1997) few natural habitats remain for breeding amphibians. Native frogs, for the most, have had to adapt to breeding in artificially-constructed wetlands that often bear little resemblance to the original natural wetlands of the region: they are typically habitat poor and are frequently inhabited by the exotic fish, *Gambusia holbrooki* (Chapters 2 and 3).

In Australia the introduced predatory mosquitofish, *Gambusia holbrooki*, has been shown to be capable of killing native amphibian larvae and it has been implicated in the decline of several native anurans (for a review see Gillespie & Hero 1999). However, as the fish mainly occurs in areas that have been disturbed or modified, it is often difficult to determine whether the observed negative associations between anuran populations and *G. holbrooki* are directly related to the presence of the introduced fish, habitat modification or the combination of the two stressors. The impact of habitat change upon the persistence of amphibian populations needs to be determined independently from the impact created by the presence of non-native fish (Gillespie & Hero, 1999). Anurans often aggregate and call at artificially-constructed wetlands in Western Australia (Chapter 2), but tadpole abundance of some species is often lower than that found in nearby natural wetlands of comparable size (Chapter 3). In this study I asked whether the lower abundance of tadpoles in artificially-constructed wetlands

was directly related to the presence of the introduced predator *G. holbrooki* using field and laboratory based experimental approaches with a common native frog, *Crinia insignifera*.

There have been numerous studies on the role that introduced predators play in the decline of amphibian species. Critics of these studies often state that the experiments focus on laboratory trials which provide no alternative food source for the predator and as a result the predator has no alternative but to eat the provided prey species (Goodsell & Kats 1999). In addition, these studies frequently provide little information on the impact of syntopic native predators of the target species, and hence it is difficult to gauge the comparative, ecological significance of predation by *G. holbrooki* (Gillespie & Hero 1999).

Here I contrast the results of laboratory and field experiments testing the impact of *G. holbrooki* and native invertebrate predators on tadpole survival in the frog *Crinia insignifera*. The laboratory experiment incorporated two levels of alternative prey and compared the impact of the introduced predator, *G. holbrooki*, with that of a common native predator, the larval zygopteran, *Hemicordulia tau*, which occurs in both natural and constructed wetlands. A zygopteran was selected as a comparative predator rather than a native fish as constructed wetlands are often isolated from natural drainage systems and therefore have little chance of being naturally stocked by native fish. In contrast, *G. holbrooki* are often stocked in artificially-constructed and highly modified natural wetlands by managers and the public who believe the fish will control mosquito populations. The impact of *G. holbrooki* was also tested with a field experiment, which compared tadpole survival to metamorphosis in constructed wetlands populated with natural levels of native invertebrate predators and two fish treatments (*G. holbrooki* present and *G. holbrooki* absent). The combination of field and laboratory data provide a comprehensive picture of the impact an introduced predator can have on native prey in highly modified environments.

5.2 Methods

5.2.1 The impact of two predators on tadpoles under controlled experimental conditions (factorial experiment)

Tadpoles for the factorial experiments were raised in the laboratory from egg clutches gathered from 25 field collected, amplexing pairs. Tadpoles were raised as mixed clutches in the laboratory to 13-15mm in length (Gosner stages 25 and 26) before being used in the experiment. Fish (*Gambusia holbrooki*) and odonate larvae (*Hemicordulia tau*: >25mm) were collected from local water bodies, where they are syntopic with *C. insignifera* tadpoles, on the Swan Coastal Plain of Western Australia using a 0.5mm mesh sweep net. All predators were acclimatised to laboratory conditions for a minimum of 24 hours before the trial and were isolated from food for this period to standardise hunger levels.

The influence of two levels of alternative prey (*Daphnia* or no alternative prey) on the survival of tadpoles of *C. insignifera* exposed to three levels of the predator factor (i.e. no predator, *G. holbrooki* or *H. tau*), were examined, with each treatment replicated nine times. This gave a two factor fully orthogonal factorial design (2 x 3).

Each of the six treatments were randomly assigned to 54, 8L opaque, polyethylene containers (31 x 28 x 11.5cm). All containers were lined with 440ml of a natural wetland sand/mud substrate and filled with 6L of water collected from a constructed wetland. An initial, pilot study identified that shade cloth was a suitable replacement for natural leaf and plant cover, which enabled quick recovery of tadpoles in treatments. Therefore three pieces of shade cloth (10x15cm) were added to each container to mimic and provide some refuge for the tadpoles. Treatments with alternative prey had approximately 60-80 daphnia in the tubs in addition to two *C. insignifera* tadpoles. Each treatment had either a single *G. holbrooki*, a single *H. tau* or a small rock, as a control to mimic the disturbance created by predator addition. The predators were added approximately 15min after two tadpoles had been randomly assigned to each treatment. The experiment was conducted in a controlled temperature room for 24 hours commencing at 09:00 on a 12 hour light (20°C): dark (16°C) cycle simulating natural light and temperature conditions.

5.2.2 *The impact of an introduced predator on tadpoles in the field*

Field trials were conducted in six field trial constructed wetlands (approximately 10m x 25m x 1.5 deep. These ponds were independent of the ponds sampled for chapters 2 and 3) in the Capel region (33.33S, 115.34E) of south-western Australia from May to November 2002. All of the ponds were constructed in 1997 for the purpose of conducting experimental field fish trials. The ponds were never used for this purpose. In 1998 *G. holbrooki* was accidentally introduced to three ponds and allowed to establish. Between 1997 and 1999 trees and sedges were planted in the riparian zone around each pond. Woody debris was placed in and around the ponds. Since this date fringing plants, submerged aquatics and populations of native invertebrates have been allowed to establish. In 2002, three of the ponds remained *G. holbrooki* free and all six ponds supported populations of native odonates and other native invertebrate predators, grazers and detritivores.

In May 2002 each pond was surrounded by 20-30cm high drift fence and all frogs within the pond enclosures were located by searching in around the pond, under vegetation and logs and removed. Removal of frogs and the presence of the fence prevented any incidental breeding by *C. insignifera* or other ground dwelling frogs. The fence did not prevent airborne predators from accessing the ponds and some arboreal species of frog were able to access all ponds for breeding.

A total of 660 tadpoles of *C. insignifera*, were collected from a nearby constructed wetland in August 2002. Immediately after collection, tadpoles were randomly assigned to each of the six ponds (n=110 tadpoles/wetland) and released. Ponds were visually monitored by torch light at night on several occasions to determine the development of tadpoles but were otherwise left undisturbed until tadpoles were estimated to be approaching metamorphosis (i.e. development of front legs).

In November 2002 each pond was searched using repeated 30 min searches in and around ponds, under woody debris and amongst vegetation within each pond enclosure. Juveniles of *C. insignifera* are highly conspicuous during daylight hours shortly after metamorphosis (Smith, pers. comm.) and this in combination with the sparsely vegetated shorelines of the ponds enabled an effective capture of the majority of individuals. Williamson and Bull (1996) noted that the high activity levels of

juveniles of *C. signifera* made them particularly conspicuous and easily captured by hand. As each pond was surrounded by a 15 x 30m drift fence it was assumed that individuals could not escape from the patch before being counted. Searches were repeated until no more metamorphs were located during two consecutive 30min searches and the numbers captured approached the total number of tadpoles added to the ponds. All metamorphosed tadpoles were counted to give a survival rate for each treatment. Once all metamorphosed frogs had been collected, each pond was swept ten times with a 340x220mm, 0.5mm (mesh size) sweep net to determine if any tadpoles remained in the pond and to give an estimate of fish abundance in each of the three fish treatment ponds. As no *C. insignifera* tadpoles were found in the sweeps and in a number of the ponds the number of metamorphs approached the number of tadpole initially added to the ponds it was assumed that most surviving tadpoles had emerged and metamorph counts were an accurate estimation of tadpole survival. A randomly chosen subsample of metamorphs from each pond were measured (snout-vent length: SVL) and weighed to determine if metamorphosis size was influenced by the presence of *G. holbrooki*.

5.2.3 Data Analysis

Factorial Experiment: The impact of two predators on tadpoles under controlled experimental conditions

The proportions of tadpoles surviving from the nine replicates of the factorial experiment were transformed [$\arcsine((\text{proportion tadpoles surviving})^{0.5})$] to equalise variance. The transformed data were then analysed as a two factor fully orthogonal ANOVA design.

Field Trial: The impact of an introduced predator on tadpoles in the field

Tadpole survival, measured as the number of tadpoles which survived to metamorphosis in constructed ponds were analysed untransformed in a single factor ANOVA. Metamorph weight and SVL were each analysed separately using a nested ANOVA. Individual ponds were treated as randomly chosen experimental units and

were nested within predator. Predator (fixed) was the between subject factor in the model.

All analyses were conducted using the statistical package JMP version 4.0. Metamorph ANOVA's were conducted using the Restricted Maximum Likelihood Method (REML). Using this method the variance components in the model are shown as a ratio to the error variance and as a portion of the total variance. This method helps negate the impacts of unequal sample sizes as the standard error has been correctly scaled (Quinn & Keogh 2002).

5.3 Results

5.3.1 *The impact of two predators on tadpoles under controlled experimental conditions*

There were no significant interactions in the two factor ANOVA (Table 5.1). Predator type significantly influenced tadpole survival (Table 5.1). Survival was highest in treatments with no predator (1.57 rad. (100%), ± 0 s.e., $n = 18$), lowest in treatments with *G. holbrooki* (0.26 rad. (6.7%) ± 0.13 s.e., $n = 18$) and intermediate in treatments with odonates (1.09 rad. (78.7%) ± 0.14 s.e., $n = 18$, see Fig. 5.1). The presence of alternative prey had no significant influence on tadpole survival (Table 5.1).

5.3.2 *The impact of an introduced predator on tadpoles in the field*

One pond was excluded from the analysis as one pair of *C. insignifera* was discovered in one of the pond enclosures during the metamorph searches (the pair had either not been removed from the pond initially or had managed to evade the fence) and had bred in the pond. Therefore, three replicates of the control treatment (no fish) and only two replicates of the fish treatment (*G. holbrooki*) were analysed.

The survival of *C. insignifera* in ponds containing *G. holbrooki* was significantly lower than in ponds without a fish predator (Table 5.2 and Fig. 5.2). Only $10.5\% \pm 7.5$ s.e. ($n = 2$) of tadpoles survived to metamorphose in ponds with fish compared with $95.3\% \pm 9.82$ s.e. ($n = 3$) in ponds with just natural predators present.

Comparison of the SVL and weight (Figure 5.3) of metamorphosed individuals between treatments revealed no significant difference in the weight or SVL of individuals emerging from ponds with or without fish (ANOVA_{REML}, Weight: $F_{1,3} = 0.335$, $P = 0.6041$; ANOVA_{REML}, SVL: $F_{1,3} = 0.3975$, $P = 0.5732$).

5.4 Discussion

5.4.1 The impact of two predators on tadpoles under controlled experimental conditions

I present two lines of evidence that implicate *G. holbrooki* as an important non-native predator of tadpoles. This is important because it indicates that the suitability of modified habitats for the persistence of amphibian species could be substantially compromised by the presence of non-native predators.

Under controlled experimental conditions tadpoles of *C. insignifera* were highly susceptible to predation by the introduced fish *G. holbrooki*. This result reflects similar studies undertaken throughout Australia on a number of tadpoles of endemic frog species (Morgan & Buttemer 1996; Pyke & White 2000; Reynolds 1995; Webb & Joss 1997). Webb & Joss (1997) identified that *G. holbrooki* was a likely predator of *Limnodynastes peroni*, with both fed and unfed fish noted to actively kill tadpoles in laboratory trials. However, unfed fish preyed more heavily on tadpoles than fed fish (Webb & Joss, 1997). Webb and Joss (1997) suggested that tadpoles in disturbed habitats; where insects (and hence alternative food sources) are less available, may be more susceptible to predation.

Interestingly alternative prey did not reduce the predatory impact of either predator on tadpoles of *C. insignifera*. In contrast to this, a study on the tadpoles of another native Australian anuran, *Litoria adelaidensis*, showed that the predatory impact of *G. holbrooki* was significantly reduced when alternative prey (daphnia) was present (Chapter 4). Likewise, Goodsell and Kats (1999) identified that tadpoles of *Hyla regilla* had a significantly higher survival in the presence of *Gambusia affinis* when large numbers of alternative prey was present (ie. 80 mosquito larvae: 2 mosquitofish) than when tadpoles were exposed to none or low numbers (ie. 40 mosquito larvae: 2 mosquitofish) of alternate prey.

The native, naturally syntopic, odonate predator ate very few tadpoles in comparison to treatments containing *G. holbrooki*. The significant variation in survival rates between the two treatments may reflect the variation in the hunting/foraging techniques of each predator. Larval odonates, have been described as a “sit and wait” predator that rely almost exclusively on tactile cues (Folsom & Collins, 1984). Tadpoles would therefore need to come into contact with, or in close vicinity of an

odonate before an attack. As identified by Werner & McPeck (1994) higher predation rates from odonate predators on some tadpole species in their study was due primarily to the higher activity levels of those tadpole species.

It is probable that since tadpoles of *C. insignifera* have had historical exposure to *H. tau* they may have evolved defence strategies, e.g. immobility, to avoid predation. However, as identified by Gillespie & Hero (1999) survival strategies are predator specific and are unlikely to be effective against all predators. For example, the antipredator behaviour of immobility used by a tadpole to avoid predation by an invertebrate predator such as *H. tau* is unlikely to be effective against a fish predator that uses visual or olfactory cues. *Gambusia holbrooki* are active hunters which tadpoles of *C. insignifera* have had limited, historical exposure to. It is probable that the tadpoles of *C. insignifera* lack the ability to either detect and/or avoid (physiologically or behaviourally) predation by *G. holbrooki* providing a probable explanation for the variation in death rates between the two predator treatments. Behavioural responses of the tadpoles to predators were not quantified during the trials and it is difficult to know if the tadpoles of *C. insignifera* were able to detect the fish predator or if they were undertaking any behavioural avoidance mechanisms in the presence of the mosquitofish.

5.4.2 *The impact of an introduced predator on tadpoles in the field*

Field ponds which contained *G. holbrooki*, had few tadpoles survive to metamorphosis. In comparison ponds which only contained native predators had significantly higher survival of tadpoles to metamorphosis. These results are consistent with that noted in laboratory trials and indicate that low tadpole abundances recorded in constructed wetlands may not directly be associated with the low habitat suitability of these ponds *per se* but could reflect the impact that *G. holbrooki* appears to have on the survival of tadpoles of this species. These results suggest that native predators alone are unlikely to exclude tadpoles of *C. insignifera* from constructed ponds. However, ponds containing *G. holbrooki* are less likely to provide an environment suitable for long-term successful recruitment. This result is in contrast with that reported by van de Mortel & Goldingray (1998). Population descriptions by van de Mortel & Goldingray (1998) indicated that despite numerous laboratory trials showing a strong negative correlation between Mosquitofish and tadpole survival of *L. aurea* (Morgan & Buttemer 1996; Pyke & White 2000), tadpoles of the species had been reported to survive in relatively

high numbers to metamorphosis in ponds containing high densities of Mosquitofish. van de Mortel & Goldingray (1998) did not however have any indication of the number of eggs laid relative to the final metamorphic recruitment and therefore could not provide an indication of the predation rate experienced at this site.

van de Mortel & Goldingray (1998) suggested that the habitat complexity at the site may have afforded sufficient shelter to enable tadpoles of *L. aurea* to escape predation by *G. holbrooki*. Provided that sufficient breeding occurs at a pond it is likely that recruitment will continue. But, declining recruitment may eventually reduce the availability of breeding adults to unsustainable levels. Research has indicated that there is often a substantial time lag between habitat isolation and resulting population effects. Hitchings and Beebee (1998) noted that there has been an approximate 60 year time lag between habitat loss and urban population decline of the once relatively abundant *Bufo bufo* in the United Kingdom. It is therefore possible that in the long-term, populations of *C. insignifera* may decline in highly modified environments unless habitats free of introduced predators are made available. Management strategies that prevent the addition or advocate the removal of *G. holbrooki* from currently inhabited wetlands are likely to improve the suitability of constructed wetlands as amphibian breeding habitats.

5.5 Conclusion

Under controlled experimental conditions in the laboratory *C. insignifera* were highly susceptible to predation by *G. holbrooki*. Results of the field experiment confirmed laboratory findings in showing that *G. holbrooki* can have a significant impact on survival of tadpoles of *C. insignifera* to metamorphosis in constructed wetlands and this impact is higher than what would be experienced by tadpoles in constructed wetlands with only native predators. These findings have important implications for the management of constructed wetlands and for the persistence of many frog species. Removal of *G. holbrooki* from existing wetlands and exclusion from those to be created will provide an important first step in ensuring that constructed wetlands can act as surrogate breeding habitats in areas where natural habitats have been removed.

ACKNOWLEDGEMENTS

I would like to thank F. Doyle, A. Bentley and R. Warber of the Capel Wetlands Centre for assistance with the establishment of the field component of the experiment. Thanks to Dr. M. Smith and Dr J.D. Roberts for assistance with the manuscript. I would also like to thank the Capel Wetlands Centre for assisting with the funding for this project. This research was undertaken as part of a PhD at the University of Western Australia with the assistance of Australian Postgraduate Award. All work conducted was done with approval from the University of Western Australia Animal Ethics Committee (AEC approval # 00/100/064) and the Department of Conservation and Land Management, Western Australia (Permit # SF SF003880).

Table 5.1: Summary of the two-factor ANOVA for the laboratory experiment.

Source	DF	Mean of Squares	F Ratio	Prob > F
Predator	2	31.5735	36.368	<0.0001*
Alternative Prey	1	0.5597	2.579	0.1149
Predator x Alternative prey	2	0.5940	0.684	0.5093
Residual	48	0.2170		

* indicates significant at the 0.001 level

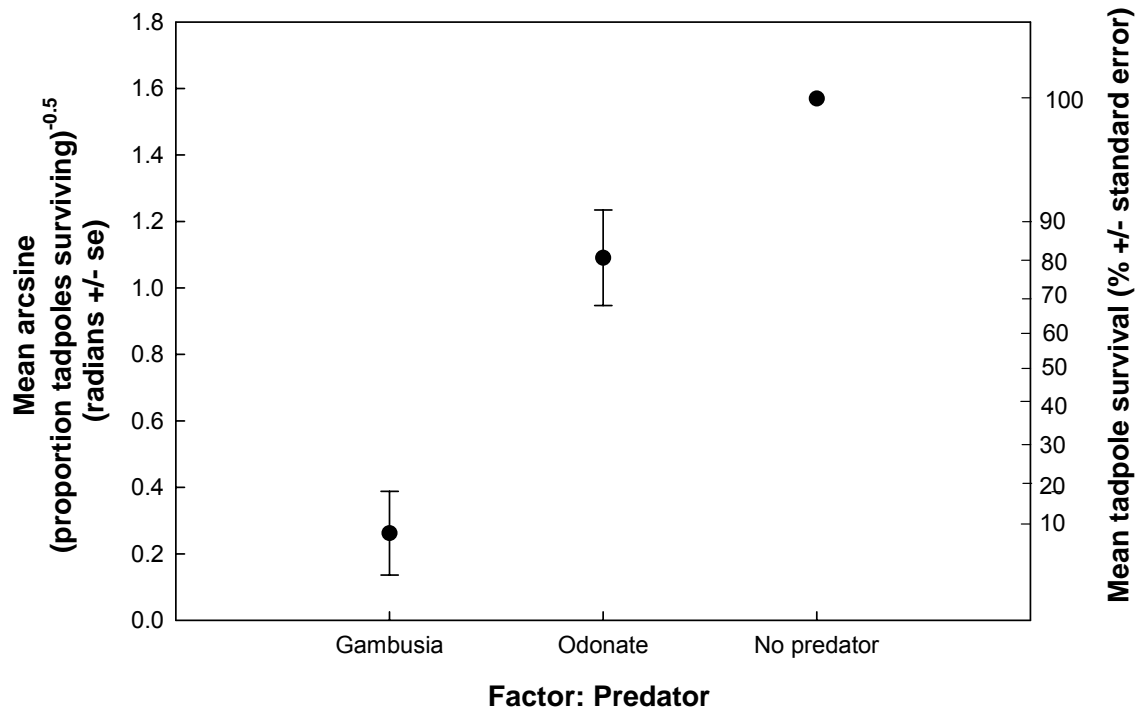


Figure 5.1: Impact of the three predator treatments (n =18) on the survival of tadpoles of *Crinia insignifera* in laboratory experiments.
Error bars = standard error.

Table 5.2: Summary of the ANOVA for the field experiment. Tadpole survival was measured as the number of tadpoles which survived to metamorphosis.

Note: only 5 of the initial 6 ponds were used in the analyses, therefore in control ponds, $n = 3$ and in ponds with *G. holbrooki* present, $n = 2$.

Source	DF	Mean of Squares	F Ratio	Prob > F
Predator	1	8636.033	37.4846	0.0088
Residual	3	230.39		
Total	4			

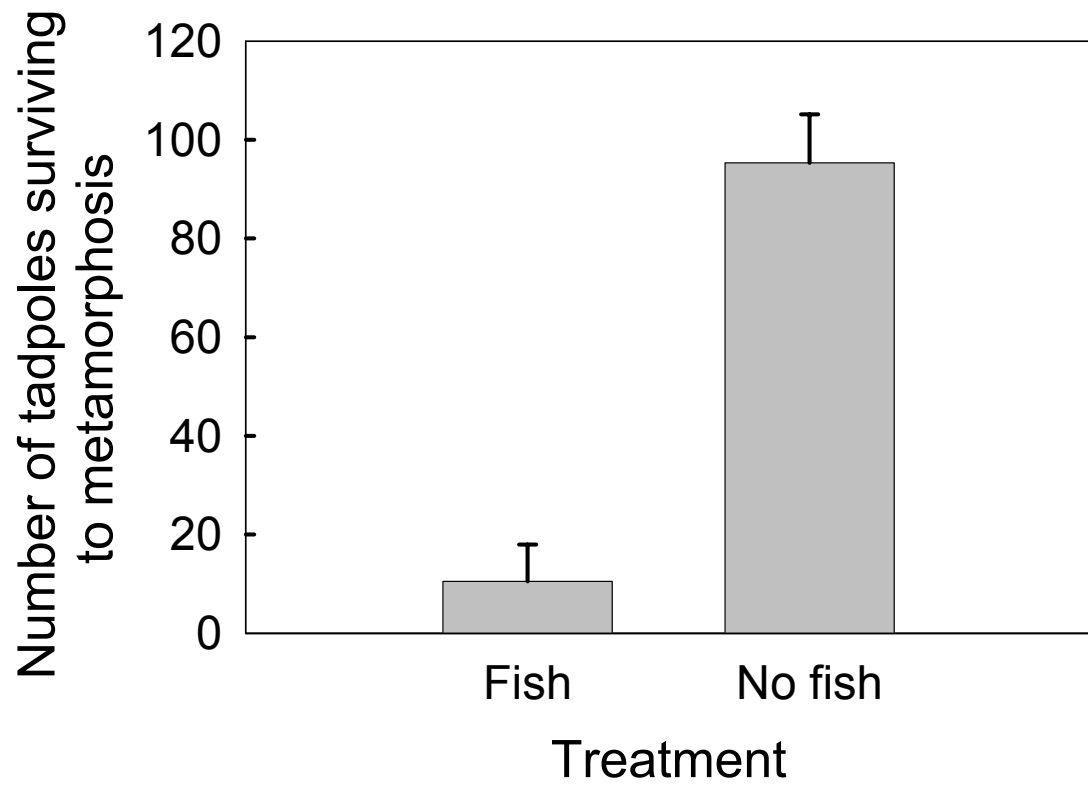


Figure 5.2: Impact of *Gambusia holbrooki* on survival of tadpoles to metamorphosis in constructed wetlands.

Note: only 5 of the initial 6 ponds were used in the analyses, therefore in control ponds, $n = 3$ and in ponds with *Gambusia* present, $n = 2$. Error bars = standard error.

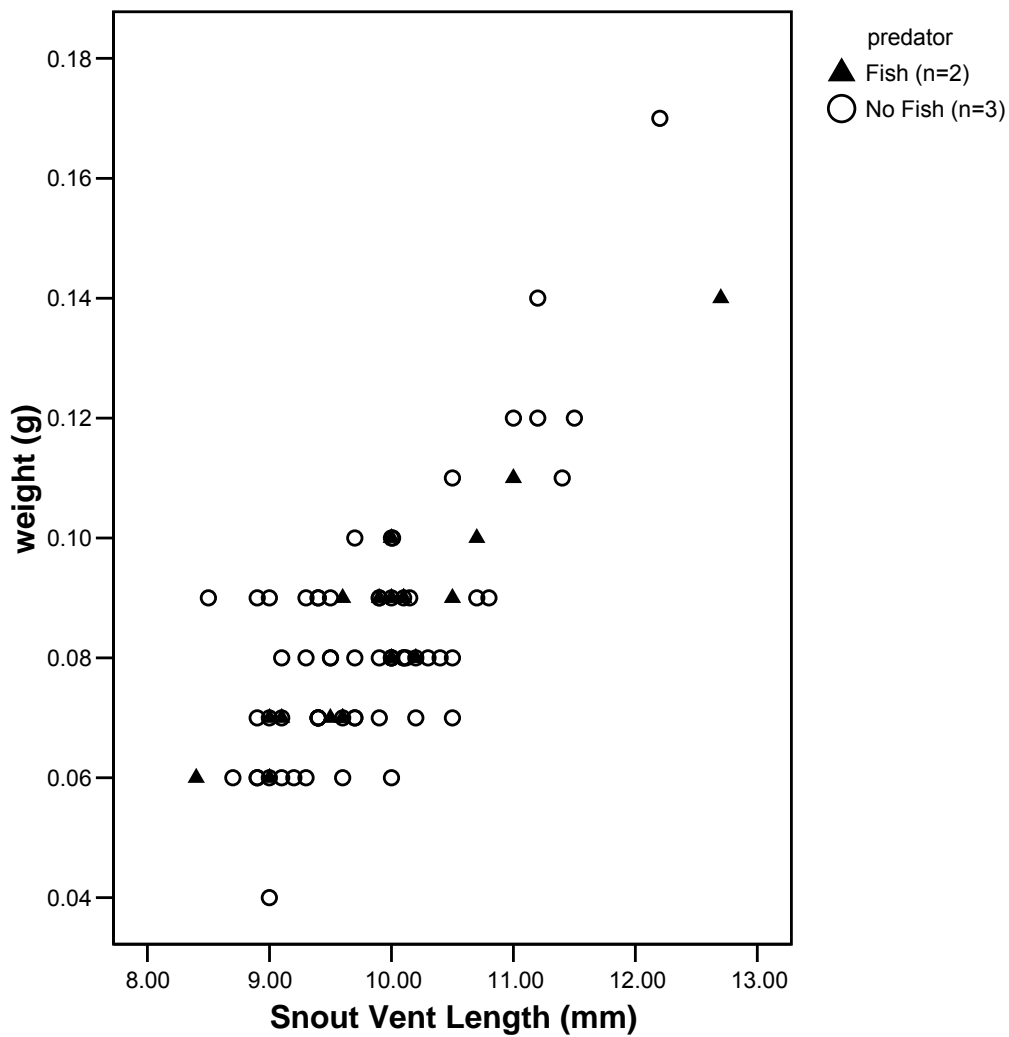


Figure 5.3: Weight to snout vent length ratio of *C. insignifera* metamorphs emerging from the field trial ponds.

Note: metamorphs from only 5 of the initial 6 ponds were used in the figure.

6.0 Impact of a native and introduced fish on the survival of tadpoles of *Crinia insignifera* (Moore, 1954) in complex habitats.

Abstract

The impact of the introduced mosquitofish on native amphibian species has been studied extensively in Australia. However, most studies have been anecdotal or based on survey data and have failed to determine the relative impact of native predators or the role that habitat-complexity can play in mediating predator-prey relationships. This paper reports on the results of a factorial design which crossed eight levels of habitat complexity with three levels of the factor predator to determine the relative impact of a native fish (*Galaxias occidentalis*) and an introduced fish (*Gambusia holbrooki*) on the survival of tadpoles of *Crinia insignifera*. A three-way interaction between water colour, cover and predator revealed that predator impact can be strongly influenced by components of habitat complexity: the impact of *G. holbrooki* on tadpoles was reduced in the presence of cover but only in clear water. In contrast tadpoles survival was highest in clear water and no cover treatments when exposed to the native fish *G. occidentalis*. Studies that investigate habitat components independently may overlook potentially important predator-prey-habitat interactions.

Keywords: Habitat complexity, predator-prey interactions, *Gambusia holbrooki*, *Galaxias occidentalis*, introduced fish.

6.1 Introduction

In the absence of evolutionary exposure to a specific predator, prey may not have suitable defence mechanisms that will enable them to coexist with novel predators. Survival strategies are generally predator specific and are unlikely to be effective against all predators (Gillespie & Hero 1999). For example, the antipredator strategy of immobility used by some tadpoles against invertebrate predators (Chovanec 1992; Werner & McPeck 1994) is unlikely to be effective against a fish predator that uses visual cues (Werner and McPeck, 1994). Likewise strategies that are effective against one fish species may not be effective against all, particularly novel, fish predators (Gillespie and Hero, 1999). The introduction of an exotic predator may therefore be highly detrimental to native species.

Introduced fish have been implicated in the decline of native amphibian populations world wide (Gillespie 2001). There are, however, few studies that explore the relationships between introduced fish predators, amphibians and their habitat sufficiently (Gillespie & Hero 1999; Goodsell & Kats 1999). For example, many studies fail to look at the impact of introduced predators relative to native predators and studies often examine the impact of an introduced predator in isolation of factors that may alter predator-prey interactions in natural communities (e.g. Baber & Babbitt 2003). The effect that a predator has on a target species in a container with clear water, no refuge and no alternative prey may be much higher than in an environment where alternative prey, water colour and/or physical structure are present. Single-factor laboratory studies may not be sufficient to predict the overall impact of an introduced predator and while they can often demonstrate that an introduced predator is capable of killing the prey species (Gillespie and Hero, 1999) they may be of limited use in developing effective management strategies.

In order to address this issue and further investigate the potential impacts of a common introduced fish species in south-western Australia, I designed an experiment that examined the potentially complex interactions that may occur between two or more habitat components in altering predator-prey interactions. Using laboratory trials I exposed tadpoles of a common native Western Australian anuran, *Crinia insignifera* Moore 1954, to two fish predators, the introduced mosquitofish, *Gambusia holbrooki*, and the native Western Minnow, *Galaxias occidentalis*. Although native fish are often isolated from constructed wetlands it was noted that wetland managers may consider the

potential of stocking native fish in constructed wetlands as an alternative to the introduced mosquito fish (F. Doyle pers. com.). It was therefore probable that tadpoles could be exposed to this native predator in constructed wetlands. Understanding the impacts of the native fish predator compared with that of the introduced fish on tadpole survival would improve the knowledge available to wetland managers intending to develop constructed wetlands as wildlife habitat areas.

The trials included eight levels of habitat complexity spanning cover, water colour and alternative prey; habitat components that vary substantially in south-western Australian wetlands. The aims were to: (1) compare the impact of *G. holbrooki* and the native fish, *G. occidentalis*, on the survival of tadpoles and (2) to determine the influence of different levels of habitat complexity on the ability of *G. holbrooki* and *G. occidentalis* to prey upon tadpoles.

6.2 Methods

6.2.1 Fish Predators

The mosquitofish, *Gambusia holbrooki* was introduced to Australia in 1925 as a biological control agent for mosquito larvae and the species now commonly occurs in the permanent fresh water bodies of most mainland states of Australia (Faragher & Harris 1994; Lloyd et al. 1986). *Gambusia* spp. are relatively small (26-62mm Pen & Potter 1991b) diurnal feeders and rely entirely on visual cues to detect, track and attack prey (Swanson & Cech 1996). The mosquitofish is most likely to inhabit warm, slow flowing or still waters, particularly shallow water with submerged vegetation which provides concealment from predators (Casterlin & Reynolds 1977; Lloyd et al. 1986). *Gambusia* spp. are opportunistic omnivores with a diet primarily composed of terrestrial insects, aquatic invertebrates and some zooplankton (Arthington 1989; Lloyd et al. 1986). Webb and Joss (1997) described *Gambusia* as a voracious predator that may consume large quantities of mosquito larvae and they have been known to eliminate *Daphnia* and other invertebrate populations (Hurlbert et al. 1972). *Gambusia* spp. may also have contributed to the decline of several species of frog in Australia (Gillespie and Hero, 1999) and the fish has been implicated in the decline of amphibian populations in other parts of the world (e.g. Gamradt & Kats 1996; Goodsell & Kats 1999).

The native Western Minnow, *G. occidentalis* is a small (95-163mm Pen & Potter 1991a), entirely carnivorous fish that eats a wide variety of organisms from planktonic crustaceans through to large aquatic organisms and terrestrial invertebrates (Pen & Potter 1991a). The species often occurs syntopically with *G. holbrooki* in Western Australia however, studies in a south-western Australian river have shown that its feeding niche is more restricted than that of *G. holbrooki* (Pen & Potter 1991b). Pen and Potter, (1991a) noted that a large proportion of *G. occidentalis'* diet is made up of small terrestrial fauna and the fish has been known to leap from the water to catch flying invertebrates (Pen 1999). *G. occidentalis* is a diurnal fish that generally swims in schools (Pen, 1999) and is highly adapted to fast flowing water. As it is one of the few native predatory freshwater fish that persists in significant numbers in wetlands of south-west Australia (Allen 1982), it was anticipated that *G. occidentalis* would provide a suitable gauge of natural predation rates of fish on tadpoles to which the impacts of *G. holbrooki*, could be compared.

6.2.2 *Crinia insignifera*

Observations suggest that the abundances of tadpoles of *C. insignifera* are often low in wetlands containing *G. holbrooki* (Reynolds 1995; chapter 3). This implies that the species may be susceptible to predation by *G. holbrooki*. However, as *G. holbrooki* are generally restricted to wetlands with a permanent water regime, which are often highly disturbed, it is difficult to dissociate the tadpoles' response from other habitat variables that may also operate in highly disturbed wetlands. As many of the natural wetlands in the region have now been removed (c. 75%: Environment Australia 1997) there is increasing pressure on highly modified or constructed wetlands to provide suitable breeding habitat. Increased predatory impact on *C. insignifera* in the remaining habitats could threaten its future persistence in the study region. Understanding, the impact of two relatively similar predators on this common species will provide important information on the impact that the mosquitofish may have on tadpole abundance.

There is little published data on the biology and ecology of tadpoles of *C. insignifera*. Physically the tadpoles are relatively small (4-8mm long at hatching: Main 1957), cryptically coloured and they have the capacity to modify body colour to suit the background or substrate (Reynolds 1995). Studies on a similar tadpole species, *C.*

signifera, from eastern Australia indicate that the tadpoles of this species are predominately quiescent and benthic (Peterson et al. 1992; Richards & Bull 1990). According to Skelly (1997), *C. signifera* tadpoles spend less than 10% of their time being active. Most other tadpoles are several times as active (Skelly 1997) and this suggests that *C. signifera*'s ability to coexist with predators in more permanent habitats stems in large part from its extreme inactivity.

6.2.3 *The impact of two fish predators on tadpoles under controlled experimental conditions (factorial experiment)*

Tadpoles of the frog *C. insignifera* sized between 13 and 15mm in total length (Gosner stages 25 and 26) were collected using a 0.5mm mesh sweep net from a single, natural, wetland near Capel, Western Australia (33.33°S, 115.34°E). Tadpoles were kept in small tanks and fed *ad libitum* prior to the experiment. A total of four tadpoles were randomly allocated to each experimental container. Fish were collected from wetlands in the Capel region of Western Australia. All fish were acclimated to laboratory conditions for a minimum of 72 hours before the trial and were denied food for 24 hours prior to the experiment to standardise hunger levels. No predator was used in the experiment more than once to prevent predators acclimating to the experimental setup.

The influence of two levels of cover (cover or no cover), two levels of water colour (colour or no colour) and two levels of alternative prey (*Daphnia* or no *Daphnia*) on the survival of four tadpoles of *C. insignifera* exposed to three predator treatments (no predator, *G. holbrooki* or *G. occidentalis*), were examined over 24 hours in 96 containers on two different trial days (i.e. four replicates of each treatment/day, with each treatment replicated 8 times in total over the 2 days). This gave a five-factor fully orthogonal factorial design (2 x 2 x 2 x 3 x 3).

Each of the 24 treatments were randomly assigned to 96, 8L opaque, polyethylene containers (31 x 28 x 11.5cm) lined with 440ml of natural wetland substrate. The containers were refilled daily with 6L of water collected from either clear ("no colour", secchi depth: >1000mm) or natural tannin stained ("colour", secchi depth: 200mm) wetlands in the Capel region. The tannin stained water is likely to be different from the clear water in components other than just colour, such as chemical composition, and therefore the response of tadpoles and/or predators may not

necessarily be due to the single component of colour. However, as all tested species occur in the south-west of Australia in both water types, testing for a response was deemed valid given its potential as a management tool (e.g. adding tannin to water). Each container was fitted with a 32 x 29cm net cover to prevent *G. occidentalis* from leaping out of their containers.

Six pieces of woven shade-cloth (10x15cm) were used in the "cover" treatments to simulate aquatic vegetation. A pilot trial using *C. insignifera* tadpoles identified that the tadpoles will shelter under or near any structure regardless of origin (natural or man-made). From the trial it was determined that shade-cloth (i.e. woven plastic fabric) would not only provide a suitable replacement for natural vegetation, but would also enable efficient post-experiment location and removal of surviving tadpoles. The shade-cloth reduced processing time and therefore facilitated higher replication of treatments whilst reducing the risk of contaminating trials with invertebrates that may have been present in natural aquatic vegetation. Three pieces of shade cloth, mimicking approximately 60% leaf litter cover over the sediment, were placed at the base of each container and were held submerged by a small river stone. A further three pieces of unweighted shade cloth were added and remained suspended in the water column to simulate floating and emergent aquatics.

Between 60-80 *Daphnia* were added to containers allocated the "alternative prey" treatment in addition to the four *C. insignifera* tadpoles. Tadpoles were randomly allocated to each treatment approximately 15 minutes before a single *G. holbrooki*, *G. occidentalis* or small rock (to mimic disturbance created by predator addition: a control treatment) was added. The trials were run in a controlled temperature room for 24 hours commencing at 08:00 on a 12 hour light (20°C): dark (16°C) cycle to simulate natural light/dark and temperature regimes at the time experiments were conducted.

6.2.4 *Data analysis*

Despite the use of net covers on all treatments, 11 of the native minnows escaped during the trials. As it was unclear at which point during the trial the fish had exited the containers, several replicates had to be excluded from the analyses. To maintain even replicates between treatments, I used only 3 randomly selected replicates of each treatment from each day in the analyses, giving a total of six replicates/treatment.

Proportions of tadpoles which survived from the six replicates were arcsine transformed and then analysed by a five-way analysis of variance with the following factors: Day, Water Colour, Cover, Alternative Prey, and Predator. All statistical analyses were conducted using the statistical package JMP, version 4.0.

6.3 Results

There were no significant effects of the five, four or three way interactions at the 0.05 level (Table 6.1). The three-way interaction of Cover x Predator x Water Colour was significant at the 0.1 level (Table 6.1). Graphical interpretation shows that this interaction was associated with the different consumption response of *G. holbrooki* and *G. occidentalis* in the tannin coloured water in the two different cover levels (Figure 6.1a and b). As anticipated *Gambusia holbrooki* consumed significantly more tadpoles in treatments with clear water and no cover (Figure 6.2a: < 12% survival) than in tannin coloured water with no cover (c. 57% survival). However, when cover was present the consumption of tadpoles by *G. holbrooki* was higher in tannin colour water (Figure 6.2b: c. 37%) than that recorded in clear water (c. 54%). *Galaxias occidentalis* demonstrated the opposite response. Survival of tadpoles was highest in clear water and no cover treatments (Figure 6.2a: c. 86%). Tadpole survival was reduced in clear water with cover present (c. 71%) compared to clear water, no cover but was higher than in tannin coloured water with cover (Figure 6.2b: 59%). Treatments with tannin coloured water and no cover recorded the lowest mean survival (56%).

The main treatment of "Predator" also had significant effects on tadpole survival (Table 6.1), with 68.7% survival in treatments with *G. occidentalis* compared to survival of 38.5% survival in treatment with *G. holbrooki* present. However, interpretation of this effect is confounded by the three-way interaction between the factors "Predator", "Cover" and "Water Colour". If predator impacts are interpreted separately for each water colour and cover treatment combination (Figure 6.2) *G. holbrooki* consumed significantly more tadpoles in the absence of water colour than *G. occidentalis*. With tannins and no cover present consumption rates between *G. holbrooki* and *G. occidentalis* were not significantly different (0.851 rad. (57.6%) \pm 0.21, s.e., n=12 and 0.851 rad. (57.6%) \pm 0.21, s.e., n = 12, respectively). But in treatments with tannins and cover present *G. holbrooki* consumed more tadpoles than *G. occidentalis* (0.87 rad. (59%) \pm 0.2 s.e., n = 12 and 0.65 rad. (37%) \pm 0.2 s.e., n =12). An examination of the size of the effect of each significant treatment revealed that

predator impacts were approximately 4 times larger than the influence of cover and water colour. This indicates that the level of mitigation provided by cover and water colour on the survival of tadpoles was very low compared with the impact of predator presence.

6.4 Discussion

6.4.1 The impact of the Mosquito Fish on tadpole survival

Gambusia holbrooki has been implicated in the decline of populations of amphibians (Gillespie and Hero, 1999) and other native organisms when it is introduced into exotic systems (Hurlbert & Mulla 1981). Only one study known to the author has examined the impact of this introduced predator on tadpoles relative to habitat complexity. The study discovered that aquatic macrophytes could significantly reduce the impact of *G. holbrooki* on tadpoles of the native Australian anurans *Litoria aurea* and *L. dentata*, 96 and 72 hours after exposure respectively (Morgan & Buttemer 1996). However, the study was criticised because *L. aurea* has been found in sympatry with native predatory fish and therefore it was difficult to assess the relative ecological significance of *Gambusia* predation (Gillespie & Hero 1999). Few studies have compared the relative impact of *G. holbrooki* and native predators on the survival of tadpoles. This study which looked at 8 levels of habitat complexity and compared the impact of *G. holbrooki* with a native, sympatric fish indicates that two components of habitat complexity can ameliorate the impact of *G. holbrooki* and suggests that native predators in some systems may have as high an impact as *G. holbrooki* on populations of tadpoles.

6.4.2 Predator impact on tadpoles of C. insignifera in controlled conditions

In treatments without water colour and cover *G. holbrooki* clearly had a greater impact on tadpoles of *C. insignifera* than *G. occidentalis*. Given that the two tested predators have similar hunting/foraging techniques it is interesting to note the difference in prey capture rates in clear water environments. Both predators are diurnal hunters that capture prey at the surface and within the water column (Pen 1999; Pen et al. 1993; Swanson & Cech 1996), however only *G. holbrooki* has been noted to feed in the benthic zone (Pen et al. 1993). If the tadpoles of *C. insignifera* are predominately a

benthic species it is possible that the tadpole would be more vulnerable to predation from predators that feed in this zone, such as *G. holbrooki*. Alternatively, a difference in consumption between the two predators may relate to the cues that initiate antipredator behaviour by the tadpoles. Tadpoles in the presence of a familiar predator (*G. occidentalis*) may modify their behaviour to reduce predator impact (Kats et al. 1988; Lawler 1989; Petranka et al. 1987; Woodward 1983). However, in the presence of a novel predator (*G. holbrooki*) tadpoles may not perceive or recognise the predator and fail to adopt appropriate antipredator behaviour (Gillespie & Hero 1999).

6.4.3 *The role of habitat complexity in mediating the impact of fish predators on the survival of tadpoles of C. insignifera.*

Coloured water

The diurnal and visually stimulated predatory tactics of both predators (Pen 1999; Swanson & Cech 1996) suggests that both fish species should be equally influenced by a variation in the colour of the water. It was therefore anticipated that the prey capture rates of both species would be reduced in treatments with coloured water (i.e. the colour of the water may restrict visual prey location). However, a reduction in prey capture rates was only recorded for *G. holbrooki* in treatments with no cover, while prey capture rates increased substantially in the presence of *G. occidentalis* in coloured water (with or without cover). This indicates that other factors beyond visual capacity may be operating or alternatively *G. occidentalis* may be better adapted to tannin stained water than *G. holbrooki*.

As identified by Nelson and Bonsdorff (1990) the behaviour of the prey may be important in determining the influence of habitat complexity on predation rates. A behavioural shift by the tadpoles as a result of water colour, such as increased movements, may have altered the predator impact. A study investigating the habitat choice by tadpoles of *Crinia signifera* identified that there was a clear difference in the area of the water column the tadpoles occupied according to available light (Peterson et al. 1992). Generally tadpoles of *C. signifera* spent most of their time on the substrate and rarely ventured into the water column. However, the tadpoles were more likely to venture from the substrate in the light phases of the experiment (Peterson et al. 1992). As *C. insignifera* tadpoles in this experiment had been kept in water unaffected by tannin it is possible that the dark coloured water may have altered the levels of

incoming light sufficiently to stimulate activities typically undertaken during low light periods, potentially changing the tadpoles susceptibility to each predator. It is also possible that the water colour or chemicals contained there in, altered or interrupted the antipredator cues of the tadpoles, affecting tadpole vulnerability to predation.

Similarly, the coloured water may have influenced the hunting techniques of each species. As with the tadpoles both fish species had been captured and held in water unaffected by tannin colours and it is possible that the dark coloured water or chemicals within alter predatory activities. However, as the behavioural activities of the tadpoles or the fish were not monitored during the experiment, changes in activity levels in different treatments cannot be determined. As the addition of tannins to a wetland may present a potentially useful management tool to mediate the predatory effects of *G. holbrooki* on tadpoles of *C. insignifera* it will be important to investigate the role tannins could play in predator-prey interactions further.

Structural complexity

Nelson and Bonsdorff (1990) and Crowder and Cooper (1982) both noted that increasing habitat complexity, in the form of physical structures, reduced the capture efficiency of fish predators in the capture of small sticklebacks (*Pungitius pungitius*). They suggested that the structural complexity of the habitat mediates fish-prey interactions through behavioural changes of both fish and their prey (Crowder & Cooper 1982; Nelson & Bonsdorff 1990). The mixed results of predators relative to water colour, in this study, indicate a complicated interaction between habitat variables. The recorded response may suggest a number of things, including: 1) that the amount of refugium provided in the cover treatments was not sufficient to alter *G. occidentalis* capture rates to a significant level, 2) that cover is not important for some predator prey interactions, and/or 3) there are complex interactions between cover and water colour which strongly influence predator-prey interactions.

As demonstrated by Minello & Zimmerman (1983) fish prey capture rates (i.e. total number of tadpoles consumed) are not always reduced by an increase in vegetation cover. They noted that the predation rate on shrimp by two species of fish was reduced as a consequence of increased vegetation structure, while no reduction in prey capture was seen for two other fish species. Minello & Zimmerman (1983) suggest, that relative mobility and different hunting techniques used by each of the fish species in the vegetation may have influenced the different responses. *Gambusia holbrooki* has been

noted to favour densely vegetated shorelines (Garcia-Berthou 1999). This suggests the species is well adapted to hunting amongst vegetation and is potentially a very efficient predator in these environments. It would therefore follow that the capture rates of tadpoles by *G. holbrooki* should not be overly affected by the presence of cover.

Tadpole survival with cover was much higher than without, however the variation in capture rates within the coloured water (i.e. 37% survival with cover as opposed to 58% survival with no cover) illustrates complex interactions. Nonetheless, the interaction does suggest that habitat complexity in the form of cover and/or colour could potentially mediate the impacts of this predator.

In contrast, cover did not appear to influence the capture rates of *G. occidentalis* in coloured or clear water. The lack of significant variation indicates that there may have been insufficient cover to hinder the activities of this fish species and consequently prey capture rates were not significantly reduced in the presence of cover. In a natural environment, habitat complexity is often composed of multidimensional layers provided by organic debris and submerged and emergent aquatics. Although the shade-cloth was observed to be utilised in a similar manner to leaf litter by tadpoles of *C. insignifera* in a pilot trial it is unlikely that the shade-cloth could mimic all of the complexities of a natural system. For example, the shade-cloth did not provide small fissures or fine movable organic debris (silt or detritus) which might typically be discovered in and on the sediment of a natural system. Tadpoles in the field “burrow” into the silt on the surface of the sediment when disturbed (pers. obs.). As noted by Lawler, (1989) silt can effectively conceal tadpoles, especially when stirred by an animal moving quickly (e.g. fleeing from attack) in the benthic zone. Therefore, the absence of small shelter zones may have prevented natural avoidance behaviours in the tadpoles and consequently exposed them to higher predation rates than in a natural system.

Alternative prey

Alternative prey did not reduce the predatory impact of the experimental fish species significantly. However, examination of the data indicates that although prey capture rates were not reduced significantly in treatments with minnows, prey capture rates *G. holbrooki* on tadpoles were reduced by almost half in the presence of alternative prey. Reynolds (1995) identified that *G. holbrooki* consistently consumed invertebrate prey in preference to tadpoles in his study, suggesting that in environments with a low availability of alternative food sources, tadpoles may be more susceptible to

attack by *G. holbrooki* (Reynolds 1995). The lack of a substantial reduction in the capture rates of tadpoles by *G. occidentalis* is interesting, but may just reflect the surface feeding habits of the fish, which may have reduced the likelihood of the fish intercepting potential tadpole prey regardless of the presence of alternative prey.

6.4.4 *The value of laboratory experiments*

The impact of an introduced predator is often monitored in isolation. This study highlights the need to compare predator-prey interactions with native predators in complex habitats in order to ensure a more realistic assessment of a predators' ecological significance. The study also demonstrates that *G. holbrooki* has the potential to be a detrimental predator of tadpoles of *C. insignifera* under laboratory conditions, but that the impacts of *G. holbrooki* may not be greater than that of native predators in some sites. It is important to note that in this study the relative impacts of *G. holbrooki* and *G. occidentalis* were measured at equivalent densities, a scenario which is unlikely to represent that which occurs in the real world. As identified by Sarti and Allen (1978 in Pusey et al. 1989) in a survey of the wetlands of the northern Swan Coastal Plain, *G. holbrooki* is the most abundant fish species in lentic habitats. Where native fish species were present in wetland habitats, Sarti and Allen (1978 in Pusey et al. 1989) noted that they were in low densities and usually confined to inlet streams. More recently, Pusey et al. (1989) identified that in highly regulated streams with deep, relatively slow flowing water comparable with lentic habitats, *G. holbrooki* densities are approximately three times that of the density of *G. occidentalis*. This would indicate that the relative impacts of each *G. holbrooki* on tadpoles of *C. insignifera*, may actually be higher than that of *G. occidentalis*.

Skelly (1997) noted that results obtained in laboratory based experiments may not necessarily translate into natural habitats. The relatively confined and sterile environment provided by small plastic tubs, is unlikely to effectively mimic the dynamics of more natural systems. Therefore, it is important that in-field studies are undertaken to determine if the predatory impacts recorded in this study represent real world scenarios and to provide insight into mechanisms which may best manage the introduced fish species, *G. holbrooki*.

6.4.5 Potential conservation implications & management strategies

Recent estimates suggest that since European settlement, over 75% of the wetlands on the Swan Coastal Plain of south-west Australia have now been filled or drained (Environment Australia 1997). This represents a substantial loss of native breeding habitat for a number of south-western Australian amphibian species. The majority of wetlands on the Swan Coastal Plain are now either constructed or highly modified natural wetlands (Chambers & McComb 1994), which are often characterised by a species poor invertebrate community, often lack vegetation communities which stain the water and are frequently inhabited by large populations of *G. holbrooki*. If the predation rates demonstrated in this laboratory based experiment even remotely mimic the impacts of *G. holbrooki* on populations of *C. insignifera* in these wetlands, the long-term persistence of this frog species on the Swan Coastal Plain could be under threat.

Current records do not indicate that the species is declining in the area (IUCN Conservation International and NatureServe 2005), but as there has been no long term monitoring of the species, population disappearances may have gone unnoticed. The species ability to breed in ephemeral wetlands (Main 1957), which do not support populations of *G. holbrooki*, will likely ensure that the species will persist in this area, provided these habitats remain available. However, climate change, continued clearing and a general lack of awareness of landholders of the importance of natural ephemeral wetlands will all potentially threaten the availability of these habitats into the future.

Wetland management decisions which ensure the conservation of ephemeral wetlands will be vital for this and other species. However, as this study has demonstrated managers of all wetlands can take steps to ensure that permanent constructed or highly modified natural wetlands can provide suitable breeding habitat for this species. Managers should wherever possible remove or exclude *G. holbrooki*. Where this cannot be achieved, aquatic and riparian habitat development should be encouraged to stimulate the development of a diverse and abundant invertebrate community and to promote tannin staining of the water.

ACKNOWLEDGEMENTS

I would like to thank Dr R. Black for statistical advice, Frank Doyle for assistance with fish capture and Dr A. Storey for supplying the equipment. Thanks to Ben Roberts who diligently assisted with the counting of surviving tadpoles. Dr J. D. Roberts, Dr P. Davies and Dr M. Smith for assistance with the manuscript. I also would like to thank the Capel Wetlands Centre for assisting with funding for this project. This research was undertaken as part of a PhD at the University of Western Australia with the assistance of Australian Postgraduate Award. All work conducted was done with approval from the University of Western Australia Animal Ethics Committee (AEC approval # 00/100/064) and Department of Conservation and Land Management, Western Australia Licence number SF003880.

Table 6.1: Results of the five factor ANOVA conducted on the survival of *Crinia insignifera* tadpoles in the presence of three predators and varying habitat complexity (n = 3).

Source	DF	MS	F Ratio	Prob > F
day	1	0.00011	0.0004	0.9838
cover	1	0.00589	0.0221	0.8821
Predator**	2	39.12929	36.7175	< 0.0001
water colour	1	0.00956	0.0359	0.8501
alternative prey	1	0.53471	2.0070	0.1598
day x cover	1	0.07416	0.2784	0.5990
day x predator	2	0.12274	0.1152	0.8913
day x water colour	1	0.47279	1.7746	0.1860
day x alternative prey	1	0.02000	0.0751	0.7847
cover x predator	2	0.64217	0.6026	0.5495
cover x water colour	1	0.18131	0.6805	0.4114
cover x alternative prey	1	0.09983	0.3747	0.5419
predator x water colour	2	1.95279	1.8324	0.1656
predator x alternative prey	2	0.51731	0.4854	0.6169
water colour x alternative prey	1	0.70486	2.6457	0.1071
day x cover x predator	2	0.36382	0.3414	0.7116
day x cover x water colour	1	0.60042	2.2537	0.1366
day x cover x alternative prey	1	0.05230	0.1963	0.6587
day x predator x water colour	2	0.49933	0.4686	0.6273
day x predator x alternative prey	2	0.07745	0.0727	0.9300
day x water colour x alternative prey	1	0.04311	0.1618	0.6884
cover x predator x water colour*	2	2.65001	2.4867	0.0885
cover x predator x alternative prey	2	2.49550	2.3417	0.1016
cover x water colour x alternative prey	1	0.02000	0.0751	0.7847
predator x water colour x alternative prey	2	1.07113	1.0051	0.3698
day x cover x predator x water colour	2	0.83808	0.7864	0.4584
day x cover x predator x alternative prey	2	0.44773	0.4201	0.6582
day x cover x water colour x alternative prey	1	0.00293	0.0110	0.9166
day x predator x water colour x alternative prey	2	0.66700	0.6259	0.5370
cover x predator x water colour x alternative prey	2	1.69795	1.5933	0.2086
day x cover x predator x water colour x alternative prey	2	0.26679	0.2503	0.7790
Error	96	0.26642		
Total	143			

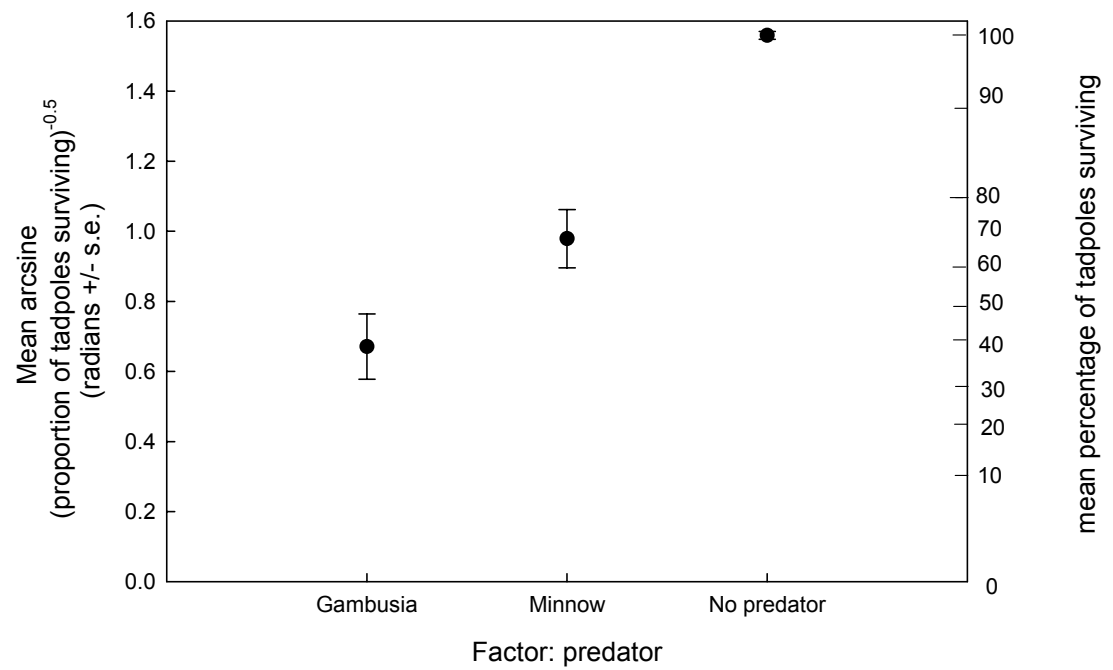
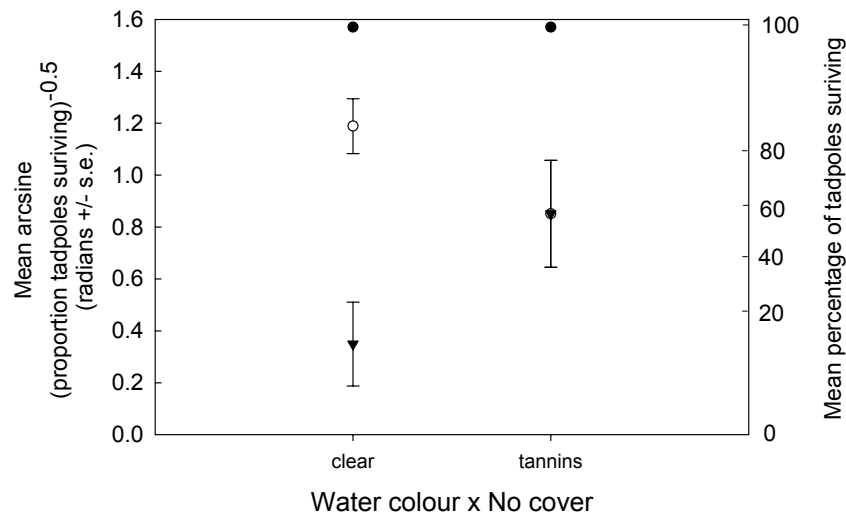


Figure 6.1: Impact of each predator treatment on the survival of tadpoles of *C. insignifera*.

Error bars = standard error, n = 48.

a.



b.

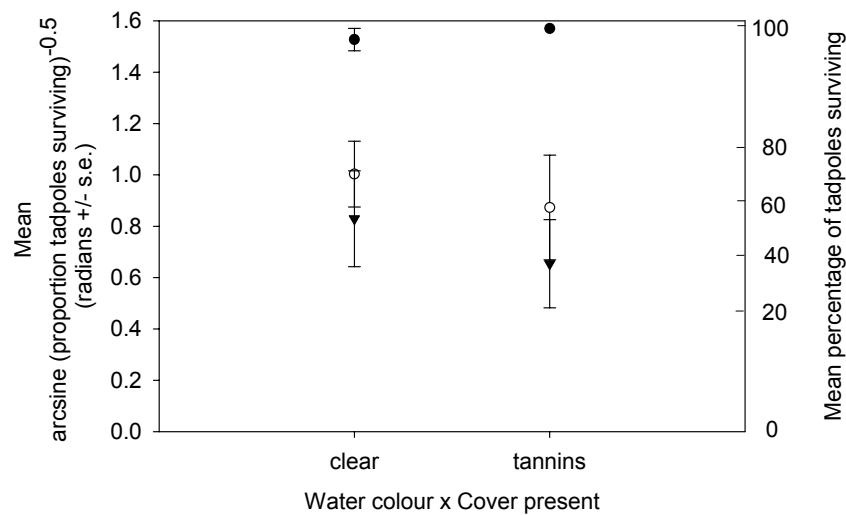


Figure 6.2: The interaction between cover, water colour and predator treatments (n =12) on the survival of tadpoles of *C. insignifera*.

a) The impact of predators on tadpoles in clear and tannin water in the absence of cover.

b) The impact of the predators in clear and tannin water with cover present.

The interaction was significant at the 0.1 level in the five-factor ANOVA. Dots (●) represent treatments with no predator, open circles (○) are treatments with the native

fish *G. occidentalis* and inverted triangles (▼) represent treatments with the exotic fish *G. holbrooki*. Error bars = standard error of the mean.

7.0 Discussion

7.1 ***Conservation of Australian anuran communities in modified landscapes***

Understanding what impacts land modification may have on the ongoing persistence of frog species is important in the long-term conservation of amphibians in highly modified environments (Hazell 2003; Rawlinson 1981). Yet, to date there has been little quantitative information on frog-habitat relationships in modified landscapes in Australia (Hazell 2003). Of the studies which have attempted to characterise amphibian communities in modified environments, few have made an explicit contrast between natural and constructed wetlands (Hazell 2003, Chapter 1; Quinn et al. 2001). This has limited the value of the findings available to support conservation and management decisions. Research in Australia has mostly focussed on eastern Australian species (see Hazell 2003 for a review) and consequently little information is available on the community response of the largely endemic west Australian frog fauna when habitats are modified. In order to redress some of the information gaps in amphibian management in modified landscapes in Australia, this study examined the following issues:

- the structure of the amphibian community in constructed and natural wetlands assessed by counts of calling frogs;
- reproductive output, measured by tadpole numbers, in natural and constructed wetlands;
- how habitat complexity of the wetlands may affect community structure and population density of the frog and tadpole communities;
- how introduced predatory fish may affect tadpole survival;
- how the impacts of an introduced predatory fish compare with the impact of predator species native to the area on tadpole survival.

7.2 Community structure

My research aimed to understand whether the amphibian community composition and the relative species abundance differed significantly between natural and constructed wetlands with the ultimate aim of understanding the role constructed wetlands may play in the maintenance of amphibian populations in highly, modified landscapes. I also examined the relationship of the amphibian community structure to habitat structure of the wetland and the surrounding environment.

The study showed that the species richness of calling males did not differ greatly between constructed and natural wetlands in the south-west of Australia, but tadpole species richness did. Community composition of both calling males and tadpoles varied substantially between the two wetland types. The higher tadpole species richness and the preference for some species to call and reproduce in natural wetlands appear, in part, to be due to habitat characteristics of the wetland and the surrounding terrestrial landscape. My results suggest that the amount of native vegetation in the terrestrial landscape and riparian zone and wetland water regime all strongly influenced the ability of a wetland to support species rich tadpole communities. Similarly, the abundance of most species (adults and tadpoles) correlated positively with the amount of native vegetation in the landscape and negatively with increasing wetland water permanence. One species of frog appeared to be influenced by wetland bathymetry with *H. eyrei* more likely to occur in ponds with shallow margins.

The higher tadpole species richness and overall abundances of tadpoles in natural wetlands compared with that recorded in constructed wetlands, illustrates the importance of natural wetlands in the maintenance of amphibian populations. As demonstrated by this study, constructed wetlands in their current form can provide habitat for a number of species but the value of that habitat in the maintenance of populations is questionable, given the relative level of reproductive activity (i.e. calling males - high) to reproductive output (i.e. tadpoles - low).

7.3 Predator impacts

I compared the impacts of an introduced predatory fish, *G. holbrooki* commonly found in constructed wetlands on two native tadpoles species to that of two native predators, odonates and a Galaxid fish. I attempted to redress the bias in current

research which has examined the impacts of introduced fish in isolation but has not compared the impacts of the introduced fish on tadpoles to that of native predators (Gillespie & Hero 1999). I also determined whether increasing levels of habitat complexity can reduce the predation impacts of *G. holbrooki* on a number of tadpole species.

Laboratory studies in combination with field studies demonstrated that the reproductive output of amphibians in constructed ponds is likely to be detrimentally impacted by the presence of the introduced fish predator, *G. holbrooki*. Predation impact was in some instances reduced by habitat quality: e.g. increasing structural diversity and water colour. Odonates had less impact than both introduced and native fish predators. In laboratory trials the native fish predator, *Galaxias occidentalis* was shown to have as high an impact as *G. holbrooki* on tadpoles of *C. insignifera* in some treatments, but the relatively low density of the native predator in wetlands compared with *G. holbrooki* is likely to limit the impact of this native predator on tadpoles.

7.4 Outcomes

Overall my results indicate that when constructed wetlands

- are in close proximity to native terrestrial vegetation cover;
- are characterised by gently sloping banks;
- have a well developed aquatic habitat structure;
- are free of *G. holbrooki* and;
- have water regimes that mimic that of natural wetlands in the region (e.g. ephemeral),

they are likely to support a diverse and reproductively viable amphibian community. Consequently, constructed wetlands, in their present form, i.e. steep sided, permanent and placed in cleared areas, are unlikely to provide viable habitat sites for many of the amphibian species found in the southwest of Australia.

7.4.1 Applicability

Results from this study are consistent with a number of international studies that identify that constructed wetlands can potentially provide habitat areas for some amphibian species (e.g. Baker & Halliday 1999; Beebee 1981; Monello & Wright 1999; Porej & Hetherington 2005). In addition, many studies have identified that the success of constructed ponds as viable sites for reproduction is often limited by the presence of introduced, predatory fish (e.g. Beebee 1981; Beebee 1997; Ildos & Ancona 1994; Porej & Hetherington 2005; Pyke & White 1996), a lack of natural terrestrial vegetation associated with the wetland (e.g. Beebee 1985; Monello & Wright 1999), steep bank slopes (Porej & Hetherington 2005) and poor availability of non-breeding habitats within or immediately surrounding the wetland (e.g. Ildos & Ancona 1994; Knutson et al. 2004; Stumpel & van der Voet 1998). In the Australian context, my study presents an important contribution for conservation and land managers by providing quantitative reproductive output information from both constructed and natural wetlands, which has enabled an objective assessment of the value of constructed wetlands as surrogate, breeding habitats for amphibian species.

Previous studies in Australia (Hazell et al. 2004; Healy et al. 1997; van de Mortel & Goldingay 1998; Watson et al. 1995) have documented the occurrence of frogs but not quantified density or analysed effective reproductive output in both constructed and natural wetlands. The results of my study indicate that monitoring adult presence at a wetland will only provide information on the ability of a species to arrive at a given location, but little evidence of the population's ability to persist and contribute to the overall landscape population. As identified by Berven (1990) fluctuations in the size of the adult populations of the wood frog, *Rana sylvatica*, were strongly associated with larval survival. Similarly, both Beebee *et al* (1996) and Semlitsch *et al* (1996) found significant correlations between the number of tadpoles surviving to metamorphoses and the number of breeding adults in the following year for a number of species. These studies (Beebee et al. 1996; Berven 1990; Semlitsch et al. 1996) have demonstrated the importance of pond habitats in the persistence of amphibian populations and illustrate the role they may play in the population dynamics of frog species.

Results from my study also highlight the importance of monitoring the reproductive outputs of species in a habitat matrix to ensure that the habitat can support a viable and persistent population. It cannot be assumed that all ponds that contain adult calling males are recruiting metamorphs. Studies which fail to incorporate a measure of reproductive output are unlikely to provide sufficient information on which conservation management decisions can be based (Van Horne 1983). The collection of information on what provides suitable habitat for effective reproduction is likely to be vital in the preservation of species into the future. Research such as mine which can provide information on both the adult and recruiting phases are therefore likely to prove valuable to the long-term conservation of amphibian communities. Future studies should not only determine the ability of constructed wetlands to provide suitable habitat for amphibian adults but should also provide information on the importance of these habitats for amphibian breeding and for the maintenance of populations at the local and landscape scale.

7.4.2 *Limitations*

This study was primarily a pond based study which enabled the effective assessment of the reproductive value of constructed and natural wetlands in a disturbed landscape. However, as identified by Marsh and Trenham (2001) amphibian spatial dynamics are more complex than that which might be inferred from studies which focus primarily on breeding ponds as habitat patches. As most adult frogs spend little time at breeding ponds (Wilbur 1984) it is likely that the quality and availability of terrestrial habitats will critically influence local and regional population dynamics. A growing body of evidence suggests that although breeding ponds can strongly influence population trends, terrestrial habitats may also play an important role in populations dynamics (Marsh & Trenham 2001). For example, Schwarzkopf and Alford (1996) identified that shelter-site quality was important in the growth of *Bufo marinus* populations. Similarly, Lamoureux and Madison (1999) demonstrated that overwintering habitat was vital in the survival of adult *Rana pipiens* in New York State. They noted that while poor choice of breeding habitat may result in unsuccessful breeding in that season, the incorrect choice or the lack of availability of overwintering habitat may be fatal (Lamoureux & Madison 1999). A review by Marsh and Trenham

(2001) identified that there is a general lack of understanding of the role terrestrial habitats play in determining the patterns of abundance of amphibians at breeding ponds.

Terrestrial habitat dynamics were not investigated in this study as my focus was on a simple question: do frogs recruit in constructed wetlands? Subsequently, no assumptions can be made about the quality and extent of habitat required for the persistence of any of the studied species beyond the pond environment. But, the results of habitat analyses (Chapter 2) suggest even moderate amounts of vegetation close to breeding ponds might improve numbers for some species. Long-term studies on the use of terrestrial habitats, rates of metamorphosis from the pond and breeding metamorph return will likely provide important information on the conservation of amphibian populations in highly modified terrestrial landscapes. Future studies should therefore consider examining the population dynamics in the terrestrial environment and metamorph recruitment to assist in providing a more complete picture of amphibian frog-habitat relationships in modified landscapes.

The methods used to determine the relative abundance of tadpoles in this study were based on the assumption that all individuals, regardless of the wetland, are equally catchable. Variations in habitat between wetlands, wetland bathymetry and individual tadpole behaviour within and between wetlands may have influenced the capture rates of each species within each wetland. In the absence of more effective sampling techniques, traps and sweeps were used in combination to minimise variations in catchability between species and wetlands. I made every effort to ensure sampling techniques, climatic conditions and the time of sampling did not vary between wetlands. Further, I did not use the relative numbers captured from each technique to estimate the total population size or the density of the tadpoles in each wetland. As identified by Shaffer et al. (1994) provided caution is made to ensure standardisation between study areas, quantitative comparisons between sites can provide effective comparisons. However, this information should be considered when interpreting findings from my study.

Water quality in constructed wetlands was not measured to determine the impact it might have on the survival and persistence of tadpoles. Numerous researchers (e.g. Boone & Semlitsch 2001; e.g. Hecnar 1995; Marco & Blaustein 1999; Pope et al. 2000; Rouse et al. 1999) have identified that water pollutants such as pesticides, pH and ammonia can strongly influence the quality of the habitat provided by wetlands for developing tadpoles. Given the nature of many of the wetlands, i.e. placed in highly modified landscapes and their origin as mining wetlands, it is probable that water

quality may have been a strong influencing factor in their suitability as amphibian breeding habitat. Logistic issues prevented consistent sampling of water quality in all wetlands in this study, however, future studies should ensure that these components are measured.

Predatory trials are often conducted exclusively in laboratories or in highly artificial tanks or containers and therefore the results have only limited applicability to that which occurs in a more natural setting. I attempted, in part, to amend this issue by also undertaking field based studies which can be more easily translated to more natural settings. However, limitations in the availability of suitable constructed ponds prevented more extensive *in situ* research within this study. Knowledge of processes that occur in more natural settings will be valuable in the control of introduced predators such as *G. holbrooki*. Therefore future research in this area should attempt to effectively translate the results from the laboratory to the outside environment through more extensive field based experiments.

7.4.3 *Management decisions and actions*

Results of this study have several implications for conservation and wetland management and for the design and development of constructed wetlands. For example, wetlands should not be considered in isolation from the surrounding landscape, it is important to consider the proximity of native terrestrial vegetation to the wetland. While at the pond level, water regime, the morphology of the banks, the presence of predators, the extent of native vegetation and aquatic habitat structure all appear to influence the communities of amphibian that actively call and reproduce there. This study emphasises the need to encourage wetland and conservation managers to develop protocols which ensure that restored and newly constructed wetlands are designed to provide a diversity of habitats which mimic that originally found in the region.

In the south-west of Australia a wetland with gradually inclined banks in combination with a locally appropriate drying and flooding regime will encourage a number of burrowing amphibian species such as *H. eyrei* and *P. guentheri*, while the presence of dense riparian vegetation is likely to encourage more arboreal species such as *L. adelaidensis*. Excluding *G. holbrooki* from wetlands is likely to increase the reproductive output of those wetlands as the reduced predator pressure may enable more tadpoles to survive. Similarly, it is probable that the exclusion of this introduced fish

may encourage species such as *G. leai* to inhabit constructed wetlands. In wetlands where *G. holbrooki* is well established and cannot be removed, the promotion of a diverse and complex aquatic environment may mediate predator impacts. I suggest that the establishment of native terrestrial habitat in association with existing constructed wetlands, during the development of constructed wetlands or alternatively establishing constructed wetlands in close proximity to existing terrestrial native habitat may maximise the reproductive potential of the wetland. As noted by Marsh and Trenham (2001), management plans that focus only on preserving wetlands will probably fail to maintain viable amphibian populations. Identifying and protecting terrestrial habitats in association with wetlands should also be a conservation priority.

As this and other studies (e.g. Guerry & Hunter 2002) have identified, the principles of wetland design which enhance the suitability of the environment to particular species is strongly influenced by the habitat requirements of the adults and their tadpoles. Therefore understanding species life histories and their reliance upon particular habitat components in the available habitat will provide extensive information on the value of constructed wetlands for the persistence of a particular species. The wetland design principles derived from the results of this study (e.g. hydroperiods that mimic the natural wetland water regime of the area, removal/exclusion of introduced predators, provision of upland habitat, etc) are therefore likely to be applicable in a wide range of areas throughout Australia and throughout the world.

7.5 Conclusions

As demonstrated by this study constructed wetlands in their current form can provide habitat for a number of species, however the value of that habitat in the maintenance of populations is questionable, given the relative level of reproductive activity (i.e. calling males) to reproductive output (i.e. tadpoles). Further, the absence of some species from constructed wetlands indicates that these wetlands in their present form do not have the capacity to support the full array of species typically found in the region. The results suggest that wetlands may be constructed in ways that maximise their conservation potential. Nonetheless, these wetlands should not be considered a substitute for the retention of natural wetlands as it is probable that constructed wetlands may never be able to support the complete suite of native amphibian species. As noted by Brock et al. (1999) the attitude that replacement is somehow a satisfactory substitute

for lost wetlands is a threat to the future conservation of natural wetlands. Conservation of natural wetlands and natural terrestrial habitats should be paramount for conservation efforts throughout Australia as these areas are likely to support a wide array of species that may never be able to adapt to constructed wetlands. But as this study has highlighted, habitat creation in highly modified regions may potentially play a valuable role in maintaining amphibian populations, particularly during drought periods, when natural wetlands may not receive surface water. As noted by Hazell et al. (2004) constructed wetlands should only be viewed as potential supplementary habitat zones for anuran species and not as a replacement for natural wetlands.

This study was one of the first studies in Australia that provides quantitative information on how amphibian communities use and reproduce in constructed wetlands compared with natural wetlands in the same region. It addresses some of the information gaps in amphibian conservation, with the intention of providing wetland and land managers with practical information on how to develop management plans which can be designed to include amphibian conservation goals. The study has highlighted that constructed wetlands can and do support some species of amphibians in their existing form. Further, in regions such as the Swan Coastal Plain, of south-west Australia where natural wetlands are now scarce, constructed wetlands may represent important alternative breeding habitats for amphibians. However, the relatively low reproductive output in constructed wetlands suggests that these wetlands need to be developed differently if they are to provide more optimal amphibian habitat. Informed design and management of constructed wetlands may assist in improving the availability and quality of breeding habitat and these wetlands may potentially support more viable and diverse amphibian communities. Wetland designers should recognise the importance of developing a diversity of wetland types and land managers need to acknowledge the importance of retaining natural wetlands including ephemeral waterbodies in association with native vegetation to ensure the persistence of native Australian anuran species.

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