

# **Conservation Management of Two Threatened Frog Species in South-Eastern New South Wales, Australia**

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Submitted in total fulfillment of the requirements of the degree Doctor of Philosophy  
at the University of Canberra

June 2007



## Abstract

The decline and extinction of amphibian species over the past three decades is widely acknowledged as one of the greatest biodiversity crises of modern time. Providing convincing data to support hypotheses about these declines has proved difficult, which has greatly restricted the development and implementation of management actions that may prevent further amphibian declines and extinctions from occurring. In this thesis, I present research that was undertaken as part of the recovery programs for the southern corroboree frog (*Pseudophryne corroboree*), and the Booroolong frog (*Litoria boorooolongensis*); two species that underwent very rapid declines in distribution and abundance during the 1980's. More specifically, I investigated potential causal factors in the declines of both species using experimental and correlative studies, and examined the mechanisms by which one threatening process (chytridiomycosis) may be causing continued decline and extinction in *P. corroboree*. I also examined the implications of population dynamics for monitoring *L. boorooolongensis*, and suggest a possible monitoring strategy that may reliably facilitate the implementation of recovery objectives for this species. I also tested one possible reintroduction technique aimed at preventing the continued decline and extinction of *P. corroboree* populations.

In Chapters 2 and 3, I present the results from a series of experiments in artificial enclosures designed to examine whether the tadpoles of *L. boorooolongensis* are susceptible to predation by co-occurring introduced predatory fish species; brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), European carp (*Cyprinus carpio*), redfin perch (*Perca fluviatilis*), and mosquito fish (*Gambusia holbrooki*). I demonstrated that the tadpoles of *L. boorooolongensis*, and a closely related species *Litoria lesueuri*, were palatable to non-native trout species, but not to two native predatory fish species, *Gadopsis bispinosus* and *Galaxias olidus*. A pond breeding frog species included in this experiment, *Limnodynastes tasmaniensis*, was palatable to both the native and non-native fish species. In a separate experiment I also demonstrated that the tadpole of *L. boorooolongensis* is palatable to the three other introduced fish species examined in this study; *C. carpio*, *P. fluviatilis*, and *G. holbrooki*. In three of the experiments, the provision of rock within enclosures as a potential refuge habitat did not afford protection to *L. boorooolongensis* tadpoles from

predation by any of the five introduced fish species examined. While all the introduced fish species tested here did consume *L. booroolongensis* tadpoles, the results also suggested that chemical unpalatability might afford some level of protection against some of these fish species. Firstly, the addition of alternative prey items in one of the experiments reduced the proportion of tadpoles consumed, suggesting that *L. booroolongensis* may not be a preferred prey item. Secondly, the proportion of tadpoles consumed varied greatly among the different fish species examined, suggesting differing levels of palatability. Overall, this study supports previous research in suggesting that chemical unpalatability may be an important strategy for the tadpoles of riverine frog species in south-eastern Australia to avoid predation by native fish species, and that this strategy is less effective against introduced fish species. While *L. booroolongensis* currently persists in streams inhabited by a number of introduced fish species, this study supports the likelihood that these species are having a negative impact on populations of *L. booroolongensis* in the wild.

In Chapter 4, I present the results of a study aimed at examining potential monitoring techniques for *L. booroolongensis*. The results of a mark-recapture exercise demonstrated that *L. booroolongensis* may exhibit large fluctuations in abundance from one year to the next, and through a prospective power analysis approach, I demonstrated that it would be difficult to confidently identify population trends of interest using either indices or estimates of abundance for this species. An assessment of the capacity to identify the presence or absence of *L. booroolongensis* using night-time spotlight surveys demonstrated the high detectability of this species using this technique, at both the scale of 300-meter sections of stream and individual breeding areas (typically less than 10-meters of stream). This study suggests that the monitoring objectives of the *L. booroolongensis* recovery program would be most effectively achieved using presence/absence surveys at different scales.

In Chapter 5, I present the results of a field survey aimed at determining the current distribution and habitat requirements of *L. booroolongensis* in the South West Slopes region of New South Wales. Of the 163 sites I surveyed across 49 streams, I located *L. booroolongensis* along 77 of these sites from 27 streams. Based on population and habitat connectivity, this study identified 18 populations of *L. booroolongensis* that

are likely to be operating as independent populations. Twelve of these populations are not represented in conservation reserves, but rather occur along streams that flow through the agricultural landscape. A broad scale habitat analysis identified a positive relationship between extent of rock structures along the stream and the occurrence of *L. booroolongensis*, and a negative relationship between the proportion of canopy cover and this species' occurrence. At the breeding habitat scale, this study identified a positive relationship between the presence of breeding males and; number of rock crevices in the aquatic environment, extent of emergent rocks, and proportion pool. This analysis also detected a negative relationship between occupancy and water depth. These results confirm previous work suggesting the importance of rocky stream habitats to the persistence of *L. booroolongensis*, but also suggest how disturbance processes, such as increasing sedimentation and weed invasion, may reduce the suitability of rocky structures as breeding sites.

In Chapter 6, I investigated current levels of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) infection in corroboree frog populations, and used retrospective screening of museum specimens to assess the possibility that this pathogen was implicated in the initial decline of the corroboree frogs. Using histology, I did not detect any *B. dendrobatidis* infections in corroboree frog populations prior to their decline, however using the same technique, moderate levels of infection were detected in post-decline populations of both species. Real-time PCR screening of skin swabs identified much higher overall infection rates in post-decline populations of *P. corroboree* (between 44% and 59%), while significantly lower rates of infection were observed in *P. pengillyi* populations (14%). These results suggest that the initial and continued decline of the corroboree frogs may well be attributed to the emergence of *B. dendrobatidis* in populations of these species.

In Chapter 7, I investigated how *B. dendrobatidis* may be causing the continued decline of *P. corroboree* through the presence of an abundant reservoir host for this pathogen. I found that populations of adult *C. signifera* in sub-alpine bogs carry high *B. dendrobatidis* infection rates (86%), but appear unaffected by this infection. An experiment involving the release of *P. corroboree* tadpoles into 15 natural pools resulted in metamorphs from seven of these pools testing positive for *B. dendrobatidis*, with all these individuals dying soon after metamorphosis. These

results support the possibility that *B. dendrobatidis* infection in *P. corroboree* populations is being facilitated by the presence of large numbers of infected *C. signifera* in the shared environment.

Chapter 8 presents the results of a population augmentation study for *P. corroboree*. I investigated the extent to which increasing recruitment to metamorphosis may result in population recovery in this species. This was undertaken by harvesting eggs from the field and rearing them through to mid stage tadpoles over the winter period prior to being released back to their natal ponds in spring. While I was able to increase recruitment to metamorphosis by an average of 20 percent, this did not result in a noticeable influence on the subsequent adult population size, as both manipulated and non-manipulated sites declined over the course of this study by an average of 80 percent. I observed a positive relationship between natural recruitment to a late tadpole stage and subsequent adult male population size, however there was considerable variation associated with this relationship. The relationship between recruitment and subsequent population size at the augmentation sites was consistent with the relationship observed at the non-manipulated sites. These results suggest that recruitment to metamorphosis may not be the most important life stage restricting the population recovery of *P. corroboree*, but that mortality during post-metamorphic stages may be more important in regulating current population size. Hence, further attempts to use captive rearing to increase *P. corroboree* populations in the wild should focus on the release of post-metamorphic frogs.

Overall, this thesis demonstrates the value of quantitative research to the implementation and progress of threatened species recovery programs. While this research will specifically contribute to the recovery programs for *L. booroolongensis* and *P. corroboree*, it more broadly contributes to the understanding and capacity to respond to the concerning levels of amphibian extinctions currently occurring throughout the world.



## Acknowledgements

The research undertaken in this thesis was made possible through the provision of funding from the following organisations; Australian Research Council, NSW Department of Environment and Climate Change, University of Canberra, Co-operative Research Centre for Freshwater Ecology, NSW Fisheries, Murray Catchment Management Authority and Amphibian Research Center. I am indebted to staff in the NSW National Parks and Wildlife Service offices in Tumut and Khancoban for their efforts in assisting many aspects of this work. Chapters 6, 7 and 8 are the result of continued support and guidance by the Corroboree Frog Recovery Team.

I am very thankful to all those who provided me with field assistance and company throughout this research; Sean Doody, Michelle Walter, Rod Pietsch, Mike Smith, Lachlan Farrington, Alex Knight, Alex Quin, David Bourne, Martha Reese, Dieuwer Reynders, Russel Traher, David Judge, Rajani Rhy, Colin De Pagder, Craig Smith, Jessica Rossell, Michael McFadden, Yoko Shimizu, Ty Mathews and Jamie Molloy. Claire Steel is owed particular thanks for undertaking much of the husbandry for Chapter 7. I am particularly thankful to Mike Smith and Michael Scroggie for their assistance with the statistical analyses in Chapters 2 and 5. Jason Baldwin and Tony Day (NSW Fisheries) provided considerable assistance in many aspects of this project. I also thank Bob Faragher and Roy Witstanly (NSW Fisheries) for capturing the fish used in Chapter 3, and also Brian Free and the Tumut Acclimatisation Society for the use of their shed. Rick Speare and Diana Mendez undertook the histology and screening of specimens in Chapter 6, and provided much advice and comment on this chapter. I am particularly appreciative to all the property owners and managers I visited during the surveys in Chapter 5, who allowed me access through their land, and shared their wealth of knowledge about the streams in the South West Slopes region. Kylie Durrant and Cherie White provided much local knowledge and advice that greatly facilitated the surveys undertaken in Chapter 5. I also thank the following for fruitful advice and discussions at various stages through my thesis; Tony Tucker, Murray Evans, Sean Doody, Arthur Georges, Graeme Gillespie, Nick Cleemann and Glen Johnson.

I am especially thankful to the following people for their contributions to this thesis and my capacity to undertake it. Firstly, I probably wouldn't have embarked on this project if it wasn't for the encouragement of Scott Keogh and Paul Doughty – unfortunately Plan A didn't quite work out. My supervisor Will Osborne provided constant support and advice during all phases/directions of my PhD work, and was particularly helpful during the final write-up. Michael Smith has been an invaluable source of support, advice and amusement during this thesis. I am particularly indebted to Mike Saxon and Rod Pietsch for their support as colleagues and supervisors during this research – they have definitely inspired me to keep pursuing a career in this field. I am greatly indebted to Gerry Marantelli for his innumerable contributions to this thesis and his tireless efforts in our broader endeavors - I hope one day we celebrate success! My family (Mum, Dad, Jonathan, Meggsie, Allie, Steven and Dorrie) provided much valuable support during periods when I was away from home.

The second half of my PhD journey has been a relatively painless exercise thanks to the love and fulfillment in my life from Dieuwer, Ollie and Manu. In addition to the direct support and sacrifices they made for my research, they importantly gave me the capacity to put this thesis into perspective.



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# Chapter 1

## Introduction

### 1.1 Amphibian Declines

#### 1.1.1 *The Global Amphibian Decline Phenomena*

Prior to 1990 there had been very little attention given to specific conservation issues for amphibian species (Duellman and Trueb 1986, Beebee 1996). Where conservation concerns had been raised, the threats were typically obvious, usually being habitat degradation or restriction to a small geographic range (Beebee 1996). The general perception was that trends in the conservation status of amphibians were no different to those observed in the other major vertebrate faunal groups (May *et al.* 1995). This perception was challenged in 1990 when several publications announced that amphibian declines and extinctions were occurring around the world at an alarming rate (Barinaga 1990, Blaustein and Wake 1990, Phillips 1990, Vitt *et al.* 1990). Because these claims were largely based on anecdotal evidence, they were received with some scepticism. Questions were raised as to whether the declines were simply natural fluctuations in abundance (Blaustein *et al.* 1994, Pechmann and Wilbur 1994), or if they did represent real declines and extinctions, were they representative of a specific global phenomenon impacting on amphibians (Pound *et al.* 1997).

The concerns raised about global amphibian declines generated increased attention that was focussed on the conservation of many amphibian species suspected to be in a state of decline. This included targeted surveys and monitoring for species suspected to have declined, which confirmed that substantial recent declines had been occurring, with some species apparently disappearing altogether (McDonald 1990, Crump *et al.* 1992, Richards *et al.* 1993, Gillespie and Hollis 1996, Hollis 1995, Osborne 1989). While it was obvious that not all amphibian species had entered a state of dramatic decline (Alford and Richards 1999), a quantitative assessment of the conservation status of amphibian species across the globe did conclude that over the past few decades amphibian declines had been occurring at a rate greater than that experienced

by birds or mammals (Stuart *et al.* 2004). A summary of the conservation status of the world's amphibians presented by Stuart *et al.* (2004) identified 34 species as becoming recently extinct, 88 species as possibly extinct, and one third of all the worlds amphibian species as threatened. Within Australia, 50 amphibian species have been identified as having undergone major declines in recent years, of which eight are considered most likely to be extinct (Hero *et al.* 2006).

### **1.1.2 Causal Factors of Recent Amphibian Declines**

The suggestion that amphibian species were suffering a 'global decline phenomenon' was particularly intriguing and concerning because many of the species reported to have declined occurred in areas of relatively pristine habitat within conservation reserves, where there had been no obvious environmental perturbations (Alford and Richards 1999). The correlative and experimental research that followed gained support for a number of hypotheses as to the possible causes of recent amphibian declines, which included; increased ultraviolet radiation (Blaustein *et al.* 1994, Blaustein *et al.* 1995), exotic predators (Bradford *et al.* 1993), habitat modification (Hecnar and M'Closkey 1996, Hecnar 1997), increased acidity and toxicants (Bishop 1992), and disease (Laurance *et al.* 1996, Lips 1998).

To date, the most substantial and convincing research into the causes of mysterious amphibian declines supports the hypothesis that emerging infectious disease has played an important role in many of these recent declines (Berger *et al.* 1998, Daszak *et al.* 1999). In particular, there is much evidence that the amphibian chytrid fungus, *Batrachochytrium dendrobatis*, is associated with many of the observed amphibian declines across the world, particularly in moist upland environments (Berger *et al.* 1998, Bosch *et al.* 2001, Lips *et al.* 2006). While the debate continues over the origins of this pathogen, and its role in broad scale amphibian declines (McCallum 2005, Rachowicz *et al.* 2005), a recent study from Panama provided very compelling evidence of the capacity for *B. dendrobatis* to cause population declines across a range of amphibian species and over a broad geographic area (Lips *et al.* 2006). The strength of the data presented by Lips *et al.* (2006) was that they were able to monitor the real time impacts of *B. dendrobatis* as it moved through different amphibian

communities in Panama, as opposed to other studies that have related retrospective data to historic declines (McCallum 2005).

Another important area of research into causal factors of recent amphibian declines has been into possible synergistic interactions between different threatening processes (Blaustein and Kiesecker 2002). For example, Kiesecker *et al.* (2001a), identified interactions among three processes, whereby global climate change would increase the frequency of reduced water level in breeding pools, which would subsequently increase exposure of *Bufo boreas* embryos to UV-B radiation, which in turn increases the embryos susceptibility to infection with the fungus pathogen *Saprolegnia ferax*. Studies identifying interactions among different factors are important in our understanding of amphibian declines, because the demography of a species in decline is more likely to be driven by multiple factors rather than just one. Moreover, identifying the range of factors and their interactions causing population decline would undoubtedly strengthen the capacity to develop a management response to reverse the population trend.

### ***1.1.3 Conservation Management of Threatened Amphibian Species***

The primary aim of a threatened species recovery program is to identify the causal factors of the species decline, and develop and implement management actions aimed at suppressing or removing the threatening processes (Dickman 1996). Two examples of how this has been successfully achieved for amphibian species are the recovery programs for the Majorcan midwife toad, *Alytes muletensis*, which is restricted to a small area on the Balearic Islands (Beebee 1996, Bloxam and Tonge 1995) and the natterjack toad, *Bufo calamita*, which declined from a large portion of its range in Britain (Beebee 1996, Denton *et al.* 1997). In both these cases, a combination of identifying the causal factors of decline, habitat restoration, captive breeding and translocation were used to increase the distribution and abundance of these species and enhance their overall population viability (Bush 1994, Bloxam and Tonge 1995, Beebee 1996). The importance of identifying the causal factors of decline in the recovery process was well illustrated in the case of the *B. calamita* recovery program, as management strategies were initially unsuccessful until the factors that caused the decline in this species were identified and remedied (Denton *et al.* 1997).

Our understanding and approach to amphibian conservation has also greatly benefited from metapopulation studies of amphibian species (Marsh and Trenham 2001). A number of studies have found that amphibian populations interact across the landscape such that extinction and re-colonisation of local populations are relatively common events, being facilitated through the dispersal of juvenile and adult frogs (Hanski and Gilpin 1991, Sjogren 1991, Gibbs 1993). It thus follows that the management of individual populations requires taking into consideration connectivity with other populations across the landscape (Hanski and Gilpin 1991). Hence, effective conservation of populations requires conserving not only the immediate habitat surrounding breeding sites but also the habitat connecting different sites (Green 1997, Alford and Richards 1999, Semlitsch 2000). Similarly, the development of monitoring programs requires following not only changes in the number of individuals within a populations, but also changes in the number of populations across the broader landscape (Hecnar and M'Closkey 1996, Green 1997).

The conservation management of amphibians has also greatly benefited from the recent development and application of molecular techniques to population genetics. Apart from elucidating patterns of population connectivity across the landscape (e.g. Shaffer *et al.* 2000), several studies have demonstrated the potential implications of reduced genetic variation to individual fitness. Hitchings and Beebee (1998) found that reduced heterozygosity in *Bufo bufo*, as a result of genetic drift, correlated with reduced survivorship during the larval stage. Similarly Rowe *et al.* (1999), found that populations of *Bufo calamita* that were more isolated had reduced levels of heterozygosity and slower larval developmental rates. Kraaijeveld-Smit *et al.* (2006) were able to demonstrate that after many generations in captivity the Majorcan midwife toad (*Alytes muletensis*) lost genetic variation and the capacity to respond to a predator. These studies provide strong evidence for the need to apply techniques to maintain genetic variation in amphibian species that have declined to low population size, or are fragmented across the landscape.

#### **1.1.4 Management Response to Recent Amphibian Declines**

Given the difficulties in identifying the causal factors of more recent amphibian declines (Alford and Richards 1999), it is not surprising that despite extensive

research over the past two decades, there are limited examples of positive management outcomes in responses to these declines. This is of considerable concern as many species continue to decline, and more extinctions appear inevitable in the short term (Lips *et al.* 2006). It is also apparent that responding to many amphibian declines following the principles for amphibian conservation set out in recent publications (Green 1997, Semlitsch 2000, Semlitsch 2002), which involves using current knowledge of amphibian biology to refine accepted scientific approaches to dealing with declining species, would not have been effective at preventing the rapid decline and apparent extinction of unique species like the golden toad (*Bufo periglenes*) (Crump *et al.* 1992) or the gastric brooding frogs, (*Rheobatrachus silus* and *R. vitellinus*) (Tyler 1991).

Given the potential for amphibian species to become extinct with very little or no warning, Mahony *et al.* (1999) identified the need for a more prompt safeguard reaction to amphibian declines. This approach relies on a more precautionary and pre-emptive philosophy, and involves securing in captivity species that display either the early signs of population decline, or are predicted to be susceptible to decline. Mahony *et al.* (1999) suggests that once a species has been secured in captivity, further steps, such as those suggested by Green (1997) and Semlitsch (2000), can then be pursued to address their fate in the wild. It is without doubt that the development of management systems for preventing further loss of amphibian species in the wild is currently one of the great challenges in modern day threatened species conservation.

## **1.2 Study Species 1; The Booroolong Frog (*Litoria booroolongensis*)**

### ***1.2.1 General Description***

The Booroolong frog (*Litoria booroolongensis* Moore 1961) is a medium-sized frog belonging to the Family Hylidae. Females may attain 54 mm snout-vent length while males are smaller, attaining 42 mm (Moore 1961). The dorsum may be grey, olive, or reddish-brown with rather indistinct black reticulations and scattered salmon-coloured flecks. The dorsal skin texture is usually slightly warty and the ventral surface is pale and finely granular. Small dark flecks may be present on the throat. A faint thin, black stripe is often present along the snout and passing through the eye, curving slightly over a small distinct tympanum to the shoulder. However, this stripe may be completely absent in some individuals. The backs of the thighs may be dark brown or covered in a yellow and black reticulated pattern. The fingers are free from webbing, while the toes are strongly webbed. Webbing extends to the base of all discs except the second toe (Moore 1961, Barker *et al.* 1995, Cogger 1996).

The tadpole of *L. booroolongensis* is free-swimming and adapted for living in a riverine environment (Anstis *et al.* 1998, Anstis 2002). The body is elongated and flattened, with a rounded snout, and well developed tail musculature. Individuals attain a total length of 50 mm prior to metamorphosis. The eyes are dorso-lateral and the mouth is ventral. The dorsal body colour is uniform rusty-brown with some darker mottling, continuing along the tail musculature as two longitudinal stripes. A conspicuous dark brown band is present across the urostylar region. The venter has an almost uniform gold sheen, with some darker patches. The oral disc is large, and a band of oral papillae surround the entire margin. There are two rows of anterior labial teeth, and three posterior rows (Anstis *et al.* 1998).

### **1.2.2 Distribution and Habitat**

*Litoria booroolongensis* is restricted to New South Wales (NSW) and north-eastern Victoria, predominantly along western-flowing streams of the Great Dividing Range, from 200 to 1300 meters above sea level. The species occurs from catchments draining the Northern Tablelands to the Murray River in the Southern Highlands of NSW and north-eastern Victoria (Figure 1.1; Heatwole *et al.* 1995, Anstis *et al.* 1998, Hunter and Gillespie 1999, Gillespie and Hunter 2000).

*Litoria booroolongensis* is associated with permanent streams in wet and dry forest, woodland, and cleared grazing land (Anstis *et al.* 1998, Gillespie 1999). The species occurs in dissected mountainous country, tablelands, foothills and lowland plains (Anstis *et al.* 1998, Gillespie 1999). Adults tend to occur on or near cobble banks or bedrock structures within stream margins, or near slow-flowing connected or isolated pools (Anstis *et al.* 1998). These areas also tend to have some vegetation cover, such as ferns, sedges or grasses. Frogs shelter under rocks or amongst vegetation near the ground along the edge of the stream.

Eggs are deposited in either shallow slow flowing sections within the stream channel or isolated rock pools along the margins of streams (Anstis *et al.* 1998). The egg clutch is a rigid gelatinous clump, adhered to rock or in crevices (Anstis *et al.* 1998). Tadpoles have been observed in slow-flowing sections of streams, or in pools (Anstis *et al.* 1998, Gillespie 1999). The tadpoles are benthic and have been found on rocks and detritus on the streambed (Anstis *et al.* 1998).

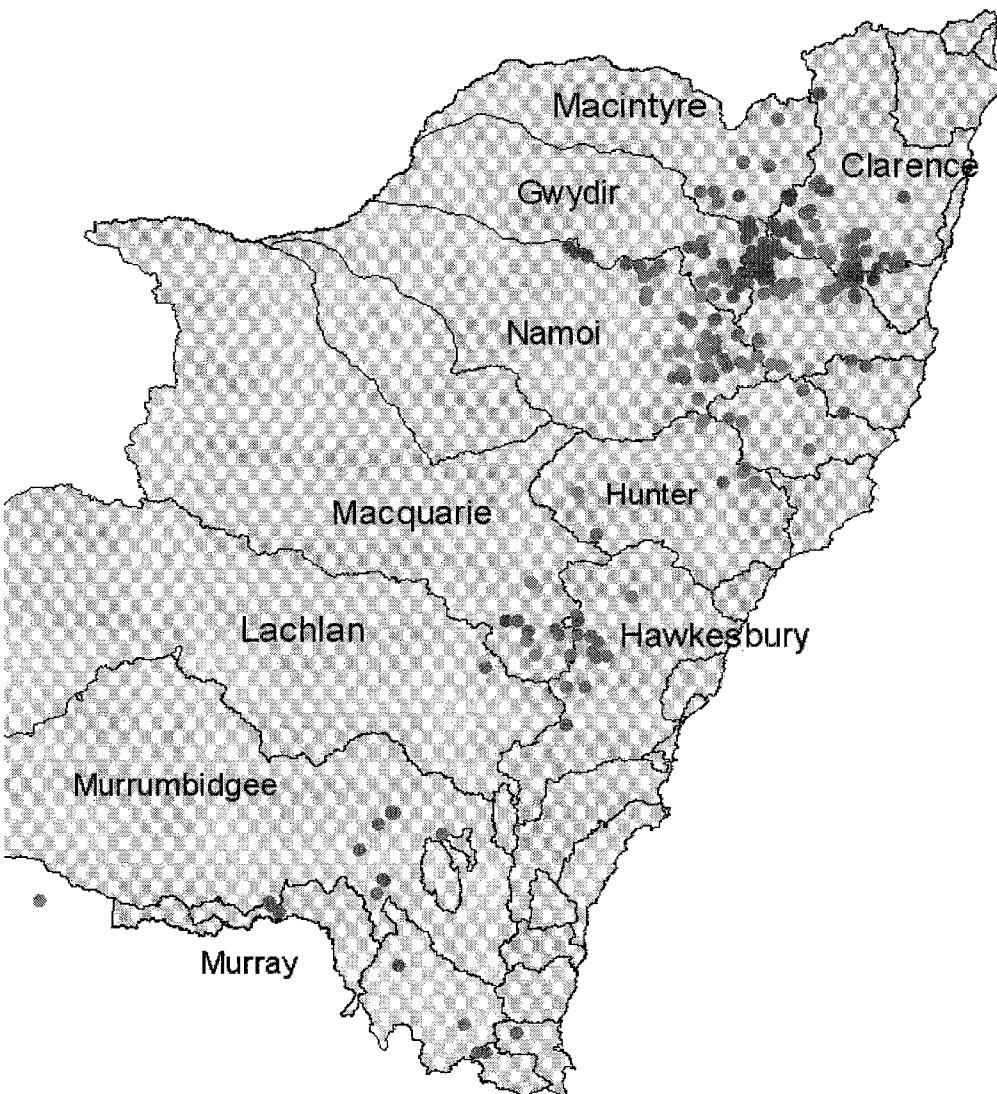


Figure 1.1. Distribution of *Litoria booroongensis* in relation to major water catchments in New South Wales. Locality records are based on NSW Wildlife Atlas records.

### **1.2.3 Life History and Demography**

The life-history of *L. booroolongensis* is strongly centered around riparian environments. Male *L. booroolongensis* call from rocky habitats along permanent streams from mid spring through to mid summer (Anstis *et al.* 1998). Mating and egg deposition also take place along these rocky habitats, with the eggs either being deposited within the stream channel or in disconnected streamside pools (Anstis *et al.* 1998). There is virtually nothing known about the population demography of *L. booroolongensis*.

### **1.2.4 Decline and Management**

The decline of *L. booroolongensis* over the past two decades has been most noticeable in the northern section of its former known range (Gillespie 1999, 2000). Prior to the mid 1980's *L. booroolongensis* was both common and in high abundance along streams on the New England Tablelands (Anstis *et al.* 1998, Heatwole *et al.* 1995), however it can no longer be located in much of this region (Gillespie 2000). *Litoria booroolongensis* has also disappeared from streams in the central and southern parts of its former range (Gillespie 1999, 2000). Based on the level of decline and range contraction observed over the past two decades, *L. booroolongensis* has been listed as Endangered in New South Wales under NSW *Threatened Species Conservation Act 1995*. To date, the conservation management of *L. booroolongensis* has been in its infant stages; only broad surveys and general habitat assessment has been undertaken (Gillespie 1999, 2000).

## **1.3 Study Species 2; The Southern Corroboree Frog (*Pseudophryne corroboree*)**

### ***1.3.1 General Description***

The corroboree frog (*Pseudophryne corroboree*) (Anura: Myobatrachidae) was described by Moore (1953) from a single specimen collected at Round Mountain within Kosciuszko National Park (Colefax 1956). Until recently, only one species of corroboree frog was recognised (Cogger 1992), however, Wells and Wellington (1985) provided a brief argument for recognising the northern form as a separate species, which they named *P. pengilleyi*, with the southern form retaining the name *P. corroboree*. This taxonomic distinction was supported by a geographic analysis of variation in morphology and calls of both corroboree frog species (Osborne *et al.* 1996).

Both *P. corroboree* and *P. pengilleyi* are distinctive and easily recognised from other frog species because of their striking dorsal colour pattern consisting of bright yellow or green longitudinal stripes alternating with black stripes (Osborne *et al.* 1996). The ventral surface is boldly marked with black, yellow and white blotches. A large flat femoral gland is present on each hind limb, and the inner metatarsal tubercle is low and round. Adults reach a length of between 25 and 30 mm. There are a number of differences between *P. corroboree* and *P. pengilleyi*, including considerable genetic divergence (Roberts and Maxson 1989, Osborne and Norman 1991), differences in colour-pattern, morphology and advertisement call (Pengilley 1966, Osborne *et al.* 1996), and skin biochemistry (Daly *et al.* 1990).

### **1.3.2 Distribution and Habitat**

The distribution of *P. corroboree* is restricted to an area of 400 km<sup>2</sup> within an altitudinal band between 1300 m and 1760 m in the Snowy Mountains Region of Kosciuszko National Park (Osborne 1989) (Figure 1.2). Within this area, the broadest part of the species range is in the Jagungal Wilderness area around Mount Jagungal. However, arms of its distribution extent south-east to Smiggin Holes and north to the Maragle Range area (Osborne 1989).

The breeding habitat typically utilised by *P. corroboree* comprises pools and seepages associated with a range of vegetation communities, including sphagnum bogs, wet tussock grasslands and wet heath (Osborne 1990). Within these sites, Osborne (1990) found that *P. corroboree* generally prefers pools that are shallow, have relatively large surface area, low water flow rates, and have a long hydroperiod. Since adults and subadults of both *P. corroboree* and *P. pengilleyi* have been found under logs and other debris in woodland habitat during the non-breeding season (Will Osborne, pers. com.), it is likely that this species also moves out of the breeding habitat and possibly over-winters in the adjacent woodland.

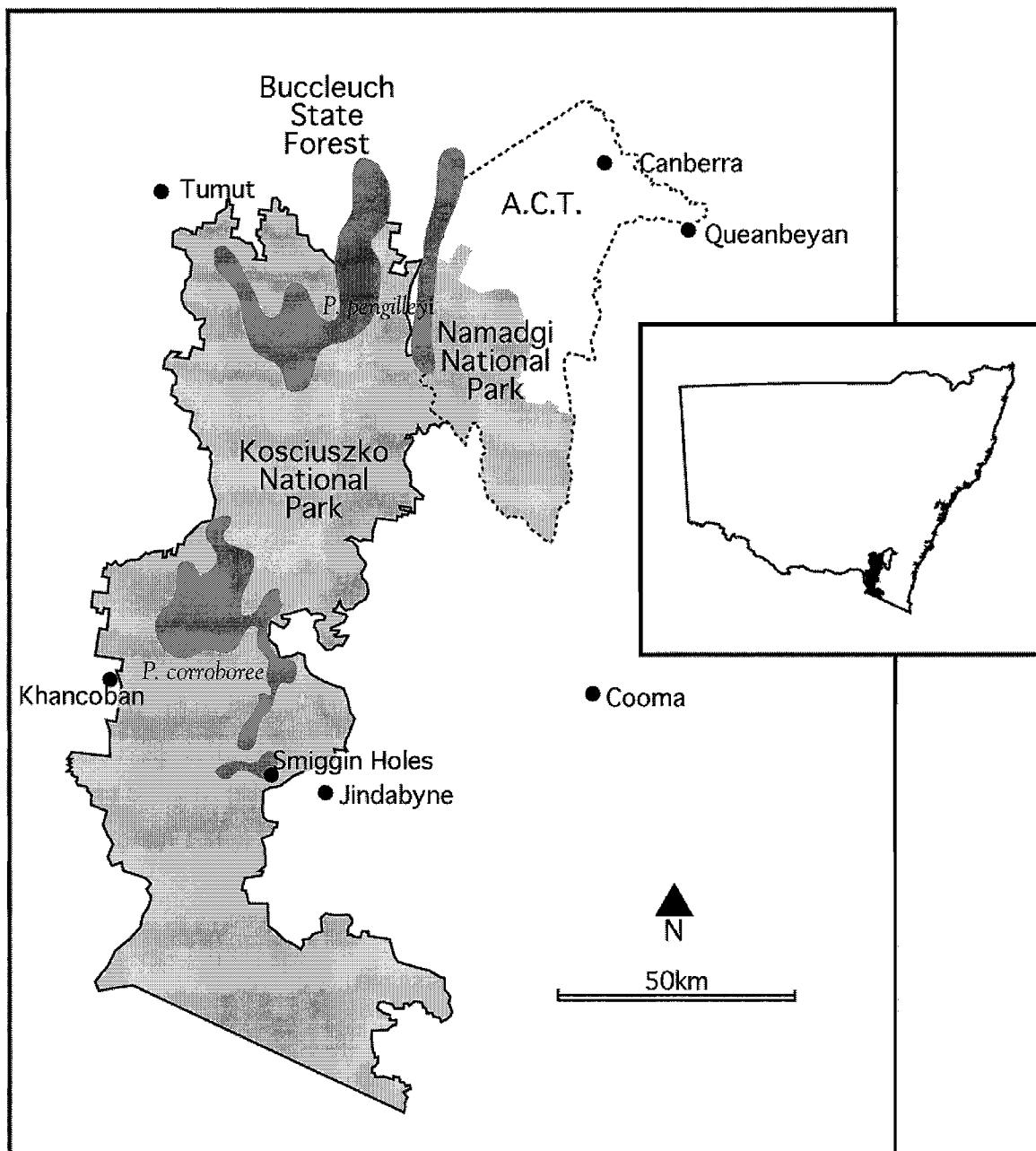


Figure 1.2. The distribution of the two corroboree frog species, *P. corroboree* and *P. pengilleyi*, in relation to nature conservation reserves in south-eastern New South Wales.

### **1.3.3 Life History and Demography**

Gaining information on the population demography of *P. corroboree* has been a major focus of the Recovery Program for this species since 1997. Information on survivorship from egg laying to metamorphosis was obtained for individual clutches across several sites between 1997 and 1999. This study determined average survivorship from egg to metamorphosis for this species to be 20 percent in the absence of early pool drying (Hunter 2000). Early pool drying (i.e. drying of the pools before tadpoles reach metamorphosis) during drought years typically caused 100 percent failure of recruitment to metamorphosis for that year (Hunter 2000). The distribution of nest survivorship for this study was positively skewed, with the majority of nest sites attaining very low recruitment while a small proportion of nests attained very high recruitment (Hunter 2000).

Age to sexual maturity and longevity for *P. corroboree* have been determined using skeletochronology. Age to first reproduction was four years from metamorphosis for the majority of individuals, with a small proportion of individuals attaining sexual maturity in three years (Hunter 2000). There is currently no information on survivorship from metamorphosis to sexual maturity for *P. corroboree*. Annual survivorship estimates for the adult life stage are restricted to information attained on the male breeding population (Hunter 2000). Using skeletochronology the age structure for the breeding adult male population was determined for several populations over several years (Hunter 2000). The oldest individual identified using this technique was nine years old (Hunter 2000). Based on the proportion of individuals in different age classes from one year to the next, annual survivorship for adult males was between 50 and 60 percent (Hunter 2000).

Information on the breeding sex ratio for this species has been attained through comparing the number of males calling to the estimated number of females based on the number of eggs present in nest sites. This comparison has been undertaken over the past nine years and typically suggests that there are more females than males (Hunter 2000). There is however, much variation in the estimated ratio of females to males, particularly at low population size, with some populations appearing to have no females present as no eggs were located in nest sites (Hunter 2001).

#### **1.3.4 Decline and Management**

While detailed surveys were not undertaken for *P. corroboree* prior to the 1980's, anecdotal reports suggest that *P. corroboree* was in high abundance in the Snowy Mountains region during the 1950's and 1960's (Colefax 1956, Pengilley 1966). A detailed survey of the distribution and abundance of *P. corroboree* during the mid 1980's found the species to be in extremely low abundance across a large proportion of its range (Osborne 1989), including areas where it had previously been observed in high numbers. It was concluded that *P. corroboree* had suffered a major decline in abundance (Osborne 1989). At this stage, both *P. corroboree* and *P. pengilleyi* were considered to be a single species, and even though the abundance of *P. corroboree* was very low, it was not considered endangered because *P. pengilleyi* still occupied a relatively large range and still maintained reasonably high abundance in certain areas (Osborne 1989).

Following the surveys conducted during the mid 1980's by Osborne (1989), a long-term monitoring program was established so that future changes in the abundance of *P. corroboree* could be documented (Osborne *et al.* 1999). During the first two seasons of this program, between 1986 and 1987, a rapid decline was observed at many of the monitoring sites, including sites in the Mount Jagungal region that had previously maintained reasonably large populations (Osborne 1989, Osborne *et al.* 1999). The following 10 years of the monitoring program documented the gradual decline and extinction of the frog at many of the long-term monitoring sites, and by 1998 only four of the 23 monitoring sites still contained breeding males (Osborne *et al.* 1999). This decline, and the recognition of the Snowy Mountains populations as a distinct species, resulted in *P. corroboree* being listed as critically endangered under the 1994 IUCN criteria (Tyler 1997). This species has subsequently been listed as Critically Endangered under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*.

In 1996 a recovery plan was written for *P. corroboree* that outlined a three-year process to aid the recovery of this species (Osborne 1996). The recovery plan outlined a number of management actions, including a population enhancement experiment, captive breeding program, pool manipulation and feral pig control (Osborne 1996). This document also outlined research actions that included testing

causal factors of decline, assessing the current distribution and abundance, and determining ecological and demographic characteristics such as current levels of mortality for different life-history stages (Osborne 1996). While many of these actions have subsequently been undertaken, the causal factors of decline remain unknown and effective management actions to halt the ongoing decline of this species have not been developed.

## **1.4 Research Objectives**

The research presented in this thesis was undertaken as part of the recovery programs for both *L. booroolongensis* and *P. corroboree*. The specific objectives associated with this research are outlined in each results chapter. The overall objectives of this research were to:

1. Assess the potential impact of exotic predatory fish species on *L. booroolongensis*.
2. Determine the distribution and critical habitat requirements of *L. booroolongensis* on the South West Slopes of New South Wales.
3. Examine the influence of variance components on the capacity to monitor populations of *L. booroolongensis* using different techniques.
4. Investigate the potential role of *B. dendrobatidis* in the decline of *P. corroboree*.
5. Determine levels of infection with *B. dendrobatidis* attained during the *P. corroboree* tadpole stage, and assess the possibility of using artificial pools to prevent infection.
6. Examine the potential for the common eastern froglet, *Crinia signifera*, to be acting as a reservoir host for *B. dendrobatidis*.
7. Assess the potential to prevent the continued decline and extinction of remnant *P. corroboree* populations through increasing recruitment to metamorphosis.

## **Chapter 2**

### **Experimental Examination of the Potential for Three Non-Native Fish Species to Prey on the Tadpole of the Endangered Booroolong Frog (*Litoria booroolongensis*)**

#### **2.1 Introduction**

Interactions between aquatic predators and tadpoles are often important in influencing the distribution and abundance of frog species (Alford 1999). Tadpoles co-existing with aquatic predators often display defense mechanisms to reduce predation, such as chemical unpalatability (Wassersug 1971, Kats *et al.* 1988, Werner and McPeek 1994), cryptic colouration (Wassersug 1971), protean flight (Taylor 1983), and reduced mobility (Woodward 1983). These defense mechanisms are likely to be the result of co-evolution between predator and prey (Kats *et al.* 1988, Alford 1999), and are often predator or predator group specific (Kats *et al.* 1998). Such defense mechanisms may be less effective against novel predators (Gillespie 2001), and as such it is not surprising that studies have documented declines of amphibian species following the introduction of non-native predatory fishes (Sexton and Phillips 1986, Denoel *et al.* 2005). In addition to direct predation, non-native fishes may also affect native amphibian species through competition (Bradford *et al.* 1993), population fragmentation (Shaffer *et al.* 2000), and the transmission of harmful parasites and pathogens (Kiesecker *et al.* 2001b).

Within Australia a number of exotic predatory fishes have been released into streams and other waterways, typically for the establishment of recreational fisheries or release from the aquarium pet trade (Lintermans 2004). These exotic fish liberations are likely to be impacting on the Australian frog fauna (Gillespie and Hero 1999), with experimental studies demonstrating the potential for introduced trout (*Salmo trutta* and *Oncorhynchus mykiss*) and mosquito fish (*Gambusia holbrooki*) to prey on the tadpoles of a number of Australian frog species, including several species listed as nationally endangered (Morgan and Buttemer 1996, Gillespie 2001, Hamer *et al.* 2002). Apart from these studies, very few other non-native fish species now

established in Australian waters have been assessed for their potential to be impacting on native frog populations (Gillespie and Hero 1999). This is despite the large number of Australian frog species that have declined in recent years (see references in Campbell 1999).

Two species of exotic fish that occur in areas occupied by threatened or endangered species of frog in Australia, but which have not been assessed for their potential to impact on frog populations, are European carp, *Cyprinus carpio*, and redfin, *Perca fluviatilis* (Gillespie and Hero 1999). While adult *C. carpio* are generally considered filter feeders (Koehn *et al.* 2000), mobile aquatic invertebrates may constitute a significant proportion of their diet as juvenile fish (Khan 2003), and so they might also prey on tadpoles. *Perca fluviatilis* is a likely candidate for preying on tadpoles in the shared aquatic environment, as this species has been shown to be primarily predatory in feeding habit (Morgan *et al.* 2002). Assessing the potential for these exotic fish species to prey on the tadpoles of native frogs is important for identifying the range of processes impacting on threatened frog species, and to elucidate potential cumulative or synergistic interactions among these processes (Kiesecker *et al.* 2001a).

In this study I undertook a controlled experiment in artificial enclosures to determine the propensity for three exotic fish species (European carp *C. carpio*, redfin perch *P. fluviatilis*, and mosquito fish *Gambusia holbrooki*), to prey on recently hatched tadpoles of the Booroolong frog, *Litoria booroolongensis*. I also investigated whether alternative prey or habitat complexity influenced the level of predation on tadpoles. *Litoria booroolongensis* is a riverine frog species that occurs predominantly along streams flowing west of the Great Dividing Range in south-eastern Australia (Anstis 2002). Over the past two decades *L. booroolongensis* has undergone a major decline and range contraction (Gillespie and Hines 1999), and is currently listed as Endangered in New South Wales under the *Threatened Species Conservation Act 1999*. A number of factors have been proposed as contributing to the recent decline of this species, including habitat degradation, disease and predation by introduced fish species (Gillespie and Hines 1999). Since many of the former and extant populations of *L. booroolongensis* occur along streams with established populations of *C. carpio*, *P. fluviatilis*, and/or *G. holbrooki*, determining the potential impact of these non-

native fish species is fundamental to our understanding of the processes threatening this frog species.

## 2.2 Methods

### 2.2.1 Obtaining Tadpoles and Fishes

Young tadpoles of *L. booroolongensis* were obtained for this study by locating recently laid egg masses in the field. Egg masses were located by lifting loose rocks in shallow water adjacent to rocky sections of stream where male *L. booroolongensis* were observed congregating for breeding. Three egg masses were located for use in this study from the Goobragandra River (AMG: 626800 6087900). The egg masses were collected from the stream and placed in a 200-liter tub where they remained until hatching. River rocks with algal growth were also collected from the stream and placed in the tub with the egg masses to provide a food source for the tadpoles once they had hatched. The tadpoles were grown for two weeks (until they were between 10 and 12 mm in snout-tail length) before commencement of the experiment.

The fish used in this study were dip-netted at night from stream and pond environments. The *C. carpio* used in this study were obtained from Killimicat Creek (AMG: 513500 6103300) and ranged between 6 and 15 cm in total length. The *G. holbrooki* used in this study were captured from the Tumut community wetland (AMG: 610500 6093700) and ranged between 4.5 and 6 cm in total length. The *P. fluviatilis* used in this study were captured from the Blowering Reservoir (AMG: 615750 6069300) and ranged between 5 and 9 cm in total length. The fish were captured between 24 and 48 hours before being used in this experiment and immediately placed in 200-liter tubs at a maximum density of six fish per tub.

### 2.2.2 Experimental Apparatus and Design

This experiment was conducted in Tumut, New South Wales. The experimental enclosures used in this study were 200-liter grey polyethylene tubs. The tubs were positioned outdoors in eight rows of 12 tubs in an area that received filtered light through the canopy of several large trees. Each tub was filled with tap water and

spiked with 500 ml of pond water, and then left for seven days prior to initiating the experiment. Before the experiment was initiated, the experimental treatments were randomly allocated to each tub. The experimental design was a nested design with four treatments (habitat/alternative food, no habitat/alternative food, habitat/no alternative food, no habitat/no alternative food) for each of the three fish species (*C. carpio*, *P. fluviatilis*, *G. holbrooki*) and a control set of treatments with no fish. This produced a total of 16 different treatment combinations, with six replicates per combination. Each replicate had 20 tadpoles and only one fish (the species varied depending on the treatment allocation), or no fish for the control replicates.

The habitat used in this study was twenty river cobble-stones (between 10 and 20 cm in diameter), which were placed on the bottom of the tub to mimic the rock environment typically occupied by the tadpoles of *L. boorooolongensis* (Anstis *et al.* 1998). The alternative prey used in this study was ten chironomids and ten mosquito larvae. Tadpoles were introduced to each tub and allowed to settle for a 24-hour period before the fish were introduced to their respective tubs. The experiment was run for 48 hours before the fish were removed from the tubs and the number of remaining tadpoles, chironomids, and mosquito larvae counted.

### **2.2.3 Statistical Analysis**

The results of this experiment were analysed using a Bayesian logistic regression model, with the proportion of tadpoles consumed in each treatment nested within each fish species. To examine the influence of the habitat and alternative prey treatments, models of tadpole consumption with and without these treatments (total of five models) were compared to each other based on their Deviance Information Criterion (DIC) (Spiegelhalter *et al.* 2002). Models that had a higher DIC value by greater than two were considered substantially inferior to models with a lower DIC value (similar to the criteria recommended by Burnham and Anderson 2002 for comparing AIC values). This analysis was undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003) which fits Bayesian statistical models using Markov Chain Monte Carlo (MCMC) methods. Uninformative priors were used for all model parameters and convergence of the algorithm was checked by visual examination of the output of three replicate Markov chains with differing starting values (Brooks &

Gelman 1998). Final inferences for the mean and 95 percent credible intervals for each treatment combination were derived by discarding the first 10000 iterations from two chains and retaining the next 90000.

## 2.3 Results

Tadpoles of *L. booroolongensis* were consumed by all the fish species tested in this experiment (Figure 2.1). The greatest difference in the proportion of tadpoles consumed was due to fish species, with *C. carpio* consuming the greatest number of tadpoles, followed by *G. holbrooki*, and *P. fluviatilis* consuming the least (Figure 2.1). A comparison of the DIC values for the five models examined suggested that the addition of the alternative prey (mosquito larvae and chyrinomids) substantially reduced the proportion of tadpoles consumed (Table 2.1). This effect of alternative prey reducing the proportion of tadpoles consumed was observed for *G. holbrooki* and *P. fluviatilis*, whereas the *C. carpio* consumed all *L. booroolongensis* tadpoles whether or not alternative prey were present (Figure 2.1). The provision of rock did not substantially influence the proportion of tadpoles consumed in this experiment (Figure 2.1, Table 2.1). Within each fish species, a substantially greater number of items were consumed in treatments containing the alternative prey (Figure 2.2).

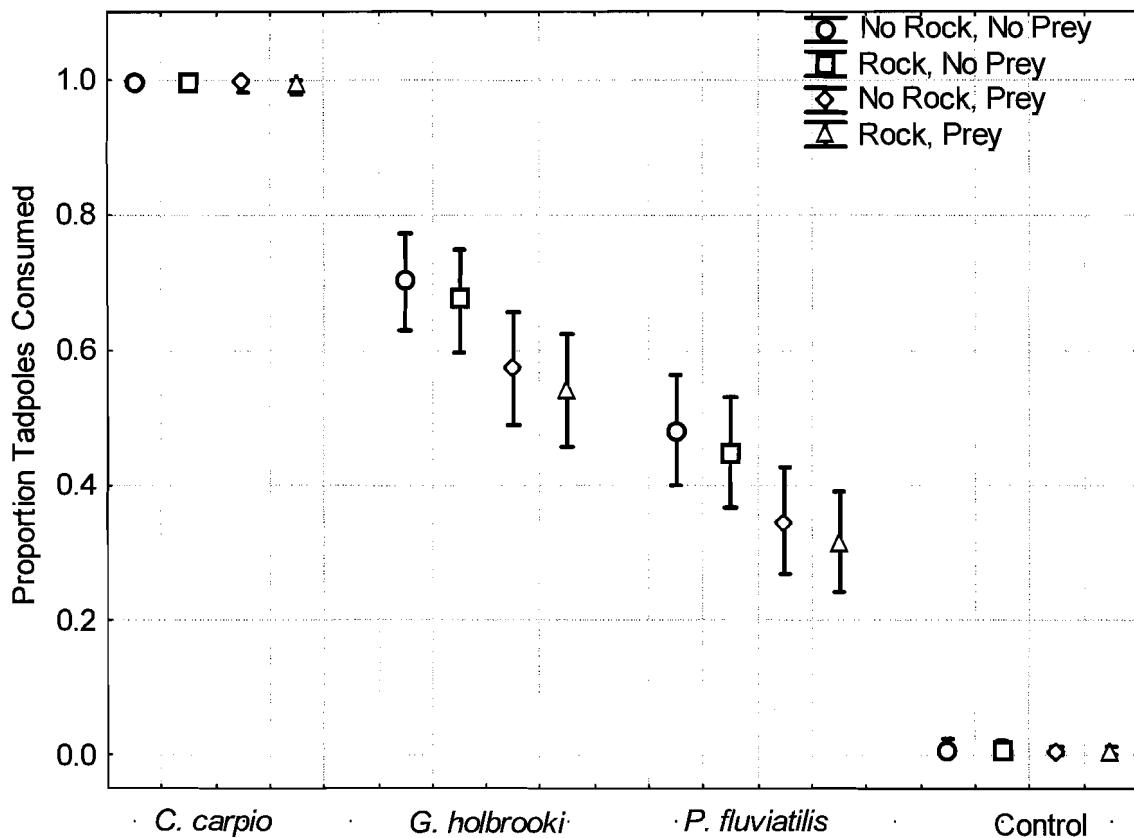


Figure 2.1. Posterior distributions from the Bayesian logistic regression analysis.  
The point is the mean and the error bars are 95% credible intervals.

Table 2.1. Deviance Information Criteria for the five Bayesian logistic regression models examined.

<b>Model</b>	<b>DIC</b>	<b>ΔDIC</b>
Carp + Gambusia + Redfin + Prey	187.728	0
Carp + Gambusia + Redfin + Rock + Prey	188.608	0.880
Carp + Gambusia + Redfin + Rock + Prey + Rock*Prey	190.288	2.560
Carp + Gambusia + Redfin	194.370	6.642
Carp + Gambusia + Redfin + Rock	195.850	8.122

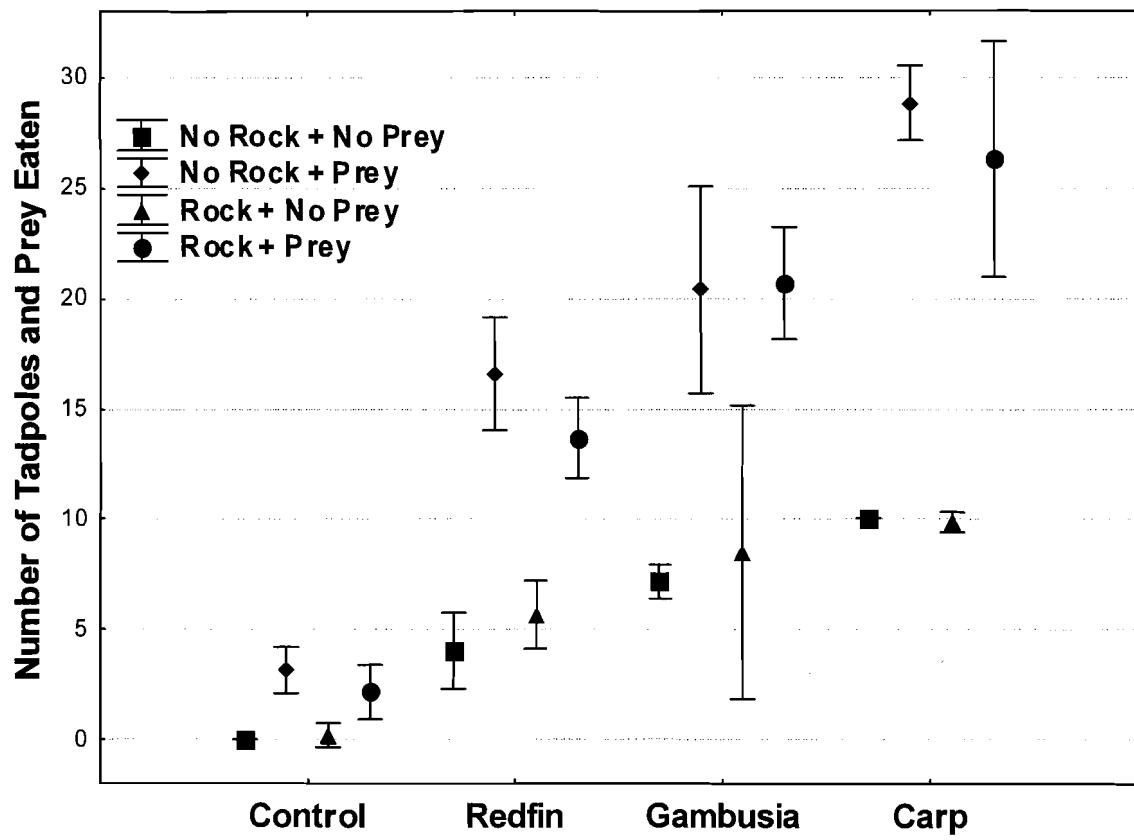


Figure 2.2. Summary of total number of prey items not recovered for the different treatments in this experiment (mean and 95% credible intervals).

## 2.4 Discussion

This study demonstrated that *G. holbrooki*, *P. fluviatilis* and *C. carpio* can prey on the tadpoles of *L. booroolongensis*. Previous studies have demonstrated the capacity for two closely related *Gambusia* species (*G. affinis* and *G. holbrooki*) to consume amphibian tadpoles; through experiments undertaken in artificial enclosures (Komak and Crossland 2000, Hamer *et al.* 2002, Baber and Babbitt 2004), and in the natural environment (Goodsell and Kats 1999). In addition to direct predation, *Gambusia* sp. can also cause non-lethal effects on tadpoles through physical damage (Lawler *et al.* 1999). Given the results of this study, it is likely that *G. holbrooki* prey on *L. booroolongensis* tadpoles in the natural environment as they occupy the same general microhabitats (shallow, relatively slow-flowing sections of stream, Faragher and Lintermans 1997), and *G. holbrooki* occurs in high densities when recently hatched *L. booroolongensis* tadpoles are present in the stream (from November to January, author pers. obs.).

This is the first study in Australia investigating the potential for *P. fluviatilis* and *C. carpio* to prey on amphibian tadpoles. The finding that *P. fluviatilis* has the capacity to prey on *L. booroolongensis* tadpoles is not surprising as other studies have demonstrated the predatory feeders (Morgan *et al.* 2002). While adult *C. carpio* are generally filter feeding in habit (Koehn *et al.* 2000), the very high level of predation by juvenile *C. carpio* in this study is not surprising given the results of field studies that have found mobile aquatic invertebrates in the stomach of wild juvenile *C. carpio* (Vilizzi 1998, Khan 2003). These results suggest that *C. carpio* should not be overlooked as a potential predator of highly mobile aquatic organisms, particularly if they are within the size range of prey items for juvenile *C. carpio*.

The greatest influence on the variation in the number of tadpoles consumed in this study was due to the different fish species examined (Figure 2.1). One possible explanation for this is that *L. booroolongensis* tadpoles vary in palatability to the different species of fish. Gillespie (2001) found that introduced brown trout (*Salmo trutta*) consumed the tadpoles of four different frog species at different rates and attributed this variation to differences in palatability. Similarly, the tadpoles of *L. booroolongensis* appear to avoid predation from at least two native predatory fish

species using chemical unpalatability (see Chapter 3), so chemical defences expressed by the tadpoles of *L. booroolongensis* may afford varying degrees of protection against predation from different exotic fish species. While size differences between the species of fish used in this experiment may also be expected to influence the number of tadpoles consumed, there were no clear patterns associated with fish size and number of tadpoles consumed as the *G. holbrooki* consumed more tadpoles than the larger *P. fluviatilis* used in this study. The behaviour of the fish, or their capacity to settle in the captive environment after being caught from the wild, may have also contributed to these results as the *P. fluviatilis* appeared more sedentary and nervous in the experimental tubs compared to the *G. holbrooki* and *C. carpio*.

The reduction in the proportion of tadpoles consumed by *P. fluviatilis* and *G. holbrooki* when alternative prey were available (Figure 2.1) may be due to the tadpoles of *L. booroolongensis* not being a preferred food item for these two fish species, or that the fish were limited in the amount of food they could consume. The latter explanation seems unlikely since the number of food items consumed by the different fish species in this experiment was considerably greater for the treatments with alternative prey (Figure 2.2). Alternatively, predator avoidance strategies used by *L. booroolongensis* may have been more effective when other prey items were present in the enclosure.

Since rock habitats (cobble banks and bedrock) are the primary habitat occupied by *L. booroolongensis* tadpoles in the natural stream environment (Anstis *et al.* 1998), the failure for the provision of rocks in this experiment to significantly reduce tadpole predation (Figure 2.1) may indicate a limited capacity for this species to avoid predation by these exotic fish species. Results from other studies suggest that the ability for tadpoles to use habitat to avoid predation may vary among amphibian species (Baber and Babbitt 2004). The capacity for a tadpole to express a particular predator avoidance strategy when exposed to an exotic predator is likely to depend on the avoidance strategies used to avoid being eaten by native predators. As mentioned above, the tadpole of *L. booroolongensis* appears to use chemical unpalatability to avoid predation from at least two native fish species (see Chapter 3), and thus may be inherently limited in expressing behavioural strategies to avoid predation by non-native fish species.

With respect to the potential impact that these non-native fish species may be having on *L. booroolongensis* in the wild, extrapolating the results of this study to population level impacts is somewhat limited (Lawler *et al.* 1999, Meissner and Muotka 2006). Nevertheless, given the co-occurrence of one or more of these fish species in the majority of extant *L. booroolongensis* populations (Gillespie 1999), if any of these fish species are negatively impacting on *L. booroolongensis*, then the overall impact is likely to be substantial. Moreover, while *L. booroolongensis* is currently persisting in streams occupied by these exotic fish species (author pers. obs.), there may be complex interactions between these predatory fish species and other threatening processes that mask obvious associations with the presence of *L. booroolongensis*. For example, the impact of *G. holbrooki* may only be significant when there is also substantial habitat disturbance along the stream as the capacity for this fish species to occupy the stream environment appears to increase with increasing riparian habitat disturbance (Faragher and Lintermans 1997). In the case of *C. carpio*, this species has only recently arrived in many of the streams occupied by *L. booroolongensis* along the Southern Tablelands of New South Wales (within the past 20 years, Koehn 2000), and so the ultimate impact of this exotic fish may not be apparent at this stage from correlative studies.

These non-native fish species may also be impacting on *L. booroolongensis* in an indirect manner, such as acting as a vector for harmful pathogens (Kiesecker *et al.* 2001b), or contributing to habitat disturbance. *Perca fluviatilis* is recognised as one of the primary vectors for the epizootic haematopoietic necrosis virus, which has had a substantial impact on native fish populations in eastern Australia (Langdon and Humphrey 1987). The capacity for *C. carpio* to increase suspended sediment loads in the stream environment (Fletcher *et al.* 1985) is likely to disrupt the breeding habitat requirements of *L. booroolongensis* through increasing sediment deposition in the rock crevices used by this species as oviposition sites (Anstis *et al.* 1998).

Despite the large number of Australian frogs undergoing concerning levels of population decline over the past three decades (see references in Campbell 1999), very few of the exotic fish species established in Australian waters have been examined for their potential impacts on sympatric threatened frog species (Gillespie and Hero 1999). This study has demonstrated the capacity for three non-native fish

species to be impacting on *L. booroolongensis*. While the potential impacts of these exotic fish species on *L. booroolongensis* have not been examined in the natural environment as yet, with respect to the conservation management of *L. booroolongensis* it should be assumed that negative impacts are likely. In particular, any actions that may extend the range of these exotic fish species along streams supporting populations of *L. booroolongensis* should be considered a threatening process. Given the demonstrated capacity for exotic fish species to negatively impact on frogs at the population level (Vredenburg 2004), assessing the potential impacts of exotic fish species on Australia's threatened frog fauna is fundamental to the effective conservation management of these species.

## **Chapter 3**

### **An Experimental Examination of the Propensity for Introduced Trout to Prey on the Tadpole of the Endangered Booroolong Frog (*Litoria booroolongensis*)**

#### **3.1 Introduction**

Predatory fish species can regulate amphibian populations through predation on the egg and tadpole life stages (Alford 1999). The capacity for some amphibian species to breed in aquatic environments occupied by predatory fish is typically the result of coevolution between predator (fish) and prey (tadpole) resulting in tadpole defence mechanisms (Kats *et al.* 1988, Sih *et al.* 1988, Werner and McPeek 1994). A number of experimental studies have shown that tadpole defence mechanisms are often predator or predator group specific, and may have limited effectiveness against fish species for which there has been minimal coevolution (Kats *et al.* 1988, Gillespie 2001). Hence, it is not surprising that the introduction of a non-native predatory fish species may result in the decline of native frog populations (Denoel *et al.* 2005).

The release of non-native fish species may also have indirect impacts on amphibian populations. Fish predation may exclude some amphibian species from habitats or sections of streams, resulting in greatly reduced and fragmented populations which are more prone to loss of genetic variation or local extinction (Bradford *et al.* 1993, Shaffer *et al.* 2000). Moreover, some diseases can infect both fish and amphibians, so the movement of fish to different water bodies may inadvertently result in the spread of pathogens that are harmful to amphibians (Kiesecker *et al.* 2001b). Water used to transport fish may also contain pathogens that are harmful to amphibians, even if the fish themselves are not infected (Johnson and Speare 2003). Fish may also compete with tadpoles for resources and so indirectly reduce their fitness (Resartarits 1995).

Because of their popularity as recreational angling species, several fish species within the family salmonidae have been extensively liberated outside their natural range. Salmonids are highly efficient predators, and so not surprisingly, their recent

introduction into waterways in various parts of the world has been associated with the decline of native frog species (Mathews *et al.* 2001, Lowe and Bolger 2002, Bosch *et al.* 2006, Orizaola and Brana 2006). The capacity for non-native salmonids to regulate the population density of a native amphibian species was particularly well demonstrated by Vredenburg (2004), who found that the removal of two salmonids from high altitude lakes in California resulted in the rapid recovery of *Rana mucosa* populations in those water bodies. While this study demonstrated a clear negative impact from the introduced salmonids, it is also clear from correlative distributional studies and controlled experiments in artificial environments that susceptibility to salmonid predation may vary greatly among amphibian species (Gillespie 2001, Orizaola and Brana 2006).

For over a century two salmonid species, brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*, have been widely released into rivers and dams in south-eastern Australia, and have established self-sustaining populations (McDowall 1996). Predation by introduced trout has been identified as a potential causal factor in the decline of one frog species in south-eastern Australia, the spotted tree frog, *Litoria spenceri*, through a combination of field observations, palatability experiments, and an in-stream experiment (Hunter and Gillespie 1999, Gillespie 2001). Since several other threatened frog species occur in streams where *O. mykiss* and *S. trutta* have become established, it has been strongly recommended that further studies examining the impact of introduced trout on other threatened frog fauna in south-eastern Australia be undertaken (Gillespie and Hero 1999).

Included in the list of riverine frog species that have undergone recent population declines in south-eastern Australia is the Booroolong frog, *Litoria booroolongensis* (Gillespie and Hines 1999). Recent surveys for *L. booroolongensis* demonstrated that this species has declined from more than half of its historic known distribution over the past two decades (Gillespie 1999, Gillespie 2000). Currently, the majority of known extant populations for *L. booroolongensis* exist along western flowing streams on the Southern and Central Tablelands of New South Wales, however declines in this region have also been documented and many of the persistent populations occur at very low densities along highly degraded streams (Hunter and Gillespie 1999, Gillespie 1999). *Litoria booroolongensis* is currently listed as Endangered in New

South Wales (NSW *Threatened Species Conservation Act 1995*), indicating that “it is likely to become extinct unless the circumstances and factors threatening its survival or evolutionary development cease”.

Several factors have been proposed as contributing to the decline of *L. booroolongensis*, including; habitat degradation, disease caused by infection with the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, and predation by introduced fish species (Gillespie and Hines 1999). The hypothesis that trout is negatively affecting *L. booroolongensis* has important social and economic implications, as recreational trout fishing is a major industry in south-eastern Australia. Thus, adequately testing this hypothesis is important so that the management of *L. booroolongensis* can be implemented in a manner that meets obligations to threatened species legislation and addresses public interest in the future of trout fishing. The research presented here represents the first stage in assessing whether introduced trout are significantly affecting extant populations of *L. booroolongensis*. The broad aim of this research was to undertake controlled experiments in artificial enclosures to gain insight into possible processes operating in the natural stream environment. The specific aims of this research were to:

1. Test the palatability of hatchling tadpoles of *L. booroolongensis* to both introduced trout and two sympatric native predatory fishes.
2. Test whether the provision of rock microhabitat affords protection from trout predation to hatchling and mature tadpoles of *L. booroolongensis*.
3. Test whether juvenile trout that are field reared or hatchery reared have different propensities to prey on hatchling *L. booroolongensis* tadpoles.

## 3.2 Methods

### 3.2.1 Fish and Tadpole Study Species

The primary aim of this study was to assess the potential susceptibility of *L. booroolongensis* to introduced trout. Tadpoles of two other frog species were also incorporated into this study; rocky river frog (*Litoria lesueuri*) and spotted marsh frog (*Limnodynastes tasmaniensis*). Because *L. lesueuri* was incorporated into an earlier study assessing the impact of introduced trout on south-eastern Australia riverine frogs (Gillespie 2001), including *L. lesueuri* in the present study allows comparisons to be made with the earlier study. *Litoria lesueuri* has also shown limited signs of population decline or range contraction (Gillespie and Hines 1999) and so given its close relatedness to *L. booroolongensis* (Anstis *et al.* 1998) the relative palatability of these two species to introduced trout is of interest. *Limnodynastes tasmaniensis* was used in this study as a palatable control species, as this species breeds in pond environments (Barker *et al.* 1995) and so would be less likely to possess fish predation avoidance strategies.

In addition to the use of brown trout, *Salmo trutta*, and rainbow trout, *Oncorhynchus mykiss*, two native fish species were also incorporated into this study; two-spined blackfish, *Gadopsis bispinosus*, and mountain galaxias, *Galaxias olidus*. These two native predatory fish species commonly occur in sympatry with *L. booroolongensis* (Gillespie 1999, 2000). Hence, testing their potential to prey on *L. booroolongensis* tadpoles is important for assessing the potential contribution of fish predation in the population regulation of this species prior to the introduction of trout.

The following experiments were undertaken in an open-walled shed managed by the Tumut Acclimatisation Society located near the Tumut River within Tumut Township. The water supply for this facility was pumped directly from the Tumut River with no treatment or processing.

### **3.2.2 Obtaining Experimental Tadpoles and Fish**

Tadpoles used in these experiments were obtained through the collection and rearing of three freshly laid egg masses for each of the three species incorporated into this study. Egg masses of *L. booroolongensis* were obtained from the Goobragandra River (AMG: 629300 6083300) while egg masses of *L. lesueuri* were obtained from Killimicat Creek (AMG: 614500 6102800). Egg masses of *Limnodynastes tasmaniensis* were obtained from rice fields in the Griffith district of New South Wales. All egg masses were collected within the same week so that the timing of hatching and size of tadpoles upon commencing the first experiments was similar between each of the species. After transportation from the field collection site, each egg mass was placed in an individual nine litre container filled with fresh river water. Upon hatching, the tadpoles were placed in 200-litre tubs, with each species being housed in a separate tub, and feed ad libitum a combination of fresh squid and frozen endive. The tadpoles were then reared for one week in these tubs prior to the commencement of experiments one and two. At the commencement of these experiments the three species of tadpoles were at Gosner stage 24 (Gosner 1960) and measured between 3 and 4 millimetres in body length. Tadpoles of *L. booroolongensis* were further reared in the 200-litre tubs for use in experiment three. This involved continued feeding as described above. A 20 percent water change was undertaken once every three days. At the commencement of experiment three the *L. booroolongensis* tadpoles were between Gosner stage 31 and 35 (Gosner 1960) and measured between 13 and 18 millimetres in body length.

The wild fish used in these experiments were collected using a backpack electro-fishing unit. Upon capture the fish were immediately transported to the experimental tubs. The juvenile *S. trutta* and the adult *G. bispinosus* were collected from the Goobragandra River (AMG: 629300 6083300) and Stony Creek (AMG: 629800 6083500). The adult *G. olidus* were collected from Brungle Creek (AMG: 6230100 6103900). The one and two year old trout used for experiment three were collected from Stony Creek (AMG: 629800 6083500) and from Walls Creek (AMG: 623500 6086100). All the fish collection sites either contain extant populations of *L. booroolongensis* or are connected and in close proximity to populations of this frog species.

### **3.2.3 Experimental Apparatus and Design**

#### *Experiment one:*

This experiment involved comparing the palatability of three different hatchling tadpole species to three different fish species. Each treatment had seven replicates and was randomly assigned to an enclosure after all the enclosures had been set-up. Each replicate only had one individual fish, with the species varying according to the treatment allocation. This made a total of 63 enclosures for the entire experiment. The experimental enclosures were nine-litre clear plastic tanks (17 cm wide, 19 cm high, 28 cm long) with a mesh lid to prevent the fish from escaping. The bottom half of the tanks were covered on the outside with brown contact paper so that the fish were not exposed to constant visual stimulus from outside the enclosures. Each enclosure had a flow through water system whereby water entered the top of the enclosures, at a rate of 90 litres per hour, and drained through mess-covered holes at the back of the enclosures (mesh diameter 0.5 millimetre). The water entering the enclosures dropped from a height of five centimetres to create a small amount of turbulence to enhance oxygen levels in the water. The water temperature remained reasonably constant at 19 degrees Celsius through the duration of the experiment. The fish were randomly allocated to enclosures and given a settling period of 24 hours prior to commencement of the experiment. After this settling period, ten randomly chosen tadpoles of one of the three frog species were introduced to each enclosure according to the treatment allocation. The experiment was run for 48 hours. Upon terminating the experiment, the fish were removed from the enclosures, measured using vernier callipers for their body length, and the number of tadpoles remaining in each enclosure counted.

#### *Experiment two:*

This experiment involved comparing levels of predation on hatchling *L. booroolongensis* tadpoles by juvenile *S. trutta* that were hatchery reared versus wild reared, and whether this predation was reduced when rocks were placed in the enclosures. Each fish treatment had ten replicates and were randomly assigned to an enclosure after all the enclosures were set-up. The ‘no fish’ treatments had five replicates and were used to control for the possibility that tadpoles may have died due

to reasons other than fish predation, or that not all the tadpoles could be recovered. This made a total of 50 enclosures for the entire experiment. The experimental enclosures were 200-litre dark grey polyethylene tubs (dimensions = 450 mm height, 600 mm wide, 900 mm long). Each enclosure had a flow through water system whereby water entered the top of the enclosures, at a rate of 90 litres per hour, and drained through a stainless steel mesh opening at one end of the enclosure (mesh diameter = 0.4 millimetre). The water entering the enclosures dropped from a height of 15 centimetres to create turbulence to enhance oxygen levels within the tubs. The rock treatment involved placing 20 river cobbles with a diameter of between 10 and 20 centimetres in the bottom of the enclosure. The fish were randomly allocated to enclosures according to their respective treatments and given a settling period of 24 hours prior to commencement of the experiment. After this settling period, twenty randomly chosen hatchling *L. booroolongensis* tadpoles were assigned to each enclosure. The experiment was then run for 72 hours. Upon terminating the experiment, the fish were removed from the enclosures, measured using vernier callipers for their body length, and then placed in a large holding tub. The 'rock' treatment enclosures then had their rocks removed and the number of tadpoles in each tub was then counted. This involved repeated searches and dip net removal of tadpoles until two searches had been undertaken without any further tadpoles being found. This process was necessary because the enclosures were large and it was easy to miss the small hatchling tadpoles during individual searches.

*Experiment three:*

This experiment involved assessing the propensity for adult *O. mykiss* to prey on large *L. booroolongensis* tadpoles and whether the provision of rocks in the enclosures afforded protection to the tadpoles. There were ten replicates for each of the rock and no-rock treatments, and five replicates for the no fish treatment. A total of 30 enclosures was used in this experiment. The enclosures and apparatus used in this experiment were the same as those used in experiment two (refer above), with two modifications. The first was that air was bubbled through the water of each enclosure to provide greater oxygenation of the water to cater for the larger fish. The second was that mesh coverings were placed over each enclosure to prevent the fish from escaping (mesh diameter = 15 millimeters). Again, after the fish were randomly

allocated to enclosures, they were given a 24-hour settling period before ten randomly chosen tadpoles were introduced to each enclosure. The experiment was run for 72 hours. Upon terminating the experiment, the fish were removed from the enclosures, measured using vernier callipers for their body length, and then placed in a large holding tub. Rocks were then removed from the rock treatment enclosures and the number of tadpoles remaining in each tub counted.

### ***3.2.4 Statistical Analysis***

Differences in the proportion of tadpoles consumed by each fish species in experiment one was analysed using a Bayesian logistic regression model, with the proportion surviving for each tadpole species being nested within each fish species. For experiment two, a Bayesian logistic regression model was used to compare the proportion of tadpoles surviving according to the 3 by 2 factorial design for fish type (hatchery, wild or no-fish) and habitat (rock or no-rock). Similarly, a Bayesian logistic regression model was used to compare the proportion of tadpoles surviving in experiment three according to the 2 by 2 factorial design for fish type (fish or no-fish) and habitat (rock or no-rock). These analyses were undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003) which fits Bayesian statistical models using Markov Chain Monte Carlo (MCMC) methods.

Uninformative priors were used for all model parameters and convergence of the algorithm was checked by visual examination of the output of three replicate Markov chains with differing starting values (Brooks & Gelman 1998). Final inferences for the mean and 95 percent credible intervals for each treatment combination were derived by discarding the first 10000 iterations from two chains and retaining the next 90000.

### 3.3 Results

The results for experiment one identified differences in the proportion of tadpoles consumed within and among each fish species (Figure 3.1). Within each fish species the proportion of *L. lesusuri* and *L. booroolongensis* tadpoles consumed was similar, with *S. trutta* consuming more of these two tadpoles species than either of the two native fish species (*G. bispinosus* and *G. olidus*) (Figure 3.1). Both *G. bispinosus* and *G. olidus* typically consumed only one or none of the two riverine tadpole species (*L. lesusuri* and *L. booroolongensis*). Within all three fish species, the proportion of tadpoles consumed was greater for the pond breeding species (*L. tasmaniensis*), than for the two river breeding species (*L. lesusuri* and *L. booroolongensis*), with *S. trutta* consuming more *L. tasmaniensis* tadpoles than the other two fish species (Figure 3.1).

The results for experiment two observed no difference in survivorship for the *L. booroolongensis* tadpole when rock habitat was provided in the tubs (Figure 3.2). There were significant differences in tadpole survivorship among the different fish treatments, with hatchery *S. trutta* to consuming more tadpoles than the wild *S. trutta* (Figure 3.2). As with experiment two, experiment three also observed no effect of rock habitat on tadpole survivorship, and also a significant decrease in the proportion of *L. booroolongensis* tadpoles surviving in the presence of the fish (in this case adult *O. mykiss*) (Figure 3.3).

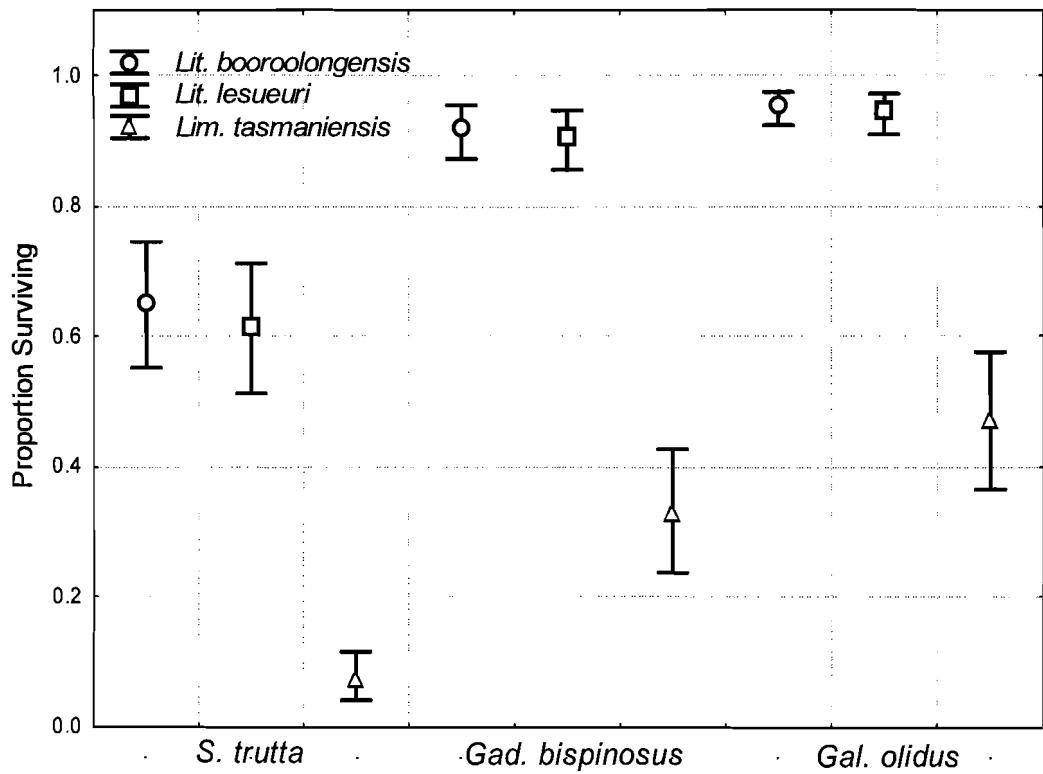


Figure 3.1. Posterior distributions from the Bayesian logistic regression model comparing the proportion of three tadpole species surviving in the presence of three fish species. The points represent the mean and error bars represent the 95% credible intervals.

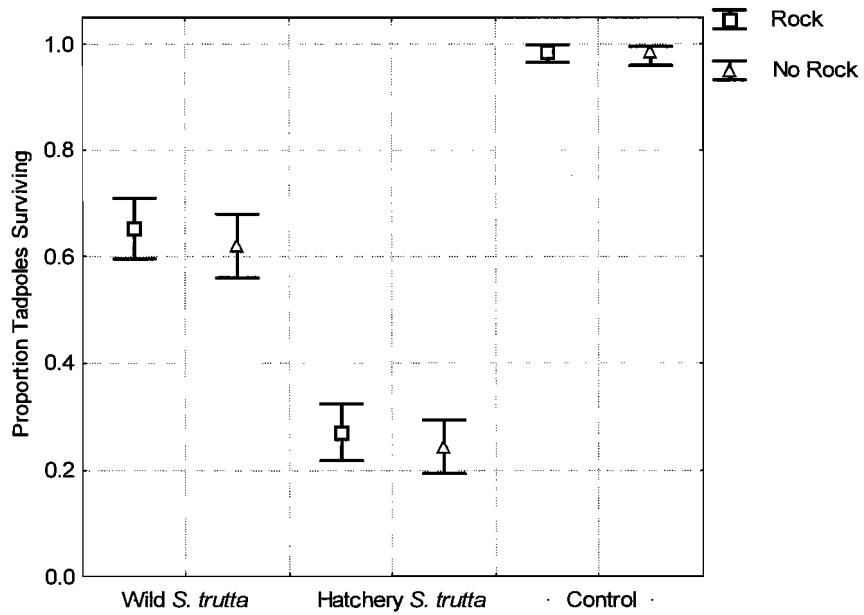


Figure 3.2. Posterior distributions from the Bayesian logistic regression model comparing the proportion of *L. booroolongensis* tadpoles surviving in the presence of wild versus hatchery reared *S. trutta* with rock and no-rock treatments. The points represents the mean and error bars represent the 95% credible intervals.

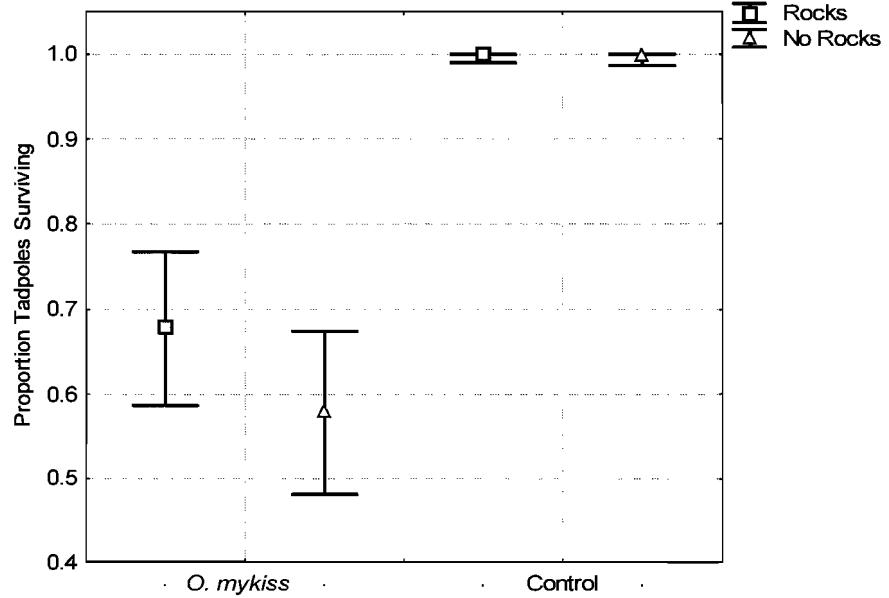


Figure 3.3. Posterior distributions from the Bayesian logistic regression model comparing the proportion of *L. booroolongensis* tadpoles surviving in the presence of adult *O. mykiss* when rock is provided and not provided. The points represents the mean and error bars represent the 95% credible intervals.

### 3.4 Discussion

The results of this study are consistent with the findings of Gillespie (2001) in that very few tadpoles of the riverine species (*L. booroolongensis* and *L. lesueuri*) were eaten by the two native fish species (*G. bispinosus* and *G. olidus*), but a high proportion were eaten by the introduced trout species (*S. trutta*) (Figure 3.1). In addition to this, the pond breeding frog species, *L. tasmaniensis*, used in this experiment was consumed by all three fish species (Figure 3.1). Since the native fish consumed the *L. tasmaniensis* tadpoles in this experiment, it is reasonable to assume that the tadpoles of *L. booroolongensis* and *L. lesueuri* possess defence mechanisms that are effective against predation from these native fish but not from the juvenile *S. trutta*, at least in a simple tank environment. It is likely that this defence mechanism is chemical unpalatability, as there was very limited capacity to express behavioural strategies to avoid predation in the small experimental tanks. Chemical unpalatability in tadpoles is a common defence strategy used by amphibians that breed in water bodies occupied by predatory fish species (Kats *et al.* 1988, Werner and McPeek 1994). It is also typical that tadpole defence mechanisms may be effective against predation from sympatric native fish species but ineffective at deterring predation from fish species for which they have had no long-term history of co-existence (Kats *et al.* 1988, Gillespie 2001).

Another feature of the results of this study was that hatchling tadpoles of *L. booroolongensis* and *L. lesueuri* had similar levels of palatability to *S. trutta* (Figure 3.1). It is likely that the *S. trutta* had the capacity to consume more tadpoles since the number of *L. tasmaniensis* tadpoles consumed by *S. trutta* in this study was significantly greater than for either species of riverine tadpole. This suggests that predation on the two riverine tadpoles by *S. trutta* was inhibited to some extent. While there was considerable variation between replicates in the number of tadpoles eaten for both riverine species (Figure 3.1), indicating the potential for differences in palatability to go undetected, the similar level of predation on *L. booroolongensis* and *L. lesueuri* is not surprising given the close relatedness of these two taxa (Anstis *et al.* 1998). Gillespie (2001) found the palatability of *L. lesueuri* to *S. trutta* was less than that for the tadpoles of two other south-eastern Australian species he examined, and suggested that this may indicate reduced vulnerability of *L. lesesuri* to trout predation

in the wild. Gillespie (2001) also suggested that this might explain the greater persistence and abundance of *L. lesueuri* in upland streams than these other species. If this is correct then the results of the present study suggest that *L. booroolongensis* may also have lower susceptibility to trout predation than other riverine frog species in south-eastern Australia. Certainly, the tadpoles of both *L. lesueuri* and *L. booroolongensis* are often observed in high abundance in streams known to support high densities of trout (author pers. obs.). While the potential for trout to consume the tadpoles of these two species may be similar, this may not necessarily equate to a similar level of influence on the adult population. *Litoria booroolongensis* has lower fecundity and a shorter life-cycle than *L. lesueuri* (Gillespie 2001, author unpub. data), and so would potentially respond differently at the population level to a given level of tadpole predation. Similarly, susceptibility to trout predation at the population level may also vary between these two species depending on the degree to which they are susceptible to other factors such as disease or habitat degradation.

The results of this study also found that hatchery reared trout consume more *L. booroolongensis* tadpoles than wild trout in the experimental environment (Figure 3.2). These results should only be used to infer the potential for behavioural differences between hatchery and wild *S. trutta*, rather than the likelihood that the hatchery fish are more voracious tadpole predators. It is certainly possible that this result was an artefact of the methodologies used in this study. The capture method for the wild *S. trutta* of electro-fishing may have influenced these results, as the hatchery *S. trutta* were not subjected to this procedure. Moreover, the wild *S. trutta* may have also found the experimental enclosures a more foreign environment than the hatchery fish, which had been reared in artificial tubs all their life.

Since the proportion of tadpoles consumed in Experiments two and three were similar, and the provision of rock did not afford protection to the tadpoles in either experiment (Figures 3.2 and 3.3), this study failed to detect an ontogenetic shift in *L. booroolongensis* tadpoles for two possible defence strategies against introduced trout predation (unpalatability, seeking refuge). This comparison is somewhat compromised by the use of different trout species between the experiments, which was due to limitations on the availability of the different fish species at the time the experiments were conducted. The lack of a significant difference in the level of

predation between the treatments of rocks and no-rocks in either experiment (Figures 3.2 and 3.3) suggests that either seeking refuge is not a behavioural defence strategy utilised by *L. booroolongensis* tadpoles to avoid fish predation, or that their use of this strategy is not effective against either trout species.

The implication that trout may be impacting on populations of *L. booroolongensis* has been inferred previously from studies on other frog species (Gillespie and Hero 1999). The results of this study suggest that it is very likely that trout would be preying on *L. booroolongensis* tadpoles in the natural stream environment, particularly since what appears to be the primary defence mechanism for avoiding predation by native fish species (chemical unpalatability) has limited effectiveness for preventing trout predation. However, the potential implications of trout predation at the population level for *L. booroolongensis* is not clear as this species has persisted in many streams in the presence of trout for over a century. This does not discount the potential for the impact of trout to be either cumulative or operate synergistically with other threatening processes (Alford and Richards 1999, Kiesecker *et al.* 2001a), making correlative patterns in the field less obvious. Given the dramatic decline experienced by *L. booroolongensis* in recent years (Gillespie and Hines 1999), a prudent approach to the conservation management of this species would be to avoid actions that either introduce or increase the density of trout in water bodies supporting extant populations of this endangered frog species.

## **Chapter 4**

### **Evaluating Monitoring Strategies for the Endangered Booroolong Frog (*Litoria booroolongensis*)**

#### **4.1 Introduction**

In the early 1990's it was announced that amphibian species from many parts of the world were undergoing concerning rates of decline and extinction (Blaustein and Wake 1990, Phillips 1990). This was met with much criticism that these claims were unsubstantiated and that owing to the capacity for amphibian species to exhibit large fluctuations in abundance, these apparent declines could not be differentiated from natural fluctuations (Blaustein *et al.* 1994b, Pechmann and Wilbur 1994). This debate highlighted the need to implement rigorous monitoring programs to gain a greater understanding of amphibian population demographics and temporal fluctuations in abundance, particularly for species suspected to have undergone recent declines (Pechmann and Wilbur 1994, Marsh 2001). Apart from gaining the necessary information to interpret temporal changes in population abundance, monitoring is also an important component of determining the specific factors driving population fluctuations. With respect to threatened species recovery programs, monitoring is the cornerstone for assessing management strategies aimed at population recovery, or determining the impact of perceived threatening processes (Dickman 1996, Yoccoz *et al.* 2001).

While population monitoring is an integral component of wildlife conservation and management, implementing an effective monitoring program can be logically and technically difficult to achieve. Biological factors influencing variance components of monitoring results need to be considered, as they have the capacity to obscure trends of interest, or lead to false conclusions (Reed and Blaustein 1995, Thompson *et al.* 1998). This is typically the case for amphibian populations, because they often display large temporal fluctuations in population size (Pechmann *et al.* 1991, Meyer *et al.* 1998, Green 2003), and may also exist as metapopulations where local extinction of areas/patches across the landscape occurs frequently (Sjogren-Gulve 1994, Skelly

*et al.* 1999). The nature of temporal trends in amphibian population numbers is also an important consideration, because amphibian populations may be declining more frequently than increasing, even when the overall trend is stable (Meyer *et al.* 1998, Alford and Richards 1999). Hence, developing an effective monitoring program for a particular amphibian species requires an understanding of the species' temporal and spatial population dynamics. If this information is lacking, then the initial implementation of a monitoring program should incorporate an appropriate demographic study to address this deficiency.

A major logistical constraint in undertaking monitoring programs is the limitation imposed by a lack of available resources. Such constraints often influence the methods employed in monitoring programs. The key issue here is that cheaper monitoring methods are typically less accurate and have associated biases and assumptions. One approach to monitoring wildlife is to use index measures of abundance such as counts of individuals or their signs such as footprints (Caughley 1977). There has been considerable debate regarding the appropriateness or effectiveness of indices of abundance, with some authors claiming they should never be used in monitoring programs (Anderson 2001, Schmidt 2004). Much of this criticism centres on the typically unrealistic assumption of constant detectability (Schmidt 2003, 2004). However, Bart *et al.* (2004) pointed out that constant detectability is not necessarily critical for effective trend estimates, but rather that there are no temporal trends in detectability across important sampling units. Regardless of the debate over detectability, indices of abundance continue to be one of the most common techniques used in wildlife monitoring, particularly in the monitoring of amphibian populations (Alford and Richards 1999).

In recent years there has been an increasing focus on the application of presence/absence surveys to determine occupancy rates across a large number of sites as a means to monitoring temporal trends and the conservation status of animals (MacKenzie *et al.* 2002, MacKenzie and Royle 2005). One advantage of this approach is that the results may be more applicable to the overall status or population trends for a particular species, especially if the species operates in metapopulations where local population dynamics may not be indicative of the species population trends across the landscape (Hencar and M'Closkey 1996). Another advantage is that

presence/absence surveys typically require fewer resources and less specialised expertise to implement when compared with methods involving capture and marking of individual animals. For these reasons, site occupancy modelling is becoming increasingly applied and advocated for the monitoring of amphibian species (Knapp *et al.* 2003, Trenham *et al.* 2003, Dodd and Dorazio 2004, Schmidt 2005).

Included in the list of Australian frog species that have undergone recent population declines is the Booroolong frog, *Litoria booroolongensis* (Gillespie and Hines 1999). *Litoria booroolongensis* is a riverine frog species that was known to occur predominately along the western fall of the Great Dividing Range in New South Wales, Australia (Anstis 2002). Over the past two decades *L. booroolongensis* has declined from a substantial portion of its former known range (Gillespie and Hines 1999), and the species is currently listed as endangered in New South Wales under the *Threatened Species Conservation Act* 1995. An important feature of this species persistent populations is that they occur along streams in various states of habitat modification; from completely cleared country used for stock grazing to relatively undisturbed areas within National Parks (see Chapter 5). As such, an important component of the recovery program for *L. booroolongensis* is to determine the population status/trajectory for this species in areas subjected to a variety of land management practices.

In this study, I investigated techniques that may be used to survey for, and monitor, populations of *L. booroolongensis*. I used mark-recapture techniques to investigate temporal trends in estimates of this species' abundance and detection probability. I used this information on temporal trends in abundance to compare our capacity to detect population trends over time when using abundance estimates (mark-recapture) and indices of abundance (raw counts). I also used a site occupancy model to investigate the capacity to use spotlight surveys to determine the presence/absence of *L. booroolongensis* at the scale of 300-metre stream sections, and also within discrete breeding habitats along the stream. Given the capacity for large variation among amphibian species in demographic parameters (Duellman and Trueb 1986), assessing the merits of using different monitoring techniques is an essential initial stage in implementing a recovery program for threatened amphibian species.

## **4.2 Methods**

### **4.2.1 Study Area**

This study was undertaken between 1999 and 2004. Study sites were within the Murrumbidgee catchment on the South West Slopes of New South Wales. The mark-recapture of individual frogs was undertaken along three 500-metre transects on three streams; Brungle Creek, Goobragandra River and Mountain Creek (Figure 4.1). Due to the property owner restricting my access, the Mountain Creek transect was only sampled during 2000 and 2001. The Goobragandra River transect was then incorporated into this study as a substitute for not being able to sample the Mountain Creek site. The assessment of night-time spotlighting to determine the presence/absence of *L. booroolongensis* was undertaken along fifteen 300-metre transects spread across five streams; Brungle Creek, Goobragandra River, Adelong Creek, Gilmore Creek and Bombowlee Creek (Figure 4.1).

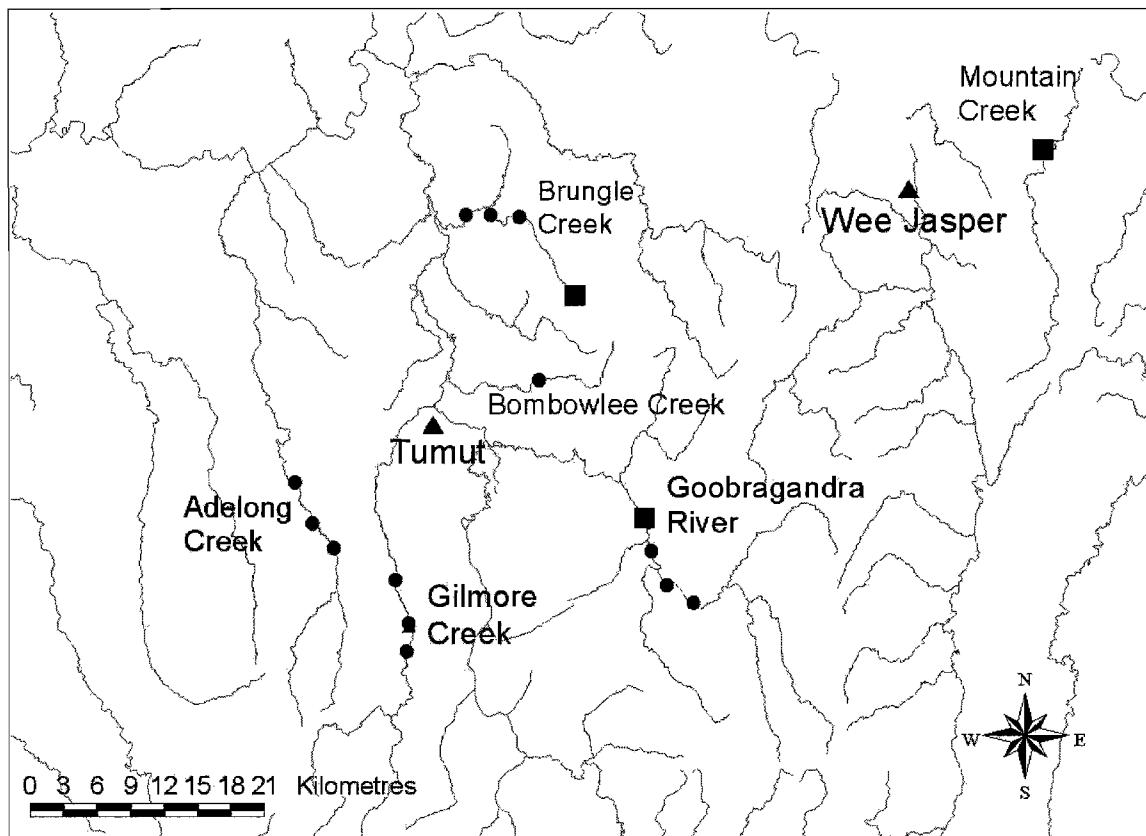


Figure 4.1. Map of study site localities. Solid squares are the localities of areas where mark-recapture was undertaken, while solid circles are the localities where an assessment of presence/absence surveys was undertaken.

#### **4.2.2 Mark-Recapture**

Mark-recapture was used to estimate the number of male and female *L. booroolongensis* present along three 500-metre stream transects. Each transect was visited on four separate occasions through the breeding season (start of November to mid January), with each census being three weeks apart. Censuses were only undertaken under conditions considered conducive to detecting this species; temperatures greater than 10 degrees, not raining, and not within three days after substantial rain events that caused raised water levels. Each census involved walking the stream transect at night and searching by spotlight along the immediate riparian zone to within four meters of the waters' edge for active *L. booroolongensis*. Frogs were hand-captured and new individuals given a unique mark by removing combinations of toes (maximum of three toes) (following Hero 1989). Frogs were handled wearing a new pair of latex gloves, and the scissors sterilized in 90 percent ethanol after toe-clipping each new frog. Male and female adult *L. booroolongensis* were differentiated by the presence of raised and pigmented nuptial pads on the males inner finger. After processing, each frog was released at the point of capture.

#### **4.2.3 Presence/Absence Surveys**

To assess the effectiveness of undertaking nocturnal spotlight surveys for determining the presence or absence of *L. booroolongensis*, repeated surveys were undertaken along 15 transects from five streams. Each transect was a 300-metre section of stream and was surveyed on five separate occasions; three within the breeding season (start of November to mid January) and two within six weeks after the breeding season (mid January through February). Surveys involved walking the section of stream at night and searching by spotlight for *L. booroolongensis* within the immediate riparian zone to within four metres of the waters' edge. The number and location of male frogs and tadpoles were recorded using a GPS and the area marked with flagging tape.

Using the repeat survey data, the detectability of *L. booroolongensis* using spotlight surveys was assessed at two scales; the transect/site level and at the breeding area level. A potential breeding area was defined as any discreet section of rock habitat

(cobble bank or bedrock), that encompassed a section of stream bank greater than one metre in length. A breeding area was identified as occupied if one or more males (during the breeding season) or tadpoles and metamorphs (within six weeks after the breeding season) were located in that specific area. All surveys were undertaken by the author and were undertaken during conditions considered suitable for detecting *L. booroolongensis* (not raining, not within five days of a significant rain event, and not when air temperatures were below 10 °C). Air temperature, date and time were recorded at the start of each census.

#### **4.2.4 Statistical Analysis**

There were two specific aims for the analysis of the mark-recapture data. The first was to investigate whether there was significant variation in detection probabilities between years, sites and sex, and the second was to estimate the male and female breeding population size so as to investigate temporal variation in abundance. This was undertaken using the Jolly-Seber open population model (Seber 1982). Pre-defined models were fitted to the data that accounted for variation in capture probability and survival being influenced by year, site or sex, or a combination of these, which gave a total of 32 pre-defined models. Each of the models was ranked according to Akaike's Information Criterion (AIC), with the estimated population sizes being derived from the model-averaged estimate for the top nine models (Burnhan and Anderson 2002). These procedures were undertaken using the *POPAN* module in Program MARK (Cooch and White 2006).

The temporal variation in abundance observed at Brungle Creek (the site with the most years of data) was used to investigate our capacity to identify significant declines or increases in abundance for *L. booroolongensis*. This was undertaken by simulating declining and increasing populations of *L. booroolongensis* and assessing the power to detect these trends using linear regression. Declines were simulated at constant annual rates of 10, 20, 30....90 percent declines over a ten and fifteen-year period with a constant coefficient of variation, while increases were simulated at constant annual rates of 50, 100, 150....400 percent increases over a ten and fifteen-year period with a constant coefficient of variation. The power to detect a trend over this time period for a given rate of decline was assessed using Monte-Carlo

simulations with 5,000 iterations to determine the proportion of occasions (power) a significant trend was detected. The natural logarithm of the results of each simulation was used in the linear regression analysis, and the alpha level of significance for this analysis was set at 0.10 for a one-tailed test for the simulated declining populations, and 0.05 for a two-tailed test for the increasing populations. The alpha level of significance was relaxed for the declining population since it is desirable to reduce the potential for a type two error in assessments of population decline for threatened species (Thompson *et al.* 1998). These simulations were undertaken using the open-source statistical packages *R* version 2.3.0 (R Core Development Team, 2006).

Our capacity to detect population declines was assessed using the temporal variance associated with results of the mark-recapture analysis and also the raw counts for the number of male and female frogs observed during each census (index of abundance). The variance for each Jolly-Seber population estimate was obtained from the standard errors for each of these estimates using the delta method (Cooch and White 2006). The temporal variance associated with all the Jolly-Seber population estimates, and the raw counts, was obtained via an iterative approach (Burnham *et al.* 1987) using equation 2;

$$1 = \frac{\sum_{i=2004}^{1999} w_i (\hat{N}_i - \bar{N})^2}{(n - 1)} \quad \text{Equation 2}$$

where  $\hat{N}_i$  is the estimation of abundance for each year,  $\bar{N}$  is the weighted mean of all population estimates,  $w_i$  is the weighted variance for each population estimate, and  $n$  is the total number of population estimates (years) for the study.

To estimate detection probabilities and occupancy rates for the spotlight surveys along the 300-metre transects, a Bayesian occupancy model was fitted to the repeat survey data using Markov Chain Monte Carlo methods (Mackenzie *et al.* 2006). Separate estimates of model parameters were propagated for the two census periods (within and after the breeding period) because breeding site occupancy was expected to change due to the potential for some sites not to have attained successful recruitment to the tadpole/metamorph stage. I used uninformative priors in the model and based our parameter estimates on 100,000 chains, with the first 10,000 being

discarded. This analysis was undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003).

## 4.3 Results

### 4.3.1 Population Size Estimates

Of the 32 Jolly-Seber open population models examined, the greatest support was for the model where survivorship was held constant, but recapture probability varied between males and females (Table 4.1). Relative to this model, there was very little support for the other models examined, suggesting that differences in detection probabilities among years and sites were not significant (Table 4.1). The results of the model averaging for the top nine models suggested that the recapture probability was on average 10 percent greater for males than for females (0.54 versus 0.44 respectively; Table 4.2).

The population size estimates for the three study sites suggested that breeding populations of *L. booroolongensis* may fluctuate greatly from one year to the next, particularly for the male population (Figure 4.2). The timing of fluctuations in population size between years was reasonably similar among the three study sites with peaks in the number of males being recorded in 2000 and 2003 for each site (Figure 4.2). The index of abundance (frog counts for each census) also displayed large annual shifts in breeding population size (Figure 4.3), with the timing and pattern of fluctuations being similar to that observed for the Jolly-Seber population estimates (Figures 4.2 and 4.3).

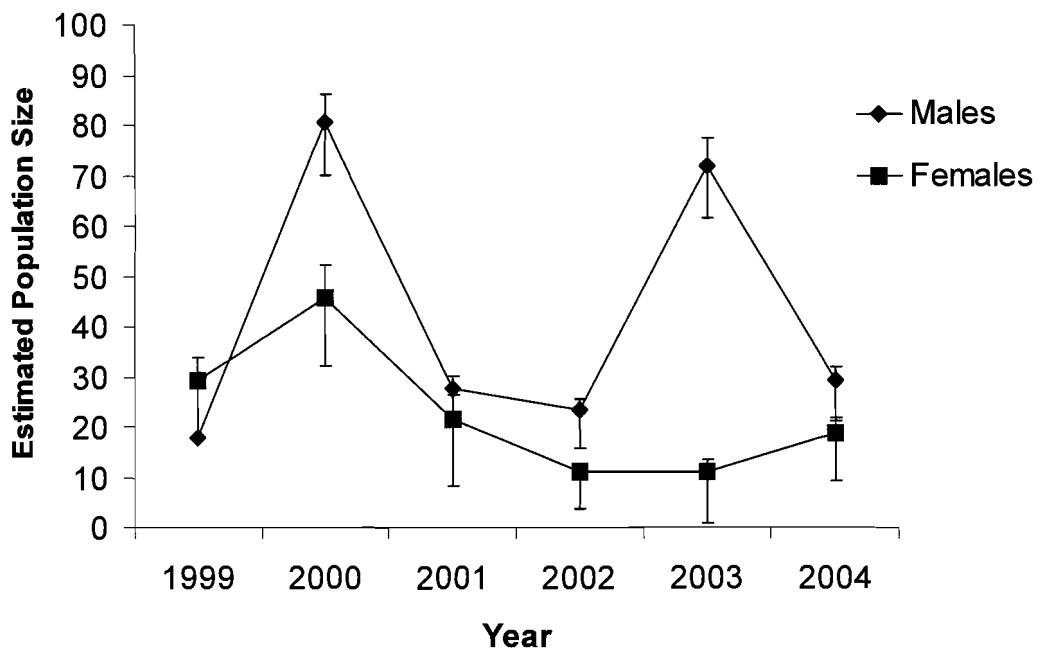
Table 4.1. The nine most parsimonious Jolly-Seber models, ranked according to their Akaike's Information Criterion (AIC) values, used to examine the relationship between sex (s), year (t), and population (g) on survivorship (phi) and probability of detection (p) in three *L. booroolongensis* populations.

Model	$\Delta$ AIC	AICc	Model	Likelihood	No.	Deviance
{phi(.), p(s), pent(t)}	2322.100	0.000	0.98638	1.0000	57	602.531
{phi(s), p(.), pent(t)}	2331.990	9.890	0.00702	0.0071	62	601.379
{phi(.), p(.), pent(t)}	2333.764	11.660	0.00289	0.0029	58	611.994
{phi(s), p(s), pent(t)}	2334.278	12.180	0.00224	0.0023	66	594.765
{phi(.), p(g), pent(t)}	2335.427	13.330	0.00126	0.0013	66	595.914
{phi(g,s), p(.), pent(t)}	2339.141	17.040	0.00020	0.0002	70	590.665
{phi(g,s), p(.), pent(t)}	2344.852	22.750	0.00001	0.0000	70	596.377
{p(s), phi(tg), pent(t)}	2411.844	89.740	0.00000	0.0000	147	478.054
{phi(s), p(g,s), pent(t)}	71782.998	69460	0.00000	0.0000	31	70119.377

Table 4.2. Model averaged estimates for the parameters of male and female *L. booroolongensis* capture probability and survivorship. These averages are based on the top nine Jolly-Seber models and associated AICc Weights presented in Table 4.1

	Mean	Standard Error	Lower 95% C.L.	Upper 95% C.L.
Survivorship (Phi)	0.8245566	0.019018	0.7841227	0.8587811
Male Probability of Detection (p)	0.5453787	0.0224105	0.5012144	0.5888404
Female Probability of Detection (p)	0.4454304	0.0328298	0.3823372	0.5103327

(a)



(b)

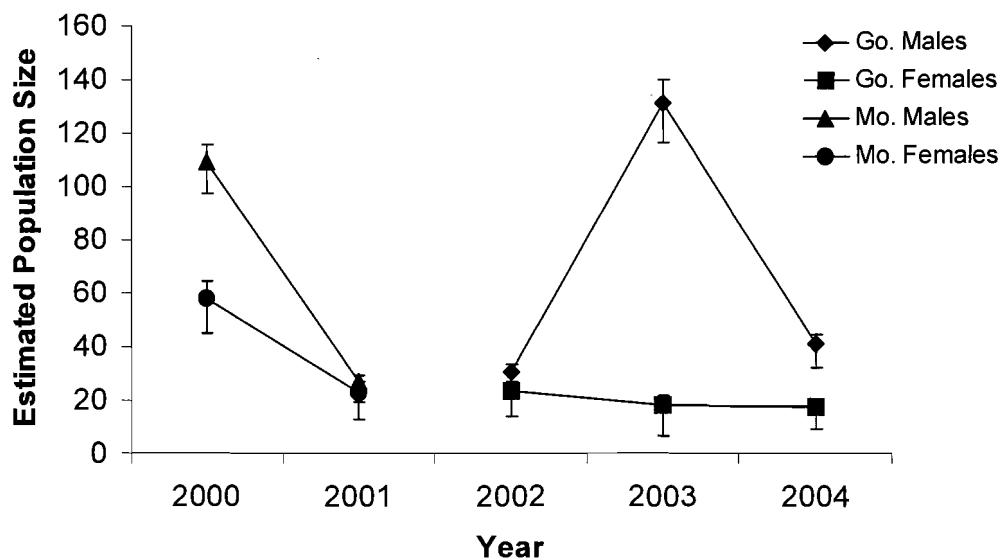
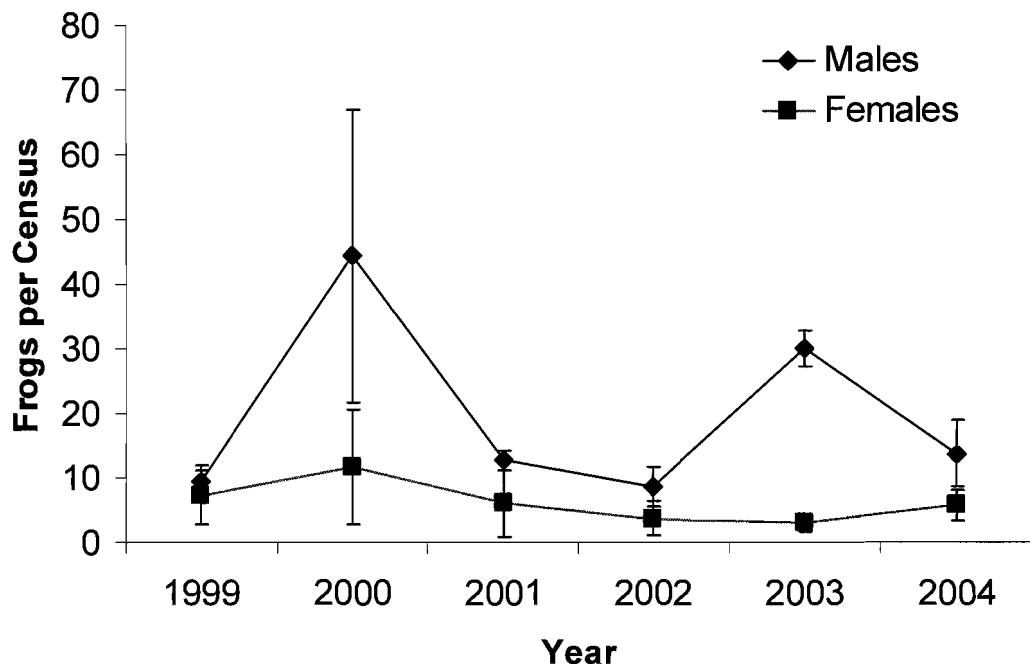


Figure 4.2. Annual population estimates for adult male and female *L. booroolongensis* from mark-recapture, for a) the Brungle Creek transect, and b) the Mountain Creek (Mo) and Goobragandra River (Go) transects. The point is the mean estimate and the error bars are the upper and lower 95 percent confidence limits.

(a)



(b)

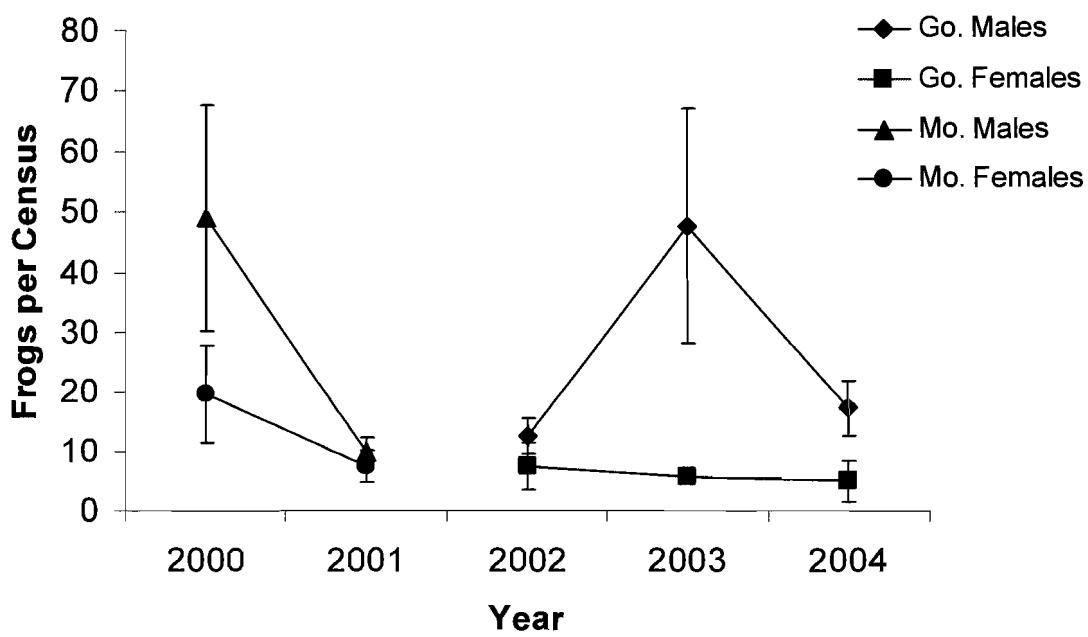


Figure 4.3. Annual population indices for adult male and female *L. booroolongensis* from raw counts, for a) the Brungle Creek transect, and b) the Mountain Creek (Mo) and Goobragandra River (Go) transects. The point is the mean estimate and the error bars are the upper and lower 95 percent confidence limits.

#### **4.3.2 Capacity to Detect Population Trends**

The power analysis of the simulated population declines suggested that there was only enough power when using mark-recapture population estimates to confidently identify a decline in the female population if the population declined by 80 or 90 percent over a 15 or 10-year period respectively (Figure 4.4). For the male population, there was limited capacity to detect even a 90 percent decline over a 15-year period (Figure 4.4). The results of the power analysis for the index of abundance (raw counts) also suggested that an adequate level of power to detect a decline in the female population would only be attained if the population was declining by at least 80 percent over a 15-year period (Figure 4.4). Similarly, this analysis suggested that there was very little capacity to identify a decline in the adult male population after fifteen years using raw counts (Figure 4.4). The results for the increasing populations were similar, as this study suggested that enough power to detect an increasing population would only be attained for the female population using population estimates if the population increased by at least 350 percent over 15 years (Figure 4.5).

#### **4.3.3 Presence/Absence Surveys**

*Litoria boorooolongensis* was detected during each of the five surveys undertaken on each of the 15 stream transects. Hence, the detectability of *L. boorooolongensis* at the site level (300-metre stream transect) using spotlight surveys during this study was perfect ( $p = 1$ ), for both surveys undertaken during the breeding season and surveys undertaken within six weeks after the breeding season. The results for the breeding area occupancy modeling suggested that the detectability of males along breeding areas using spotlight surveys is high ( $p = 0.86$ ), and that it was likely that the three spotlight surveys undertaken within the breeding season identified all rock areas occupied for breeding by *L. boorooolongensis* along the steam transects (Table 4.3). The average number of males observed per census for individual breeding areas was 3.2 (max = 10, min = 1). The results for the post-breeding season spotlight surveys suggested that identifying areas where successful recruitment occurred was also reasonably effective using only two censuses (Table 4.3). Tadpoles and metamorphs were only detected on or adjacent to rock structures where males were observed during the breeding season.

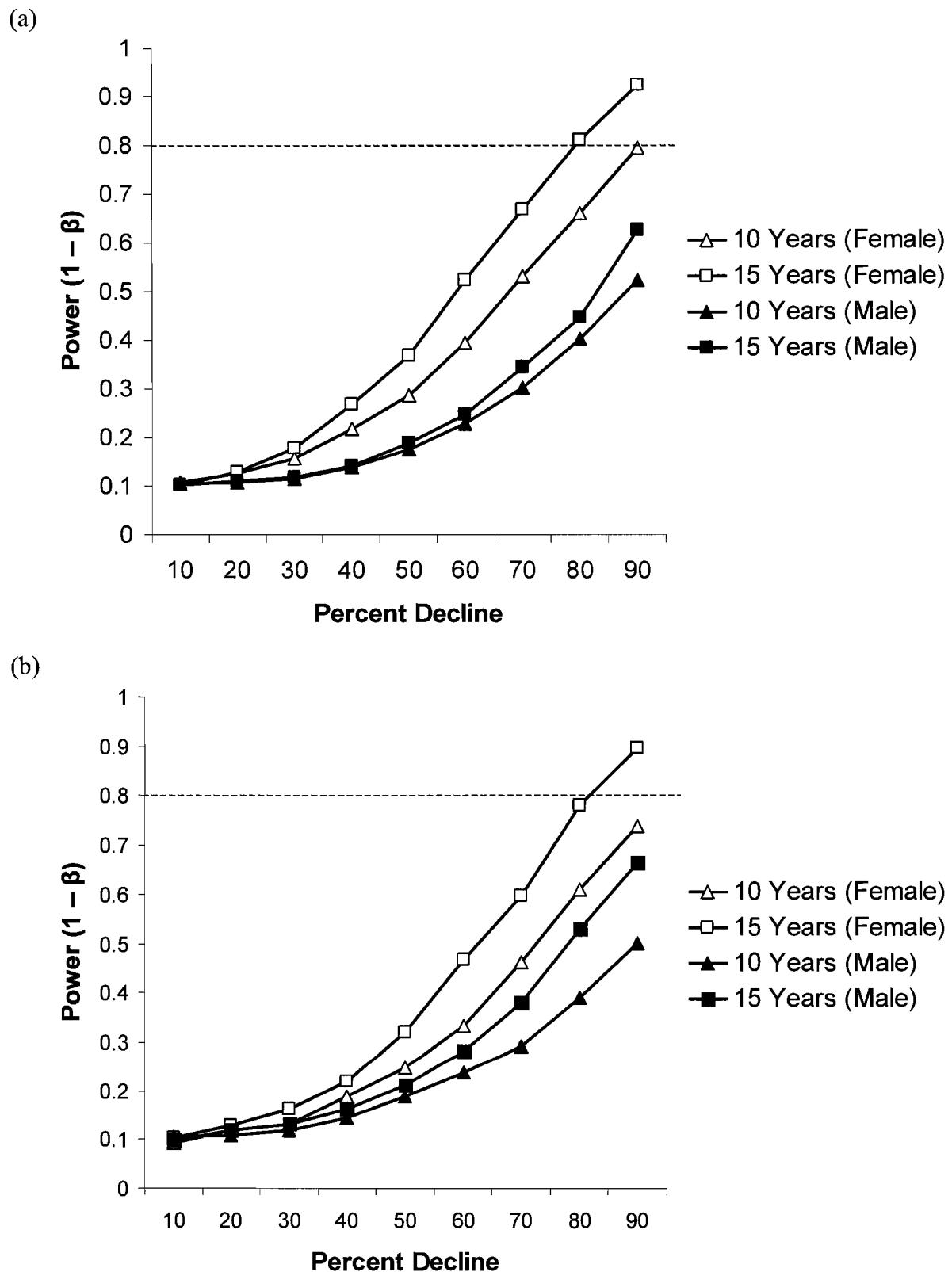
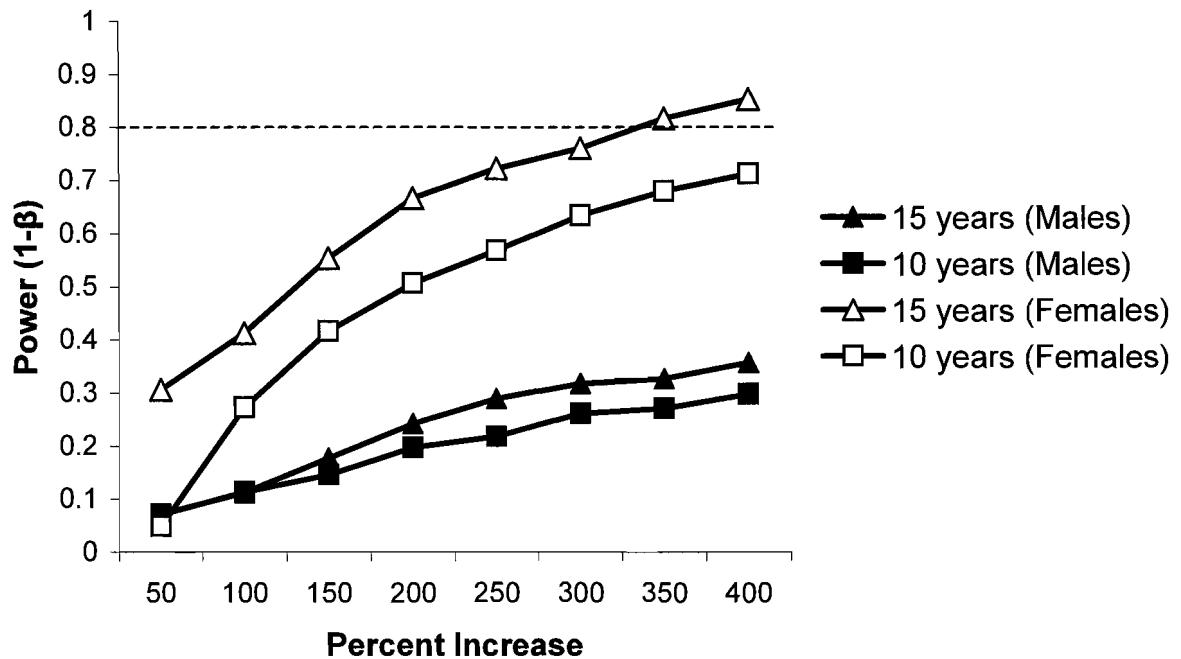


Figure 4.4. Power to detect a population decline for *L. booroolongensis* using, a) mark-recapture population estimates, and b) raw counts of frogs along the 500-metre transect.

(a)



(b)

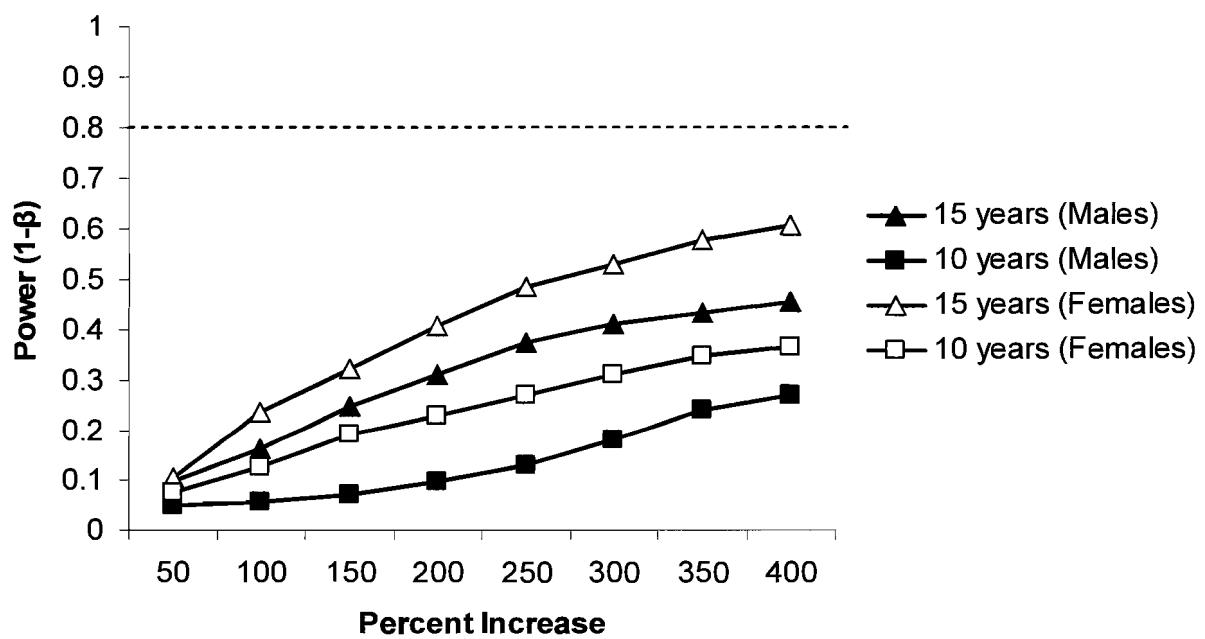


Figure 4.5. Power to detect a population increase for *L. booroolongensis* using, a) mark-recapture population estimates, and b) raw counts of frogs along the 500-metre transect.

**Table 4.3.** Posterior distributions for site occupancy model parameters fitted to the repeated surveys of *L. booroolongensis* breeding habitat during the breeding period (male surveys), and immediately after the breeding period (tadpole and metamorph surveys).

<i>Variable</i>	<i>Mean</i>	<i>SD</i>	<i>95% Credible Interval</i>
<b>Male Surveys:</b>			
Detectability	0.86	0.03	(0.80, 0.91)
Occupancy	0.31	0.04	(0.24, 0.38)
Estimated No. Sites Occupied	38.13	0.37	(38, 39)
<u>Observed No. Sites Occupied</u>	<u>38</u>		
<b>Tadpole and Metamorph Surveys:</b>			
Detectability	0.79	0.08	(0.65, 0.91)
Occupancy	0.15	0.03	(0.10, 0.21)
Estimated No. Sites Occupied	17.96	1.33	(17, 21)
<u>Observed No. Sites Occupied</u>	<u>17</u>		

## 4.4 Discussion

### 4.4.1 Temporal Trends in Abundance

The results of this study demonstrated the capacity for *L. booroolongensis* to exhibit large fluctuations in the male population size from one year to the next (Figure 4.2). This is consistent with the results of other amphibian studies that have observed large temporal fluctuations in abundance (Pechmann *et al.* 1991, Meyer *et al.* 1998, Green 2003). Since the coefficient of variation for population fluctuations is likely to increase as the length of the study increases (Marsh 2001), the extent of population fluctuations exhibited by *L. booroolongensis* is likely to be substantially greater than this study identified. The large annual fluctuations in abundance exhibited by the male *L. booroolongensis* populations is likely to be related to this species high annual adult mortality (> 90% per year, author unpub. data) and variable recruitment through to sexual maturity. The relatively reduced fluctuations exhibited by the *L. booroolongensis* female population may be partly due to differences in age to sexual maturity between the sexes. Female *L. booroolongensis* in these relatively low altitude populations take two years to reach sexual maturity, as opposed to one year for the males (author unpub. data), and hence factors influencing seasonal variation in recruitment to sexual maturity are balanced over two seasons for the female population.

While this study did not specifically identify what is driving fluctuations in *L. booroolongensis* abundance, an interesting feature of these results is that the fluctuations appear to be synchronised across different catchments (Figure 4.2). If this observation is a feature of *L. booroolongensis* population dynamics, then it suggests that fluctuations in this species are strongly influenced by factors operating across the broader landscape, such as climatic features. Synchronised fluctuations in abundance across broad geographic areas, referred to as the Moran effect (Moran 1953), have been documented for amphibians elsewhere and attributed to weather patterns and broad landuse changes (Houlihan and Findley 2003, Trenham *et al.* 2003, Gibbs *et al.* 2005). Determining the factors influencing fluctuations in *L.*

*booroolongensis* populations would greatly benefit the interpretation of monitoring results for this species.

#### **4.4.2 Trends in Detection Probabilities**

The mark-recapture analysis undertaken in this study suggests that sex has the greatest influence on detection probability for *L. booroolongensis*, and that there is little evidence for significant differences in detection probability among sites or years (Table 4.1). While variation in habitat structure among sites is likely to influence detection probabilities, failure to observe this for *L. booroolongensis* in this study may not be surprising. This is because even though the vegetation structure among the three study sites varied from almost complete cover to almost none, the rocky habitats occupied by the breeding males are very uniform in structure among sites. Similarly, while factors influencing breeding activity may result in significant seasonal or census variation in detection probability for amphibians (Schmidt 2005), the spotlighting method of detecting *L. booroolongensis* is not dependent on breeding activities such as calling.

The differences in detection probability between male and female *L. booroolongensis* identified in this study is likely to be due to their differences in distribution along the stream. While the majority of males congregate along rocky sections of stream during the breeding season, where they are easily spotlighted, females are more evenly distributed along the stream (author unpub. data), and may also occupy areas of the riparian zone away from the immediate stream bank. Hence, females are more likely to occupy areas of the riparian zone where they would be more difficult to detect.

#### **4.4.3 Using Abundance Estimates to Detect Population Trends**

Given the large annual fluctuations in abundance identified in this study (Figure 4.2), it is not surprising that the results of the power analysis suggest that we would have limited capacity to confidently detect a decline or increase in *L. booroolongensis* populations by monitoring individual abundance unless the rate of decline was very large. If the intention of the monitoring program is to serve as an early warning signal of population decline, then these results suggest that monitoring *L. booroolongensis*

abundance would not be effective. Because many amphibian species exhibit large fluctuations in abundance, it is well acknowledged that monitoring their populations using estimates of abundance is often problematic, as appears to be the case for *L. booroolongensis* (Reed and Blaustein 1995, Hayes and Steidl 1997, Marsh 2001).

Interestingly, the capacity to detect a trend was similar between estimates of population abundance using mark-recapture and the index of population abundance using raw counts (Figures 4.4 and 4.5). Similarly, the pattern of population fluctuations was also similar between the index and estimates of abundance (Figures 4.2 and 4.3). While this is somewhat academic due to the limited capacity to detect overall trends, it does suggest that raw counts are comparable to estimates of abundance for identifying shifts in population size from one year to the next for *L. booroolongensis*. The major problem associated with using indices of abundance in monitoring programs relates to variability in detection probabilities (Anderson 2001, Schmidt 2003). Undoubtedly, detection probabilities will vary to some degree within and among populations of a species, however, what is most important is that there are no temporal trends in detection probability, and that variation in detection probability has less influence on trends of interest than other variance component such as actual variation in abundance (Bart *et al.* 2004). Compared with the large annual variation in abundance observed in this study (Figure 4.2), the variation in detection probability for *L. booroolongensis* certainly appeared to have minimal influence on our capacity to detect trends in abundance. However, while this study suggests that raw counts may be used to monitor shifts in the abundance of *L. booroolongensis*, it should be acknowledged that using mark-recapture provides greater capacity to identify which life-history stages are contributing significantly to observed changes in abundance, and thus have greater capacity to identify the external factors causing these shifts (e.g. Scherer *et al.* 2005).

#### **4.4.4. Detecting Population Trends Using Presence/Absence Surveys**

The results of the repeated surveys suggest that spotlighting at night is an effective technique for determining the presence or absence of *L. booroolongensis* along sections of stream, both during and immediately after the breeding season for this species (Table 4.3). In addition to this, spotlight surveys within the breeding season

were also effective at identifying breeding areas along the stream. While surveys undertaken within six weeks after the breeding season for *L. booroolongensis* were effective at identifying sections of stream where successful recruitment to the tadpole and metamorph stage had occurred, the number of sites identified in this manner as breeding areas was only half the actual number of breeding areas identified during the breeding season (Table 4.3). This is likely to be due to variation in recruitment success among different areas along the stream.

The high probability of detection at the site level for *L. booroolongensis* is consistent with the mark-recapture results for this species (Table 4.2). An individual male probability of detection of 0.54 suggests that there would only have to be 3 males present along the survey transect, or breeding area, to be 95 percent confident that the species would be detected during an individual census. These results suggest that spotlight surveys undertaken for *L. booroolongensis* should accurately determine the presence or absence of this frog species during the majority of survey occasions (ie. very low rate of false negatives).

Given the capacity to accurately determine the presence/absence of *L. booroolongensis* using spotlight surveys, this technique is likely to be an effective means to monitor temporal trends in the distribution of *L. booroolongensis*, at both the landscape scale and breeding habitat scale. That said, we currently have limited data on temporal variation in site occupancy at different scales for *L. booroolongensis*, and so any monitoring program for this species using presence/absence surveys should be reviewed regularly to ensure that the number of sites being monitored is adequate for addressing the objectives of the program. Using presence/absence surveys to monitor a species population trends across the landscape, as opposed to monitoring trends in individual abundance, has a range of advantages. Apart from the issue of efficiency and available resources, determining the influence of seasonal or observer bias in monitoring results requires greater effort for estimates of individual abundance as opposed to presence/absence surveys. It is also a technique that can be more readily implemented by general field staff, a factor that may prove beneficial in ensuring the ongoing implementation of a monitoring program.

While *L. booroolongensis* was detected on every occasion during the breeding season, it should not be assumed that spotlight surveys would always have a perfect detection rate for this species. It should be reiterated that surveys in this study were undertaken during and within six weeks after the breeding season, and under ideal environmental conditions suitable for frog activity and for detecting this species (air temperatures greater than 10 °C, dry conditions, and not within four days of major rainfall events). Low temperatures, wet conditions, high water levels or surveying outside the optimal period would all be expected to either reduce *L. booroolongensis* activity or presence on the stream, or reduce the capacity to detect this species along the stream. In addition to this, even if surveys are undertaken under suitable conditions and at the right time of year, detectability will decrease as the species abundance decreases in the survey area (Royle and Nichols 2003). Hence, if *L. booroolongensis* is present along a section of stream in very low densities it may not be detected.

Two other important issues to consider when assessing survey/monitoring methodologies is the potential for observer bias (Brown *et al.* 2006) and annual variation in detectability (Schmidt 2005). Since the repeated surveys undertaken in this study were undertaken by the one person, and all within the same season, I was not able to assess how different observers or seasons may influence detection probability of the species. Any monitoring program for *L. booroolongensis* using presence/absence surveys should address this issue through repeated surveys of individual sites, as this would allow for ongoing assessment of detection probabilities.

#### ***4.4.5 Recommendations for Monitoring *L. booroolongensis****

It is critical to the design and implementation of a monitoring program for threatened species that there is a clear understanding of what the specific objectives of the monitoring is. If the intention of the program is to serve as an early warning system to population decline, then an alarm needs to be broadcast with sufficient time for management to adequately respond. In other situations, monitoring may be undertaken as a means to assessing specific actions or treatments, in which case an adequate level of power to detect changes in abundance of a specified amount is required. With respect to *L. booroolongensis*, there are currently two primary objectives for monitoring this species:

1. Determine current overall population trends for this species.
2. Determine the viability of populations occurring along streams subject to different management regimes.

For both these objectives, it is desirable that the monitoring program be able to identify shifts in the abundance of *L. booroolongensis* across a broad geographic area. This will require monitoring many sites across many streams and so from a resource perspective, addressing these two monitoring objectives using presence/absence surveys would be most efficient and achievable, particularly if the monitoring protocol involved visiting a proportion of sites only once every two or three years. Since my research suggests that we have a limited capacity to identify trends in abundance for *L. booroolongensis* using population estimates, presence/absence surveys will also be more likely to achieve the statistical requirements of this program.

## **Chapter 5**

### **Survey, Habitat Assessment and Conservation of the Endangered Booroolong Frog (*Litoria booroolongensis*) on the South West Slopes of New South Wales.**

#### **5.1 Introduction**

Continuing declines and extinctions are an ongoing conservation concern for many amphibian taxa across the globe (Alford and Richards 1999, Lips *et al.* 2006). To date, there have been few positive conservation outcomes for amphibians and in many cases options appear to be limited to drastic solutions like captive breeding to prevent extinction (McCallum 2005). Of the 213 frog species recognised in Australia, 50 are considered threatened or extinct in accordance with the IUCN Global Amphibian Assessment guidelines (Hero *et al.* 2006). Included in this list is the Booroolong frog (*Litoria booroolongensis*, Moore 1961); a medium sized riverine species that was historically known to occupy permanent rocky streams along the Great Dividing Range of South Eastern Australia (Anstis 2002). The altitudinal range of *L. booroolongensis* has been recorded between 200 and 1300 meters above sea level (NSW Wildlife Atlas) and prior to 1990 *L. booroolongensis* was considered both common and secure (Tyler 1992). By the mid 1990's, however, it was apparent that the species had declined from much of its range (Heatwole *et al.* 1995, Gillespie and Hines 1999), which formed the basis for the listing of *L. booroolongensis* as endangered under the New South Wales *Threatened Species Conservation Act 1995*. Subsequent targeted surveys for *L. booroolongensis* confirmed that this species had declined from more than 50 percent of its former known range (Gillespie 1999, 2000) and noticeably, a high proportion of persistent populations occurred in the South West Slopes region of New South Wales (Gillespie 1999, 2000). As this area includes the southern extreme of the distribution of *L. booroolongensis*, it is potentially important for the species longer-term persistence (Hampe and Petit 2005). Additionally, *L. booroolongensis* is often the only obligate river breeding frog species in many of the

montane and foothill streams in this region, and hence fills an important ecological niche in these habitats.

While recent surveys have identified the persistence of *L. booroolongensis* populations along a number of streams (Gillespie 1999, 2000), there is currently limited information on the extent of these populations. Further, little is known about the specific habitat requirements of the species, beyond their general association with rocky stream habitat (Anstis 2002). Identifying habitat features associated with the persistence of *L. booroolongensis* is important for assessing hypotheses as to why this species has declined from much of its range in recent years. More importantly for the immediate conservation management of *L. booroolongensis*, it would provide guidance for the management of this species habitat (Hazel 2003). This is particularly important for *L. booroolongensis* as many of the persistent populations of this species occur along freehold land which is the focus of riparian restoration, and also other land management practices which may be detrimental to this species' populations.

In this study I undertook detailed surveys for *L. booroolongensis* to determine the current distribution of this species in the South West Slopes region of New South Wales. I also undertook an analysis of habitat variables associated with the occurrence of this species at both regional and within breeding habitat scales. The results of this study will be used to identify potentially threatening processes for *L. booroolongensis* and provide targeted management and habitat restoration recommendations for the conservation of this species. This study also demonstrates the value of undertaking targeted surveys and habitat assessments for poorly known species believed to be of conservation concern.

## 5.2 Methods

### 5.2.1 Study Area, Site Selection and Survey Methods

Surveys were undertaken for *L. booroolongensis* in the South West Slopes region (hereafter referred to as SWS) of New South Wales, Australia along permanent streams containing rocky bank habitats. Surveys were undertaken at night by spotlighting for eye-shine along the stream. A minimum of 500 meters was surveyed

at each site by walking upstream and spotlighting along all emergent areas of the stream to within four meters of the waters edge. This survey technique is highly efficient at determining the presence/absence of *L. booroolongensis* (see Chapter 4). Frogs were only hand-captured if their identification required confirmation. This was always undertaken at sites where the frog had not been previously recorded. Each frog was handled wearing a new pair of disposable rubber gloves and individuals were typically handled for less than 30 seconds.

### **5.2.2 Regional Habitat Use**

A comparison was undertaken to assess patterns in the distribution of *L. booroolongensis* along the SWS. This involved measuring and comparing habitat variables where *L. booroolongensis* was and was not detected. The following variables were measured for each 500-meter section of stream incorporated into this comparison:

*Altitude*: Taken from 1:25,000 map sheet.

*Length of Cobble Bank*: The total length of cobble bank along the 500-meter section of stream. A cobble bank was defined as a section of stream bank greater than one meter with a continuous cover of loose rock.

*Length of Bedrock*: The total length of bedrock bank along the 500-meter section of stream. Bedrock was defined as a section of stream bank greater than one meter with a continuous cover of solid rock that is embedded in the ground.

*Proportion Canopy*: Average percent canopy above the stream bank for ten evenly spaced points along the stream. The percent canopy cover for each point was estimated by taking a vertical digital image, using a Nikon Coolpix 995, with the angle of the image being 30 degrees from the vertical along the longest axis of the image, and then overlaying a grid of 100 evenly spaced points, with the number of points falling on vegetation being used as the percentage canopy. For streams less than two meters across, the image was taken from the center of the stream, while streams with a width greater than two meters, an image was taken for each side of the stream.

*Water Depth:* Average water depth of ten measurements taken at evenly spaced points along the 500-meter section of stream. Each water depth measurement was the maximum depth determined along a straight line from one side of the bank to the other for that point on the stream.

*Percentage Rapid:* Average proportion of rapid from ten measures taken at evenly spaced points along the 500-meter section of stream. Each point was a ten meter section of stream and the measure was a visual estimate of the proportion of that section of stream where the water surface was broken (white water) due to the velocity and movement of the water.

### **5.2.3 Within-Breeding Habitat Use**

Patterns in the breeding habitat use of *L. booroolongensis* were investigated by comparing rocky structures along the stream occupied by calling/breeding males, to unoccupied rock structures. This was undertaken along fifteen, 300-meter transects across six streams, which were surveyed on three separate occasions during the breeding season for this species (November to mid January). Surveys involved walking the stream transects at night and spotlighting for *L. booroolongensis* in the manner outlined above. A section of rock bank was identified as breeding habitat if there was at least one male in breeding condition present (identified by swollen and pigmented nuptial pads). The number and location of male frogs was recorded using a GPS and/or marking the site with flagging tape. The non-occupied rock habitats used in this comparison where the nearest rock habitat where no male *L. booroolongensis* were observed. The rock habitats were divided into two broad categories based on the two predominate types of rock bank structures: Cobble banks, which were defined as a section of stream bank greater than one meter in length with a continuous cover of loose rock, and bedrock banks, which were defined as a section of stream bank greater than one meter in length with a continuous cover of solid rock that is embedded in the ground. The following habitat variables were measured at each occupied and non-occupied rock habitat:

*Aspect:* Direction of the rock bank facing into the stream.

*Proportion Canopy:* A single measurement taken from the center of the rock bank using the techniques outlined above for the regional habitat comparison.

*Emergent Width:* The length of in-stream habitat immediately adjacent to the rock bank that contains areas of emergent rock.

*Terrestrial Width:* The length of the rock bank structure.

*Proportion Pool:* The proportion of stream immediately adjacent to the rock bank, to a distance of one meter from the waters edge, which had still or very slow moving water.

*Proportion Riffle:* The proportion of stream immediately adjacent to the rock bank, to a distance of one meter from the waters edge, which had moving rip.

*Rapid Length:* The proportion of stream immediately adjacent to the rock bank, to a distance of one meter from the waters edge, which had still or very slow moving water.

*Water Depth:* Water depth along the coble bank or bedrock was measured at four evenly spaced points along the bank and 50cm into the stream from the water edge. The average of the four measurements was used in the analyses.

*Number of Aquatic Crevices:* A crevice was defined as a space under or between rock where a 2.5cm wide, 1cm high and 3cm long piece of metal could be freely inserted, but which was no higher than 3cm. This area was considered a representation of the area that could be used by *L. booroolongensis* for egg deposition. Regardless of crevice length, continuous crevices in bedrock or under individual rocks were only counted as one crevice. Number of crevices was measured within a 50cm by 50cm quadrat. These quadrats were randomly placed within one meter of the water edge with a quadrat position allocated to every one-meter section of stream bank. The random position of the quadrat within the one meter section being determined using a random numbers table to identify an X and Y coordinate. The number of crevices used in the analysis was the mean number of crevices across the quadrats for that particular bank.

#### **5.2.4 Statistical Analysis**

Logistic regression analyses were used to explore the relationships between the presence/absence of *L. booroolongensis* and the habitat predictor variables. Separate analyses were run for the regional scale and for the bedrock and cobble habitats from the within breeding area level. I made the specific assumption that the habitat preferences of populations did not, on average, vary across the region and accordingly, I did not expect there to be any spatial autocorrelation. For the analysis of breeding habitat, I linked each breeding habitat to its nearest non-occupied habitat to investigate any relationship between the paired data. Correlations among variables were examined and one variable from any strongly correlated pairs (correlation coefficient:  $r < 0.5$ ) was removed from subsequent analyses. All models were fitted with WinBUGS version 1.4 (Spiegelhalter *et al.* 2003) which is a software package for fitting Bayesian statistical models to data using Markov Chain Monte Carlo (MCMC) methods. Vague or uninformative priors were used for all model parameters and convergence of the algorithm was checked by examining the output of three replicate Markov chains with differing starting values (Brooks & Gelman 1998). To further minimise intercorrelation among variables, each value was subtracted from the mean value for the variable. Final inferences were made by discarding the first 5000 iterations from two chains and retaining the next 45000. Variables were considered to have a strong effect if their 95% confidence intervals did not cross zero. By using Bayesian methods, I was able to generate predictions of changes in presence/absence with particular predictor variables as would be expected to occur over a representative range of values.

## 5.3 Results

### 5.3.1 Survey Results

A total of 163 sites were surveyed across 49 streams on the SWS. The majority of these sites were surveyed between the start of November and mid January, with a small number of sites ( $n=24$ ) being surveyed after this period. All surveys were conducted within the period deemed suitable for detecting this species. *Litoria boorooolongensis* was detected along 77 of the sites surveyed from 27 streams (Figure 5.1). Based on population and habitat connectivity, *L. boorooolongensis* was found in 18 distinct areas/populations (Table 5.1). Based on the upper and lower localities where *L. boorooolongensis* was recorded, the estimated length of stream occupied along the sections of stream found to contain *L. boorooolongensis* varied greatly, with 4 streams estimated to have relatively short (three kilometer or less) sections of stream occupied by this species (Table 5.1).

Of the sites surveyed during this study, 19 were localities where this species was historically recorded. Fourteen of these localities were identified during surveys undertaken for *L. boorooolongensis* in 1999 (Gillespie 1999), while the other five localities were pre-1990 records for this species (NSW Wildlife Atlas, Caughley and Gall 1985). This study failed to locate *L. boorooolongensis* along sections of Killimicat, Bombowlee and Horse Creeks where Gillespie (1999) located this species in 1999, however this species was observed elsewhere along Bombowlee and Horse Creeks during this study, but not elsewhere along Killimicat Creek. This study also failed to locate this species along all sections of the Tumut River, Yarrangobilly River and Basin Creek where this species was recorded prior to 1990, however I did locate this species elsewhere on the Yarrangobilly River.

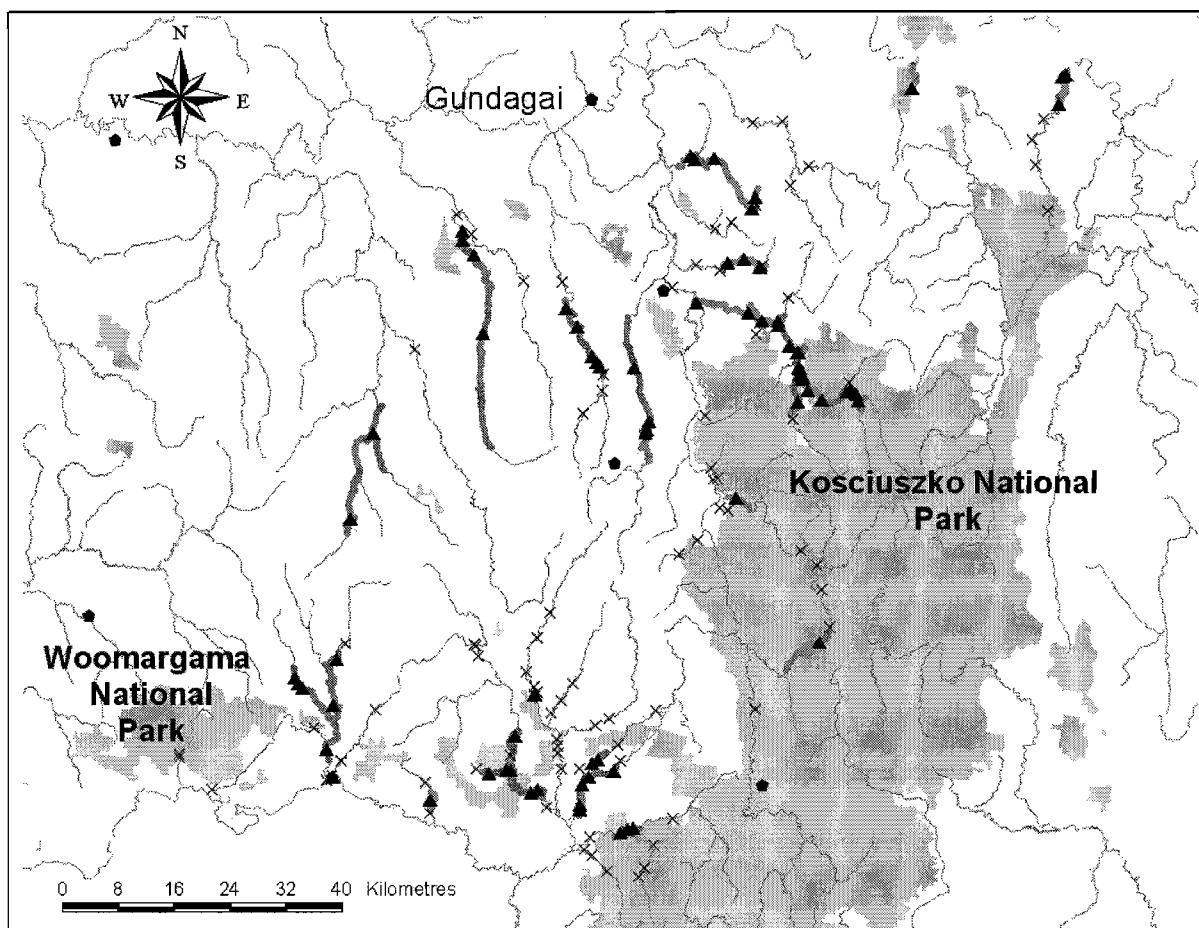


Figure 5.1. Map of the South West Slopes region of New South Wales showing survey localities and sections of stream likely to support extant populations of *L. booroolongensis*. Crosses are survey sites where *L. booroolongensis* was not located. Closed triangles are sites where *L. booroolongensis* was located. Shaded sections of the landscape are areas of conservation reserves while shaded sections of stream are areas where *L. booroolongensis* is expected to occur based on the survey results and presence of suitable habitat.

Table 5.1. Summary of *L. booroolongensis* populations in the South West Slopes region for estimated length of stream occupied (E.L.O.), and estimated proportion of each population represented in conservation reserves.

Population	Streams within each population	E.L.O. (km)	Percent of population in Nature Reserve.
<b>Jingellic Ck.</b>	Jingellic Ck.	7.5	0
	Coppabella Ck.	13.0	0
	Lankeys Ck.	9.5	0
<b>Horse Ck.</b>	Horse Ck.	0.05	0
<b>Ournie Ck.</b>	Ournie Ck.	1.0	0
<b>Manus Ck.</b>	Manus Ck.	19.5	85
	Sappling Yards Ck.	5.5	90
<b>McCabe Ck.</b>	McCabe Ck.	0.1	0
<b>Maragle Ck.</b>	Maragle Ck.	20.0	0
	Maragle Back Ck.	4.0	0
<b>Tooma R.</b>	Tooma R.	6.5	100
<b>Goobragandra R.</b>	Goobragandra R.	40.0	50
	Sandy Waterfall Ck.	5.0	0
	Stony Ck.	0.5	0
	Peak R.	10.0	80
<b>Mountain Ck.</b>	Mountain Ck.	4.5	0
<b>Macpherson Swamp Ck.</b>	Macpherson Swamp Ck.	4.0	50
<b>Brungle Ck.</b>	Brungle Ck.	15.5	0
<b>Bombowlee Ck.</b>	Bombowlee Ck.	8.0	0
<b>Gilmore Ck.</b>	Gilmore Ck.	23.0	0
<b>Adelong Ck.</b>	Adelong Ck.	18.0	0
<b>Yaven Yaven Ck.</b>	Yaven Yaven Ck.	30.0	20
<b>Umbango Ck.</b>	Umbango Ck.	12.0	0
	Carabost Ck.	15.0	0
<b>Jounama Ck.</b>	Jounama Ck.	7.0	100
<b>Yarrangobilly R.</b>	Yarrangobilly R.	10.5	100

### **5.3.2 Broad Habitat Associations**

Broad habitat variables were measured at a total of 81 sites, with *L. booroolongensis* being identified as present at 41 of these sites. Three variables in particular explained variation in the presence/absence of *L. booroolongensis*; bedrock length, cobble length and percentage canopy cover (Figure 5.2). Presence was positively associated with bedrock and cobble bank length and negatively with canopy cover (Figure 5.3).

### **5.3.3 Breeding Habitat Associations**

For the within breeding habitat analyses, I examined frog occupancy in two habitat types; bedrock and cobble areas. Three variables explained most of the variation in *L. booroolongensis* presence in the bedrock habitat; number of crevices (positive), number of pools (positive) and water depth (negative; Figures 5.4 and 5.5). For the cobble habitat analysis, four variables related strongly to the presence of *L. booroolongensis*; emergent rock, number of crevices, number of pools and water depth (Figures 5.6 and 5.7).

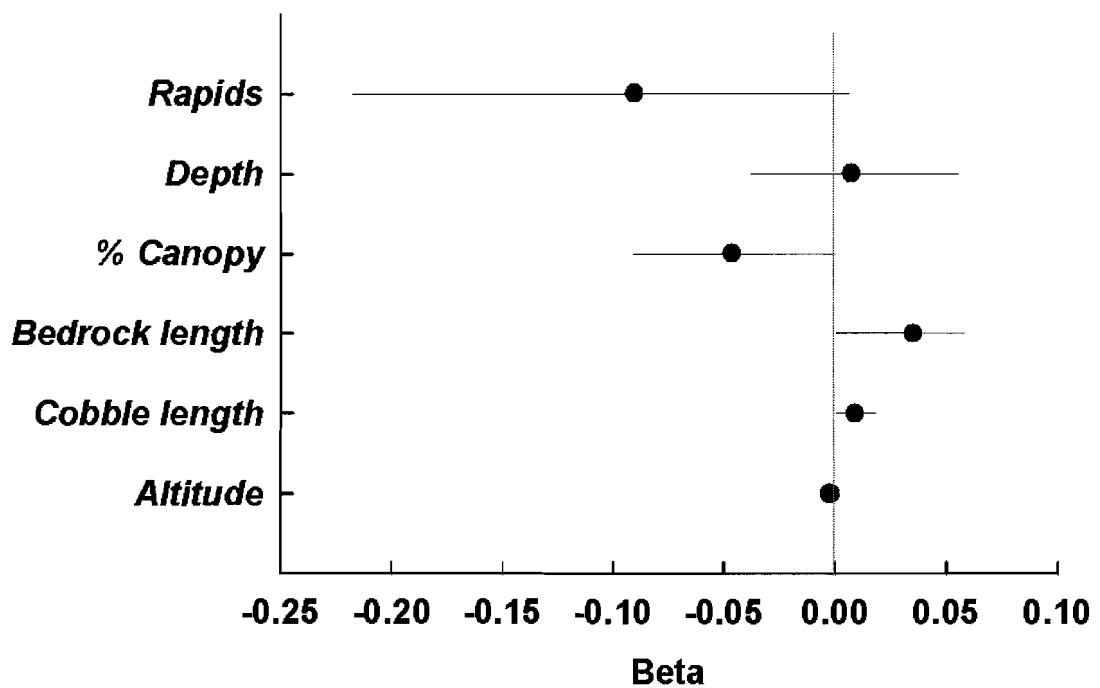
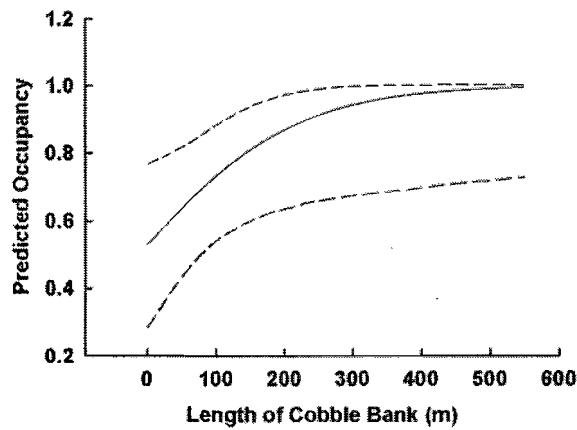
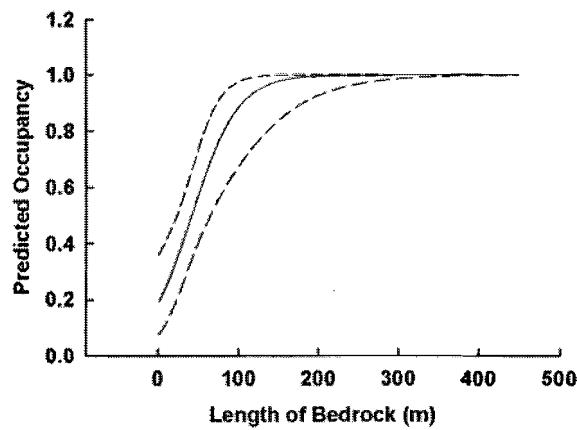


Figure 5.2. Beta values and 95% confidence intervals (CI) for each regional predictor variable. Intercept values have been omitted to improve resolution.

(a)



(b)



(c)

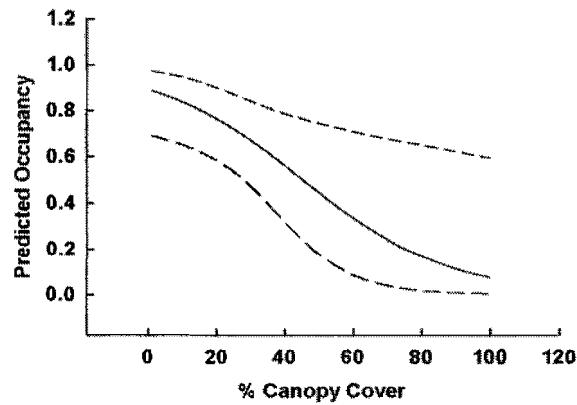


Figure 5.3. Fitted curves for probability of occupancy versus (a) cobble bank length, (b) bedrock length and (c) percentage canopy cover. Dashed lines indicate 95% credible intervals.

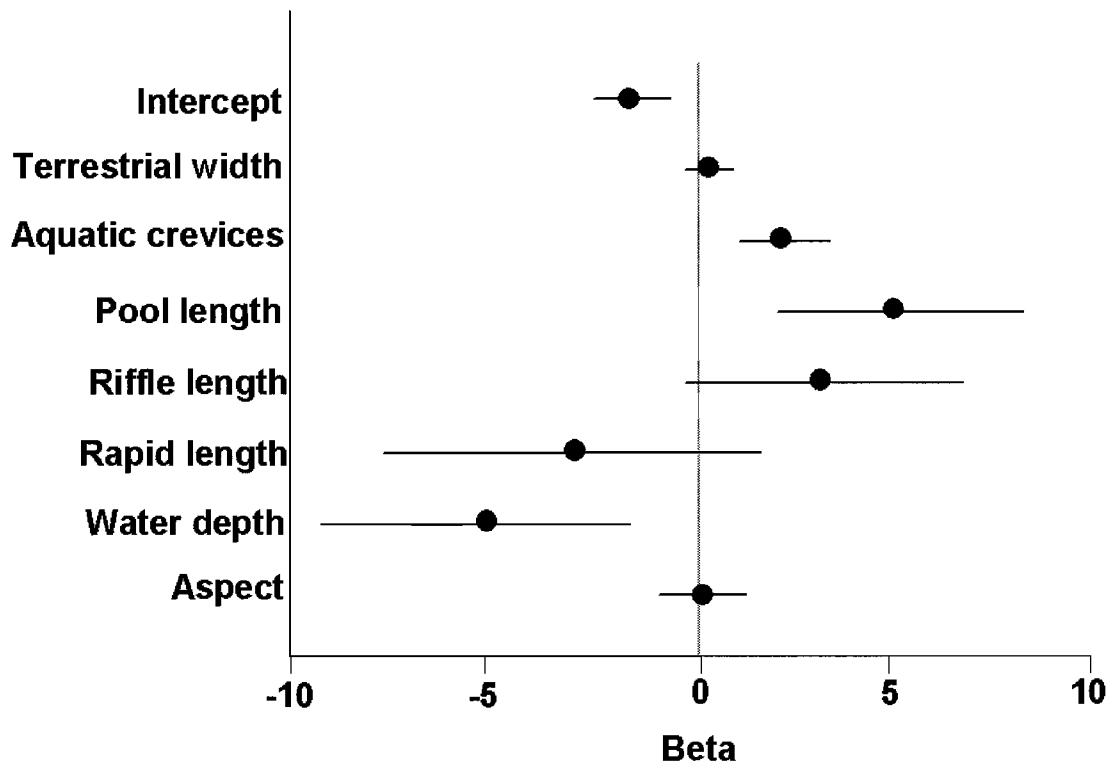
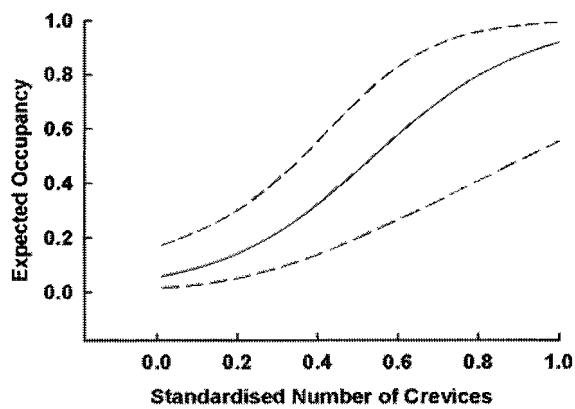
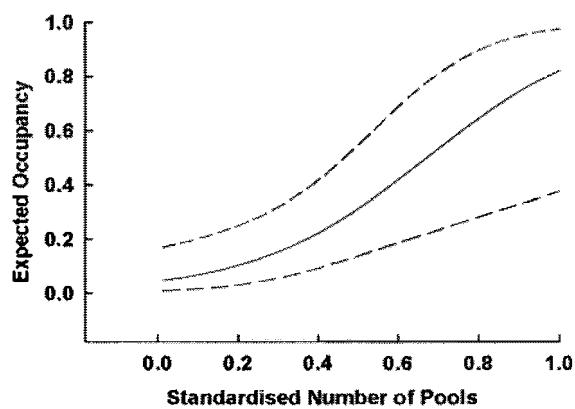


Figure 5.4. Beta values and 95% credible intervals (CI) for each among bedrock habitat predictor variable.

(a)



(b)



(c)

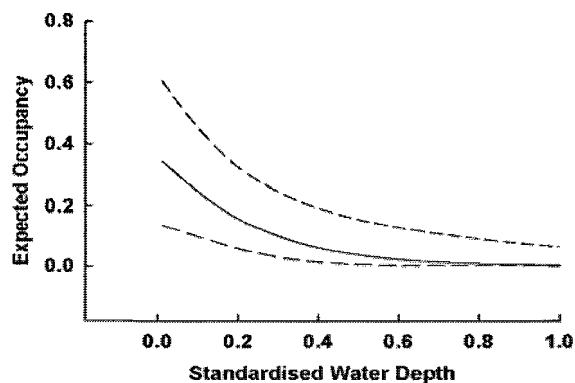


Figure 5.5. Fitted curves for the relationship between expected occupancy and (a) the number of crevices, (b) pool length and (c) water depth in the bedrock habitat. Dashed lines indicate 95% credible intervals.

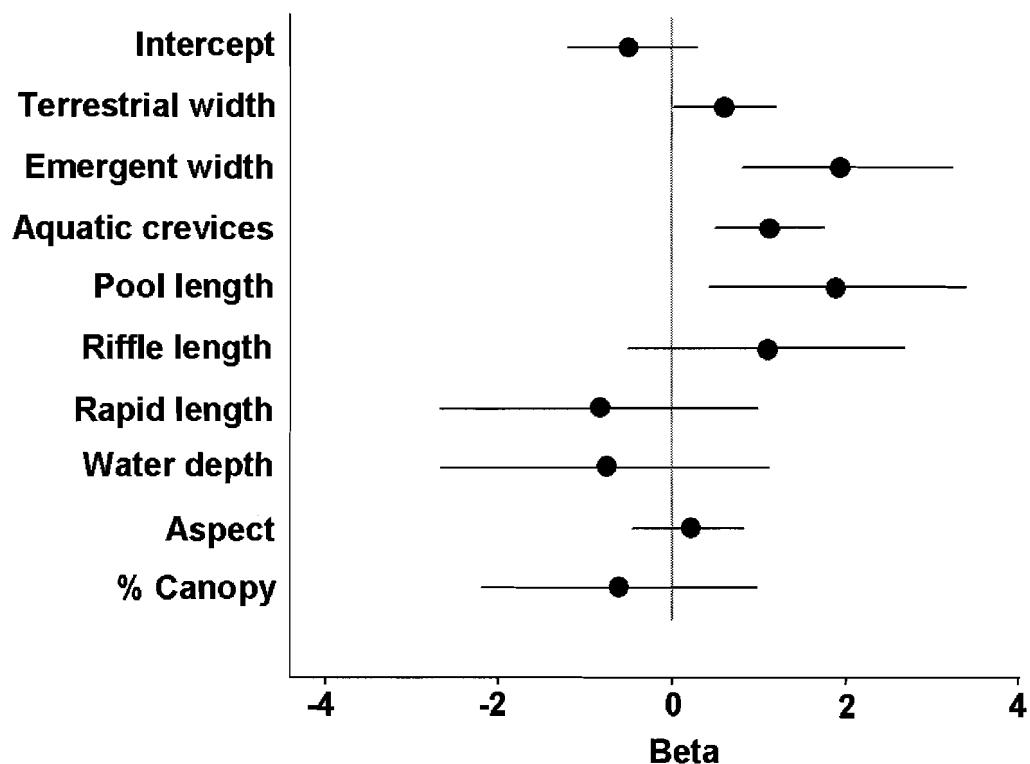


Figure 5.6. Beta values and 95% credible intervals (CI) for each among cobble habitat predictor variable.

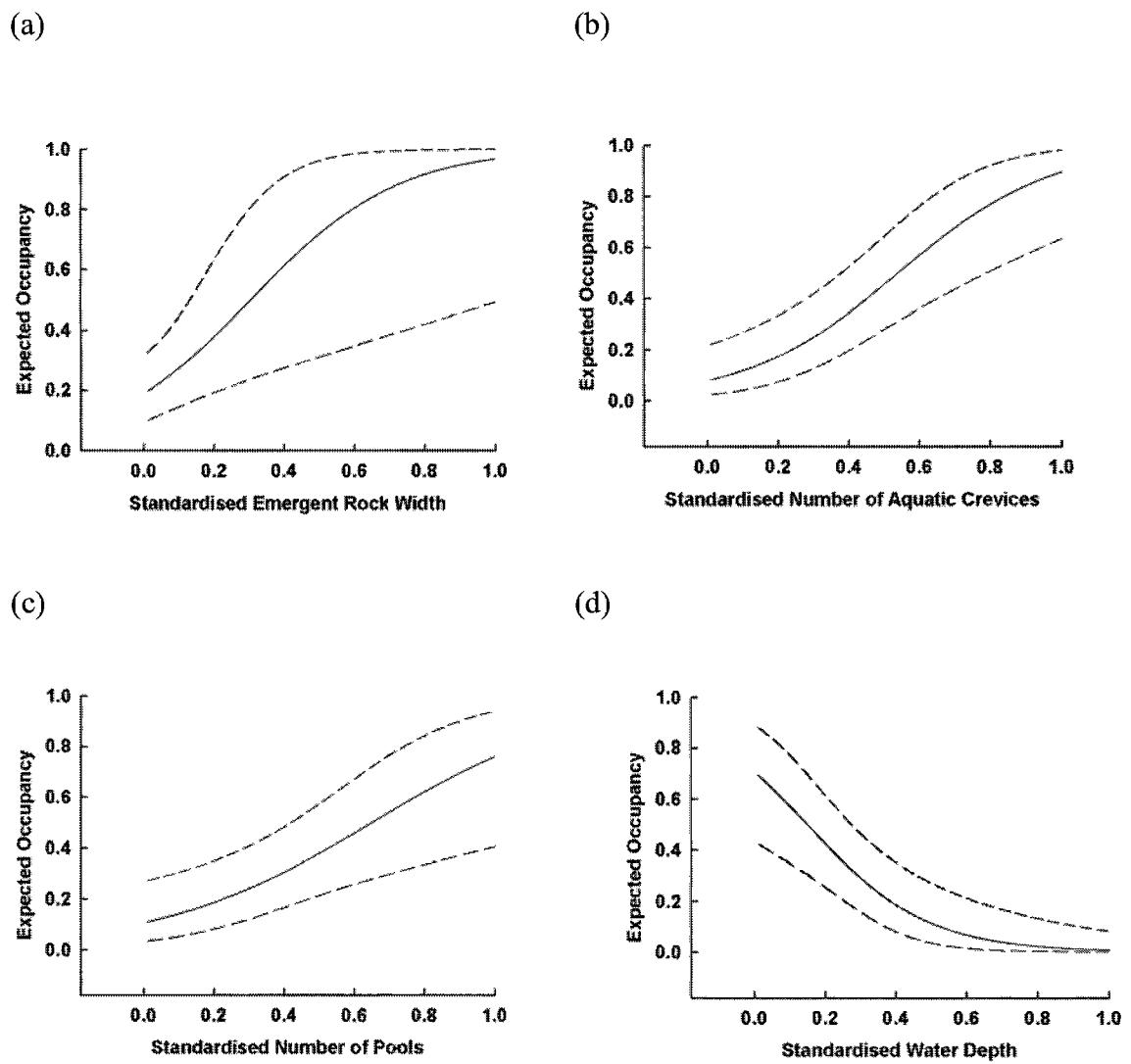


Figure 5.7. Fitted curves for the relationship between expected occupancy and (a) emergent rock, (b) the number of crevices, (c) the number of pools and (d) water depth in the cobble habitat. Dashed lines mark 95% credible intervals.

## **5.4 Discussion**

### ***5.4.1 Distribution and Conservation Status of the Booroolong Frog on the SWS***

This survey has substantially increased our understanding of the distribution of *L. booroolongensis* on the SWS. Based on habitat and population connectivity, this study has identified 18 populations of *L. booroolongensis* separated from each other by greater than 3 km of unsuitable habitat (Figure 5.1). While local amphibian populations often interact as metapopulations (Sjogren-Gulve 1994, Skelly *et al.* 1999), it is likely that there is minimal migration between the areas of occupancy identified in this survey, and that they are effectively operating as independent populations. This is because the habitat between the occupied sections of stream has been highly modified such that *L. booroolongensis* would be unlikely to migrate through these areas. Population genetic studies of other stream-breeding amphibians in eastern Australia have shown genetic subdivision between relatively close watersheds, particularly if they are separated by unsuitable or degraded habitat (eg. McGuigan *et al.* 1998, Donnellan *et al.* 1999). In the absence of a population genetic study of *L. booroolongensis*, the discrete populations identified in this study should be considered important management units for the conservation of this species.

Assessing temporal trends in the spatial distribution of *L. booroolongensis* within the SWS is limited at this stage due to the lack of locality records for this species prior to the last decade. Failure to locate *L. booroolongensis* along several sections of stream where this species was historically found suggests that this species may have undergone recent declines in this region. The local extinction from these sites would certainly be consistent with our understanding of the ecology of this species. The disappearance of *L. booroolongensis* from the Tumut River is likely to be due to the greatly altered flow regimes of this river resulting from the development of the Snowy Mountains Hydro Electric Scheme, as this river now runs with high water flows and low temperatures through much of summer. While sections of Basin Creek appeared to have extensive suitable habitat, this stream has been ephemeral in recent years due to drought, and so would not be expected to support a population of *L. booroolongensis*. This is because *L. booroolongensis* has a very short life-cycle such

that two consecutive years of failed recruitment due to stream drying would be expected to extirpate this species from that section of stream (author unpub. data).

There also appears to have been more recent population declines in the distribution of *L. booroolongensis* on the SWS. A resurvey of streams where *L. booroolongensis* was recorded in 1999 (Gillespie 1999) failed to locate the species along three streams. As with Basin Creek, these streams have extensive suitable habitat, but completely dried in the areas where *L. booroolongensis* was previously recorded in two consecutive summers between 2001 and 2003. While these apparent local extinctions may represent natural fluctuations in this species distribution and abundance, they also suggest the potential population sensitivity of *L. booroolongensis* to processes that increase stream ephemerality.

#### **5.4.2 Broad Habitat Associations**

The results of the broad habitat analysis for *L. booroolongensis* are consistent with our understanding of this species general ecology. The life-cycle of *L. booroolongensis* appears particularly centered around rocky structures along the stream. Males congregate for breeding around rock structures, females deposit eggs in the crevices of rock structures within the stream or in adjacent rock pools (Anstis *et al.* 1998), and all frog stages can be found sheltering under rocks along the stream both within and outside of the breeding season (Anstis *et al.* 1998, author pers. obs.). Since not all rock habitats are suitable as breeding sites for *L. booroolongensis*, the positive association for the presence of this species and increasing amount of rock habitat may be associated with an increasing probability that some of the rock habitat will be suitable for breeding, or that enough suitable habitat will be available to sustain a viable population.

The negative association between the presence of *L. booroolongensis* and canopy cover (Figure 5.3), is likely to relate to the thermoregulatory requirements of this species. The tadpoles of *L. booroolongensis* are often observed congregating in shallow sections of water (author pers. obs.), presumably to attain warmer body temperatures to enhance growth and development. While *L. booroolongensis* frog stages are typically nocturnal in their foraging and breeding activities, this species

may thermoregulate via selection of daytime retreat sites, as is often observed in nocturnal ectotherms (e.g. Webb and Shine 1998).

Despite recent declines, and being listed as an endangered, *L. booroolongensis* has managed to persist along sections of stream that have been highly modified. Many of the stream sections where *L. booroolongensis* is currently persisting have been completely denuded of native riparian vegetation and are open to stock access. This is very different to the response of other threatened riverine frog species along the eastern ranges of Australia, which appear to be susceptible to even moderate levels of habitat alteration (Gillespie and Hollis 1996, Parris 2001). The capacity for *L. booroolongensis* to cope with high levels of habitat disturbance along the stream may be due to this species primary habitat requirement being rock structures, as rock structures may remain intact even after all riparian vegetation has been removed, at least in the relatively short term.

While the persistence of *L. booroolongensis* along highly modified streams is intriguing, it should not be assumed that these populations are viable in the medium to long-term. Disturbance and associated habitat modification along many of these streams is an ongoing process, with habitat changes potentially moving in a direction not conducive to the longer-term persistence of *L. booroolongensis*. In addition to this, populations of *L. booroolongensis* along modified sections of stream may be greatly reduced in density, more fragmented, and hence more susceptible to stochastic influences (Caughley and Gunn 1996).

#### **5.4.3 Breeding Habitat Associations**

The positive association between breeding habitat and number of aquatic crevices (Figures 5.4 and 5.6) is consistent with the oviposition site requirements of *L. booroolongensis*. This species oviposits its eggs in rock crevices immediately adjacent to areas where breeding males are calling along rocky sections of stream bank (Anstis *et al.* 1998). The tadpole of this species also uses aquatic rock crevices for shelter. Hence, any process resulting in the loss of those crevices is likely to impact on *L. booroolongensis* populations. Increased sediment loads entering the stream are likely to reduce the availability of rock crevices as sediments may fall out of suspension and fill these crevices. High sedimentation in the stream also has the

potential to impact on the tadpole stage through reducing food availability, which may result in slower development and reduced fitness (Gillespie 2002).

One process likely to greatly reduce the availability of aquatic rock crevices along streams is willow (*Salix* sp.) infestation. This is because willows form dense surface root mats (Jayawardana *et al.* 2006) which, when in the close vicinity of rock habitats along the stream, would engulf and fill all available rock crevices. Willows may also impact on *L. booroolongensis* by completely shading the stream environment, which greatly reduces options for thermoregulation for both the tadpoles and frogs.

The positive association between the presence of *L. booroolongensis* and extent of pool, and negative association with water depth (Figures 5.4 and 5.6), is likely to be due to factors influencing larval development and survivorship. Water flow can be an important aspect of the ecology of riverine frog species, because high velocity water flows, particularly following rain events, can wash away egg clutches and tadpoles (Kupferberg 1996). The tadpole of *L. booroolongensis* is not torrent adapted (Anstis *et al.* 1998), and so streams that have the capacity for high water velocity may not be suitable for this species' larval survival. Slower flowing sections of stream would also be expected to favour tadpole development, as less energy would be required for swimming or maintaining position in the stream. In addition to this, shallower sections of stream may offer greater thermoregulation potential, and also restrict the range of possible aquatic predators of *L. booroolongensis* eggs and tadpoles.

#### **5.4.4 Conclusions and Management Recommendations**

An important feature of the current distribution of *L. booroolongensis* on the SWS is that 12 of the 18 discrete populations identified in this study are not represented within conservation reserves, but rather occur along streams flowing through privately owned and managed land in the agricultural landscape (Figure 5.1). Thus, adequately conserving *L. booroolongensis* on the SWS will rely on ensuring the persistence of this species along streams subjected to a variety of agricultural practices. It is important that an assessment of this species population response and viability under different management practices is undertaken to ensure that widely implemented practices are conducive to the persistence of this species. This should be undertaken

within a framework of rigorous monitoring such that the results are used to justify the continuation or modification of current land management practices (Hazel 2003).

One riparian management strategy that is currently being trialed along sections of stream supporting populations of *L. booroolongensis* is the restriction of stock access and improvement of native riparian vegetation cover. This management strategy is likely to greatly benefit *L. booroolongensis* through providing a buffer against stream erosion and sediment inflow (Prosser *et al.* 2001). However, while restricting stock access and increasing riparian vegetation is desirable for a range of environmental reasons (Pusey and Arthington 2003, Naiman and Decamps 1997), it should not be assumed that populations of *L. booroolongensis* would automatically respond positively to such actions. For example; the natterjack toad, *Bufo calamita*, recovery program found that this species preferred ponds in an early successional stage of vegetation growth, and the complete exclusion of cattle was ultimately to the detriment of this threatened toad species persistence at breeding sites (Denton *et al.* 1997). Similarly, cattle grazing was shown to maintain a more desirable hydroperiod in vernal ponds for the tiger salamander in North America (Pyke and Marty 2005). Hence, the broader application of this management approach for the conservation of *L. booroolongensis* should only be implemented after initial trials determine the population response of this species to be neutral or positive.

The apparent susceptibility of *L. booroolongensis* to stream drying has implications for the management of this species. A number of management practices in and around river systems may increase the potential for stream drying where *L. booroolongensis* currently occurs. This particularly relates to the direct pumping of water from streams during drought periods, and an increase in the proportion of catchments shifting from stock grazing to softwood plantations (Keenan *et al.* 2004). Climate change may prove to be the greatest threat to the persistence of *L. booroolongensis* along many streams, as this is likely to result in increased severity and frequency of stream drying.

Of critical importance for ensuring the persistence of *L. booroolongensis* populations is the protection of this species rocky breeding habitats along the stream. This particularly requires minimising sediment loads entering the stream and controlling invasive weeds. Increased sediment loads is likely to result from; poor road

construction and maintenance, inappropriate use of heavy machinery in the riparian zone, erosion due to livestock or vegetation removal and soil ripping over large areas. Since willow (*Salix* sp.) and blackberry (*Rubus* sp.) infestation may greatly reduce the available breeding habitat for *L. booroolongensis* along the stream, a control/eradication program for these weeds is likely to be a very effective immediate conservation strategy for this endangered species throughout much of its current range.

Given the broad scale decline recently experienced by *L. booroolongensis* (Gillespie and Hines 1999), the goal for the management of this species on the SWS should be to maintain viable populations for all existing populations/management units in this region. This may be difficult to achieve as several populations appear to be occupying very short sections of stream (Figure 5.1), and therefore are likely to be susceptible to local extinction due to environmental and demographic stochasticity (Caughley and Gunn 1996). Nevertheless, the majority of *L. booroolongensis* populations identified in this study occur along lengths of stream greater than three kilometres (Table 5.1), for which there are reasonable prospects to develop and implement adequate land management practices to ensure this species longer-term viability.

## **Chapter 6**

# **Presence of the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) in Threatened Corroboree Frog Populations in the Australian Alps**

### **6.1 Introduction**

Over the past fifty years, extinctions and declines of amphibian species worldwide have been occurring at an alarming rate (Stuart *et al.* 2004). Within Australia, eight frog species have not been seen in the wild since their initial observed declines, and a further 32 species have declined to levels warranting listing as either vulnerable or endangered (Hero and Morrison 2004). These declines have primarily occurred along the eastern ranges of Australia, from the alpine regions in the southern highlands to the wet tropics rainforest regions of North Queensland (see reviews within Campbell 1999). While frog declines in North Queensland have primarily been documented for upland riverine species (McDonald and Alford 1999), in south-eastern Australia both riverine and pond breeding species have exhibited rapid population declines from both high and low altitudes (Mahony 1999, Gillespie and Hines 1999, Osborne *et al.* 1999). Of particular concern is that many of the declines have occurred rapidly from areas of relatively pristine habitat within national parks and other reserve systems (Alford and Richards 1999, Osborne *et al.* 1999).

Research into the causes of frog declines within Australia has identified number of potentially contributing factors, including; introduced fish species (Gillespie 2001), habitat disturbance (Hero and Morrison 2004), increased drought frequency (Osborne 1989), increased UV-B radiation (Broomhall *et al.* 2000), the widespread use of agricultural chemicals (Hamer *et al.* 2004), and disease (Berger *et al.* 1998). The most substantial evidence for the cause of amphibian declines from relatively pristine upland environments is that they are the result of an outbreak of a disease known as chytridiomycosis, caused by infection with the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Berger *et al.* 1998, Skerratt *et al.* 2007). It is hypothesised that this pathogen was recently introduced into the Australian

environment (Berger *et al.* 1998), possibly from South Africa (Daszak *et al.* 2003, Weldon *et al.* 2004), which may explain why some species appear to be particularly susceptible to this pathogen. Both genetic (Morehouse *et al.* 2003, Morgan *et al.* 2007) and pre-decline screening for infection (Berger *et al.* 1998) supports the novel pathogen hypothesis. Although the retrospective data is considered by some to be insufficient to settle this issue (McCallum 2005, Rachowicz *et al.* 2005), a prospective study demonstrated that population declines of upland amphibians in Panama followed the arrival of *B. dendrobatidis*, supporting the epidemic wave hypothesis originally proposed on retrospective data (Lips *et al.* 2006).

Two closely related frog species that have undergone recent declines in south-eastern Australia are the northern and southern corroboree frogs, *Pseudophryne corroboree* and *Pseudophryne pengilleyi* respectively. Both these species occur in high montane and sub-alpine bog environments, where they typically breed in small ephemeral pools and seepage lines (Osborne 1989). Because the decline of these species initially coincided with a drought period during the early 1980's, it was suggested that drought causing early pool drying and tadpole mortality was the primary mechanism for the observed declines (Osborne 1989). However, despite subsequent periods of suitable climatic conditions, these species have continued declining across much of their range (Osborne *et al.* 1999). *Pseudophryne pengilleyi* is currently listed as nationally vulnerable, while *P. corroboree* is listed nationally as critically endangered. If current population trends continue, *P. corroboree* is likely to become extinct in the wild within the next five to ten years (David Hunter unpublished data).

This study was undertaken as an initial investigation into whether patterns of the emergence of infection with *B. dendrobatidis* in populations of both corroboree frog species is consistent with the possibility that this pathogen contributed to the initial and continued decline in these species. Histological techniques were used to assess the presence of *B. dendrobatidis* before and after population declines were first observed using preserved museum specimens and material collected from the field. Subsequently, skin swabs and real-time PCR was used to assess prevalence of infection in both species. Real-time PCR is a more sensitive test for *B. dendrobatidis* than histology in experimentally infected frogs (Boyle *et al.* 2004) and in wild frogs (Kriger *et al.* 2006; Speare *et al.* 2005), but was not available prior to 2004.

## **6.2 Methods**

### ***6.2.1 Study Species***

The southern corroboree frog, *Pseudophryne corroboree*, and northern corroboree frog, *P. pengilleyi*, are closely related species that breed in high montane and sub-alpine bog environments in south eastern New South Wales, Australia. Male corroboree frogs call from terrestrial nest sites around the edge of ephemeral pools that are typically dry during the summer breeding period. Females lay their eggs in these nest site, and the eggs develop through to a hatching stage and then enter diapause and await sufficient rains in autumn and winter to flood the nest site and allow the eggs to hatch the tadpoles to move through to the main pool. The tadpoles are then free swimming and feeding tadpoles until metamorphosis (Anstis 2002).

### ***6.2.2 Field Sampling***

The positions of male nest sites were determined using the shout-response technique, which involves shouting loudly at breeding habitat, to which corroboree frogs respond with their threat call. The position of responding males is then determined using triangulation, and marked with flagging tape for later inspection to locate the male. To avoid disturbing the males during the core breeding period, attempts to locate males were undertaken towards the end of the breeding season. The swabbing procedure involved holding the frog by the back legs and wiping the frog three times on each of the feet, hands, inside and outside of the thighs, stomach and back region. After the swab sample was taken, a digital photograph of the belly and throat pattern for each frog was also taken so as to check for recaptured individuals during future sampling. The swabs were stored in a cool location (esky with ice and then fridge) until delivery to the CSIRO Animal Health Laboratory in Geelong. The swabs were screened for the presence of *B. dendrobatidis* DNA using Taqman real-time PCR assay (see Boyle *et al.* 2004 for details of this procedure).

The following procedures were undertaken to minimise disease transmission between sites and between individuals within sites. Before entering the sites, all equipment that came into contact with frogs (both directly and indirectly) was sterilised with 90

percent ethanol. Each individual frog was handled using a new pair of disposable rubber gloves and a new plastic snap lock bag. Both items were immediately discarded after the frog was processed and a new set used for the next frog. Between processing individual frogs scissors were sterilised using 90 percent ethanol.

#### ***6.2.3 Histology and screening of toe material***

Preserved toe material from museum specimens collected prior to 1980, and toe material collected from extant populations between 1997 and 2000 were examined for infection with *B. dendrobatidis* using histology. The toes collected between 1997 and 2000 were decalcified in 15% formic acid for 20 hours and then embedded vertically in paraffin wax. The toes obtained from museum specimens were decalcified in 10% formic acid for 48 hours and then embedded horizontally in paraffin wax. The difference in the initial processing was due to the field-collected toes being originally processed for skeletochronology analysis. The toes were sectioned using a wax microtome to create ribbons of 5 µm sections. For the vertically embedded toes 15 to 20 transversal sections were taken, while for the horizontally embedded toes eight longitudinal sections were taken from the middle of the toe. The ribbons of sections were placed into a water bath mixed with 2% laboratory grade gelatine, and then mounted on a microscope slide. After drying sections were stained using the routine Mayer's haematoxylin and eosin procedure. The slides were then mounted with a 60mm cover-slip using D.P.X mounting fluid. The slides were assessed for *B. dendrobatidis* infection by visually scanning the areas of *stratum corneum* and *stratum granulosum* for each toe section using a light microscope set at 200 times magnification, and then 400 times magnification to confirm the presence of *B. dendrobatidis* zoosporangia (following recommended methods by Berger *et al.* 1999).

#### ***6.2.4 Statistical Analysis***

Uncertainty around the total proportion of adults testing positive for infection with *B. dendrobatidis* was estimated using a Bayesian approach with uninformative priors. The 95% credible intervals were propagated using Markov Chain Monte Carlo methods with 100,000 samples after the first 10,000 samples were discarded. This

was undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003).

### 6.3 Results

All time periods were not evenly represented by the archived specimens since there were no corroboree frog specimens collected between 1979 and 1991 available in the museums. The earliest museum specimen I sampled was collected in 1963. Of the 122 pre-1980 museum archived corroboree frog specimens sampled for infection with *B. dendrobatidis* using histology, no specimens tested positive for infection, whereas, of the 23 archived museum corroboree frogs sampled that were collected between 1991-93, 14 (61%) tested positive (Table 6.1). Of the 389 corroboree frog specimens collected from the field between 1997-2000 and sampled using histology, 14 tested positive for infection with *B. dendrobatidis* (Table 6.1). Because there was no overlap in the 95% credible intervals, differences between pre-1980 and post-1980 infection rates were considered significant for the archived museum specimens (Table 6.1). Significant differences were also observed between the post-1980 archived specimens, and the specimens collected from the field between 1998 and 2000 (Table 6.1).

Table 6.1. Results for the histological screening of individual *P. corroboree* and *P. pengilleyi* across populations before and after observed decline in these species

Period	Number of Sites	Number of Samples	Number Positive	Proportion Infected	95% Credible Intervals
<b><i>P. corroboree</i></b>					
<b>Archived</b>					
1960-69	2	56	0	0	0 - 0.06
1970-79	3	7	0	0	0 - 0.38
1980-89	NA				
1990-99	2	12	9	0.75	0.47 - 0.91
<b>Field</b>					
1998-2000	5	105	9	0.09	0.05 - 0.15
<b><i>P. pengilleyi</i></b>					
<b>Archived</b>					
1960-69	3	48	0	0	0 - 0.07
1970-79	2	11	0	0	0 - 0.27
1980-89	NA				
1990-99	3	11	5	0.45	0.21 – 0.69
<b>Field</b>					
1998-2000	12	284	5	0.02	0.01 - 0.04

The overall levels of *B. dendrobatidis* infection determined by real-time PCR screening of skin swabs were very high across remnant population of *P. corroboree* in both 2005 and 2006 (Table 6.2). Given the considerable overlap in 95% credible intervals, the difference in the proportion of individuals infected between 2005 and 2006 were not considered significant. Comparisons were not undertaken among populations because of the low number of individuals present/sampled in each population. The overall level of infection observed across *P. pengilleyi* populations in 2006 using real-time PCR screening was significantly lower than infection rates observed across *P. corroboree* populations during 2005 and 2006 (Table 6.2 and 6.3). High levels of inhibition of the PCR assay was observed across samples in 2006, with the highest inhibition being observed in the *P. pengilleyi* populations (Tables 6.2 and 6.3). Inhibition was not observed in 2005 because samples were diluted until the influence of the inhibitors was negligible (Hyatt et al. 2007).

Table 6.2. Results for the Amphibian Chytrid Fungus sampling undertaken across remnant *P. corroboree* populations in 2005 and 2006. Note - The calculation for proportion positive and 95% credible intervals (95%CI) excluded inhibited samples.

Site	Number of Samples	Number Inhibited	Number Testing Positive	Proportion Positive	95% Credible Intervals
(2005)					
Jagumba	4	0	3		
Big Dargals 2	6	0	6		
Far Dargals	10	0	3		
Far Dargals 2	4	0	1		
Upper Jagumba	2	0	1		
Manjar	3	0	0		
Far Manjar	1	0	0		
Hell Hole	1	0	1		
Ogilives Montane	3	0	0		
Snakey	5	0	2		
Upper Snakey	2	0	1		
TOTAL	41	0	18	0.44	0.30 - 0.59
(2006)					
Upper Jagumba	8	3	4		
Upper Snakey 2	1	0	1		
Dargals Flat	4	2	1		
Dargals Mountain	1	0	0		
Dargals Saddle	4	0	3		
Snakey	6	2	2		
Upper Snakey	2	0	1		
Far Manjar	1	0	0		
Maragle	2	0	1		
Far Dargals	8	0	5		
Far Dargals 2	4	0	2		
Hell Hole	4	1	2		
Upper Ogilives	2	2	0		
TOTAL	47	10	22	0.595	0.43 - 0.74

**Table 6.3.** Results for the Amphibian Chytrid Fungus sampling undertaken across *P. pengilleyi* populations in 2006. Note - The calculation for proportion positive and 95% credible intervals (95%CI) excluded inhibited samples.

Site	Number of Samples	Number Inhibited	Number Testing Positive	Proportion Positive	95% Credible Intervals
Bogong Peaks	11	0	2		
Big Plain C	4	0	0		
Big Plain C	4	1	0		
Big Plain E	9	8	1		
Big Plain A	7	3	0		
Devils Peak	3	3	0		
Brumby Flat	3	1	0		
Pabral Rd	2	2	0		
Cooleman	15	7	0		
Brindabella	15	10	1		
Barnets Rd	15	13	0		
Broken Cart	15	14	0		
Micalong Swamp	21	11	4		
Nottingham Rd	14	11	0		
Swamp Ck.	11	11	0		
TOTAL	149	95	8	0.14	0.08 - 0.26

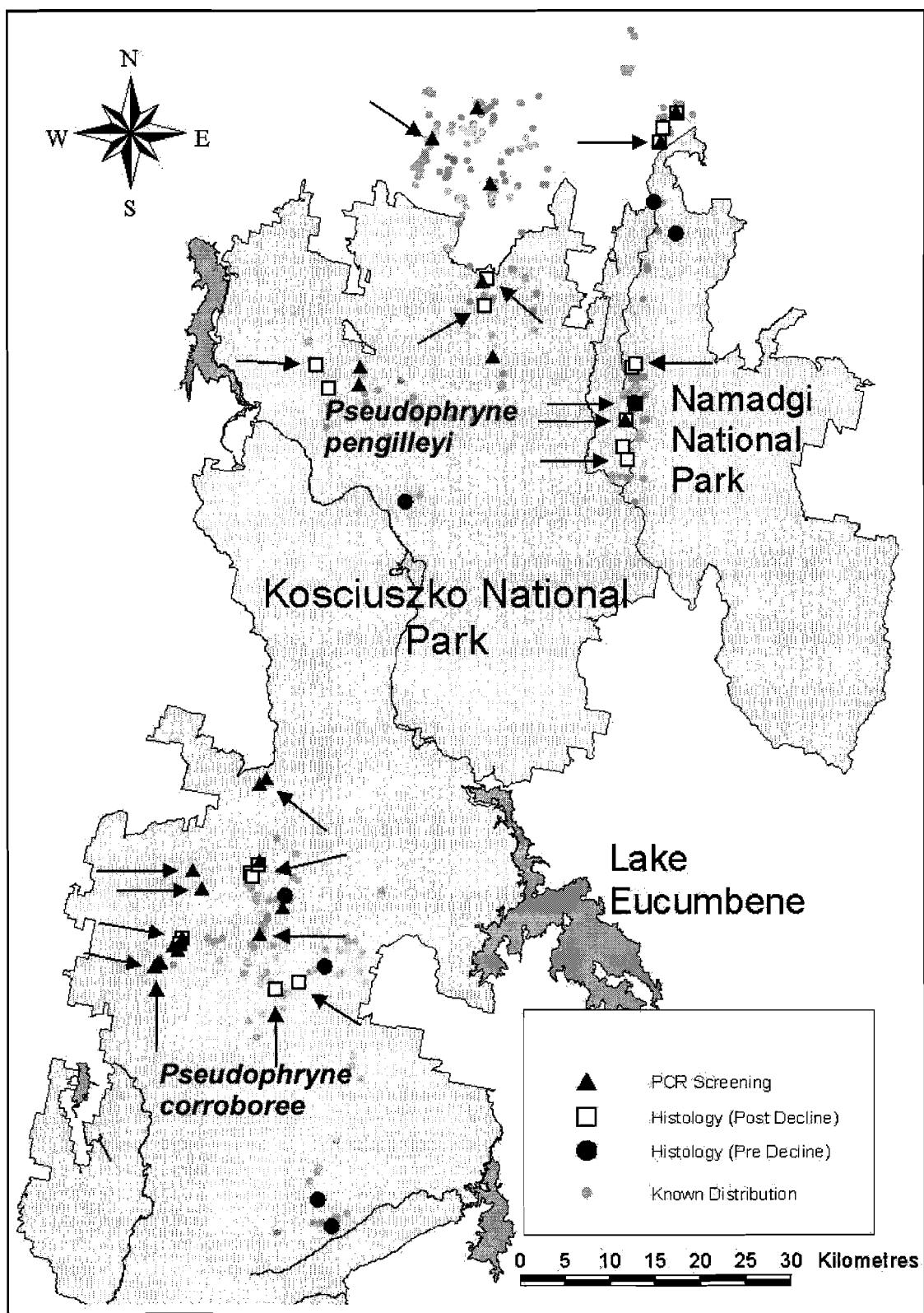


Figure 6.1. Location of sites sampled for *B. dendrobatidis* infection in *P. corroboree* and *P. pengilleyi* populations. Arrows point to sites where positive infection was recorded.

## 6.4 Discussion

### 6.4.1 Distribution and Levels of Infection in Extant Corroboree Frog Populations

This study has identified the presence of *B. dendrobatidis* across the distribution of both corroboree frog species (Figure 6.1), with considerable variation in infection levels between the two species and among the different sampling periods (Tables 6.1, 6.2 and 6.3). An important feature of these results is the high level of infection observed in very small populations of *P. corroboree* (Table 6.2). These results have several possible explanations. High infection at low densities may be expected if the pathogen is relatively benign to the host species (Anderson 1979). If *P. coorooboree* is susceptible to *B. dendrobatidis*, then this level of infection could be expected if other factors are enhancing infection rates, such as the presence of non-susceptible reservoir host species (Gog *et al.* 2002, McCallum 2005). Confidently interpreting these results would require information on how infection with this pathogen influences *P. corroboree* survival in the field. *Pseudophryne corroboree* certainly appears to be susceptible to this pathogen in captivity (David Hunter unpublished data), however the response of corroboree frogs to infection with this pathogen in captivity may not be indicative of its response in the wild owing to environmental factors.

Another important feature of these results is the significantly lower levels of infection observed in extant *P. pengilleyi* populations compared to extant *P. corroboree* populations (Tables 6.2 and 6.3). This is interesting because monitoring data suggests that many of these *P. pengilleyi* populations have not displayed population declines in recent years to the same extent as *P. corroboree* populations, or *P. pengilleyi* populations at higher altitudes (Osborne *et al.* 1999). If *B. dendrobatidis* is involved in the continued decline of corroboree frogs, then the different population trajectories observed among different areas may be attributed to variation in rates of infection with *B. dendrobatidis*. While this result may be an artefact of sampling variation associated with fluctuations in detectable infection rates (Berger *et al.* 2004, Kriger and Hero 2006), this may also be a plausible hypothesis for a number of reasons. During the breeding season in summer, lower altitude *P. pengilleyi* populations

occupy a warmer and drier environment than *P. corroboree* populations or high altitude *P. pengilleyi* populations (Osborne 1990), which are conditions less conducive to *B. dendrobatidis* spread and pathogenicity (Woodham *et al.* 2003, Johnson and Speare 2003, Berger *et al.* 2004). The potential mechanisms for lower infection in low altitude *P. pengilleyi* populations, or reduced pathogenicity, should be further investigated as it may provide critical information about the ecology of *B. dendrobatidis* in corroboree frog populations.

While I observed significant differences in overall infection rates among the three main sampling periods when *B. dendrobatidis* was detected (Tables 6.1, 6.2 and 6.3), biological explanations for much of this are confounded by the different screening techniques I used among these periods (i.e. histology of archived specimens, histology of field material, PCR screening). PCR has been shown to be a more sensitive test for *B. dendrobatidis* than histology in experimentally infected frogs (Boyle *et al.* 2004, Hyatt *et al.* 2007) and in wild frogs (Kriger *et al.* 2006), and it is possible that the different histological techniques used between the field collected and archived specimens may have also influenced detectability of infection. With regards to sampling procedures, an important aspect of the results of this study was the high proportion of real-time PCR samples expressing inhibition (Table 6.2 and 6.3). Hyatt *et al.* (2007) suggested that one factor increasing the probability of inhibition was the presence of foreign material, such as dirt, on the swabs. This may explain our results, because *P. pengilleyi* swabs from populations where high rates of inhibition were observed typically had greater quantities of dirt on them as a result of the frogs at these sites more often occupying partially earthen nest sites, as opposed to the vegetation nest sites at other sites (personal observations). While repeated dilutions of inhibited samples may reduce the influence of these inhibitors, as was undertaken for the 2005 *P. corroboree* samples, this procedure may increase the rate of false negative results (Alex Hyatt personal communications), and also increases the cost per sample.

#### **6.4.2 Presence of the Amphibian Chytrid Fungus pre and post decline**

This study did not detect the presence of *B. dendrobatidis* in corroboree frog populations prior to observed declines in these species, which began in the early

1980's (Osborne 1989). This is consistent with the results of other Australian studies which have undertaken retrospective screening of preserved frog specimens (Berger *et al.* 1998, Aplin and Kirkpatrick 2000), with the earliest record of *B. dendrobatidis* in Australia being 1978 (Berger *et al.* 1999). Berger *et al.* (1998) outlined several possible explanations for not detecting *B. dendrobatidis* in frog populations prior to observed declines, including that this pathogen only recently spread through the Australian environment. Determining whether *B. dendrobatidis* is a novel pathogen in Australia is fundamental to understanding and responding to frog declines attributed to this pathogen, because the hypothesis that it is endemic and only recently attained increased virulence implies that other factors are likely to be involved and need to be identified. While genetic data (Morehouse *et al.* 2003, Morgan *et al.* 2007) and pre-decline screening for infection supports the novel pathogen hypothesis, this evidence was not considered sufficiently robust to adequately address this hypothesis by some authors (McCallum 2005). The prospective study by Lips *et al.* (2006) has weakened arguments against chytridiomycosis not manifesting as an epidemic wave in chytrid-free areas (Skerratt *et al.* 2007).

As was pointed out by McCallum (2005), the strength of evidence of retrospective screening is limited in supporting the novel pathogen hypothesis. The level of sampling undertaken in this study was only statistically confident in detecting the presence of *B. dendrobatidis* if the levels of detectable infection were greater than 0.06 percent, which overlaps with the 95 percent credible intervals for the post-decline infection rates I detected using histology (Table 6.1). Hence, I am limited in suggesting that *B. dendrobatidis* was not present in the environment prior to these declines. Interpreting pre and post decline comparisons are confounded by a range of unknown factors, including the potential for infection to vary greatly between both seasons and years (Berger *et al.* 2004), and the potential for high rates of false negatives because of the limited sensitivity of histological screening for infection (Kriger *et al.* 2006). Despite the limitations for supporting the novel pathogen hypothesis, retrospective screening is a very powerful technique for rejecting this hypothesis, as only one positive sample prior to observed declines is required. Hence, retrospective screening should continue to be undertaken as a means to furthering our understanding about the emergence of *B. dendrobatidis* in frog populations.

#### **6.4.3 Disease hypothesis and the decline of the Corroboree Frogs**

The pattern of decline of corroboree frogs has similarities with the decline of other frog species for which *B. dendrobatidis* has been suggested as the primary causal agent. The initial decline observed for the corroboree frogs involved a reduction in the breeding adult population size that would have required an increase in adult mortality, not just failed recruitment to metamorphosis (Hunter 2000). Hence hypotheses implicating factors causing mortality during the post-metamorphic stages are more parsimonious than hypotheses involving only failed recruitment to metamorphosis (Scherer *et al.* 2005). In addition to this, the apparent altitudinal relationship in the observed corroboree frog declines, in combination with the fact that the high altitude populations are in cooler and moister habitats, is also consistent with the pattern of decline observed in other frog species where *B. dendrobatidis* has been implicated (Berger *et al.* 1998, McDonald *et al.* 2005).

Unlike other studies where *B. dendrobatidis* has been associated with declining frog populations (Berger *et al.* 1998, Lips 1999, Lips *et al.* 2006), sick and/or dead frogs infected with *B. dendrobatidis* have not been located during the monitoring of declining corroboree frog populations. Even if moribund frogs were present in declining corroboree frog populations, locating these individuals may not be expected because these species are typically concealed within vegetation and sick individuals are unlikely to respond to the survey technique (shout/response). The fact that this study sampled apparently healthy individuals reduces at least one form of sampling bias associated with comparing infection levels between different periods, areas or species (McCallum 2005).

There remains some conjecture over the origins of *B. dendrobatidis* and its role in the decline of frog species along the eastern ranges of Australia (McCallum 2005, Rachowicz *et al.* 2005). It may not be possible to adequately determine the role of this pathogen in the initial decline of corroboree frogs, because sampling during the early 1980's was not undertaken. Both corroboree frog species now have endemic chytridiomycosis, a situation similar to that which occurred after the epidemic wave in rainforest frogs in central and north Queensland (Retallick *et al.* 2004, McDonald *et al.* 2005). Species in central and north Queensland that did not decline rapidly to extinction (Schoegel *et al.* 2005) appear now to have stabilised and represent an

epidemiological pattern of initial declines, partial recovery and stability. This pattern may be occurring for low altitude populations of *P. pengilleyi*. However, I hypothesise that *P. corroboree* and high altitude populations of *P. pengilleyi* are showing a different pattern with a progressive decline, initially rapid, but now at a slower rate (Osborne *et al.* 2007, Hunter *et al.* 2006). This epidemiological pattern will lead to extinction unless the cause can be addressed.

Hence, it is important that research is undertaken to determine whether *B. dendrobatidis* is contributing to the continued decline of these species and if it is, to take measures to counteract it. Since other frog species in the Australian Alps region underwent similar rapid population declines starting in the early 1980's (Osborne *et al.* 1999), a coordinated approach to assessing the influence *B. dendrobatidis* on declining alpine frog species would be a more appropriate context. This would not only benefit the implementation of the corroboree frog recovery program, but would make an important contribution to assessing the broader implication of *B. dendrobatidis* to recent frog declines along the eastern ranges of Australia.

## **Chapter 7**

### **Significance of Reservoir Hosts in Amphibian Declines caused by the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*)**

#### **7.1 Introduction**

Amphibian declines and extinctions have been occurring at an alarming rate over the past five decades (Stuart *et al.* 2004). While there are a number of causal agents implicated in these declines (see Alford and Richards 1999 for review), the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, which causes the disease chytridiomycosis, is most notable for its widespread association with a large number of these declines (Berger *et al.* 1998, Daszak *et al.* 2003). Amphibian declines attributed to chytridiomycosis have occurred on every major continent (Berger *et al.* 1998, Rachowicz *et al.* 2005), and across a variety of environments from tropical rainforests to alpine areas (Berger *et al.* 1998).

While there is a growing literature associating *B. dendrobatidis* with rapid frog declines, conclusive data on the origins of this pathogen and modes of transmission and infection in the wild are lacking (McCallum 2005, Rachowicz *et al.* 2005). Determining whether this pathogen is novel in environments where declines have occurred is important, for if it is an endemic organism that has recently acquired increased pathogenicity, co-factors are more likely to be involved and need to be identified (McCalum 2005). Both genetic data (Morehouse *et al.* 2003) and pre-decline screening for infection (Berger *et al.* 1998) support the novel pathogen hypothesis, however, these data are considered insufficient to settle this issue (McCallum 2005, Rachowicz *et al.* 2005). The lack of information on host vectors and rates of infection are likewise leaving major gaps in our understanding of how *B. dendrobatidis* could be a primary agent in the observed declines as the speed and comprehensive spread of infection across the landscape appears implausible for a water-borne pathogen (McCallum 2005).

Another unresolved issue with respect to the impact of *B. dendrobatidis* is the mechanisms by which this pathogen could be causing species to decline to critically low densities or even extinction. The capacity for pathogens to cause wildlife populations to decline is of particular interest to epidemiologists and conservation biologists. This is because simple host/pathogen models predict that a highly virulent pathogen will have limited capacity to cause population decline because infected individuals would be expected to die before infecting others (Anderson 1979). Hence, factors other than just the interaction between the susceptible host and the pathogen are typically required for a virulent pathogen to spread through a wildlife population and cause significant population decline (McCallum 2005). A commonly observed factor enhancing the capacity for a pathogen to spread through a susceptible population is the presence of non-susceptible reservoir host species in the shared environment (Gog *et al.* 2002).

While reservoir hosts have been suggested as potentially important in the spread of *B. dendrobatidis* through declining frog populations (McCallum 2005), interactions between declining and non-declining species resulting in increased infection have not been demonstrated in the wild. Investigating the potential for non-declining frogs that are sympatric with declining species to be acting as a reservoir host for this pathogen is fundamental to the conservation management of species being impacted by this pathogen. This is because one potential management strategy for reducing the impact of disease in wildlife populations is the reduction of reservoir host densities in critical habitats (Caley and Hone 1994, Lloyd-Smith *et al.* 2005). In addition to this, knowing when major points of contact and cross infection are occurring between susceptible and non-susceptible species would also benefit the development of reintroduction strategies for critically endangered species so those critical periods can be avoided.

This study investigated the potential for the common eastern froglet, *Crinia signifera*, to be acting as a reservoir host for *B. dendrobatidis* in sub-alpine bog environments used by *P. corroboree* in Kosciuszko National Park. I also investigated the potential for tadpoles and subsequent metamorphs of *P. corroboree* to become infected with *B. dendrobatidis* upon being returned to their natural breeding pools, and whether this

infection was reduced if the tadpoles were placed in artificial pools (tubs) with natural silt substrates and water from the same site.

## 7.2 Methods

### 7.2.1 Study Sites and Species

The common eastern froglet, *Crinia signifera*, is a small myobatrachine found throughout much of south-eastern Australia (Barker *et al.* 1995). Within Kosciuszko National Park, this species is located at all elevations and breeds in a range of wetlands from small ephemeral pools to large permanent dams. Above 1300 meters altitude this species typically breeds following snow melt in spring. Females lay approximately 210 eggs directly into the water, which hatch and develop as free swimming and feeding tadpoles until metamorphosis approximately six weeks later (Anstis 2002).

The southern corroboree frog, *Pseudophryne corroboree*, is a small myobatrachine species restricted to the Snowy Mountains region of Kosciuszko National Park (Osborne 1989). The majority of extant populations of this species are located within the Jagungal Wilderness Area (Osborne 1989). This species is listed as Critically endangered under the *Commonwealth Environment Protection and Biodiversity Conservation Act* (1999), and is currently the focus of an intensive recovery program. The breeding season for *P. corroboree* is the middle of summer from early January to mid February, during which time their breeding pools are typically dry (Anstis 2002). The male *P. corroboree* call from a terrestrial nest site, which is where the female lays her eggs. At the end of the breeding season the male vacates the nest and the eggs are left to develop through to a hatching stage, at which point they enter diapause and await sufficient rainfall to fill the pools and flood the nest site which stimulates the eggs to hatch (Anstis 2002). Once the eggs have hatched, they then move through to the main pool where they develop as a free swimming and feeding tadpole until metamorphosis the following December (approximately 11 months after being laid) (Anstis 2002).

This study was undertaken within the distributional range of *P. corroboree*, in the Snowy Mountains Region of New South Wales. The locations of experimental sites are presented in Figure 7.1.

### **7.2.2 Swabbing Adult *C. signifera***

In November 2005, adult frogs of *C. signifera* were sampled for infection with *B. dendrobatidis* across seven sites within the current range of *P. corroboree* (Figure 7.1). Adult *C. signifera* were located by visual searches around breeding pools during the day, and then hand captured for swabbing using a sterile medical swab before being released at the point of capture. All swabbing for a particular site was undertaken on one day and the site was surveyed systematically to ensure frogs were only captured once. The swabbing procedure involved holding the frog by the back legs and wiping the frog several times on each of the feet, hands, inside and outside of the thighs, and on the belly. The swab was then stored in a cool environment until screening. Each frog was captured and swabbed wearing a new pair of disposable rubber gloves to prevent cross contamination between individuals.

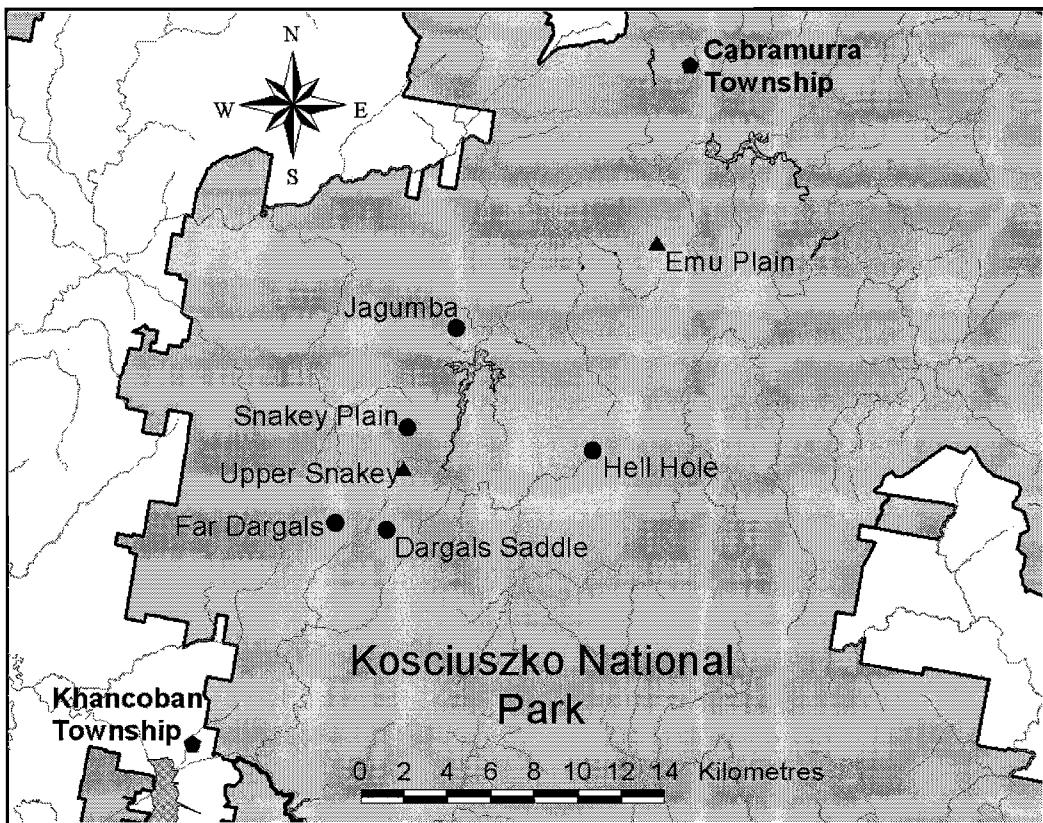


Figure 7.1. Location of sites where swabbing of *C. signifera* was undertaken (closed circles and triangles) and where *P. corroboree* tadpoles were returned to natural and artificial pools (closed circles).

### **7.2.3 Field Reintroduction/Infection Experiment**

The reintroduction experiment involved releasing *P. corroboree* tadpoles into three natural and three artificial pools at each of five sites (Figure 7.1) for the duration of their spring development period. Just prior to metamorphosis, the tadpoles were recaptured and brought back into the laboratory for completion of their development to metamorphosis, and then subsequent rearing to assess post metamorphic survivorship and *B. dendrobatidis* infection status. The *P. corroboree* tadpoles used in this study had been collected from the experimental sites as eggs in autumn, hatched and maintained in the laboratory over the winter period (at the ARC), and then released at the experimental sites in early spring. Refer to Hunter *et al.* (1999) for specific husbandry procedures prior to tadpole release. This process was undertaken to ensure sufficient numbers of tadpoles would be present in the pools in spring for this study. It was also undertaken to ensure that the tadpoles released into the artificial pools were free of infection with *B. dendrobatidis* at the time of release.

The criterion used to choose the natural pools within experimental sites was that they had been used by *P. corroboree* for breeding at least once in the past three years. This ensured that the pools were a suitable environment for the released tadpoles and that they represented the natural situation in terms of their susceptibility to infection.

The artificial pools were 200-liter grey polypropylene tubs positioned along the main channel of the bog. Each tub had a constant flow through of natural water from the mainstream channel at a rate of approximately 20 litres per hour. The stream water was gravity fed into one side of each enclosure and allowed to flow out of a mesh covered opening on the other side of the enclosure. A 2 cm layer of silt was placed on the bottom of each tub to provide a natural food source for the tadpoles. The tops of the tubs were a minimum of 30cm from the ground and positioned such that they could not be accessed by *C. signifera* frogs. The tubs were established just prior to snowmelt and allowed to settle for two weeks before the introduction of *P. corroboree* tadpoles.

On the 26 of September, twenty tadpoles were released into each of the 3 natural pools at the five experimental sites. Ten tadpoles were also released into each of the three artificial pools at these five sites. Only half the numbers of tadpoles were

released into the artificial pools because it was assumed that a greater proportion of these tadpoles would be recovered due to the less complex habitat structure and lack of aquatic invertebrate predators. The tadpoles were between Gosner stages 27 and 30 at the time of release (Gosner 1960).

Development of the tadpoles in the natural and artificial pools was monitored fortnightly by visual inspection. When the most developed tadpoles were approaching metamorphosis, tadpole sampling was undertaken to recover surviving tadpoles from all the natural and artificial pools (27<sup>th</sup> of November). This procedure involved dip-net removal of tadpoles from the natural pools until two censuses had been undertaken with no further tadpoles being found. Each census involved the careful dip-net removal of tadpoles until five minutes had elapsed without any further tadpoles being found. Dip-netted tadpoles were placed in five-litre food grade buckets that had water and a one-centimeter layer of silt from the pool being sampled. These surveys were also used to determine the presence or absence of *C. signifera* tadpoles. If they were present in the pool, ten *C. signifera* tadpoles were also dip-netted and placed in the buckets to be reared with the *P. corroboree* tadpoles so as to maintain the interaction between these two species. A maximum of twenty *C. signifera* tadpoles from each pool were also preserved in 100% ethanol for PCR screening for infection with *B. dendrobatidis*. Once all the experimental pools and tubs had been surveyed within a site, the buckets were placed in large plastic bags and transported by helicopter or 4WD vehicle back to a hut at the Tooma Reservoir, where they were stored in a cool location. After all the experimental sites had been surveyed, the *P. corroboree* tadpoles were measured for body length using vernier calipers, and scored for their Gosner developmental stage. The buckets with tadpoles were then placed in polystyrene boxes with a small amount of ice and transported by road to the Amphibian Research Center in Melbourne (approx. six hour drive).

Upon arriving at the Amphibian Research Center, the tadpoles, silt and water were placed in ten-liter plastic tanks, within a constant temperature room set at 16 °C ( $\pm$  2°C), for rearing through to metamorphosis. Each tank contained all the tadpoles, both *P. corroboree* and *C. signifera*, collected from a particular natural or artificial pool and were fed fish flakes and frozen endive, in addition to the natural food items in the silt substrate brought in from the field. The tanks were then randomly

positioned on a four-tiered shelf. Each tank had a 30 percent water change with carbon and sediment filtered water every two to three days. The tanks were checked every two to three days for metamorphosing individuals.

Metamorphs were removed from the tank and individually housed in 850ml plastic containers with a small amount of moist sterile sphagnum moss. These containers were randomly placed on a table in the same constant temperature room as the tadpole tanks. The metamorphs/juvenile frogs were feed on small crickets. The juvenile frogs were checked every two to three days to ensure that their containers were moist, and whether the frog was still alive. Containers were re-moistened using reverse osmosis water, and the enclosure changed for a new enclosure with new moss every two months. Any frog found to be dead were preserved in 70 percent ethanol for *B. dendrobatidis* screening. The experiment was terminated in late May, at which point surviving frogs were swabbed to determine *B. dendrobatidis* infection status.

#### **7.2.4 PCR Screening for *B. dendrobatidis* Infection**

The *C. signifera* adult swabs and tadpoles from the field, plus the dead *P. corroboree* metamorphs and swabs of live metamorphs for both species, were sent to the CSIRO Animal Health Laboratories in Geelong, and screened for the presence of *B. dendrobatidis* DNA using Taqman real-time PCR assay (Boyle *et al.* 2004). The hind feet of the dead *P. corroboree* and the mouthparts of the *C. signifera* tadpoles were used in the PCR screening as these areas typically harbour zoospores in infected frogs or tadpoles (Marantelli *et al.* 2004).

#### **7.2.5 Statistical Analysis**

Uncertainty around the proportion of *C. signifera* adults testing positive for infection with *B. dendrobatidis* was estimated using a Bayesian approach with uninformative priors. The 95% credible intervals were propagated using Markov Chain Monte Carlo methods with 100,000 samples after the first 10,000 samples were discarded. This was undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003).

Differences in the proportion of post metamorphic survivorship was analysed using a Bayesian logistic regression model, with the proportion surviving for the natural pool and artificial tub treatments being nested within each site. Because I was also interested in survivorship differences among the artificial tubs, natural pools where chytrid was not detected, and natural pools where chytrid was detected, I also pooled the data in these groups and compared their survivorships using a Bayesian logistic regression model. I undertook this analysis for survivorship from tadpole release to field collection, tadpole release to most metamorphosis, and also post-metamorphic survival. I also investigated differences in body length and Gosner stage between treatments using a Bayesian nested ANOVA, where the treatments of natural pool and artificial tub were nested within each site.

These analyses were undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003) which fits Bayesian statistical models using Markov Chain Monte Carlo (MCMC) methods. Uninformative priors were used for all model parameters and convergence of the algorithm was checked by visual examination of the output of three replicate Markov chains with differing starting values (Brooks & Gelman 1998). Final inferences for the mean and 95 percent credible intervals for each treatment combination were derived by discarding the first 10000 iterations from two chains and retaining the next 90000. Treatments were considered to be significantly different if their 95 percent credible intervals did not overlap.

### **7.3 Results**

Of the 109 *C. signifera* tadpoles preserved from the field from five sites, only one tadpole was identified as infected with *B. dendrobatidis*. Of the 138 adult *C. signifera* swabbed in the field from seven sites, 86 percent tested positive for infection with *B. dendrobatidis*. The results for individual sites and the 95% credible intervals for the overall proportion infected are presented in Table 7.1.

Of the 300 tadpoles placed in natural pools and 150 tadpoles placed in artificial tubs, 112 tadpoles were recovered from the natural pools and 120 tadpoles were recovered from the tubs for subsequent rearing through to a juvenile (post-metamorphic) frog stage at the Amphibian Research Center. No metamorphs from the artificial tubs were identified as infected with *B. dendrobatidis*. Seven of the 15 natural pools had metamorphs testing positive for infection. Of the natural pools identified as infected with *B. dendrobatidis* (as determined by the presence of at least one infected metamorph), 21 of the 22 dead metamorphs from these pools tested positive for infection with *B. dendrobatidis*. None of the *P. corroboree* metamorphs remaining alive at the end of this study tested positive for infection with *B. dendrobatidis*.

Table 7.1. Results from the swabbing and PCR screening of adult *C. signifera* for *B. dendrobatidis* infection.

Site Name	Number Sampled	Number positive	Proportion Infected	95% Credible Interval
Jagumba	11	11	1	
Hell Hole	2	2	1	
Emu Plain	4	4	1	
Upper Snakey	33	31	0.94	
Dargals Saddle	13	9	0.69	
Snakey Plains	48	41	0.85	
Far Dargals	27	21	0.78	
<b>Totals</b>	<b>138</b>	<b>119</b>	<b>0.86</b>	<b>(0.79, 0.91)</b>

While there was considerable variation in post-metamorphic survivorship between treatments and among sites, the results of the Bayesian logistic regression model identified greater survivorship for the artificial tubs within all five sites, with the 95 percent credible intervals only slightly or not overlapping within three of the sites for the artificial tub and natural pool treatments (Figure 7.2). The results for the Bayesian logistic regression model suggested that the proportion of tadpoles surviving from release to collection was similar for the data pooled by artificial tubs, natural pools with chytrid, and natural pools where chytrid was not detected (Figure 7.3). Post-metamorphic survivorship was significantly different among these groups, with the tadpoles from the artificial pools having the greatest survivorship (Figure 7.3). No tadpoles from natural pools with chytrid infections survived the post metamorphic period during this experiment (Figure 7.3). The results of the Bayesian nested ANOVA for tadpole development and size detected no relationship for these response variables with either pool or site (Figure 7.4). A visual inspection suggested no differences in the sizes or developmental stages of tadpoles from natural pools where chytrid was recorded compared with the tadpoles from the artificial tubs or pools where chytrid was not recorded (Figure 7.4).

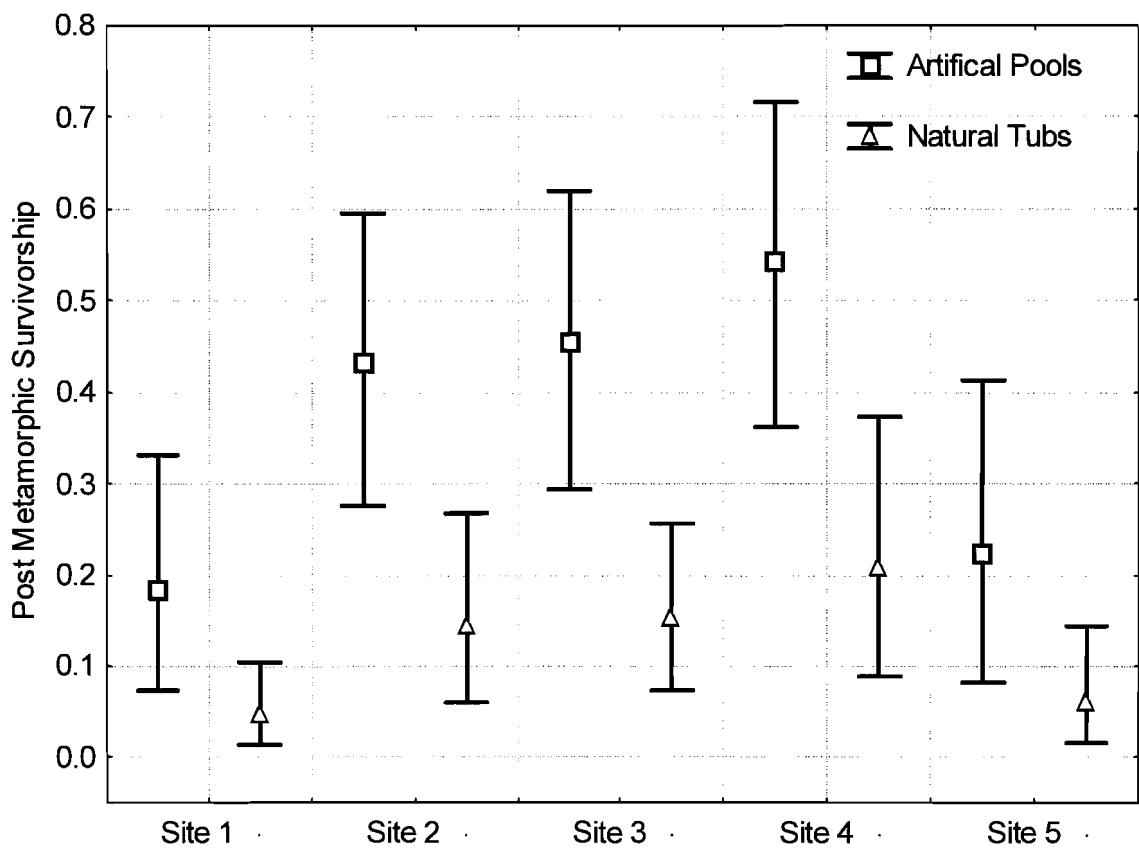


Figure 7.2. Post metamorphic survivorship from the Bayesian logistic regression for the tadpoles from artificial tubs and natural pools within each of the five experimental sites. The points represents the mean and error bars represent the 95% credible intervals.

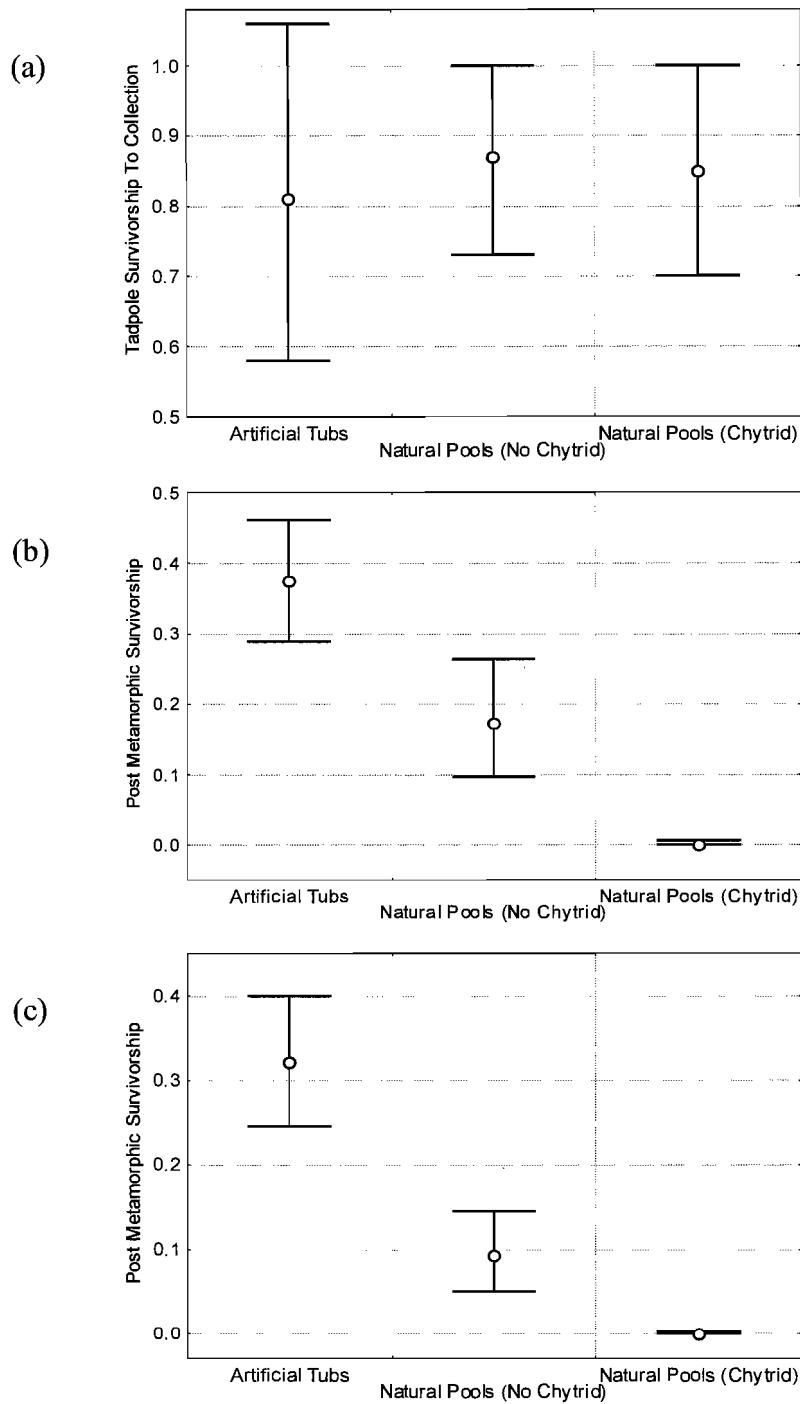
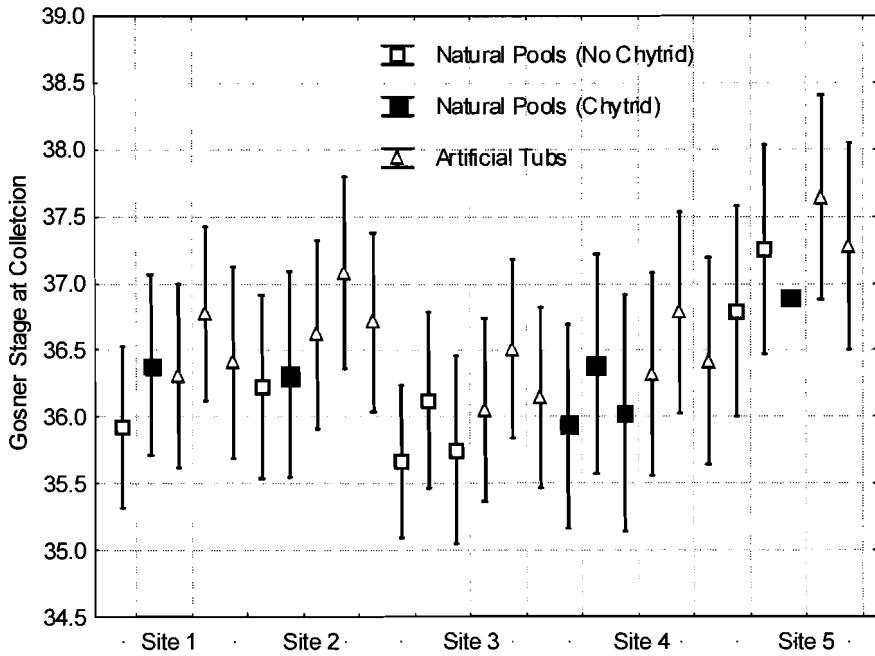


Figure 7.3. Results of the Bayesian logistic regression analysis for (a) tadpole survivorship from release to collection, (b) survivorship from collection to post metamorphosis, and (c) postmetamorphic survivorship. The points represent the mean and error bars represent the 95% credible intervals.

(a)



(b)

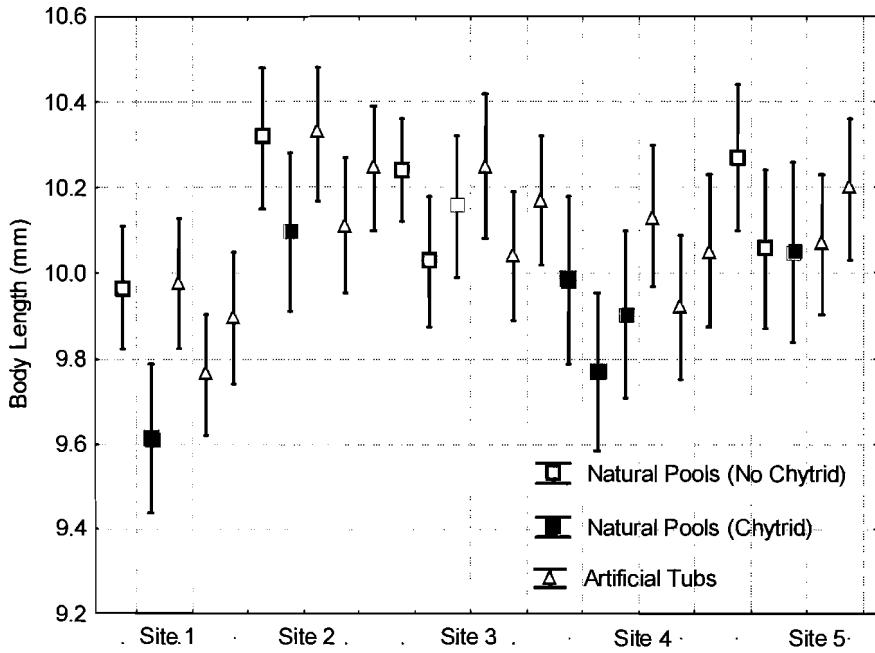


Figure 7.4. Results of the Bayesian nested ANOVA for (a) development stage, and (b) body length at the point of tadpole collection. Points represent the mean and error bars represent the 95% credible intervals. Note, the infection status of the natural pools was not incorporated into the analysis, but is presented for visual comparison.

## 7.4 Discussion

### 7.4.1 Infection of *P. corroboree* with *B. dendrobatidis*

The extremely high *B. dendrobatidis* infection rates observed in *C. signifera* in this study suggests that this pathogen is enzootic in populations of this species in sub-alpine bog habitats in Kosciuszko National Park. *Crinia signifera* is the only frog species occurring in sub-alpine and alpine environments in Kosciuszko National Park that has not undergone a noticeable decline over the past three decades. The extent to which infection with *B. dendrobatidis* increases mortality in this frog species in the wild is unknown, however this level of infection in apparently healthy populations suggests that this pathogen has minimal impact on this species. The implications of these findings is that *C. signifera* is a substantial reservoir host for *B. dendrobatidis* in subalpine bog environments in this region. These findings are similar to results for infection rates in the American bullfrog, *Rana catesbeiana*, which has also been hypothesised as being relatively resistant to this pathogen and operating as a major vector and reservoir host species across the landscape (Daszak *et al.* 2004, Hanselmann *et al.* 2004).

This study also demonstrated a high propensity for *P. corroboree* tadpoles to become infected with *B. dendrobatidis* in natural pools. Since the number of *P. corroboree* tadpoles released into each experimental site would be equivalent to a breeding population of less than ten adults, this result suggests a lack of any meaningful density dependant relationship between *P. corroboree* and tadpole infection with *B. dendrobatidis*. This finding is irrespective of whether *B. dendrobatidis* causes increased mortality in *P. corroboree*. High infection rates in *P. corroboree* despite low population density is particularly ominous with respect to the potential for *B. dendrobatidis* to impact on susceptible amphibian species, because it indicates the potential for this pathogen to drive susceptible species through to extremely low population size.

One very likely cause of this infection is the co-habitation of these sub-alpine pools by infected *C. signifera*. While *C. signifera* tadpoles were not detected in all natural pools in this study, adult *C. signifera* were observed in all the natural pools where

tadpoles were released during this study. My results suggest that post-metamorphic *C. signifera* may be more important as a reservoir for *B. dendrobatidis* than tadpoles because very few tadpoles of this species tested positive for infection. This is an interesting result as tadpoles of other species can be highly susceptible to *B. dendrobatidis* infection (Fellars *et al.* 2001, Rachowicz and Vredenburg 2004, Knapp and Morgan 2006), and in some cases infections in the tadpole stage can be much greater than in the terrestrial frog stage (Woodhams and Alford 2005). One possibility is that the tadpoles of *C. signifera* self cure or avoid infection, possibly through their behaviour of basking in warm shallow sections of pools as *B. dendrobatidis* is sensitive to even moderately warm temperatures (Longcore *et al.* 1999, Woodhams *et al.* 2003, Berger *et al.* 2004). While this study did not demonstrate that *C. signifera* was the only host species maintaining *B. dendrobatidis* in *P. corroboree* breeding pools, in the absence of other hosts for this pathogen, the results of this study would not be unexpected given the presence of *C. signifera* in these small sub-alpine bog pools.

The lack of infection in the tadpoles that were reintroduced into the tubs is an interesting result, as *B. dendrobatidis* can be easily transported in water or moist substrates (Johnson and Speare 2003, Johnson and Speare 2005). It certainly seems possible that small quantities of zoospores may have been introduced into the tubs initially, as a small number of *C. signifera* eggs were accidentally released in two tubs during the initial setup. These tubs certainly excluded tadpole and terrestrial frog stage *C. signifera*. Whatever the reasons for the *P. corroboree* tadpoles remaining free of infection, the capacity to maintain tadpoles in the field under reasonably natural conditions, but without becoming infected, has potential applications to future reintroduction efforts for this species.

With regards to other frog species acting as a reservoir host for *B. dendrobatidis*, McCallum (2005) made the following testable predictions; 1, the fungus should be less pathogenic in reservoir non-declining species than in the declining species, 2 the fungus should occur in lower prevalence in the declining species, and 3 the reservoir should continue to exist at sites where endangered species have either declined or disappeared. It appears from this study that *P. corroboree* may be more susceptible to *B. dendrobatidis* than *C. signifera*. Apart from the extremely high levels of infection

in apparently healthy populations of *C. signifera*, the only frogs remaining alive at the end of this study that tested positive for *B. dendrobatidis* were two *C. signifera*. Infection rates in the adult populations of these two species certainly suggest that *P. corroboree* has a lower prevalence of infection than *C. signifera* populations (refer to Chapter 6). And with respect to the third prediction, *C. signifera* remains persistent across the entire former range of *P. corroboree* (author pers. obs.).

An unexpected result of this study was the high juvenile frog mortality for pools and tubs where no *B. dendrobatidis* infection was observed, and this mortality was significantly greater for the tadpoles from the natural pools (Figure 7.3). This is unlikely to be due to *B. dendrobatidis* causing mortality but not being detected, because the rate of detection in the pools known to have *B. dendrobatidis* was very high (95 percent). It is also unlikely that this mortality was due to poor husbandry techniques, as these techniques have been used with great success for this species at the Amphibian Research Center over the past ten years. It cannot be discounted that this mortality was due to another pathogen. Given the possibility that this mortality was due to factors originating from the field, the causes of this mortality should be further investigated.

#### **7.4.2 Implications for the *P. corroboree* Recovery Program**

The implications of this study for the *P. corroboree* recovery program depend on the extent to which infection with *B. dendrobatidis* causes increased mortality in this species. Information is currently lacking on how infection with *B. dendrobatidis* influences survivorship of *P. corroboree* in the wild, however if the results from this study are any indication then *P. corroboree* appears to be highly susceptible. If *P. corroboree* is susceptible to *B. dendrobatidis*, then the capacity for high infection rates despite extremely low population size may explain the continued decline and impending extinction of this species from its relatively pristine environment in the Jagungal Wilderness area of Kosciuszko National Park (Osborne *et al.* 1999, Hunter 2000).

This study may also explain why an attempt to increase the size of *P. corroboree* breeding populations via the reintroduction of tadpoles failed. While this program increased recruitment to metamorphosis, it failed to increase the size of *P. corroboree*

breeding populations (see Chapter 9). Based on the results of this study, it is likely that a high proportion of tadpoles released back to the field would have become infected with *B. dendrobatidis* and probably died soon after metamorphosis. Further reintroduction efforts for *P. corroboree* should focus on the rearing and releases of post-metamorphic frogs, as this would avoid at least one period shown to result in high infection with *B. dendrobatidis*. Alternatively, tadpoles could be returned to the wild into pools that exclude potential reservoirs of *B. dendrobatidis*, such as the artificial pools used in this study.

#### **7.4.3 Reservoir Hosts and Frog Declines Due to *B. dendrobatidis***

A number of authors have suggested the potential importance of reservoir hosts in the decline of amphibians due to infection with *B. dendrobatidis* (Daszak *et al.* 1999, McCallum 2005, Woodhams and Alford 2005). The ecology of both amphibians and *B. dendrobatidis* are conducive to a multi host/single pathogen system resulting in increased infection of susceptible species with increasing diversity and abundance of non-susceptible/reservoir host species. Firstly, *B. dendrobatidis* is a generalist pathogen, being capable of infecting many different amphibian species (Berger *et al.* 1998). Moreover, the results of this study (Table 7.1) and others (Daszak *et al.* 2004, Hanselmann *et al.* 2004) suggest that some amphibian species can maintain very high infection levels (> 80%) with little or no apparent mortality, which increases the potential significance of these species as reservoir hosts as very few contacts between species would be required for cross infection to occur.

Secondly, regardless of the density of individuals across the broader landscape, many amphibian species often congregate at breeding sites (Duellman and Trueb 1986), resulting in close contact between susceptible and non-susceptible species. This is an important issue as it can potentially result in a complete lack of any density thresholds for infection levels, and hence allow *B. dendrobatidis* to drive susceptible species to extinction, or to levels where they are vulnerable to demographic and environmental stochasticity (Caughley and Gunn 1996). The potential for a lack of any relationship between density and infection rates was demonstrated in this study for *P. corroboree*, and was likely to have resulted from the shared use of an aquatic breeding environment with a reservoir host species.

Thirdly, *B. dendrobatidis* has a free-swimming zoospore stage that can live off frog hosts for up to seven weeks in the aquatic environment before infecting a new host (Johnson and Speare 2003). This allows cross species infection to occur in the aquatic environment without direct contact between individuals. In addition to this, because tadpoles can become infected with *B. dendrobatidis* in their keratinised mouthparts (Berger *et al.* 1998, Fellers *et al.* 2001), the period of overlap and potential cross infection between species includes the tadpole stage. In the case of this study, *P. corroboree* and *C. signifera* breeding seasons do not overlap, but the *P. corroboree* tadpole and metamorphic stage does overlap with the *C. signifera* breeding season.

#### **7.4.4 Conclusions and Further Research**

The potential for *B. dendrobatidis* to drive amphibian population declines is likely to be greatly enhanced by the presence of non-susceptible reservoir host species that share the aquatic breeding environment. For amphibian species believed to be in a state of decline due to a chytridiomycosis epidemic, identifying potential reservoir hosts for *B. dendrobatidis* is essential for developing management strategies aimed at reducing the impact of this pathogen. This is because reducing infection rates in a susceptible species may be most effectively achieved through a reduction in the density of non-susceptible host species (Caley and Hone 1994, Lloyd-Smith *et al.* 2005). Reducing the density or eradicating reservoir hosts would certainly be a powerful test of the significance of reservoir hosts in infection rates and declines caused by *B. dendrobatidis*.

If reservoir hosts play an important role in *B. dendrobatidis* induced declines, then susceptible species would be expected to contract to areas where they no longer occur in sympatry with the reservoir hosts. A useful exercise that may provide insight into the potential role of reservoir hosts would be to compare the contracted range of declining species to the distribution of potential reservoir host species. Furthermore, susceptibility to population declines caused by *B. dendrobatidis* may not only relate to a species' physiological or immunological characteristics (Woodham *et al.* 2006), but also to its breeding ecology (i.e. do they attend communal breeding sites).

As was outlined by McCallum (2005), understanding the mechanisms and rates of spread of *B. dendrobatidis* in the field is fundamental to assessing the hypothesis that this pathogen may be the primary cause of many amphibian declines. The interactions investigated in this study are likely to be occurring in other amphibian communities containing threatened frog species. Given the potential difficulties in responding to disease threats in the wild, this information is necessary in the search for management actions that may reduce the impact of this disease on wild amphibian populations.

## **Chapter 8**

### **Augmenting Recruitment to Metamorphosis Fails to Increase the Size of Breeding Populations of the Critically Endangered Southern Corroboree Frog (*Pseudophryne corroboree*)**

#### **8.1 Introduction**

The reintroduction of animals from captivity into the wild is often undertaken in threatened species recovery programs. Most commonly, reintroductions are undertaken as a means to bolstering populations threatened with extinction, or as an attempt to re-establish species in areas where they have recently gone extinct. While in theory animal reintroduction programs have much to offer species conservation programs, their application is often at great financial expense and success is anything but guaranteed (see Griffith *et al.* 1989, Snyder *et al.* 1996, Fischer and Lyndenmayer 1999, for reviews). Amphibians are one group of animals for which there appears to have been limited success at reintroduction (Dodd and Seigel 1991). Nevertheless, captive breeding and ultimately reintroduction back into the wild is currently perceived as a strategy to countering the alarming rate of amphibian declines and extinctions that have occurred across the world over the past three decades (Norris 2007).

A criticism of many amphibian reintroduction programs, and a possible reason for why they typically fail, is that they are often implemented before the causal factors of decline are identified and mitigated (Dodd and Seigel 1991). The importance of understanding and ameliorating threatening processes was particularly well demonstrated with the natterjack toad (*Bufo calamita*) recovery program in England where substantial success at re-establishing or enhancing populations was only achieved after the factors that caused the declines were understood and mitigated (Denton *et al.* 1997). Another criticism of amphibian reintroduction programs is that there is often inadequate follow up monitoring to assess the success of such programs. Developing general guidelines for implementing amphibian reintroductions requires

adequate post-release monitoring and assessment, and publication of the results regardless of whether the program was successful at increasing or re-establishing populations.

This paper reports an attempt to increase the size of southern corroboree frog, *Pseudophryne corroboree*, adult breeding populations by increasing recruitment to metamorphosis via captive rearing and reintroduction of tadpoles. *Pseudophryne corroboree* is a critically endangered frog species restricted to high montane and sub-alpine habitats in the Snowy Mountains region of Kosciuszko National Park, south-eastern Australia (Osborne *et al.* 1996). Since the mid 1980's, *P. corroboree* has been in a continued state of population decline (Osborne 1989, Osborne *et al.* 1999). By 1999, *P. corroboree* had become locally extinct from more than 85 percent of areas from where it was historically known to occur (Hunter 2000).

Like many amphibian species across the world that are declining from apparently pristine environments (Alford and Richards 1999, Stuart *et al.* 2004), the factors causing the decline of *P. corroboree* have not been clearly identified. Several other amphibian species across the alps region of south-eastern Australia have experienced a similar decline, suggesting that these declines may in part be due to a common causal factor that operates broadly across the landscape (Osborne *et al.* 1999). Because the initial decline of *P. corroboree* coincided with a period of severe drought, the initial decline of this species was suspected to be due to successive years of failed recruitment to metamorphosis (Osborne 1989, Osborne *et al.* 1999). Increased levels of UV-B radiation have also been suggested as a potential causal factor in the decline of this species, because the tadpoles of another declining species from Kosciuszko National Park (alpine tree frog, *Litoria verreauxii alpina*) was shown to be susceptible to ambient levels of UV-B radiation (Broomhall *et al.* 1999). More recently, the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, has been implicated in the decline of this species as high infections have been observed in declining populations, and the timing and pattern of decline is consistent with the decline of other species for which this pathogen has been suggested as the causal factor (Berger *et al.* 1998, refer to Chapter 6).

As part of the recovery program for *P. corroboree* a population augmentation program was initiated for this species in 1997 (Hunter *et al.* 1999). The aim of this

program was to determine the extent to which recruitment to metamorphosis was limiting this species' capacity for population recovery. This was undertaken by harvesting eggs from the field, and rearing them in captivity over the winter period prior to releasing them back to their natal pools in spring. I compared field and captive-reared survivorship over two seasons, and compared the adult male population trajectory over a five year period between sites where I increased recruitment, and sites where recruitment was not manipulated. I also investigated the relationship between natural recruitment to a late tadpole stage and subsequent adult male population size, and compared this relationship to the results for the sites where I artificially increased recruitment. This study tested one possible approach to preventing further population declines in *P. corroboree*, and has provided further insight into the nature of the continued decline in this species.

## 8.2 Methods

### 8.2.1 Study Species

The southern corroboree frog, *Pseudophryne corroboree*, is a small myobatrachine species restricted to the Snowy Mountains region of Kosciuszko National Park (Osborne 1989). The breeding season for *P. corroboree* is the middle of summer from early January to mid February. The males call from terrestrial nest sites, typically along the edge of bog pools, which is where the females lay their eggs. Females lay on average 26 eggs, which develop in the terrestrial nest site until Gosner stage 27 (Gosner 1960), at which point they enter diapause and await flooding of the nest site in autumn or early winter to stimulate hatching (Pengilley 1966, Osborne 1991). After hatching, the tadpoles then move into the main pool, where they then develop for a further nine to ten months before attaining metamorphosis the following summer (Pengilley 1992, Osborne 1991). It then takes a further four years for the frogs to reach sexual maturity (Hunter 2000).

### 8.2.2 Study Area

The area where this research was undertaken is within or immediately adjacent to the Jugungal Wilderness Area of Kosciuszko National Park. The location of the study

sites/populations is presented in Figure 8.1. These sites are within an altitudinal range of 1350 and 1650 meters, and are sub-alpine or high-montane sphagnum bogs.

### **8.2.3 Egg Collections and Captive Rearing**

The location of nest sites was determined by locating males during the breeding season in January. Males were located using the shout-response technique (Osborne 1989), which involves a person shouting loudly within an area of suitable habitat, to which male *P. corroboree* respond with their threat call. Augmentation sites were surveyed between three and five times during the peak-breeding period to maximise the number of nests located. Male call sites were marked and then inspected immediately after the breeding season to locate nests for egg collection. All eggs were removed from the nest and the number of live and dead eggs counted. The eggs were then placed in sphagnum moss filled containers and immediately transported by car to the Amphibian Research Center (ARC) in Melbourne (an approximately six hour drive).

Upon arriving at the ARC, the eggs were placed in containers with sterilised water, which in turn were placed in a water-bath and maintained at thermal profiles similar to that in natural nest sites. The eggs were maintained in a 100 percent humid terrestrial state. The entire system was enclosed within a controlled temperature room with ambient temperature set to approximate recorded field temperatures based on data collected previously from natural nest sites.

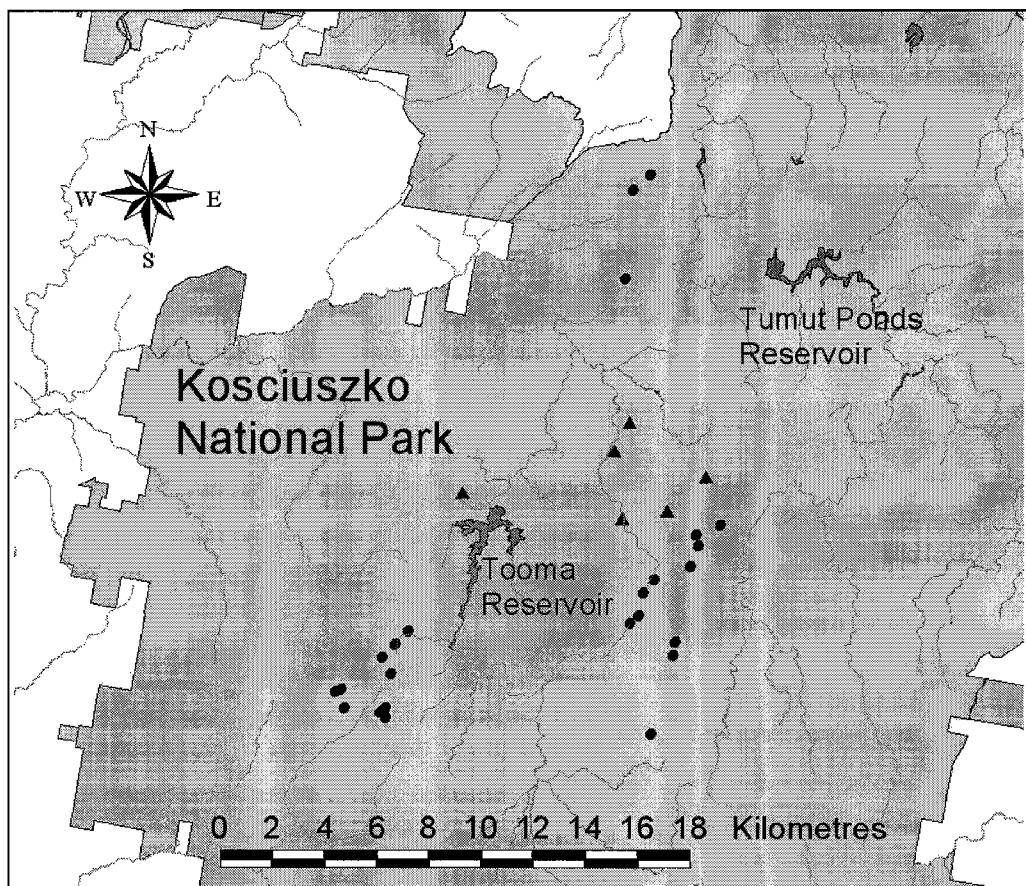


Figure 8.1. Location of population augmentation sites (closed triangles) and control sites where no augmentation and tadpole surveys were undertaken (closed circles).

Upon hatching, the tadpoles were moved to glass aquaria (1000 X 300 X 750mm) within the constant temperature room. Tadpoles from each pool were housed together. To mimic the field environment, individual tadpole enclosures contained only material obtained from the natal pool of the respective tadpoles. All water used was filtered by reverse osmosis then buffered with aquarium salt to approximate mineral content of natural alpine bogs. Tadpoles were housed at below recorded field densities (Hunter 2000) with no more than 50 in each 180-liter tank. A drip irrigation system provided for water exchange at a rate of approximately 20 percent per day.

After sufficient snow melt had occurred in spring to allow access to the bog pools, the captive-reared tadpoles were transported back to the field and released into their natal pools. Tadpoles were transported back to the field in five litre buckets containing waters and silt material from their rearing tank. A maximum of 50 tadpoles were placed in each bucket. A 30% water change and half an hour temperature equilibration period was undertaken before the tadpoles were released into the pools. Fortnightly inspection of pools was undertaken to ensure the tadpoles reached metamorphosis prior to pool drying in early summer.

#### ***8.2.4 Comparing Field Survivorship to Metamorphosis***

A comparison of field and captive-rearing survivorship to metamorphosis was undertaken at three sites over two years. This was undertaken by splitting each clutch by half so as to captive rear one half and leave the other half in the field. Eggs left in the field were returned to their nest after counting, and plastic three sided enclosures (1.0 m x 0.5 m x 0.4 m) constructed to link each nest site with an enclosed portion of the adjacent pool. This was undertaken so that after hatching, the tadpoles would move into the enclosed section of the pool where they could be counted to determine nest survivorship. The base of each enclosure was embedded deeply in the silt and moss in the pool, and extended into the moss bank beyond the position of the nest. The three walls of the enclosure had large, rubberised fiberglass mesh-covered openings to allow for water movement between the enclosure and the rest of the pool. All materials used were inert and resistant to ultraviolet radiation.

After snow had melted in mid-spring the captive-reared tadpoles were returned to the field where they were released into field enclosures (as described above)

immediately adjacent to the enclosure with the corresponding half clutch left in the field. To allow for a direct comparison of survivorship within clutches, all tadpoles released into enclosures represented individuals from the same clutch. For assessing survivorship differences between the captive-reared and field-reared tadpoles after snow melt, a census of tadpole numbers in each enclosure was conducted once a fortnight until metamorphosis in mid summer. Tadpole counts were conducted at night by torchlight. The number of tadpoles in an enclosure was assessed by dip-net removal until five minutes had elapsed without further tadpoles being found. Given the relatively small area within these enclosures, this census was assumed to have captured nearly all tadpoles present. During each census, Gosner stage (Gosner 1960) and snout-vent length were recorded for five randomly chosen tadpoles from each enclosure, or fewer if fewer tadpoles were present.

#### ***8.2.5 Assessment of Tadpole Recruitment at Non-Manipulated Sites***

Tadpole surveys were undertaken in late spring across 21 non-manipulated remnant populations so as to investigate the relationship between natural recruitment to a late tadpole stage (between Gosner stage 35 and 39, Gosner 1960) and subsequent breeding population size. These surveys were undertaken in 1998, 1999 and 2000. Breeding pools were identified by the presence of males during the breeding season, which were located using the shout-response technique. Due to the difficulties in accurately determining the abundance of tadpoles in large fens and continuous seepage lines, only populations where males were breeding in discrete pools were incorporated into this study (enclosed pools with less than a 5 m<sup>2</sup> surface area). Tadpole development was monitored across a range of sites to ensure correct timing of surveys, which in the three years of tadpole surveys was within the last three weeks of November. In each year, all sites were surveyed within a ten-day period.

The abundance of tadpoles within breeding pools was determined through dip-net removal of tadpoles until five minutes had elapsed without any further tadpoles being found. The search procedure involved three phases so as to maximise the number of tadpoles located. The first involved carefully dip-netting visible tadpoles so that silt and other loose debris was not stirred into the water column. After all visible tadpoles were removed, vegetation and other pool substrates were gently prodded and moved

so as to flush out any further tadpoles, again without stirring debris into the water column. Once no more tadpoles were detected using these procedures, dip-net sweeps were used to flush out tadpoles in more cryptic positions. On completion of the census, the tadpoles were counted and then placed back into the pool.

Because my tadpole surveys were unlikely to catch all the tadpoles present in a pool, I estimated the proportion of tadpoles that would be captured in a single census so that I could adjust my tadpole counts. This was undertaken through removal sampling of tadpoles using the tadpole survey methodology outlined above, but the tadpoles were retained after each census until five censuses were completed. A minimum resting period of half an hour was allowed between each census. This process was undertaken across nine pools in 1999 and thirteen pools in 2005.

#### ***8.2.6 Monitoring Breeding Population Size***

The breeding population size at sites where recruitment to metamorphosis was increased, and the non-manipulated sites, which included sites where tadpole surveys had been undertaken, was monitored using the shout-response technique described above. A minimum of three censuses were undertaken at each site to maximise the number of males located. During each census the position of male call sites was marked with flagging tape. The total number of males located was used as the male population size for subsequent comparisons.

### **8.2.7 Screening for Amphibian Chytrid Fungus Infection**

In each year of the tadpole releases, two tadpoles from each captive-rearing tank were screened, using histological techniques, for infection of the mouthparts with the amphibian chytrid fungus, *B. dendrobatidis*. All tadpoles tested negative for *B. dendrobatidis* infection (L. Berger pers. com.).

### **8.2.8 Statistical Analysis**

Differences in survivorship and time to metamorphosis between the captive-reared and field tadpoles were explored by estimating the within clutch mean difference for these two measures. This was undertaken using a Bayesian approach to estimating the mean difference. I used uninformative priors and assumed a normal distribution, with the mean and 95% credible intervals being estimated from 100,000 samples after the first 10,000 samples were discarded. These analyses were undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003).

I analysed the tadpole removal sampling using a closed mark-recapture model where recapture probability was fixed at 0 to account for the fact that tadpoles were not returned to the population, and therefore could not be recaptured (Lukacs 2006). I fitted four pre-defined models to my data that accounted for variation in capture probability being influenced by either year (group), census (time) or a combination of the two, which gave us four pre-defined models. Each of the four models was ranked according to Akaike Information Criterion (AIC), and the estimated capture probability for the first census (proportion of tadpoles caught using one census) was derived from the model averaged estimate for this parameter from the four models (Burnham and Anderson 2002). The closed population mark-recapture analysis and associated model ranking and averaging were undertaken using the closed-capture option in Program MARK (Cooch and White 2006).

I used a linear regression model to explore the relationship between the number of late stage tadpoles in a population, and the subsequent number of males. I adjusted both the number of tadpoles released and number of tadpoles detected during surveys before this analysis. The number of tadpoles released was adjusted to account for

tadpole mortality occurring between release and late tadpole stage by multiplying the number of tadpoles by the mean survivorship observed during the monitoring of released tadpoles during 1997 and 1998. I adjusted the number of tadpoles counted during tadpole surveys according to the detection probability for the first census ascertained by the removal sampling.

Because *P. corroboree* has overlapping generations (Hunter 2000), I used tadpole recruitment data over consecutive years to investigate the relationship between recruitment and adult population size. Tadpole counts for two consecutive breeding seasons (1998 and 1999) were used for the comparison of male counts in 2004, and three consecutive breeding seasons (1998, 1999 and 2000) for comparing male counts in 2005. The male counts for 2004 and 2005 were used because male *P. corroboree* take four years to reach sexual maturity (Hunter 2000). While the population age structure of *P. corroboree* may consist of more than three age cohorts (Hunter 2000), the two and three cohorts used in this analysis should comprise the majority of individuals in the adult population as there was failed recruitment to metamorphosis due to drought during the two years prior to 1998 (Hunter 2000). The following calculations were used to determine the number of tadpoles corresponding to the male counts for 2004 and 2005. These calculations are based on an estimated breeding male annual mortality rate of 50% (Hunter 2000).

$$2004 = (\text{tadpoles in 1998} * 0.5) + \text{tadpoles in 1999}.$$

$$2005 = (\text{tadpoles in 1998} * 0.25) + (\text{tadpoles in 1999} * 0.5) + \text{tadpoles in 2000}$$

I used a Bayesian approach with uninformative priors for the regression coefficients to explore the linear relationship, and associated variation, between number of tadpoles and subsequent number of males. This was undertaken using Markov Chain Monte Carlo methods, with the estimated parameters being based on 100,000 samples after the first 10,000 had been discarded. The data were Log10 + 1 transformed before analyses. These analyses were undertaken using the WinBUGS software package, version 1.4 (Spiegelhalter *et al.* 2003).

### **8.3 Results**

During both 1997 and 1998 the mean post-release survivorship to a late stage tadpole for the captive-reared tadpoles was 62 percent (S.E. 1997 = 7.4, 1998 = 5.3). This was higher mortality than the mean post-winter survivorship for the field tadpoles which was 79 percent for 1997 and 88 percent for 1998 (S.E. 1997 = 10.2, 1998 = 4.03). Overall survivorship from egg to metamorphosis is presented in Figure 8.2. During both 1997 and 1998, captive rearing increased survivorship to metamorphosis by an average of 19% in 1997 and 30% in 1998 (Figure 8.3). The within clutch difference in time to metamorphosis suggested that the captive-reared tadpoles took longer to reach metamorphosis (Figure 8.4). There was considerable overlap among clutches in the time taken to reach metamorphosis for the captive-reared and field tadpoles (Figure 8.5)

The number of captive-reared tadpoles released at the population augmentation sites varied among years and sites, with some sites having no tadpoles being released as no eggs were located in that site (Table 8.1). The population trajectory was very similar for both the recruitment augmentation sites and the non-manipulated sites (Figure 8.6). By the end of this study (2006), the majority of populations for both groups had declined by more than 80% of their numbers in 1999.

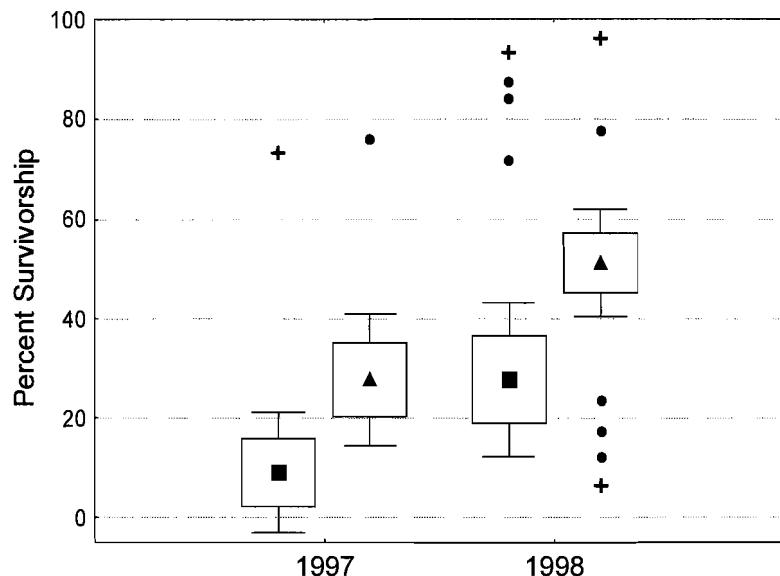


Figure 8.2. Survival from egg to metamorphosis for field clutches (closed squares) and captive reared clutches (closed triangles). The point is the mean, box is the 75% confidence limits and the error bars are the 95% confidence limits. The closed circles are outliers and crosses are extreme values.

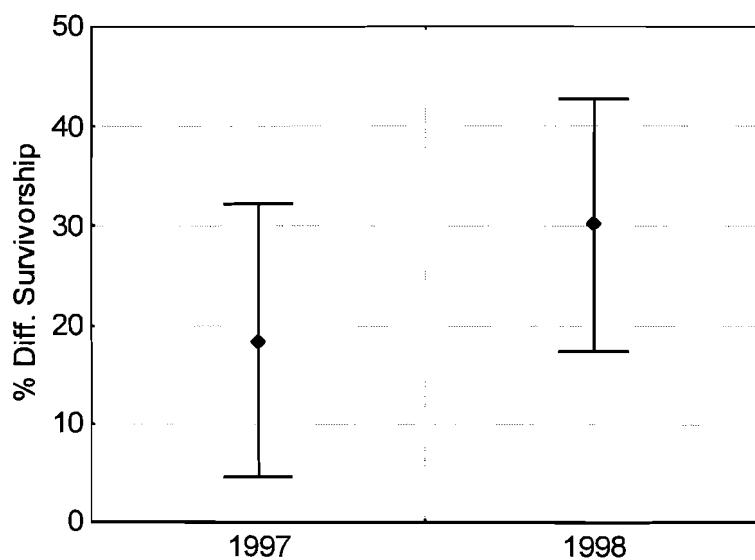


Figure 8.3. Difference in survival from egg to metamorphosis between field and captive reared clutches. The point is the mean and the error bars are the 95% credible intervals.

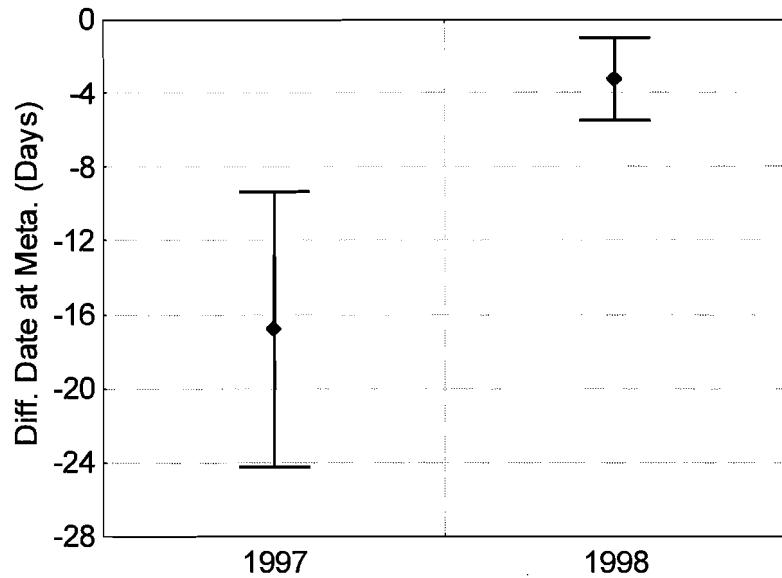


Figure 8.4. Difference in time to metamorphosis (number of days) between clutches left in the field and captive reared clutches. The point is the mean and the error bars are the 95% credible intervals.

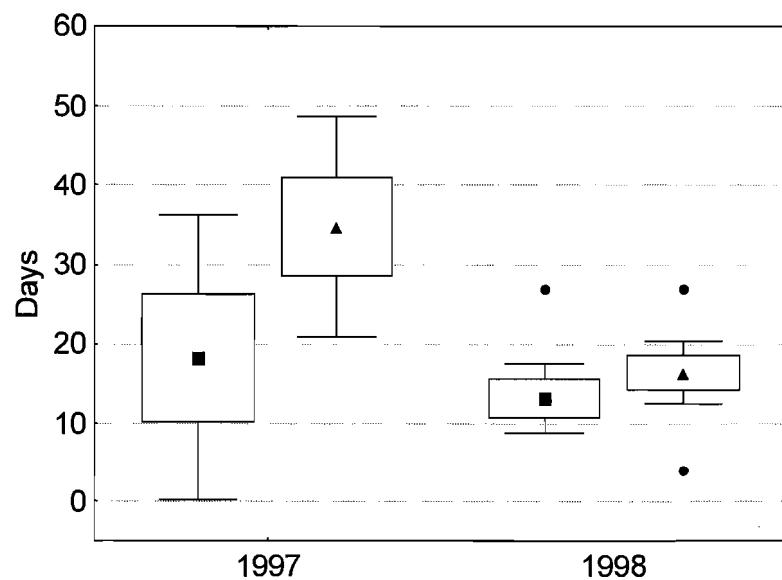


Figure 8.5. Number of days to metamorphosis after the start of December for field clutches (closed squares) and captive reared clutches (closed triangles). The point is the mean, box is the 75% confidence limits and the error bars are the 95% confidence limits. The closed circles are outliers.

Of the four models investigated for the tadpole removal sampling, the model suggesting varying capture probability between censuses while maintaining group (year) constant had the most support (Table 8.2). The model averaged results for the four pre-defined models suggested that capture probability of tadpoles within natural pools was high (0.91) (Table 8.3). During both years, no tadpoles were found during the forth and fifth census for any of the pools, suggesting that by the third census the majority of tadpoles had been captured, and the model averaged estimates for total population size were very similar to the total number of tadpoles found (Total found: 1999 = 144 tadpoles, 2005 = 110 tadpoles) (Table 8.3).

A positive relationship was observed for the linear regression model for number of late stage tadpoles and subsequent number of males for both the 2004 and 2005 data (Figure 8.7). The 95% credible intervals for this relationship were wide (Figure 8.7). The number of males corresponding with the number of released tadpoles at the augmentation sites was typically within the 95% credible interval of the linear relationship for the control sites (Figure 8.7).

Table 8.1. Number of tadpoles released at sites where recruitment to metamorphosis was augmented.

	<b>1997</b>	<b>1998</b>	<b>1999</b>	<b>2000</b>	<b>2001</b>
<b>Seldom's B.</b>	-	290	67	50	106
<b>Jugumba</b>	43	143	101	169	225
<b>Round</b>	23	-	-	26	81
<b>Ogilives A</b>	-	52	39	41	122
<b>Ogilives B</b>	-	29	67	241	-
<b>Ogilives Q</b>	-	49	205	239	353

Table 8.2. Comparison of the candidate models for the removal sampling of *P. corroboree* tadpoles. Models are ranked according to their AICc – Akaike's Information Criterion, values.

<b>Model</b>	<b>AICc</b>	<b>AICc Weights</b>	<b>Model Likelihood</b>	<b>Num. Par</b>	<b>Deviance</b>
{N, p(t), c=0}	-1787.55	0.89221	1	3	0.755043
{N, p(t+g), c=0}	-1782.25	0.06328	0.0709	6	1.3E-06
{N, p(g), c=0}	-1780.54	0.0269	0.0302	2	9.767914
{N, p(.), c=0}	-1779.7	0.01762	0.0197	2	10.6136

Table 8.3. Model averaged estimates for the parameters of capture probability during the first census and total tadpole abundance for the removal sampling of *P. corroboree* tadpoles. These averages are based on the four models and associated AICc Weights presented in Table 8.2.

<b>Parameter</b>	<b>Estimate</b>	<b>SE</b>	<b>LCI</b>	<b>UCI</b>
<b>Capture Probability (p) 2000</b>	0.91163	0.018903	0.866898	0.942328
<b>Capture Probability (p) 2005</b>	0.914073	0.018647	0.869789	0.944262
<b>Population Size (N) 2000</b>	141.4623	4.985938	131.6899	151.2347
<b>Population Size (N) 2005</b>	110	5.7E-06	110	110

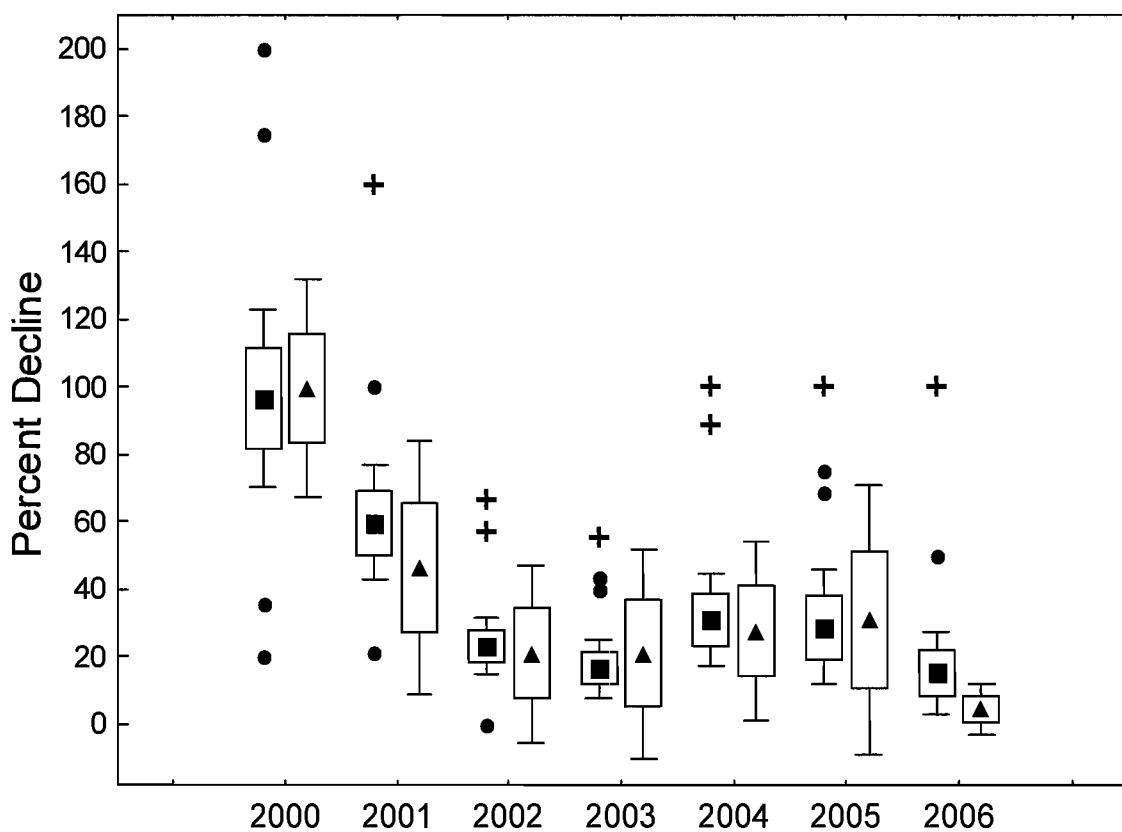


Figure 8.6. Level of decline relative to the number of males observed in 1999 at sites where recruitment to metamorphosis was augmented (closed triangles) and control sites where recruitment to metamorphosis was not manipulated (closed squares). The point is the mean, box is the 75% confidence limits and the error bars are the 95% confidence limits. The closed circles are outliers and crosses are extreme values.

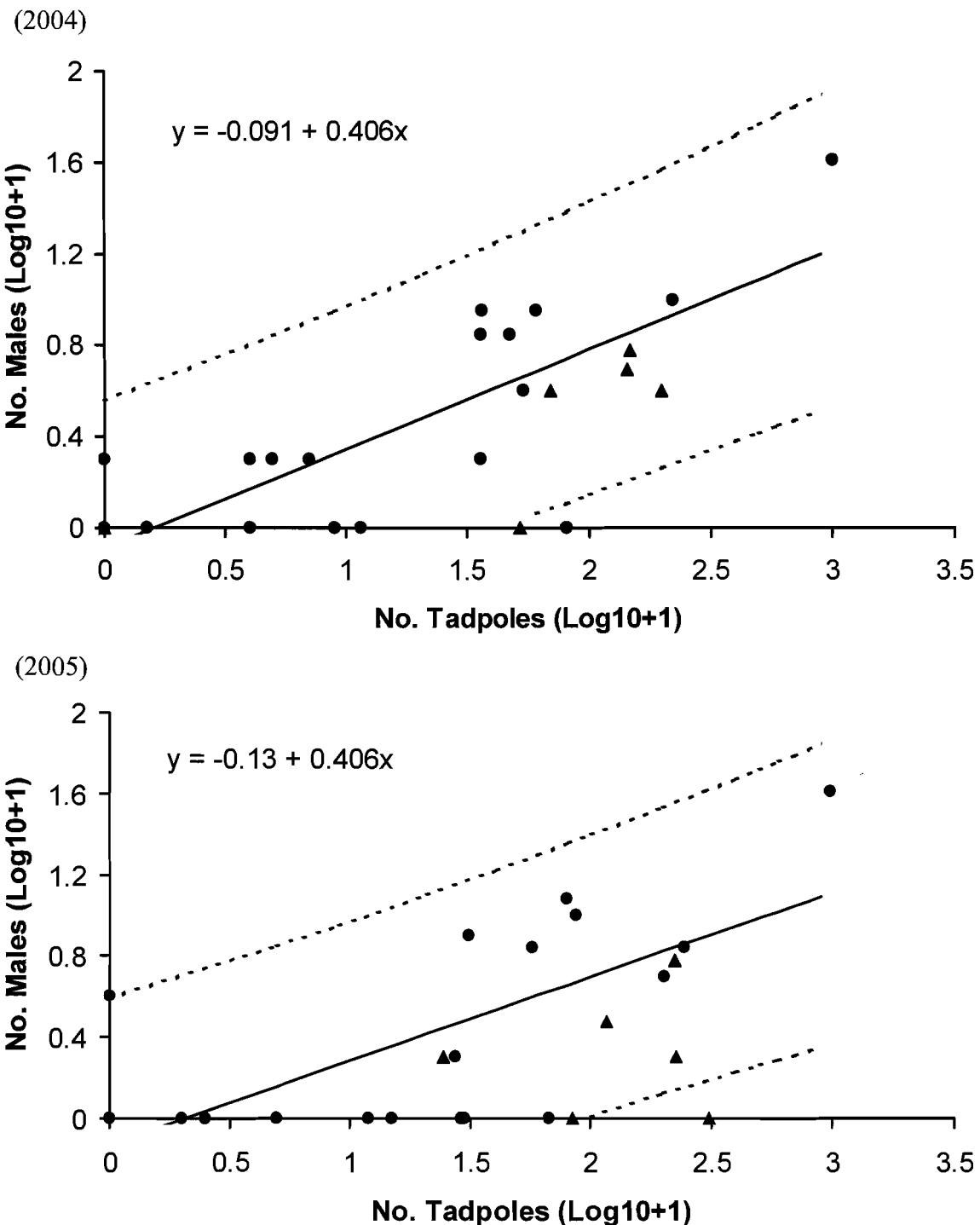


Figure 8.7. Relationship between the number of tadpoles and subsequent number of breeding males across non-manipulated sites (closed circles) for male counts during 2004 and 2005. The dotted lines are the 95% credible intervals for this relationship. The number of males relative to tadpoles released at the augmentation sites has been presented on these graphs for visual comparison (closed triangles).

## 8.4 Discussion

### 8.4.1 The Influence of Increased Recruitment on Adult Population Size

Increasing survival to metamorphosis in remnant *P. corroboree* populations did not result in either an increase in breeding population size or even a noticeable reduction in rate of decline (Figure 8.6). One explanation for this is that I did not increase recruitment to metamorphosis sufficiently to be able to detect a subsequent effect on population size. This appears likely based on the considerable variation observed between recruitment in non-manipulated populations and subsequent male population size (Figure 8.7), as this variation suggested the potential for a several fold difference in survivorship from metamorphosis to sexual maturity among sites. Hence, increasing recruitment to metamorphosis by 30 percent (Figure 8.3) was unlikely to have resulted in a noticeable or biologically significant effect on adult population size. The variation in survivorship from metamorphosis to sexual maturity observed in this study is in itself an important result, as it suggests that factors influencing post metamorphic survivorship are likely to be important in the demographic failure currently experienced by *P. corroboree*.

A number of factors may be influencing the high variation observed in the relationship between recruitment to a late tadpole stage and adult male population size (Figure 8.7), including demographic and environmental stochasticity. Demographic stochasticity is likely because many of the populations monitored were small (less than 10 calling males), and so would be expected to experience high variation in survivorship parameters (Caughley and Gunn 1996). This suggestion is consistent with other anuran studies that have observed high variation in population growth at small population size (Beebee *et al.* 1996, Carlson and Edenhamn 2000, Green 2003). Environmental stochasticity may have also contributed to this variation, as a wildfire moved through the entire distribution of *P. corroboree* in the summer of 2003. The extent to which this fire impacted on frog survivorship between different sites is likely to have varied greatly, and may have significantly reduced the overall level of frog survivorship, but unfortunately I was unable to make a meaningful assessment of this.

Another possible influence on the results of this study was the relative fitness of the captive reared tadpoles. The captive reared tadpoles certainly had reduced survivorship between their release and metamorphosis, however much of this mortality occurred immediately after release (Hunter *et al.* 1999). While the within clutch comparison indicated a difference for date at metamorphosis during both 1997 and 1998 (Figure 8.4), this difference was unlikely to be biologically important as there was considerable overlap in the actual date at metamorphosis between these two groups (Figure 8.5). Given the high variation in survival from metamorphosis to sexual maturity at non-manipulated sites, a reduction in the fitness of captive reared individuals would have needed to be substantial to have resulted in a noticeable influence on the breeding population.

An important question following from the results of this study is; could I have attained a stable or increasing *P. corroboree* population if survivorship to metamorphosis was greater? This seems unlikely given the magnitude of the continued decline at sites where I attained 50 percent survivorship from egg to metamorphosis (Figure 8.6). The capacity to increase survivorship beyond what I attained may also be limited, as 20 percent of the mortality was unavoidable because it occurred in the nest prior to egg collection (Hunter *et al.* 1999), and earlier collections would disrupt the males during the breeding period.

#### **8.4.2 Demographic Failure and Causal Factors of Decline**

The suggestion that unattainable levels of survivorship to metamorphosis would be required to increase *P. corroboree* populations suggests that the egg and tadpole stage may not be the most important life-history stage in terms of demographic failure in this species. This study suggests that mortality during post-metamorphic stages may be having a greater influence on the decline of *P. corroboree*. This would be consistent with the conclusions of several recent studies investigating the demographic sensitivity of anuran populations to mortality at different life stages. These studies suggest that while amphibian populations may fluctuate as a result of their typically variable levels of recruitment to metamorphosis, population declines of conservation concern are more likely to be the result of increased mortality occurring

during post-metamorphic frog stages (Conroy and Brooks 2003, Biek *et al.* 2002, Vonesh and Cruz 2002).

Implicating mortality during post-metamorphic frog stages with the ongoing population decline in *P. corroboree* has implications for assessing the causal factors in this species' decline. Three hypotheses have been proposed as potential causal factors of the initial and continued decline in *P. corroboree*; increased frequency of early pool drying due to climate change, increased tadpole mortality due to increased levels of UV-B radiation, and the emergence of the amphibian chytrid fungus, *B. dendrobatidis*, causing increased infection and disease in juvenile, sub-adult and adult frogs (Hunter 2000). Of these hypotheses, only the disease hypothesis is consistent with population declines involving increased mortality during the post-metamorphic stages. I observed one year of failed recruitment resulting from early pool drying across non-manipulated sites during the five years I manipulated recruitment. As with other amphibians that breed in ephemeral water bodies (Taylor *et al.* 2005), *P. corroboree* would be expected to have some resilience to years of failed recruitment.

The results of a recent field experiment (Chapter 7) have important implications for my attempts to increase *P. corroboree* breeding population size via the reintroduction of tadpoles back to their natal pools. This study demonstrated that *P. corroboree* tadpoles are at high risk of becoming infected with *B. dendrobatidis* in natural bog pools, and that in the laboratory this infection results in 100% mortality of post-metamorphic juvenile frogs (Chapter 7). These results suggest that a large proportion of the captive reared tadpoles I returned to the field would have become infected with *B. dendrobatidis* soon after release, and most likely died soon after reaching metamorphosis. They also suggest the results of this study may have been very different if the released tadpoles remained free of *B. dendrobatidis* infection prior to metamorphosis.

#### **8.4.3 Future Directions for Reintroducing *P. corroboree***

While increasing survivorship to metamorphosis within a site may not be an effective means to preventing further declines in *P. corroboree* populations, the positive relationship between tadpole recruitment and population size (Figure 8.7) does suggest the potential to use tadpole releases to establish wild populations. This would

obviously require a greater number of eggs than a site produces, which would have to be sourced from a captive breeding program for *P. corroboree* as there are insufficient animals remaining in the wild for harvesting. The establishment of a large-scale captive breeding program for *P. corroboree* should be of the highest priority for this species recovery program. If suitable numbers of tadpoles can be produced in captivity then further tadpole releases should be trialed, particularly using field techniques that reduce the rate of *B. dendrobatidis* infection prior to metamorphosis.

An alternative to releasing tadpoles would be to release post-metamorphic frog stages. Populations of the European tree-frog (*Hyla arborea*) were successfully established in Latvia following the reintroduction of juvenile frogs (Zvirgzds 1999). Similarly, several populations of Romer's tree frog (*Philautus romeri*) have also been established following the reintroduction of captive-bred frogs (Dudgeon and Lau 1999). Studies have also demonstrated the potential to successfully translocate wild frogs from one site to another, which suggests some level of resilience and capacity to adapt to a new environment in the post-metamorphic frog stage (Bell *et al.* 2004, Tocher and Pledger 2005).

While there may be important survival and breeding traits not acquired by captive reared frogs, such as natal pool imprinting and habitat mapping, the release of frogs have several potential advantages over the release of eggs or tadpoles. The most significant is the potential to bypass periods of high mortality in the field, and hence greater capacity to increase the breeding population size. With respect to *P. corroboree*, it appears that *B. dendrobatidis* may be having a significant impact on this species soon after metamorphosis, and so releasing post-metamorphic frog stages would avoid at least one period of high infection and mortality. Another important consideration for developing reintroduction techniques is determining the most financially efficient method, as available resources are likely to be a limiting factor in the broader application of any technique. While it costs substantially more per individual to produce *P. corroboree* frogs compared with tadpoles, depending on relative survival to sexual maturity, it may ultimately cost less to rear and release frogs to establish breeding populations in the wild.

One of the main criticisms of many failed reintroduction programs is that the factors that caused the species to decline in the first place have not been identified and

ameliorated (Dodd and Seigel 1991). With respect to the decline of *P. corroboree*, if *B. dendrobatidis* is the primary factor driving the continued decline in this species, then the most desirable recovery outcome would be the co-evolution of traits allowing *P. corroboree* to maintain viable populations in the presence of *B. dendrobatidis*. Such an outcome requires continued interaction between *P. corroboree* and *B. dendrobatidis*. This could be achieved via selection for less susceptible individuals in captivity, as suggested by McCullum (2005), or through maintaining interactions between these two organisms in the wild. Maintaining *P. corroboree* in the wild would reduce the potential for selection on non-target traits, which may ultimately reduce individual fitness (Gilligan and Frankham 2003, Kraaijeveld-Smit *et al.* 2006). Furthermore, monitoring the fate of released individuals may be a useful strategy to elucidate other threatening processes (Soderquist 1994). Hence, reintroduction trials for *P. corroboree* should continue to be an important focus for this species recovery program.

## Chapter 9

# General Synopsis and Implications for Threatened Frog Recovery Programs

## 9.1 Monitoring Amphibian Populations

Monitoring of species and populations is a fundamental component of conservation science (Thompson *et al.* 1998, Yoccoz *et al.* 2001), and is essential to the effective implementation of an adaptive approach to wildlife population management (Williams *et al.* 2002). Hence, the overall success of conservation efforts for threatened species is reliant on careful attention being given to the development and implementation of a scientifically sound monitoring program. This involves clearly stating the objectives of the monitoring, and designing the program based on an adequate understanding of the species population demography and variance components associated with the monitoring methods. Failure to design a robust monitoring program may have significant implications for the interpretation of results, and ultimately impact on the effectiveness of the overall program (Martin *et al.* 2007). My research demonstrated the importance of considering population dynamics during the development of a monitoring program. I found that populations of *L. boorooolongensis* fluctuate greatly in individual abundance among years, and through a prospective power analysis approach, determined that using trends in abundance at the population level is unlikely to be effective at guiding many important aspects of the conservation management of this species (Chapter 4). This result is not unique, as many amphibian species have been shown to exhibit large temporal fluctuations in abundance (Pechmann *et al.* 1991, Meyer *et al.* 1998, Green 2003), and hence careful consideration is required before using either estimates or indices of abundance in amphibian monitoring.

In recent years, site occupancy modelling has become increasingly popular for monitoring species population trends across the landscape (MacKenzie *et al.* 2002, MacKenzie and Royle 2005). My research suggested that this would be an effective approach for monitoring *L. boorooolongensis*, and would be more likely to address the

various aims of the recovery program for this species than simply monitoring individual abundance. This is based on the results of this study which demonstrated that spotlight surveys have a high probability of accurately determining the occupancy state of this species along even short sections of stream with only two censuses during the breeding season (Chapter 4).

Understanding how variance components may influence monitoring results clearly is of considerable benefit to the design of monitoring programs. Thus, an important initial phase of any threatened species recovery program is to gain this information.

Understanding the population dynamics of a species goes beyond being able to design and implement an effective monitoring program, as it is also critical for interpreting the results obtained. Correctly identifying that a significant population shift or trend is occurring is only the first step. Ultimately, if the species is in a continued state of decline then it is desirable to understand why this is occurring and to effectively respond. Identifying factors driving observed shifts in abundance is greatly facilitated through a demographic modelling approach (Morris and Doak 2002), which ideally requires detailed information on the vital demographic rates of age to sexual maturity, longevity, age/stage specific mortality schedules, and potential variation in these parameters. This was well demonstrated by Scherer *et al.* (2005) who assessed two possible hypotheses for observed declines in the boreal toad (*Bufo boreas*) using demographic modelling of mark-recapture data. Hence, the value of the information acquired through mark-recapture in this study goes well beyond the investigation of potential monitoring approaches.

## 9.2 Causal Factors of Amphibian Declines

There is now a large body of research strongly suggesting that the disease chytridiomycosis, caused by the pathogen *B. dendrobatidis*, has been the primary causal agent in many recent amphibian declines (Berger *et al.* 1998, Daszak *et al.* 1999), and is likely to continue having a devastating impact on amphibian communities (e.g. Lips *et al.* 2006). Despite this, there continues to be debate over the robustness of the evidence for the origins of *B. dendrobatidis*, and whether this pathogen is the primary cause of observed amphibian declines (McCallum 2005, Rachowicz *et al.* 2005). In this study I provided evidence that is consistent with the

suggestion that *B. dendrobatidis* only recently emerged in populations of both *P. corroboree* and *P. pengilleyi*, and that the timing of this emergence coincided with the decline of both species (Chapter 7). Hence, in the context of other research, it is certainly possible that *B. dendrobatidis* has been a major factor contributing to the decline in both species of corroboree frogs. While there are obvious limitations associated with undertaking retrospective studies for identifying the cause of historic declines (see McCallum 2005), combining the results of many such studies in a meta-analysis would provide an additional approach to addressing questions associated with the broader impact of this pathogen. Hence, undertaking current and historic screening of *B. dendrobatidis* in declining amphibian populations should be encouraged.

My research has also made an important contribution to understanding how *B. dendrobatidis* may be capable of driving susceptible species to extinction (Chapter 8). This research identified the presence of an abundant reservoir host (*C. signifera*) for *B. dendrobatidis* which shares the aquatic breeding environment with *P. corroboree*, and that a large proportion of *P. corroboree* tadpoles become infected and die soon after metamorphosis. This not only suggests a mechanism by which *B. dendrobatidis* could be driving *P. corroboree* through to extinction, but also aludes to several possible management techniques which may reduce the impact of this pathogen on *P. corroboree* in the wild. The value of this study to the broader conservation of amphibian species is that the interactions identified in this study are likely to be operating in many other amphibian communities, and so offers direction to other threatened amphibian recovery programs where disease is believed to be the key threatening process.

My research also made an important contribution to the recovery program for *L. boorooolongensis* through identifying a range of factors that are likely to be limiting or causing continued decline in populations of this species. The factors I identified as potentially impacting on *L. boorooolongensis* populations were; tadpole predation by five different exotic species of fish (Chapters 1 and 2); stream drying due to severe drought (Chapter 4); and weed invasion (Chapter 4). While these threatening processes may not have been the primary causal agent in the more recent rapid decline

of *L. booroolongensis*, they may certainly have compromised the robustness of populations of this species, rendering them more susceptible to other factors.

It is widely accepted that for most species, population declines are likely to be due to multiple factors causing mortality (Alford and Richards 1999, Semlitsch 2000, Beebee and Griffith 2005). Consequently, providing support for one hypothesis does not preclude other hypotheses from potentially being important in the decline of a species. Similarly, some factors may operate synergistically or accumulatively to cause population declines, whereby their impact is only significant at the population level when two or more factors are operating (Kiesecker *et al.* 2001a). Thus, it is important to identify as many of the major sources of mortality for a threatened species, as the successful management of the species will generally require ameliorating multiple threatening processes, or targeting processes which may be more feasible to manage (e.g. habitat degradation) in the hope that it will lessen the impact of threatening processes which are more problematic to control (e.g. disease, introduced fish). Moreover, it is a more desirable approach in recovery programs to have multiple working hypotheses, rather than a single favoured hypothesis, when attempting to identify and manage threatening processes (Conroy *et al.* 2006).

### **9.3 Amphibian Reintroduction Programs**

The IUCN recently announced that stemming the current dramatic loss of amphibian species will require *ex-situ* management of many threatened species throughout the world. Extending this focus to *in-situ* species recovery means that re-establishing wild populations will require the successful reintroduction of these species back to the wild. While this appears to be a logical action to slow down the rate of global amphibian extinctions, the published literature reminds us that amphibian reintroduction biology is anything but a proven and successful conservation tool available to threatened species recovery programs (Dodd and Seigel 1991). Progressing the application of amphibian reintroduction programs requires the implementation of such programs in a manner that allows their outcomes to be adequately assessed and reported to the broader scientific community (Dodd and Seigel 1991, Seddon *et al.* 2007). This is necessary not only for the benefit of individual programs but also so that general approaches and guidelines for amphibian

reintroductions can be developed. The *P. corroboree* tadpole augmentation program presented in Chapter 8 demonstrated the value of adequate follow up monitoring for interpreting results. This program demonstrated that while the reintroduction of tadpoles did increase recruitment to metamorphosis, this did not prevent the continued decline in *P. corroboree* populations. Most importantly, my research showed that this may not have been due to the captive-reared tadpoles not being ecologically fit, but rather that an inadequate number of tadpoles were released. Clearly, the need to understand why a program failed to re-establish a species population is a fundamental element in developing techniques that are successful.

While the primary aim of reintroduction programs is typically to bolster or establish populations, they have utility for addressing a range of recovery actions, particularly assessing factors threatening the recovery of a species (Armstrong *et al.* 1994, Soderquist 1994). By monitoring tadpole survivorship at release sites, and also non-release sites, the results presented in Chapter 8 were able to be used to gain insight into the factors driving the continued decline in *P. corroboree*. My research indicted that unrealistic levels of survivorship to metamorphosis would be required for a *P. corroboree* population to attain an overall positive rate of increase. Hence, it seems that post-metamorphic survivorship is at least partly limiting the recovery of *P. corroboree* populations, and so supports hypotheses that involve increased mortality during the terrestrial frog stages. In addition to this, the release of tadpoles into natural and artificial pools in an experimental manner allowed valuable assessment of the ecological interactions between *P. corroboree* and the pathogen *B. dendrobatidis* (Chapter 7). These studies demonstrate the capacity for reintroduction programs to have broader application to threatened species recovery programs than just the bolstering or establishment of populations.

## 9.4 Management Solutions to Recent Amphibian Declines

The decline and extinction of many amphibian species in Australia, and elsewhere in the world, has occurred over the past 25 years with very little effective management response. A number of factors have contributed to this situation, including; the rapid nature of these declines, because they often occurred in relatively pristine environments, and because for many years the causal factors of these declines remained a mystery.

Undoubtedly, the greatest challenge at present for amphibian conservation is the development of management actions that will reduce the impact of *B. dendrobatidis* on wild amphibian populations. Currently options for mitigating the impact of *B. dendrobatidis* on amphibian species is limited (McCallum 2005). One approach that may prove effective in the future is the use of biological controls in the form of competitive microbes (Harris *et al.* 2006). Another approach is simply to ensure the ongoing interaction between host and pathogen in the hope this will lead to a co-evolutionary response for either increased resistance in the host, decreased virulence in the pathogen, or a combination of both. Hence, for species like *P. corroboree* for which extinction in the wild appears inevitable, any actions maintaining the species in the wild would potentially contribute to mitigating the impact of *B. dendrobatidis* in the longer-term. While selectively breeding for resistance in susceptible amphibian species may also be undertaken in captivity, this would require considerable resources and may have other complications, such as reducing fitness in non-target traits (Gilligan and Frankham 2003, Kraaijeveld-Smit *et al.* 2006).

My research presented in Chapter 7 suggests two potential avenues for developing management actions that may reduce the impact of *B. dendrobatidis* on *P. corroboree* in the wild. The first is through removing or reducing the density of a major reservoir host for *B. dendrobatidis* (i.e. *C. signifera*), in the hope that this will reduce the incidence of infection in *P. corroboree*. While this outcome is theoretically possible and may work on a local scale, it is unlikely to be successfully implemented across a broad geographic area. Nevertheless, implementing this action would certainly be a powerful test of the significance of reservoir hosts to the continued decline of *P.*

*corroboree*, and may be a useful strategy in the short-term to increase the success of future reintroduction efforts and maintain this species in the wild.

The other possible action arising from my research that may reduce the impact of *B. dendrobatidis* in the wild is the placement of eggs/tadpoles in artificial pools that will ensure that all individuals will remain free of infection through to metamorphosis. This technique should be investigated as an alternative strategy for releasing tadpoles of this species back to the wild, rather than the approach used in Chapter 8 where tadpoles were released into natural pools where they would have been exposed to infection with *B. dendrobatidis*. Again, this is not an action that could be applied across a broad geographic area, but it may certainly prove to be a more effective means to undertaking further reintroduction efforts for *P. corroboree*.

Given the seemingly limited capacity for management to respond to the large number of recent amphibian declines, a very positive outcome of my research is the identification of threatening processes for *L. boorooolongensis* for which it should be feasible to mitigate their impacts in the relatively short-term. This particularly pertains to the impact of ongoing stream degradation through increased sediment inflow, and invasion by weeds such as blackberry (*Rubus* sp.) and willows (*Salix* sp.). It seems highly likely that a coordinated and systematic approach of protecting riparian zones and eradicating willows and blackberries in catchments where *L. boorooolongensis* currently occurs would greatly enhance the viability of this species. If implementing this action resulted in a positive outcome for *L. boorooolongensis* then it would represent a major conservation achievement during a period when ongoing amphibian declines are considered a global biodiversity crisis.

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