

# Identifying conservation and research priorities in the face of uncertainty: A review of the threatened bell frog complex in eastern Australia

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**Abstract.**—While the principles and steps to be followed in the recovery of a declining species are relatively straightforward, there is often a considerable gap between the theory and practice. One example is the decline of the bell frog complex in eastern Australia, which is comprised of three species: *Litoria aurea*, *L. castanea* and *L. raniformis*. There is considerable debate about the role of threatening processes in the decline and uncertainty in how best to develop conservation mitigation strategies for these species. To develop a clear picture of the research priorities necessary to enhance the conservation outcomes for the three species we conducted a literature review to focus on the critical gaps in ecological understanding that prevents consensus, and to set research priorities to address these gaps. Unresolved questions were identified and prioritised, and a set of priority research directions and management actions considered integral for the persistence of the species were formulated.

**Key words** – amphibians; amphibian chytrid fungus; conservation; decline; demography; management; priorities; threatened species

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## INTRODUCTION

There is one clear principle when considering the recovery of threatened species and that is first to identify the cause of decline. This is often reduced to the task of identifying the threatening process(es). Logically, once the threat has been

identified, it is necessary to take actions to mitigate the threatening process. It is also critical to monitor the outcome/s of these actions to ensure that recovery occurs and to indirectly confirm the relationship between cause-and-effect. However, while these steps are straightforward, it rarely turns

out to be so simple 'on-the-ground' in threatened species recovery and management. There are numerous explanations as to why this is so often the case; more often than not there is a lack of basic information on the biology of native animals, and the complexity of natural systems makes it difficult to determine relationships between cause-and-effect. Biologists often need first to understand the species they are dealing with, including aspects of the species' life history, ecology, behaviour, physiology and genetics. There may be no indication that a decline is occurring until it is well under way, and once this is the case it may be difficult to identify the cause of the decline. Once declines are identified the causal factors may be confounded by anthropogenic influences on natural systems such as changes to habitat, the introduction of novel predators, competitors or pollution.

To assist in the identification of cause-and-effect relationships in novel situations, conservation biologists have developed a list of candidate threatening processes through the investigation of many threatened species and from ecological principles (Sinclair *et al.* 2006). Threatening processes are divided into two basic categories: deterministic and stochastic factors (Primack 2010). Deterministic factors are those that affect the population in a constant relationship to the population's size, such as the role of competitors and predators in reducing the numbers of individuals in a population. Stochastic factors are recognised as sources of uncertainty that can affect the size of a population, such as genetic stochasticity, environmental stochasticity and demographic stochasticity. Rarely, however, is any factor affecting a population in such a simple, cause-and-effect relationship that can reliably produce a predictable result.

There is another component of investigations into identifying cause-and-effect relationships in species declines that makes the role of conservation biologists both difficult and dynamic, and that is the tension between the need for action and the need for basic research. When a species is in such decline that it has become a priority for recovery, then there will be an urgent need for actions that are on-ground and effective, while at the same time limiting the opportunity for pure research. The link between the two approaches is adaptive

management and the conduct of pilot research studies, but implementing both actions is not easy.

While the principles and steps to be followed in the recovery of a declining species are relatively straightforward, there is often a considerable gap between the theory and the practice. An example of such a gap surrounds the bell frog complex in eastern Australia. Here we are referring to three sibling species (*Litoria aurea*, *L. castanea* and *L. raniformis*) that, together, prior to the 1980's, occurred over much of south-eastern Australia (Mahony 1999). Here, the block to effective recovery lies in the identification of the reason for decline. There are two competing hypotheses: (1) the decline is due to habitat loss, degradation and fragmentation and the impact of an invasive fish predator, leading to population isolation and subsequent demographic and genetic stochasticity, or (2) the decline is primarily due to the amphibian disease chytridiomycosis, leading to demographic and genetic stochasticity. Nowhere is the complexity of this dichotomy more apparent than in considerations of the importance of habitat in recovery efforts. Almost all recovery actions for restricted populations of these frogs are based around the supplementation or the rehabilitation of habitat, or reintroduction to areas of identified historical habitat (Goldingay 2008). This approach is based on the logic that the cause-and-effect relationship of declines results from habitat degradation or isolation, and that by supplementing habitat the long-term security of populations can be achieved. Yet where this approach has been adopted on a large scale, it remains unclear why all created habitat is not used by the frog and the population density does not reach expected carrying capacity. This in turn leads to investigation of finer scale habitat variables in a search of the fundamental niche or habitat, and it begs the question as to why suitable habitat over such a large area of former distribution no longer supports populations of the frog. The alternative explanation is to see the decline as the outcome of chytridiomycosis, and to question why the disease has not affected the remaining populations. And once again, this leads to the matter of the habitat in which the species persists.

Over the past decade a number of studies have addressed the primary question of the cause of the

decline of the bell frog complex. Many studies are documented in student theses or consultancy reports, or are ongoing and are not published. Many studies have occurred where adaptive management of habitat and threatening processes has been undertaken as part of mitigation for loss of habitat to development (Goldingay 2008). Given this situation and the ongoing decline of the bell frog complex we consider it timely to produce an assessment of our understanding of the conservation biology of the bell frog complex, and to bring together the primary findings of these various studies with the objective of setting a clear framework for future studies.

### STATUS OF THREATENED BELL FROGS

The bell frog complex referred to three described species, once common in south-eastern Australia - Green and golden bell frog (*Litoria aurea*), Growling grass frog (*Litoria raniformis*) and Yellow-spotted bell frog (*Litoria castanea*). The range of *Litoria raniformis* is the most western and southern in extent, occurring in eastern Tasmania, throughout Victoria, north to southern New South Wales and west to the lower Murray River in South Australia. This overlaps with the distribution of *L. aurea*, which occurs from north-eastern Victoria, throughout coastal NSW, north to Lismore. *Litoria raniformis* and *L. aurea* have both successfully established on islands where they were introduced outside Australia, such as New Zealand and New Caledonia (Bishop 2008). Less is known of *Litoria castanea*, it is thought to have once occurred in separate populations on the northern tablelands of New South Wales and the southern tablelands of NSW and the Australian Capital Territory. However the genetic relationships of both populations of *L. castanea* with *L. raniformis* remain unresolved (Vörös *et al.* 2010). Despite the relative paucity of data on *L. castanea*, we have included the species in this review because it appears to have a similar biology to *L. raniformis* and *L. aurea* (Humphries 1979) and falls within the south-eastern bell frog group (Thomson *et al.* 2006). Conclusions are drawn from the biology of one or more species, which we consider applicable to the bell frog complex unless otherwise stated.

The range of the east Australian bell frog complex has retracted since the 1970's (Pyke and White

1996; Pyke and White 2001) and populations of all three species have disappeared from numerous historical sites. *Litoria aurea* persists in less than 40 populations, many of which are small, primarily along saline or industrial habitats on the coast (Mahony 1999). *Litoria raniformis* remains widely distributed but populations are very scattered. *Litoria castanea* was widely considered to be extinct until very recently when a population matching the morphological description of the species was rediscovered in the NSW southern highlands (Vörös *et al.* 2010). Both *Litoria aurea* and *L. raniformis* are nationally listed as 'Vulnerable' under the *Environment Protection and Biodiversity Conservation Act (1999)* whereas *Litoria castanea* is listed as 'Endangered'.

### BIOLOGY OF BELL FROGS: WHAT DON'T WE KNOW?

**Disease** – The range contraction of the threatened bell frog complex occurred rapidly, suggesting a causal agent that was able to act over short time periods was involved (Hamer *et al.* 2010). The bell frog complex disappeared from nearly all inland, high altitude areas of their respective ranges (Pyke and White 1996; Mahony 1999; White and Pyke 2008b; Hamer *et al.* 2010) alongside a suite of co-occurring frog species that did not appear to decline. This is consistent with the hypothesis that chytridiomycosis has played a role in the decline of the bell frog complex. If so, the effects of this disease must be less severe in areas where the bell frog complex has persisted. The New South Wales National Parks and Wildlife Service Draft Recovery Plans for *L. aurea*, *L. raniformis* and *L. castanea* list diseases, including chytridiomycosis as threats to the persistence of these species, and several observations of diseased individuals and causally-linked amphibian die-offs are referred to therein (Department of Environmental and Climate Change unpubl report).

The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), the pathogen responsible for chytridiomycosis has been identified in seven *L. aurea* populations (Waldman *et al.* 2001; Wassens and Mullins 2001; Penman *et al.* 2008; Stockwell *et al.* 2008; Murray *et al.* 2010) and five *L. raniformis* populations (Waldman *et al.* 2001; Murray *et al.* 2010; Vörös *et al.* 2011). Given that this pathogen is

known to be widespread throughout eastern Australia (Murray *et al.* 2010), it is likely to be present throughout these species ranges. Only the *L. aurea* population on Broughton Island was negative for the presence of *B. dendrobatidis* following swabbing surveys, and the sampling effort employed suggests it may be absent or exist at a prevalence of less than 5% (Stockwell 2011). The isolated location of Broughton Island (a small island located 2.3 km offshore in the Tasman Sea) suggests that *B. dendrobatidis* has not been introduced there and may be one of the few recorded sites known to be free from the fungus.

Bell frogs are highly susceptible to *B. dendrobatidis* and infection can result in disease and/or mortality. The experimental exposure of *L. raniformis* to *B. dendrobatidis* resulted in clinical signs of disease followed by a full recovery in 83% of individuals (Carver *et al.* 2010). By comparison, experimental exposure of *L. aurea* to *B. dendrobatidis* resulted in 100% of individuals showing signs associated with the terminal stages of chytridiomycosis (Stockwell *et al.* 2010). Multistate modelling of the Kooragang Island *L. aurea* population, showed significantly lower over-winter survival rates in infected individuals (0.10) versus uninfected individuals (0.56), which was predicted to cause the population to decline at twice the rate of an uninfected population (Stockwell *et al.* 2010). These studies indicate that large-scale unobserved seasonal die-offs may occur in populations of the bell frog complex during cold periods when both detectability and survey frequency are low. In addition, *B. dendrobatidis* has been implicated as the causal agent in the overwinter extinction of a reintroduced *L. aurea* population in the Hunter Region of NSW (Stockwell *et al.* 2008). Such die-offs and extinctions have serious implications for the ability of remaining isolated populations to persist with infection, particularly in the presence of demographic and environmental stochasticity.

The availability of management options for the *B. dendrobatidis* in susceptible populations of the bell frog complex is currently limited and models of population persistence under different management scenarios suggest that counteracting the effects of chytridiomycosis by managing other threats may be effective (Stockwell 2011). There has been considerable speculation that water solutes in sites

where the bell frog complex persists, have antifungal properties protecting populations from the effects of *B. dendrobatidis*. Suggestions have been made that the persistence of a high altitude population on the southern tablelands of NSW may be due to inhibitory effects of heavy metals in the aquatic habitat on *B. dendrobatidis* survival and virulence (Wassens and Mullins 2001; Osborne *et al.* 2008) and there is some experimental evidence to support this (Parris and Baud 2004; Threlfall *et al.* 2008).

The existence of a link between persistence of the bell frog complex in coastal environments and *B. dendrobatidis* salt sensitivities has been suggested several times (Mahony 1999; White 2006; Berger *et al.* 2009), and significant negative correlations have been found in *L. aurea* habitat between infection loads and the salinity of water bodies (Stockwell 2011). An inhibitory effect of 3-4 ‰ sodium chloride on fungal growth and infective capacity has also been confirmed experimentally (Stockwell 2011). These results suggest that *L. aurea* may currently persist in areas with a saline influence as they act as environmental refuges from the effects of *B. dendrobatidis*. The addition of salt to water bodies, both in captivity and in an experimental reintroduction site has also been found to increase *L. aurea* survival rates in the presence of *B. dendrobatidis* (Stockwell 2011), suggesting that this may be an effective management strategy. The addition of salt to water bodies is time and cost effective, remains active over time and replicates natural conditions throughout parts of the current range of the species complex. However, these beneficial effects for *L. aurea* survival need to be weighed against any negative sub-lethal effects on growth, fitness and reproductive success, as well as any effects on non-target organisms.

Because conservation efforts for declining species depend upon a thorough understanding of the factors that limit the size and distribution of populations (Sinclair *et al.* 2006), further investigations into the role of chytridiomycosis as a population regulatory factor is a research priority. This could be achieved by examining the effect of infection and disease on the vital rates that determine population size. As infection outcomes are highly dependent upon local temperatures (Berger *et al.* 2004), the density of reservoir hosts

(Rachowicz and Briggs 2007), host behaviour (Rowley 2006) and immuno-competence (Carey *et al.* 1999), impacts are likely to be site-specific and should be investigated as such. Understanding how remaining populations of the bell frog complex persist in the presence of *B. dendrobatidis* may also reveal novel management strategies, and investigations into the role of water solutes as naturally occurring antifungal agents should continue. In the absence of a means to control the effects of *B. dendrobatidis* on survival and population size of the bell frog complex, attempts to prevent and reverse declines through habitat creation efforts and/or reintroduction back into their former range are likely to have limited success.

Emerging diseases amongst the Australian anurans clearly pose a serious threat given the effect that *B. dendrobatidis* alone has had on this and many other species to date. In addition, a new myxosporean parasite has been isolated in two species of bell frog (Hartigan *et al.* 2011). This parasite attacks the brains and livers of individual frogs, leading to a reduction in motor control, general lethargy, and death. There is potential for other deadly amphibian diseases such as *Ranavirus* spp, to spread within the range of the threatened bell frog complex but this is not an immediate threat (Weir *et al.* 2012). Further research on the impacts of disease on populations of the bell frog complex and possible management actions are priorities in research.

**Habitat associations** – Several studies have examined habitat use and occupancy of *L. aurea* and *L. raniformis* in a range of landscapes in south-eastern Australia (Pyke and White 1996; Hamer 2002; Wassens *et al.* 2010; Heard *et al.* In press). There appear to be clear associations between the probability of occupancy by *L. raniformis* at a site and local habitat variables. Pond permanence (i.e. hydroperiod) and the cover of aquatic vegetation in a pond are important predictors of occupancy by *L. raniformis* (Heard 2010; Wassens *et al.* 2010), as well as seasonal flooding on occupancy in inland New South Wales populations (Wassens *et al.* 2010). However, the results of studies on the habitat requirements of *L. aurea* are equivocal, which could lead to conflicting recommendations for conservation management. Despite several studies on *L. aurea* documenting a positive effect of aquatic

vegetation on occupancy (Pyke and White 1996; Hamer *et al.* 2002a; Pyke *et al.* 2002), there is some contention surrounding the effect of predatory fish and hydroperiod on local habitat use. For example, Pyke and White (1996) found that breeding by *L. aurea* was more likely to occur in ephemeral wetlands free of predatory fish (e.g. mosquito fish *Gambusia holbrooki*), whereas Hamer *et al.* (2002a) found no significant difference in occupancy between permanent and ephemeral ponds, and between ponds where mosquito fish were present or absent. Ponds inhabited by mosquito fish may be avoided by *L. aurea* (Pyke and White 1996), possibly because mosquito fish eat the eggs and tadpoles of *L. aurea* (Morgan and Buttemer 1996). However, mosquito fish do not automatically exclude *L. aurea* (Mahony 1999; Hamer *et al.* 2002b), but the availability of habitat structure interacts with the impact of the fish as complex vegetation appears to provide sufficient refuges for the eggs and tadpoles for the two species to co-exist (White and Pyke 2008b).

These studies, and a recent review (Goldingay 2008), suggest that hydroperiod and the presence of fish are primary sources of uncertainty in the use of habitat by the bell frog complex and require further investigation. Improving the definition of hydroperiod in habitat models is necessary. This could be achieved by calculating a metric that represents fluctuations in water levels over time, or by including multiple categories of pond permanence that better represent the hydroperiod gradient (Heard 2010). There is also potential for hydroperiod and the presence of fish to interact with aquatic vegetation (Wassens *et al.* 2010), therefore we recommend experimental and field studies that include sufficient statistical power to uncouple the relationships between hydroperiod, predatory fish, vegetation and occupancy by bell frog species. Habitat creation projects based on the results of these studies would have a substantially higher chance of success.

While the effects and interactions of altered hydrological and flooding regimes on the bell frog complex remains multifaceted (particularly those in the western floodplain regions), some studies have shown that *L. raniformis* is sensitive to changes in flooding frequency, leading to possible changes in reproduction, habitat occupancy patterns, as well as

limiting dispersal (Wassens *et al.* 2008; Wassens *et al.* 2010). One study in particular found that annually flooded wetlands were more likely to support *L. raniformis* than those flooded less frequently (Wassens *et al.* unpubl. report). Alterations to hydrological and flooding regimes are thought to be key threats to *L. raniformis* and may be a significant threat to *L. aurea*. Hydroperiod may be affected by climate change and current predictions for climate change in south-eastern Australia over the next 60 years include increased temperature, decreased rainfall and changes in the timing of larger rainfall events (Lemckert and Penman In Press). The bell frog complex requires the availability of water bodies with at least moderately-long hydroperiods in order for their tadpoles to reach metamorphosis, and climate change will likely result in some currently used water bodies no longer holding water for sufficiently long periods to complete reproduction. Reductions in the number and proximity of suitable ponds will also reduce the connectivity of metapopulations.

Water temperature appears to have an important positive effect in habitat occupancy by both *L. aurea* and *L. raniformis* (Pyke *et al.* 2002; Wassens *et al.* 2010) and requires further investigation. The benefits of higher water temperatures on the fitness of *L. aurea* tadpoles are clear; tadpoles grow faster (Penman 1998) thereby reaching metamorphosis at an earlier time than tadpoles in colder water. However, water temperature may relate to habitat variables known to influence occupancy. For example, occupancy is higher in ephemeral ponds and unshaded ponds which have higher water temperatures because they are shallow and receive greater solar radiation (Lauck *et al.* 2005). Unshaded and ephemeral ponds often have higher primary productivity, which can provide greater food resources for tadpoles (Harris 1999; Werner 1999), although the dietary requirements of bell frog tadpoles are unknown for any species in the complex. It is not clear how water temperature and nutrient levels interact with pond hydroperiod to encourage the colonisation of ponds and subsequent breeding by any species of bell frog, or how these factors are related to vegetation succession at ponds (Pyke and White 1999). The management of populations of other threatened anurans, such as the pioneering natterjack toad *Bufo calamita* in the

United Kingdom, has included the maintenance of early stages of serial succession in habitat restoration projects (Denton *et al.* 1997). There is some evidence of populations of *L. aurea* becoming locally extinct at ponds over time due to vegetation succession (Pyke and White 1999), so maintaining a proportion of ponds with minimal vegetation or creating new ponds may also be beneficial in habitat restoration projects for the bell frog complex.

Terrestrial habitat is also a vital component of the long-term viability of populations of wetland-dependent amphibians (Gibbons 2003; Harper *et al.* 2009). The terrestrial periphery and terrestrial corridors surrounding wetlands are essential non-breeding habitat that sustain patchy populations and metapopulations of pond-breeding amphibians (Marsh and Trenham 2001). Terrestrial habitat links wetlands and therefore facilitates frog movement among wetlands, and provides shelter and foraging opportunities. Conversely, the intervening matrix between wetlands may comprise uninhabitable land types or barriers to movement (e.g. urban land cover and roads; (Heard 2010). Most studies on the bell frog complex have focused on the pond as a habitat patch and not included a comprehensive assessment of the relative importance of terrestrial habitats in the resource requirements of these species (e.g. dispersal, foraging and shelter). Several studies found terrestrial vegetation is a significant predictor of occupancy in *L. aurea* (Pyke and White 1996; Hamer *et al.* 2002a). Use of terrestrial habitat by bell frog species is likely to extend for at least several hundred metres beyond the pond perimeter, given the movement distances reported (Hamer *et al.* 2008). Clearly, greater emphasis needs to be given to habitat models that incorporate biologically-realistic variables relating to the quantity and quality of terrestrial habitat. For instance, many extant populations are surrounded by multiple land use types (e.g. urban and industrial) that would hamper dispersal.

Despite the uncertainties regarding important within-pond parameters for *L. aurea*, there are clear similarities in landscape variables that are important in the local and regional distribution of both *L. aurea* and *L. raniformis*. Several studies have used variations of the “distance to the nearest occupied pond” as a variable to predict occupancy (Hamer *et*

*al.* 2002a; Hamer and Mahony 2010; Heard *et al.* In press). The results show that the local distribution of bell frog species clusters around closely-spaced groups of occupied ponds. The probability of occupancy at a site declines with distance from an occupied pond, probably because bell frog species prefer to move short distances when travelling between ponds (Hamer *et al.* 2008). Alternatively, bell frogs may be attracted to existing choruses, leading to spatial cohesion within populations. For example, occupancy of ponds by *L. aurea* is higher at ponds which were occupied in the previous year, and occupancy declines with increasing distance from a breeding site (Hamer and Mahony 2010). One behavioural mechanism proposed as a proximate cause for this phenomenon is conspecific attraction, whereby dispersing individuals select waterbodies based on the presence of conspecifics and not necessarily on habitat cues (Muller *et al.* 1997). However, the role of conspecific attraction in habitat use by bell frogs is still unclear and deserves greater research attention if we are to successfully translocate individuals to created habitat. For instance, how many bell frogs does it take to establish a chorus at constructed pond? And, what role do Allee effects (Stephens and Sutherland 1999) play in determining the persistence of populations at sites? These are important questions to address because not all apparently suitable habitat is occupied by bell frogs in an area, suggesting there is a strong behavioural component regulating occupancy. Finally, the spatial autocorrelation observed in bell frog populations has implications for habitat modelling, notably in distinguishing social aggregation from environmental autocorrelation, which can lead to biased estimates of model coefficients and diminished predictive performance of habitat models (Wintle and Bardos 2006).

Two studies that assessed local extinction of populations of *L. aurea* and *L. raniformis* at waterbodies as a function of area and distance reached similar conclusions: waterbodies have a higher probability of being abandoned if they are small and are situated long distances from occupied ponds (Hamer and Mahony 2010; Heard *et al.* In press). Local colonisation of habitat by *L. raniformis* is also dependent on connectivity, based on the number of occupied ponds surrounding a pond (Heard *et al.* In press). Other important

determinants of local extinction of *L. aurea* include small population size (Hamer and Mahony 2010), while local populations of *L. raniformis* are more likely to go extinct at ephemeral, sparsely-vegetated wetlands (Heard 2010). These results offer clear directions for conservation management of bell frog populations: we need to create large, densely-vegetated wetlands within a mosaic of closely-spaced aquatic habitats to increase the probability of habitat creation projects being successful. While the role of hydroperiod has been clearly established as influencing the processes underpinning occupancy of ponds by *L. raniformis*, its relative importance in local colonisation and extinction of habitat by *L. aurea* requires further investigation. Finally, while the colonisation of waterbodies by bell frogs may be influenced by hydroperiod, identifying the proximate determinants of why habitat is selectively colonised requires greater attention (i.e. what are the benefits for population fitness?).

**Population structure and survival** – The earliest extensive study of the population ecology of east Australian bell frogs was conducted on a now extinct population of *L. aurea* and *L. castanea* north-east of Canberra (Humphries 1979). All subsequent studies were conducted in response to the decline of the threatened bell frogs. Information on natural history suggests that the bell frog group has the highest reported fecundity of the Australian anurans (Heard *et al.* In press). The survival rate of eggs in the field has not been recorded for any species of bell frog, however high fertilisation efficiency has been noted for *L. aurea* from two sites in the Sydney region where greater than 90% of eggs hatched after 2-4 days when removed to an aquarium (Christy 2000). It is important to note that removal to aquaria eliminated natural causes of mortality, and the impacts of predation and disease on wild eggs are currently unknown.

Breeding in a single population of *L. aurea* can vary dramatically between years (Bower *et al.* 2012) and anecdotal reports suggest that populations can quickly increase in number under certain conditions (Pickett *et al.* 2013). Estimates of absolute abundance of tadpoles within a pond have not been attempted for any bell frog species, and the survival rate of tadpoles is also unknown, though evidence supports high mortality, which is consistent with observations of the population dynamics of other

larval amphibians (Calef 1973). The relative abundance of a cohort of *L. aurea* tadpoles within a semi-permanent pond decreased during the first four weeks after hatching and the reduction was attributed to predation by aquatic macroinvertebrates (Hamer 1998). An ephemeral pond in New Zealand had a high density of *L. aurea* tadpoles, but few completed metamorphosis before the pond dried (Barclay 1966). The emergence of metamorphlings from ponds has been described by many authors, with counts ranging from 1 to 422 (Humphries 1979; Van de Mortel and Goldingay 1998; Daly *et al.* 2008; Hamer and Organ 2008; Osborne *et al.* 2008). These numbers also suggest a high level of mortality during the egg and tadpole stage, as a single clutch can be an order of magnitude higher than the largest number of emerging metamorphlings that have been observed.

Despite the paucity of data for the early life-history stages, much of the focus of demographic studies has been on the reproductive adult stage. The ability to mark small individuals has been limited (Christy 1996), and has resulted in most population studies focusing on adults greater than 45 mm in length, which is approximately the size of sexual maturity in males (Pyke and White 2001; Hamer and Mahony 2007; Heard *et al.* In press). However, recent advances in technology have produced tags and microchips that are now small enough to mark emerging metamorphlings; an advancement that will enable new insight to this area. Population estimates from marking studies of various bell frogs have found a large range in sizes from just six adult individuals for a single population of *L. castanea* (Humphries 1979) to 1995 individuals for a population of *L. aurea* (Hamer and Mahony 2007). Despite this large range, estimates of absolute abundance of adults are generally low, and range in the low hundreds (Humphries 1979; Goldingay and Newell 2005a; Hamer and Organ 2008).

Estimation of the annual survival rate of adult bell frogs is available from intensive and repeated marking studies. Over a three year period where all individuals were thought to be marked within the study area, the mortality rate of both *L. aurea* and *L. castanea* varied substantially; ranging from 0.05-0.42 in *L. aurea* and 0.29-0.33 in *L. castanea* (Humphries 1979). However, the small populations that were being studied meant that a single death could have a

large impact on the mortality rate, which substantially increases the variability of these estimates. More recent studies estimated survival using mark-recapture which accounts for imperfect detection and heterogeneity in capture probability of individuals. A two year mark-recapture study of *L. aurea* on Kooragang Island, NSW found a low survival rate over one breeding season (0.192) (Hamer and Mahony 2007). This was similar to the survival rate recorded for *L. raniformis* near Melbourne during one breeding season (0.135), however this varied significantly over time, with the previous breeding season experiencing a much lower survival rate (0.036), and the winter season experiencing higher survival (0.247) (Heard *et al.* In press). A different seasonal pattern of mortality was also observed for *L. aurea*, where survival rate was high during the breeding season (0.9) compared to the non-breeding season (0.1 or 0.56 depending on *B. dendrobatidis* infection status) (Stockwell 2011). Marking studies which have not used mark-recapture analyses to determine survival rates also support a low survival rate, with few recaptures occurring over successive years suggesting that few individuals are older than 1-2 years (Murphy 1995; Goldingay and Newell 2005b).

Another method for determining the survival rate within a population is the use of age class analysis (Skalski *et al.* 2005). Size classes have been used for some species of amphibian to estimate age, and therefore age structure. Hamer and Mahony (2007) determined age structure for both sexes using growth rate data; males were classed as older or younger than six months whilst females were classed as < 12 months, 12-24 months and > 24 months. Females in the oldest age class represented between 10 and 23% of the female population, again supporting a low survival rate. However, the age distribution of *L. raniformis* in the Coleambally, Victoria, as determined by skeletochronology, suggested a different demography as a large proportion of captured individuals were 2-4 years old (Mann *et al.* 2010b). Humphries (1979) reported the size distribution for both *L. aurea* and *L. castanea* over a three year period north-east of Canberra. The size of female *L. aurea* was greater than the size of female frogs reported on Kooragang Island (Hamer and Mahony 2007). Similarly, female *L. aurea* at Yuraygir National Park recorded larger average sizes than the oldest size class reported for



Kooragang Island (Goldingay and Newell 2005a). These differences in size distribution among populations might suggest two different processes: varying survival of adults or different growth rates experienced by individuals in different populations (Marunouchi *et al.* 2000).

The growth of male bell frog species to maturity appears to be rapid e.g., within three to four months (Hamer and Mahony 2007; Heard *et al.* In press), but it remains unknown at what stage females are reproductively mature. Mature eggs have been found in females as small as 53.4 mm, however this does not indicate maturity as less eggs were present than typically found in a clutch (Christy 2000). Low survival and low growth rate could contribute to a small effective population size, as few females may reach maturity in these populations. Therefore, it is important to know growth rates and age at maturity for different populations to determine whether populations with different size classes are experiencing different life history strategies. The high level of variability that appears to be present in the demographic rates of adults within single species of bell frogs, is important to determine population fluctuations and susceptibility of populations to extinction (Beissinger and Westphal 1998). Quantification of this variability can only be determined by long-term studies, as the level of variability only begins to asymptote after seven years of study, and often more (Pimm and Redfearn 1988). This has not yet been achieved for any of the remaining bell frog populations, and should therefore be a priority for future research. Studies that estimated survival over both summer and winter periods demonstrates seasonal differences (Stockwell 2011; Heard *et al.* In press). However, with just a single estimate for winter survival, it is indeterminable whether this is a result of the high level of demographic variability, or a genuine pattern in bell frog mortality.

Throughout the entire life-cycle of the threatened bell frog complex, individuals appear to be at a high risk of mortality. The combination of high fecundity, low metamorphosing emergence followed by subsequent small adult populations that experience low survival suggests that populations undergo a high level of turnover. Future studies must focus on filling gaps in the demographics of the early life-history stages and quantifying the

level of variability in demographic parameters of bell frog species. The fact that the remaining populations of this species tend to be very small (often < 100 individuals) represents a serious management issue and may be further exacerbated where they exist as a metapopulation. Small populations are prone to inbreeding effects that reduce genetic fitness and can lead to deformities of individuals (Allentoft and O'Brien 2010). Reduced genetic diversity threatens the ability of a population to adapt to changes in the environment, either natural or human induced. Most populations now exist in few or only one identifiable site that is separated from the next obvious population by tens or even hundreds of kilometres. Under such circumstances, the chances are almost zero of individuals migrating from the nearest population to meet the replacement condition that is critical to metapopulation persistence.

**Movement** – Bell frog species share a large dispersal capacity relative to other frog species (Hamer and Mahony 2007; Heard *et al.* In press), a life history trait that is synonymous with a generalist or *r* – selected species (Pianka 1970). Individuals have been recorded far from the nearest body of water, up to 1.5 km in a single night, although it is noteworthy that it was followed by the observer with the aid of a light (Pyke and White 2001). However, most studies observed little movement in bell frog species (Humphries 1979), indicative of individuals showing a relatively high level of site fidelity. In *L. raniformis*, just 13% of recaptured individuals moved between wetlands (Heard 2010) and in another population occurring in southern Victoria, most movements were between two adjacent water bodies within 20 m of each other (Hamer and Organ 2008). Average distances moved by individual *L. aurea* range between 40.7 m (Murphy 1995) and 148.5 m at Kurnell Peninsula (Christy 2000), though large distances have been observed up to 632 m (Christy 2000). Radio-telemetry studies show high variability in distances moved within a 24 hour period, which ranged from 0 m to 212 m (Wassens *et al.* 2008). The reasons for these differences in movement patterns between sampling events remains unknown, and warrants further investigation.

As with most amphibians, the movement patterns of *L. aurea* and *L. raniformis* differ seasonally. Larger

movements occur during the summer breeding season when the frogs are more active, compared to smaller movements in the winter where individuals seek shelter to avoid the cooler conditions (Christy 2000; Pyke and White 2001; Wassens *et al.* 2008). Furthermore, individuals of at least *L. aurea* are able to undertake non-breeding movements away from breeding sites once suitable conditions cease (Humphries 1979; Patmore 2001). For both *L. aurea* and *L. raniformis* there is no evidence to suggest that there is any difference in movement distances between the sexes, with studies involving both mark-recapture and radio tracking techniques reporting similar distances moved by males and females (Christy 2000; Wassens *et al.* 2008). Juveniles however have been observed moving seasonally (Humphries 1979) and it is during this life stage that the main period of dispersal probably occurs (Humphries 1979; Goldingay and Lewis 1999; Goldingay and Newell 2005b), although this remains poorly studied. Goldingay and Lewis (1999) have observed one individual juvenile 700 m from the nearest breeding pond in Port Kembla, while Van de Mortel and Goldingay (1998) noted a similar occurrence for a juvenile located 900 m from the nearest breeding site at Coomaditchy Lagoon, Port Kembla. There are currently no studies that demonstrate the biological significance of juvenile dispersal and how it relates to population ecology of bell frog species. Information on the importance of juvenile dispersal in enhancing population sizes and the potential for colonising and recolonising new or previously used sites is a priority because it will assist managers to construct habitats that are within dispersal distance and facilitate effective planning.

Rainfall appears to be a significant predictor of the movement patterns of both *L. aurea* and *L. raniformis* (Pyke and White 2001; Hazell *et al.* 2003; Hamer and Mahony 2007; Hamer *et al.* 2008; Heard 2010). Radio-tracking studies for *L. aurea* have shown that rainfall was the best indicator of movement distance (Christy 2000; Patmore 2001). Several 'dispersal events' have been observed anecdotally prior to or during periods of heavy rainfall for *L. aurea*, where observations of mortality on roads occurred during periods of rainfall (Pergolotti 1995; Goldingay and Lewis 1999). In addition, inundation events because of heavy rainfall caused adult and juvenile *L. raniformis* to move 260 m – 490 m (Heard 2010), and *L. aurea* to

move from permanent waterbodies to ephemeral waterbodies, where males called for several nights before moving back to the permanent waterbodies nearby (Hamer *et al.* 2008). The onset of these extended periods of rainfall and waterbody inundation may facilitate movement by extending the amount of time individuals can be exposed without suffering desiccation and by providing higher connectivity between waterbodies (Hazell *et al.* 2003). Understanding the significance of rainfall or other climatic variables in the facilitation of individual movement of both species may help in the management of dispersal barriers and enhancement of movement corridors.

**Genetic structure** – Early studies strongly supported a sister group relationship between *L. aurea* and *L. raniformis*, with divergence of these species from the western species of bell frogs estimated to have occurred during the late Miocene (Burns and Crayn 2006). For *L. raniformis*, Vörös *et al.* (2008) identified Australian source populations and described the current genetic diversity among populations introduced into New Zealand. Finer scale population structuring of *L. raniformis* has been investigated recently in the urban fringe landscapes of northern Melbourne (Hale 2010). Hale (2010) observed patterns suggestive of strong, distance-limited dispersal, and an apparent negative influence of urban development on gene flow with reduced neutral genetic diversity in the most urbanised areas, and clear divisions between populations separated by urban infrastructure. Microsatellite loci developed (Hale *et al.* 2011) will be a valuable tool for future research into the population dynamics of *L. raniformis*.

For *L. aurea*, there is little phylogeographic structure at the range wide spatial scale (Burns *et al.* 2007), but at a regional level *L. aurea* is highly structured, although this decreases between populations in continuous landscapes (Burns and Ferrari 2004; Burns *et al.* 2007). *Litoria aurea* (Rowe *et al.* 1998; Newman and Squire 2001; Duryea *et al.* 2009) and *L. raniformis* (Hale *et al.* 2011) have high levels of heterozygosity at microsatellite loci relative to other amphibian species, but this should be interpreted with caution as the selection of microsatellite loci typically focuses on the most heterozygous markers available. Despite isolation of several populations, high levels of heterozygosity

have been maintained throughout the species range; therefore heterozygosity may be conserved despite the increasingly fragmented environment that *L. aurea* inhabits or the loss of heterozygosity in neutral markers is not yet detectable.

Historic museum specimens have been used to compare pre-decline levels of neutral genetic diversity displayed by extinct (or presumed extinct) populations of *L. raniformis* around Melbourne with the diversity of extant populations in the region (Hale 2010). This work suggests that extinct populations did not display low levels of neutral genetic diversity and that extant populations were not highly diverse historically. This is contrary to the expected relationship between genetic diversity and population viability (Reed and Frankham 2003; O'Grady *et al.* 2006), however, neutral genetic variation does not necessarily have a direct effect on fitness (Holderegger *et al.* 2006). There have been few attempts to incorporate both demographic and genetic information to develop strategies for the conservation of amphibians. However, a recent study by Greenwald (2010) demonstrates the potential to use initial abundance and dispersal estimates to use genetic data to inform a population viability analyses (PVA). Use of PVAs would allow the assessment of the effects of urban barriers to dispersal of bell frogs, and of various components of management, including translocation and reintroduction programs.

**Monitoring and conservation** – A range of aspects of the ecology of bell frogs have been used as indicators of the status and performance of these species at a local scale, through research focused on individual remaining populations. These include the amount of habitat that is occupied and used for breeding (Hamer *et al.* 2002a; Pyke *et al.* 2002; Hamer and Organ 2008; Hamer and Mahony 2010), as well as demographic characteristics such as population size, survival, and recruitment (Hamer and Mahony 2007; Hamer and Organ 2008). Determining whether a waterbody has been used for breeding is difficult because bell frog egg masses sink and dissipate within approximately four hours of oviposition at the surface of a waterbody, which reduces visibility (Harrison 1922), and by the relatively low detectability of tadpoles (Heard *et al.* 2006). The probability of detecting *L. raniformis* tadpoles by dip-netting or by setting fish traps has

been estimated at 0.350 (95% credible interval: 0.151, 0.567) (Heard *et al.* 2006), while no such study has been conducted for *L. aurea*. It is also possible that the presence of aquatic predators, such as mosquito fish, may further reduce the detectability of bell frog tadpoles. In a study on the effect of predation of *L. aurea* tadpoles by mosquito fish, tadpoles housed in enclosures that also contained the fish restricted their movements to the bottom of the enclosure and were rarely observed in the middle depths in comparison to tadpoles which were kept in fish-free enclosures (Morgan and Buttemer 1996). This behaviour could be attributed to predator avoidance, and if tadpoles also act in this way in field situations, then studies which involve current detection techniques of tadpoles in waterbodies that contain mosquito fish or other predators may underestimate tadpole abundance and occupancy.

The difficulty in detecting bell frog eggs and tadpoles led to the use of indicators of breeding. Pyke *et al.* (2002) classified breeding sites as any waterbody where either one or more males were heard calling, where tadpoles were detected, or where five or more adults including individuals of both sexes were observed. However, these classifications are problematic as they may occur at sites which are not used for breeding and incorrectly classify sites that are used as general habitat. To address these problems and design monitoring regimes that accurately reflect the status of bell frog populations, further research is required into the detectability of tadpoles using both dip-netting and trapping methods in a range of different field settings (such as different amounts and types of aquatic vegetation, and different densities and types of tadpole predators). Conducting future studies to determine what proportion of sites where males are heard calling are actually used for successful breeding would also help to shed more light on the reproductive ecology of bell frogs, and to give an indication of whether these factors can be used as reliable indicators for monitoring breeding events.

Problems with detectability are not limited to the larval phase of the bell frog lifecycle. The probability of detecting adult *L. raniformis*, during nocturnal visual surveys of habitat was 0.696 (95% credible interval: 0.585, 0.796), and if monitoring regimes were based on single surveys for each site,

they would likely severely underestimate the proportion of sites that were occupied (Heard *et al.* 2006). The probability of detecting adult *L. aurea* using a single nocturnal visual survey has been estimated as slightly lower than that of *L. raniformis*, at approximately 0.43 to 0.56 for two consecutive breeding seasons (Hamer and Mahony 2010). These relatively low detection probabilities highlight the need for multiple surveys to be conducted for each waterbody, so that pond occupancy is not underestimated.

Captive breeding of *L. aurea* and *L. raniformis* has been successfully achieved from different populations (Browne *et al.* 2003; McFadden *et al.* 2008; Mann *et al.* 2010a) and husbandry for adult bell frog species is well practiced. However, the contribution to conservation made by captive populations has been hindered by the lack of long term success in releases of tadpoles (Stockwell *et al.* 2008; White and Pyke 2008a). The use of captive populations to provide a safety net for the conservation of the species is promising because the biology of the bell frog complex is highly fecund and fast growing. However, the ongoing costs of maintaining captive populations and the limited success from reintroductions, suggests that maintaining the persistence of wild populations is currently crucial to the persistence of the bell frog species. Understanding the failure behind reintroductions is a key priority in research and will give captive breeding much greater credence in its conservation value.

#### **PRIORITIES FOR RESEARCH AND MANAGEMENT**

The decline of the bell frog complex has resulted in considerable conservation and research effort by scientists and managers. In some cases the high profile nature of the species and local management efforts have been successful in adding offset habitat, and ensured the persistence of localised populations (Darcovich and O'Meara 2008); in other cases, the outcomes of conservation efforts such as translocations and reintroductions have been less encouraging (Stockwell *et al.* 2008). Complicating, and at times frustrating, efforts to adaptively manage the species however, can be attributed to the lack of understanding in fundamental biology of bell frog species. As a result, scientists and managers

still lack the basic tools to provide the guidelines to offset habitat, with a high degree of confidence for successful establishment. This is no small matter: many major development projects will require potentially very large sums of money to be invested in environmental mitigation where bell frog habitat is severely impacted, yet cannot predict with a high degree of confidence that the money spent will result in persistent, viable populations either at the development impact sites, or in constructed habitat offsets.

Determining the underlying factors and variables affecting population structure, dynamics and persistence at appropriate temporal and spatial scales, is crucial to implementing early management actions. These need to be aimed at arresting declines in specific populations, as well as supporting predictive modelling and hypothesis testing to inform adaptive management strategies in conservation planning with longer time horizons and broad landscape-scale management goals; the goal is persistence of populations in the short-term, and the final outcome should be persistence of populations with adaptive potential and resilience in the long-term. Yet achieving this knowledge is particularly difficult once species become rare (e.g., in the case of *L. castanea*); this provides a challenge to biologists studying bell frogs and calls upon a collaborative, innovative and multi-disciplinary approach.

Here we propose three avenues to approach the goal of improving outcomes in bell frog conservation. The first is a series of unresolved questions and gaps in our knowledge that we consider should be priorities for research. The second avenue lists under-utilised methodologies and approaches that may improve research towards filling those gaps in knowledge. Finally, we highlight management strategies we think will improve the prospects for the persistence of bell frogs in eastern Australia.

#### ***PRIORITY RESEARCH QUESTIONS***

***Disease*** – How significant was chytridiomycosis in the historical bell frog declines? What is the current influence of *B. dendrobatidis* and how does it alter population demography and limit success in reintroduction events?

**Habitat associations** – How does resource availability determine abundance and occupancy? What is the relative importance of terrestrial and aquatic habitat?

**Population structure and survival** – How do bell frogs choose breeding ponds and what reproductive strategies are used to select spawning sites? What is the role of growth rate on fecundity of adults and reproductive output? What is the contribution of the larval phase to adult fitness? How much variation occurs in the basic demographic parameters (female survival and fecundity, time to maturation, clutch frequency) required to predict population variation? Which factors drive recruitment and survival in the early life stage? How do predation, drought and disease influence survival? What is the extent of temporal variation in survival rate? How fast is population turnover – do populations replace annually and is this a post-decline phenomenon?

**Movement** – How do bell frogs find and choose habitat? What is the level of dispersal and how do different life stages contribute? How important is climate to different behaviours? What is the importance of conspecific attraction in low population sizes?

**Genetic structure** – What are the genetic benchmarks for population, conservation and landscape genetics? What is baseline allelic diversity and heterozygosity for a viable population? Do small population sizes threaten bell frog persistence? Are inter-population hybrids fitter? Is inbreeding or outbreeding depression a threat? Is there significant diversity in functional genes within and between populations? Is variation in neutral genetic markers an indicator of functional variation and diversity? Is diversity in key functional gene complexes such as MHC (major histocompatibility complex) important to population and species persistence?

**Monitoring and conservation** – Ex situ breeding, small populations, genome storage: Is there a role? How should natural captive breeding and use of assisted reproduction for captive breeding be optimised given recent reports of poor responses in bell frogs to assisted reproduction protocols such as induced ovulation? Can bell frog genomes be stored and retrieved?

**Advances in Technology and New Conceptual Approaches and Paradigms** - Use of genetic analyses (including an expanded use of next generation

genomics linked to landscape genetic modelling) for dispersal distance and effective population size and structure, metapopulation analysis, constructing recent population genetic histories; Employing Bayesian statistics for sparse data sets and an information theoretic approach to model site occupancy and habitat that incorporate imperfect detection of the species during field surveys; A physiological and structural approach to focus on basic demographic questions: skeleto physiology of museum specimens, non-invasive stress and reproductive hormone assays to measure population fitness and reproductive parameters; Integrating demographic parameters into Population Viability Analyses for extant populations; Using invasive populations of bell frogs to provide basic demographic data, and new insights into bell frog dispersal, ecology and colonisation.

**Management actions integral to the persistence of bell frog populations** – Large, connected ponds that incorporate wide terrestrial buffers are the most important habitat characteristics known; actions that fragment landscapes, and disrupt movement between ponds and habitat patches must be avoided; Documentation and meta-analysis of successful and unsuccessful reintroductions to inform and progress adaptive management; Incorporation of genetic management into the bell frog species conservation. Recognition that the persistence of as many extant populations as possible is essential to maintain genetic diversity within the species, that population size is an important factor in retaining intra-population genetic diversity, and spatial and landscape factors affecting gene flows between populations should be considered in management; The role of ex situ actions in conservation of bell frogs should be assessed and pursued as appropriate, including captive assurance populations, captive breeding for reintroduction, potential role of assisted reproduction in captive breeding, and genome storage as a management tool for reducing loss of genetic diversity.

## CONCLUSION

We recognise that a considerable amount of information on the ecology of the species complex has been completed by research students and that valuable information presented in theses were not available in the published literature, at least in the

short term (and therefore, not readily accessible by managers). Conservation priorities for these species needed to be developed in the absence of published material. We started with five goals; 1) to clarify information on the cause of decline, 2) to highlight key similarities and differences among the species, 3) to integrate findings of several studies of habitat requirements, population demography, landscape ecology, genetics and disease, 4) to identify the gaps in knowledge and focus on untested assumptions that have been made, and 5) to identify areas of research that have been overlooked. There is a need for research to address the future conservation and management of bell frog complex, and multiple avenues of basic and adaptive research are available to achieve this aim. While there is no consensus within the literature on the deterministic causes and drivers of decline of the species, a key outcome of our review has been to highlight strategic directions in adaptive and basic research to benefit threatened and declining species.

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