

Developing effective habitat restoration strategies
for the green and golden bell frog (*Litoria aurea*)
through adaptive management.

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Declarations

Originality

I hereby certify that to the best of my knowledge and belief this thesis is my own work and contains no material previously published or written by another person except where due references and acknowledgements are made. It contains no material which has been previously submitted by me for the award of any other degree or diploma in any university or other tertiary institution.

Thesis by Publication

I hereby certify that this thesis is in the form of a series of papers. I have included as part of the thesis a written statement from each co-author, endorsed in writing by the Faculty Assistant Dean (Research Training), attesting to my contribution to any jointly authored papers.

Signed:

Carla J. Pollard

Date: 11/08/2017

This thesis consists of an introduction to adaptive management and project overview, followed by five papers, of which three are published and two are unpublished manuscripts, and finally a conclusion summarising the findings of all the papers. Graphs and tables are embedded within the published papers and unpublished manuscripts. References are presented at the end of the introduction/overview and each of the five papers, rather than at the end of the thesis.

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List of publications included as part of the thesis

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Chapter 3: Pollard, C. J., Stockwell, M. P., Pickett, E. J., Garnham, J. I., Bower, D. S., O'Meara, J., Darcovich, K., John Clulow, J. & Mahony, M. J. Using adaptive management to evaluate the effectiveness of a disturbance regime in the conservation of a threatened amphibian. (*unpublished*)

Chapter 4: Pollard, C. J., Stockwell, M. P., Bower, D. S., Clulow, J., & Mahony, M. J. (2017). Combining ex situ and in situ methods to improve water quality testing for the conservation of aquatic species. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27(2), 559-568.

Chapter 5: Pollard, C. J., Bower, D. S., Stockwell, M. P., Pickett, E., Garnham, J. I., Fardell, L., Clulow, J. & Mahony, M. J. Trialling captive breeding and release methods for reversing declines in a wild frog population. (*unpublished*)

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Abstract

Traditional management of species and ecosystems is reliant on the ability to accurately predict the outcomes of management actions. Due to the high level of uncertainty in natural systems, this level of predictability is unlikely to be possible in the vast majority of management situations. Adaptive management is an alternative approach that can be effective regardless of how much is known about the species or system and the way in which it will respond to management, as actions are used as a series of experiments to test hypotheses and build knowledge about the way it functions. I used this approach to investigate the effectiveness of a range of management actions aimed at conserving the threatened green and golden bell frog (*Litoria aurea*), a species which was once common but has experienced large-scale declines since the 1970s. I found that the removal of the exotic predatory fish *Gambusia holbrooki* via pond draining was effective in greatly increasing the reproductive success of this species, and the addition of sodium chloride to increase the salinity of ponds was effective in reducing the prevalence of the pathogenic amphibian chytrid fungus, and in turn increasing *L. aurea* survival. Actions aimed at creating early-successional habitat (the removal of overgrown aquatic vegetation and shade trees) do not appear to be necessary to maintain habitat quality at sites that are dominated by urban or industrial land uses which provide incidental disturbance (though they may still be required to maintain pond function). Population supplementation via the release of captive bred tadpoles had temporary benefits for naïve occupancy and abundance, but it is likely that this strategy can only be effective in the long term if the cause of declines can be

determined and mitigated. No evidence was found to suggest that poor habitat quality is the cause of observed low occupancy at sites where *L. aurea* persists. Regardless of whether these actions were effective in meeting conservation goals in the short term, they were all valuable in the long term as they added to our understanding of the habitat requirements of this endangered amphibian and can inform and improve future management strategies.

Introduction and Overview

1 Traditional resource management

The concept of natural resource management came about during the early 1900s, when it became apparent that although natural resources may seem abundant, they are not inexhaustible (Walters, 1986). Natural resource management involves attempting to conserve resources so that they persist into the future, for two possible purposes. The first is to prevent resources from being over-exploited so that future generations can continue to use them. The second is to retain biodiversity, which makes ecosystems more resilient to disturbance, and therefore less likely to collapse (Hector et al., 1999). Protecting ecosystems is critically important, as humanity ultimately depends on them to provide services such as clean air and water.

The traditional approach to managing natural resources has been to identify a problem and then develop and implement a single action that is predicted to solve this problem. This is defined by Holling and Meffe (1996) as the ‘command and control’ approach, and they suggest that it works well if the problem is relatively simple and well-defined, and if the response of the system to the action that is implemented is predictable. Many difficulties arise, however, when this method is applied to natural systems, which are generally extremely complex and vary both spatially and temporally (Williams, 2011). Because of this complexity and variability, it is very difficult to develop a thorough understanding of the underlying processes that drive natural systems and to predict how they will respond to a particular management action. The command and control approach therefore often leads to the implementation of actions that have unexpected and undesirable effects (Holling and

Meffe, 1996). An example of this is the suppression of fire in fire-prone ecosystems, which is successful in reducing the risk of damage occurring in the short-term, but leads to an accumulation of fuel that will eventually result in a more destructive, high intensity fire (Christensen et al., 1989). A second example is the baiting of dingos in rural areas to reduce calf mortality. Although dingo abundance was lower in baited area, calf mortality was higher in comparison to non-baited areas (Ritchie, 2010). This is likely to be due to the way that high mortality in dingos disrupts their social structure, and solitary individuals kill more calves than those that are part of a pack.

Throughout the past century it has become apparent that the uncertainty surrounding natural systems and the way that they operate makes their management a difficult task (Ludwig et al., 1993). One such area of difficulty is trying to estimate the maximum sustainable yield of wild populations that are harvested by humans. Fish populations provide an example of this; they tend to fluctuate greatly over time and as a result are subject to a phenomenon called the ratchet effect (Caddy and Gulland, 1983). While the population is stable, harvesting rates also tend to remain stable, and when a sequence of years with favourable conditions occurs and the population increases, so too does the amount of harvesting. The problem arises at the end of this period of population growth when conditions return to normal, but harvesting remains at an elevated level, generally due to the increased amount of jobs that have been created and investment in infrastructure (Ludwig et al., 1993). The lack of flexibility in the management of harvested fish populations combined with the uncertainty about the processes that drive population size has lead to the over-exploitation

and collapse of many species, including the Pacific sardine (Radovich, 1982) and the Peruvian anchoveta (Ludwig et al., 1993).

Another challenge that arises from the uncertainty that is involved in natural resource management is that there is often a lack of consensus about the state of natural systems (Allen et al., 2011). Because natural systems are so complex, it can be very difficult to determine the cause (or more likely combination of causes) of problems such as the decrease in the population size of a species (Ludwig et al., 1993), or the pollution of a waterway (Henriksen and Barlebo, 2008). Disagreement about the cause of a problem inevitably leads to disagreement about the appropriate course of action that should be undertaken to fix that problem. It was argued by Ludwig et al. (1993) that waiting until a consensus is reached about the best way to manage a natural resource problem is not a viable option, because there have been many cases throughout history where natural resources have collapsed and scientific consensus about the causes has still not been reached.

Past experiences in the management of natural resources have demonstrated that managers of natural systems cannot rely on the actions that they design and implement to have a particular predicted outcome. They have also shown us that management needs to be dynamic enough to change over time in response to changes in the nature and abundance of that resource. Finally, it has become clear that it is not appropriate to wait until disagreements over the best way to manage resources are resolved, as this event may not occur until that system has been irreparably damaged. It is through the realisation of these conclusions that a different type of management, which attempts to deal with uncertainty, variability and differing opinions, was developed.

2 Adaptive management

2.1 Definitions and process

Adaptive management is an approach that involves learning about a natural system at the same time as managing it, by using management actions as a series of experiments to test different hypotheses about the way that the system functions. This concept was first described by Beverton and Holt (1957), although it was not termed adaptive management until Holling (1978) and Walters and Hilborn (1978) developed the idea further and provided a conceptual framework for it. Since its development there have been many different interpretations of what constitutes adaptive management. These interpretations range from simply using different management actions over time, to complex experimental set ups involving the use of predictive models to inform the selection of management actions, and once these are implemented, the use of monitoring programs to assess their effectiveness and finally using this information to inform future management. This lack of consensus over which types of management can be classified as adaptive and which cannot has led to a larger number of reports of adaptive management being used in real-world situations than have actually occurred (Allen et al., 2011).

Williams (2011) stated that the key feature of adaptive management is the feedback between learning and decision making. Learning contributes to management by informing future decision making about which actions to implement, whilst management contributes to learning as each intervention can be used as an experimental treatment that tests hypotheses about the system. The process of adaptive management can be divided into two broad parts:

the set-up phase, during which key components are put in place, followed by the iterative phase, in which the components are linked together in a sequential process (Figure 1, page 16). These can be further divided into the following steps (Williams, 2011):

Set-up phase

1. Engage stakeholders. It is important to communicate with all the parties that are involved with the natural system being managed. If they are given the opportunity to have their own input, stakeholders are more likely to support the adoption of adaptive management, which is then much more likely to succeed.
2. Determine objectives. There are usually multiple goals in natural resource management, and these need to be weighed up against each other in terms of perceived importance, so that the potential management actions relating to each can be prioritised.
3. List alternative management actions. Once a goal has been defined, a list of actions that are believed to have the potential to meet this goal for different reasons can be made.
4. Prioritise actions using models. Alternative management actions can be ranked in terms of which one is most likely to have the largest effect using a predictive modelling process. Models can also incorporate the cost of each action, so that actions can be prioritised based on cost-effectiveness. A good way of dealing with uncertainty about the natural system being managed is to create multiple models that are each based on a different hypothesis about the way the system operates.

5. Design a monitoring regime. Monitoring the effect of actions is a critical part of the process, as it informs the next round of decision making about whether particular actions should be continued or not, and provides information about the system being managed.

The set-up phase involves planning and designing all of the above components, and the following iterative phase is the physical part of the process, that involves carrying out these plans.

Iterative phase

1. Decide on an action. Using the modelling process described in part 4 of the set-up phase, choose one or more actions and implement it/them. The appropriate action to take will change over time, as monitoring results provide an increased understanding of the natural system and the way it responds to management.
2. Follow-up monitoring. Continually collect data relating to the response of the system to the implemented actions.
3. Assessment. Using the information gained during monitoring, evaluate the effectiveness of the actions in achieving management goals. The degree of coincidence between the predicted response of the system that was generated using models during the set-up phase and the actual response shown in the monitoring data can be used as an indicator of model adequacy. If there is little overlap between the predicted and actual response of the system to a particular action, this would indicate that the hypothesis the prediction was based on is not sound, while if the predicted

and actual responses are similar, this supports the hypothesis. In this way, the response of the natural system to different management actions provides the opportunity to learn more about the system itself.

4. Feedback. The information gained by implementing, monitoring and evaluating a management action can be used to update the beliefs and theories about the natural system that were outlined during the set-up phase. Through the process of model updating (outlined in the above assessment step), actions that are more likely to be effective can be selected for the next round of management. For example, it may be determined that a particular management action is effective in meeting the goal of increasing the size of a population, through the process of reducing predation. This finding would suggest that predation is having a large impact on that population and that other actions which are also focused on reducing predation may also be effective. In this way, evaluating the effectiveness of past management actions can lead to the selection of future actions that are more likely to be successful, which in turn gradually leads to improved management.

As the name suggests, the iterative phase of adaptive management is repeated in cycles over time. Each time a different management action (or set of actions) is implemented, tested and evaluated, the information that is learned during that cycle is used to inform the next cycle. Over time, a greater understanding of the system and how to manage it is gradually built.

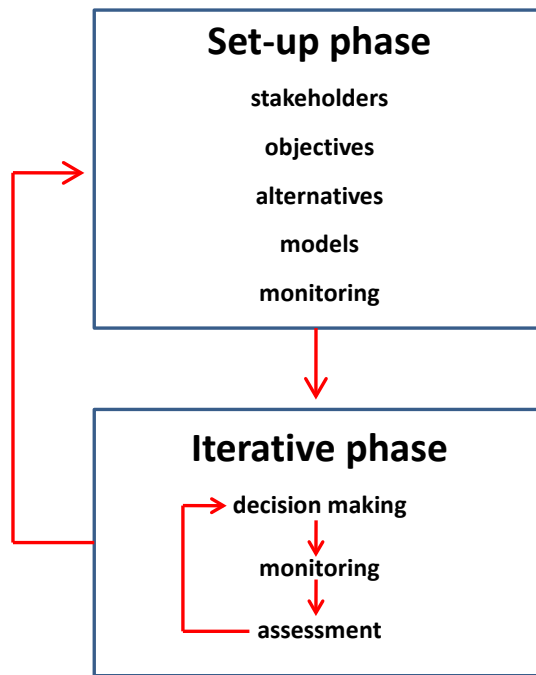


Figure 1: Flowchart of the two phases of adaptive management, the components involved in each phase and the feedback loops that connect different steps of the process. Source: Williams 2011

2.2 Buzzword or Panacea?

Although the concept of adaptive management has been around since the 1950s, there are relatively few examples of it being used to successfully manage real-world situations (Williams, 2011). In their review of case studies of adaptive management, Keith et al. (2011) found that of the total number of published cases where adaptive management was reported as being used to solve conservation problems, the majority used only some components of the adaptive management process, rather than using it in its entirety. An example of this is that structured decision making involving the use of predictive models to assist in the prioritisation of management alternatives is often used synonymously with the term adaptive management, although it is only one component of the process (Allen et al., 2011). Lynam

et al. (2010) reported the successful use of models in predicting which management alternatives were economically viable and would be most effective in their goal of reducing the amount of sediment that enters the ocean surrounding the Great Barrier Reef. A similar process was used by Marcot et al. (2001) to model wildlife population responses to a range of potential management actions.

While these papers demonstrate that structured decision making can assist natural resource managers in selecting effective actions, they are focused on the set-up phase of the adaptive management process. This is the case for much of the literature published on the use of adaptive management in conservation (Keith et al., 2011), which leads to the question, why is there such a lack of reports on the implementation of the iterative phase? This process, in which different actions are trialled in succession, is an essential part of adaptive management as it provides the opportunity to learn more about the system and gradually leads to improved management (Williams, 2011). Despite the fact that the concept of adaptive management is half a century old, and that the theoretical aspect of it has continued to be developed and refined from its initial introduction to the present day (Walters, 1986, Walters and Hillborn, 1978, Holling, 1978, Williams, 2011, McCarthy and Possingham, 2007, McLain and Lee, 1996), its implementation still provides many challenges. The over-application of the term, combined with the way that development of theory about adaptive management continues to outstrip practical applications (Keith et al., 2011) has led some to question its validity in the field of natural resource management (McLain and Lee, 1996).

2.2.1 The Pitfalls

Several limitations of adaptive management have been identified to account for its infrequent implementation. Firstly, it can be very expensive initially to implement, due to the time-intensive nature of follow-up monitoring (McLain and Lee, 1996). Monitoring the effects of management actions is beneficial and cost-effective in the long term, as it provides feedback that can be used to improve management over time. Although a particular intervention may seem anecdotally to be working, it is impossible to know this with any certainty until its effects are measured and evaluated. Unknowingly repeating a management action year after year that is not effective in achieving management goals is also expensive, and will not only result in limited conservation resources being wasted, but also potentially cause damage to the system being managed (Holling and Meffe, 1996). In some cases, however, managers do not have the resources to follow up actions with a monitoring regime that is comprehensive enough to detect system responses. In these cases, rather than using limited resources to carry out a monitoring program that is not sufficiently powerful, it would be more beneficial to direct them to other areas (Williams, 2011), such as implementing a larger number of actions, or increasing the area of land that receives interventions.

Another challenge to the implementation of adaptive management is that it involves embracing a certain amount of risk. In order to learn more about the system being managed, a range of actions must be tested and evaluated (Holling, 1978). Managers may be reluctant to do this, however, if they believe that a single action, which appears to have worked in the past, is more likely to be effective than new and untested alternatives. It was suggested by McCarthy and Possingham (2007) that options considered to be inferior should only be used

if there is a high level of uncertainty regarding whether it really is inferior, and if finding out its true effectiveness can be done relatively quickly, to minimise the potential of damage occurring if it does not have the desired effect.

While adaptive management has the potential to lead to more effective conservation of natural resources, it is important to keep in mind that it is not a suitable approach for every natural resource problem. Allen et al. (2011) argue that adaptive management should not be seen as a panacea for all the challenges that humanity is facing, as it is only appropriate in a subset of natural resource management problems, where both uncertainty and controllability of the system are high. If the system is well understood and there is little uncertainty relating to how it is best managed, then there is little reason to test novel actions and monitor the effects, although this scenario is very rare in the area of natural resources due to their complex nature (Williams, 2011). As adaptive management is dependent on the assumption that resources will respond in some way to management actions (Walters and Hillborn, 1978), this approach is also not suitable for situations where it is not possible to alter the state of the system via management.

Another condition of adaptive management is that responses to actions must be measurable, otherwise any benefit that they may have will go undetected (Williams, 2011). Even in cases where resources are sufficient to carry out intensive monitoring programs, the large amount of temporal variability that is inherent in natural systems can make it very difficult to determine whether a particular management action has had an impact on the resource. Kaji et al. (2010) applied adaptive management to the problem of maintaining sustainable populations of sika deer in Japan, with the goal of initially reducing the number

of deer by temporarily removing some of the restrictions on deer hunting. They found it very difficult, however, to determine whether reductions in population size could accurately be attributed to this change in management, due to the large fluctuation in deer numbers from year to year due to a wide range of environmental factors such as food availability and climatic conditions. Lynam et al. (2010) encountered the same type of difficulty in their study of which types of management actions are most effective in improving water quality in the Great Barrier Reef, which is dependent on a large number of land uses across a vast geographical area. They found that the positive effect of a single action aimed at reducing the amount of sediment that entered the area from one particular land use became lost amongst the accumulated variability of all the other contributing sources of pollution.

The limitations of adaptive management discussed above, consisting of the initial high cost and risks associated with this approach, its restriction to certain types of natural resource management scenarios and the challenges associated with determining the effect of specific actions, are all similar in that they are technical problems. It has also been recognised, however, that social issues relating to setting up and implementing adaptive management are frequently responsible for preventing it from being successful. Based on his review of literature focused on the challenges to the implementation of adaptive management, Johnson (1999) concluded that social challenges have played an even greater role in the lack examples of adaptive management being successfully used to conserve natural resources than technical issues or problems relating to its theory. One such problem is that if all the stakeholders are not consulted during the set-up process of adaptive management, they are

likely to feel that the new management regime does not represent their point of view, and are therefore unlikely to support it (McLain and Lee, 1996, Williams, 2011).

Another issue that has been identified is that if the responsibility of follow-up monitoring is placed solely on a single stakeholder, this can result in the withholding of data from other stakeholders, particularly if it demonstrates that an action has been unsuccessful or that the system has been damaged in some way (Johnson, 1999). Funding for conservation is limited and organisations may not be willing to reveal information that suggests money has been ‘wasted’, even though the implementation of actions that do not succeed in meeting management goals is still beneficial in providing a greater understanding of the system. This attitude highlights the large amount of pressure that is placed on environmental managers to constantly meet targets, and the fact that there is little room for error when it comes to implementing actions that are successful in meeting goals (Keith et al., 2011). It is very difficult for managers to gain enough support for conducting adaptive management in this social climate, as this approach is fundamentally built around the premise that successful management in the long term can only be achieved by implementing actions that do not work, and learning from these situations (Williams, 2011).

Keith et al. (2011) have suggested that for successful adaptive management to occur, we first need to accept that uncertainty is an inevitable part of natural resource management. They believe that there is tension between scientists and managers, as scientists often feel the need to oversell their ability to predict the outcomes of various management interventions, and that managers have a tendency to ignore scientific uncertainty when given advice about how to prioritise actions. The practice of denying the existence of uncertainty has become so

deeply entrenched in natural resource management that managers, scientists and policy makers feel that they cannot go against this culture and acknowledge that it is not possible to guarantee the success of management interventions (Keith et al., 2011). For adaptive management to be implemented on a larger scale than it is currently, there needs to be a shift in the mindset of those working in the field of natural resource management from the traditional command and control approach where natural systems are seen as predictable, linear and controllable structures, to one which accepts and embraces the uncertainty associated with these complex systems.

2.2.2 The Potential

As has been discussed in the previous section, many factors, both technical and social, have been identified as causes for the scarcity of reports of adaptive management being successfully applied. It should also be noted, however, that there is no such deficit in the amount of literature that has been published on the advancement of the theoretical side of adaptive management. The fact that the concept of adaptive management has been considered promising enough to be continuously examined, developed and improved by the scientific community for more than half a century (Holling, 1978, Walters and Hillborn, 1978, McCarthy and Possingham, 2007, Williams, 2011, Walters, 1986, McLain and Lee, 1996) is demonstrative of its perceived great potential. Allen et al. (2011) have suggested that the benefits associated with replacing traditional management approaches with adaptive management have never been appreciated more than they are currently, in the face of unprecedented global challenges. Due to the increase in the human population and in resource

consumption, the ability to effectively manage and conserve natural resources is more essential than it has ever been before.

Perhaps the largest advantage that adaptive management has to offer is the ability to deal with uncertainty. While it is required that the system being managed responds in a measurable way to each action that is implemented (Williams, 2011), it is not necessary to be able to predict the direction or magnitude of the response. The use of follow-up monitoring and assessment allow actions that do not change the system in a beneficial way to be identified quickly and removed from the future monitoring regime. For this reason, long-term success in adaptive management is not dependent on the success of every action implemented. This makes it a suitable approach for dealing with systems which are not well understood and about which little is currently known (Allen et al., 2011). It also reduces the amount of pressure placed on managers to make accurate judgements about the way that complex and dynamic systems will behave in the future, in comparison to traditional management approaches.

This does not mean, however, that the opinions of those who have had a large amount of experience working to conserve a particular natural resource, and have as a result built up a knowledge base in that area, cannot be incorporated into the adaptive management process. While the value of an action is not dependent on its success in terms of meeting management goals according to the principles of adaptive management, it still makes sense to give priority to actions that are more likely to be effective, if there is any evidence on which to base this. The process of adaptive management allows for the use of expert opinion, where it exists, in creating predictive models that form the basis of the prioritisation of alternative actions

(Henriksen and Barlebo, 2008). Individuals can use their personal knowledge as a basis for choosing the baseline values that the predictive models are built on.

If, for example, an expert believes that predation by a particular predator is a very high cause of mortality for an endangered species, they can incorporate this information as part of the input for the models that will be used to predict the effectiveness of a range of alternative actions. It is likely that actions aimed at reducing the number of individuals that are killed by this predator will have a higher predicted effectiveness than actions aimed at mitigating other threats that cause lower rates of mortality. The accuracy of the values that are used as input for predictive models can be updated at a later time using the information provided by the implementation, monitoring and assessment of a range of management actions (Williams, 2011). Because the adaptive management process facilitates learning about the system while it is being managed, inaccuracies in the initial values that were used as input for predictive models can later be identified, and gradually replaced with values that are more representative of the way that the system actually functions. Expert opinion can therefore provide a valuable starting place when it comes to beginning the adaptive management process and inaccuracies will not prevent the long-term success of management.

The modelling component of adaptive management can also be used to incorporate and resolve differences in opinion. In cases where expert opinion is divided, it is common to postpone the decision about which actions to implement until more research has been conducted and it becomes clearer which opinion is the most accurate (Holling and Meffe, 1996). The problem with this approach in natural resource management is that studies in this field tend to require a long period of data gathering, as environmental variation often makes

it difficult to detect trends in natural systems (Allen et al., 2011). Waiting until disagreements can be resolved about the best course of action to take wastes valuable time and inaction puts natural systems at risk. This can be prevented by creating multiple models, and using different values that each reflect one view of the current state of the system as input (Williams, 2011). Henriksen and Barlebo (2008) found this approach to be very useful during stakeholder meetings, when there was a disagreement over the degree to which the use of pesticides in farming affected the quality of deep ground water. Running multiple models allowed them to move ahead in the decision-making process rather than delaying it until a consensus could be reached. Multiple models can also be used if there is a lot of uncertainty surrounding one particular aspect of the system (Keith et al., 2011). By comparing models with the highest and lowest probable values relating to this aspect as input, it is possible to see whether variation in this area is likely to have a large impact on the predicted effectiveness of potential management actions.

In addition to providing a way of incorporating expert opinion into decision making and resolving differences in opinion, predictive models can be extremely useful for identifying actions that are highly unlikely to be plausible (Keith et al., 2011). Henriksen and Barlebo (2008) used modelling for this purpose in their study aimed at improving water quality by implementing strategies that would reduce the amount of pollution produced by a variety of land uses. Before beginning the modelling process, managers were planning to initiate a door knock campaign to speak with farmers face to face about whether they would consider adopting farming practices that do not pollute waterways to the same extent as traditional practices. However, when they modelled the predicted results to find how much

of an impact this intervention would have on the total amount of pollution entering waterways from all sources, they found that even if one hundred percent of the farmers that they spoke to in every catchment area changed their practices, the reduction in the total amount of pollution to enter waterways would be minimal. They therefore chose to abandon this likely ineffective and costly action before it had begun and to use their resources instead on other interventions that were more likely to be effective. This case study demonstrates that predictive modelling can be used to reduce the risk of implementing an ineffective strategy, and to direct limited resources toward strategies that are more likely to meet management goals.

Although they have many uses, it is important to recognise the limitations of predictive models, and that attempting to accurately predict the exact way that a system would respond to an action is not an appropriate way to use them (Keith et al., 2011); if this level of predictability was achievable, then the traditional command and control method would be effective and there would have been no requirement for the concept of adaptive management to have been developed in the first place. One of the main differences between traditional and adaptive management is that the success of the former is completely dependent on the ability to predict system responses, while this is not the case for the latter. In adaptive management, actions that do not meet management goals still hold value in that they provide the opportunity to build a greater understanding of the system being managed (Williams, 2011). The extent to which managers should aim to maximise either learning or meeting management outcomes remains a topic of debate, although they are not mutually exclusive. The learning that results from implementing actions that do not work leads to

improved future management (Keith et al., 2011), and these ‘unsuccessful’ actions that consume resources and lead to no direct gain can therefore be described as investments in the long-term effective conservation of resources.

2.2.3. Conclusions

Although there are many technical and social challenges that need to be overcome in order to successfully set up and implement adaptive management, this approach has the potential to provide us with a more effective way of conserving natural resources. The traditional command and control method has been in use for over a century, and this period of time coincides with the beginning of a global mass extinction event that has affected every group of vertebrates and continues to claim more species each year (Pimm and Raven, 2000). While it is impossible to tell whether this situation could have been avoided if a different approach to natural resource management had been used, it makes sense to now embrace a more flexible approach that is compatible with the dynamic, complex and often unpredictable nature of natural resource problems. Adaptive management is not a blanket cure that will provide easy answers for every conservation challenge that humanity is faced with (Allen et al., 2011), but it does have the potential to be a valuable tool in the mitigation of some of the negative impacts caused by the over-exploitation of natural resources, such as species declines (Fontaine, 2011).

3 The Green and Golden Bell Frog: An Ideal Candidate for Adaptive Management

Adaptive management has been recognised as a potentially valuable tool in the conservation of threatened species (Runge, 2011). Despite this, there are very few examples of its use in the published literature, and the majority of these describe the application of structured decision making using predictive models in prioritising potential management actions (Tyre et al., 2011, Johnson et al., 2011, Moore et al., 2011). There is therefore a need for case studies in which both the set-up and iterative phases of adaptive management are applied for the purpose of threatened species conservation. The green and golden bell frog, *Litoria aurea*, is an ideal candidate for adaptive management.

This species was once one of the most commonly encountered frogs in south-eastern Australia (Pyke and White, 2001), prior to large-scale population declines that began in NSW around the mid 1970s (Hamer and Mahony, 2007). The decline of this species was not an isolated event; it coincided with population declines of many other amphibians on almost every continent that also began at that time (Barinaga, 1990). Amphibian declines continue to occur on a global scale despite efforts to identify and mitigate their causes, and as a result amphibians have a higher proportion of species that are on the verge of extinction than any other group of vertebrates (Stuart et al., 2004). In recognition of the continuing decline in the abundance and distribution of *L. aurea* throughout NSW, the species is now listed as ‘endangered’ in this state under the Biodiversity Conservation Act 2016 and as ‘vulnerable’ nationally under the Environmental Protection and Biodiversity Conservation Act 1999 (Department of the Environment and Energy, 2018).

One of the largest remaining *L. aurea* populations in NSW is situated at Sydney Olympic Park, which covers 800 ha of land situated 14 km from the centre of Sydney. The area was used for a range of industrial purposes throughout the 1900s that greatly altered the landscape, including the development of a 16 ha quarry and the landfilling of approximately 9 million cubic metres of industrial, commercial and domestic waste (Darcovich and O'Meara, 2008). The site became available for urban renewal when the industries began to close in the 1980s, and since the presence of *L. aurea* was confirmed there in 1992, the objectives of the Sydney Olympic Park Authority, the organisation responsible for managing the area, have included measures to conserve existing habitat and to expand it through the construction of new waterbodies on remediated lands (Sydney Olympic Park Authority, 2008). The creation of suitable habitat for *L. aurea* has been largely successful, and has resulted in the expansion of its former distribution at the site, from the single population that was discovered in the disused quarry to two new self-sustaining sub-populations (Darcovich and O'Meara, 2008). An artificial wetland consisting of 22 similar clay-based ponds that were constructed in 1999 provides habitat for one of these sub-populations, while the other consists of 36 man-made ponds that vary in terms of age, size and construction materials.

Since the discovery of *L. aurea* was made at the site almost 20 years ago, a range of management actions have been implemented and recorded each year at Sydney Olympic Park, and the population has been monitored annually. Although the data collected from these activities could be used to assess the effectiveness of different interventions, this has not yet been done. The *L. aurea* population at Sydney Olympic Park is therefore an ideal candidate for benefitting from an adaptive management regime. The problem of gaining enough

resources to implement a comprehensive monitoring regime, which has been identified as one of the major stumbling blocks that can prevent the successful implementation of adaptive management (Keith et al., 2011), does not apply to this population as monitoring is already a part of the current management regime. In addition, although *L. aurea* is a relatively well-studied species, large knowledge gaps still remain in relation to the extent to which various threats negatively impact the species, and to its habitat requirements. Assessing the effectiveness of management actions aimed at reducing the impacts of these threats, and of efforts to improve habitat quality, will provide the opportunity to build a better understanding of this species and the complex ecosystem of which it forms a part. This information can in turn be used to improve the effectiveness of management of *L. aurea* not only at Sydney Olympic Park, which is listed as a key population for the persistence of the species (Department of Environment and Conservation, 2005), but also at other locations across the state where populations persist. In this way, adaptive management has the potential to improve our chances of being able to successfully conserve this species in the long-term.

The overarching goal of this thesis is to use an adaptive management approach to assess the effectiveness of actions that have been used at Sydney Olympic Park to conserve this important *L. aurea* population, and to implement new actions that have not yet been trialled. Actions were selected on the basis that they were considered relatively likely to have positive outcomes based on existing evidence and expert opinion, and their ability to answer unresolved questions relating to bell frog ecology to provide new information to improve the success of future management.

3.1 Removal of *Gambusia holbrooki* to increase reproductive success

The introduction of an invasive fish species, *Gambusia holbrooki*, has been suggested to have played a role in the widespread decline in the abundance and distribution of *L. aurea* in NSW. *Gambusia holbrooki* was first introduced into Australia from North America in 1925 in an effort to reduce the prevalence of malaria and other diseases that are transmitted by mosquitoes, via the predation of mosquito larvae by this fish (Myers, 1965). Unfortunately, it was later discovered that *G. holbrooki* also preys on the eggs and tadpoles of several Australian frog species, including *L. aurea* (Morgan and Buttemer, 1996), and predation by this fish is now listed as a ‘key threatening process’ in NSW under the state’s Threatened Species Conservation Act 1995 (Department of Environment and Conservation, 2005). The extent to which predation by *G. holbrooki* is responsible for the continuing declines of *L. aurea* remains unclear despite this being the focus of a number of studies.

Daly (1996) observed evidence of *L. aurea* breeding only in waterbodies from which *G. holbrooki* was absent at his study site at Coomonderry swamp, which is in accordance with Morgan and Buttemer’s (1996) findings that *L. aurea* tadpoles which were placed in enclosures with *G. holbrooki* were significantly less likely to survive than those that were placed in fish-free enclosures. In their study of 74 locations where *L. aurea* had been detected historically, Pyke and White (1996) found that evidence of breeding by *L. aurea* was detected significantly more frequently in ponds where predatory fish were absent than where they were present (this study also included other species of predatory fish, although there were only four sites where there were other species of predatory fish without *G. holbrooki* also being present). The results of these studies are in contrast, however, with the findings of other

research; van de Mortel and Goldingay (1998) observed over 400 metamorphs emerging from Coomaditchy Lagoon near Port Kembla when it had high densities of *G. holbrooki*, which suggests that it is possible for *L. aurea* to successfully breed in the presence of this predatory fish. There are also a number of other cases in which *L. aurea* populations have continued to persist in the presence of *G. holbrooki* (Mahony, 1999), which has led White and Pyke (2008) to hypothesise that the presence of dense aquatic and emergent vegetation in waterbodies may allow tadpoles to avoid predation by this invasive fish.

Although the extent to which predation by *G. holbrooki* reduces the reproductive success of *L. aurea* remains unclear due to disparities in the literature, it is likely to be a cause of egg and tadpole mortality in areas where these two species coexist. For this reason, one of the management goals at Sydney Olympic Park is to reduce the impact that *G. holbrooki* has on the fecundity of *L. aurea*. The action that has been put in place to achieve this goal is the draining of the artificially-created Narawang Wetland, which provides habitat for one of the three sub-populations of *L. aurea* at the site.

The first aim of this thesis is to evaluate the effectiveness of pond draining, so that this information can be used to both gain a clearer understanding of the degree to which predation by *G. holbrooki* reduces the reproductive success of *L. aurea*, and to inform future decision making about the allocation of resources to alternative management actions.

3.2 Mitigation of mortality caused by *Batrachochytrium dendrobatidis*

Another factor which has been suggested to have played a large role in the state-wide *L. aurea* population declines is infection by the pathogenic fungus *Batrachochytrium dendrobatidis*. This fungus is known to infect over 30 Australian frog species and causes high rates of mortality in some (Berger et al., 1999). Reports have been made of *L. aurea* from several remaining populations in NSW being infected by *B. dendrobatidis*, including the Sydney Olympic Park population, where it was found to have caused the mortality of at least 17 frogs (Penman et al., 2008). As a result of the declines that caused the extinction of over 80% of historic *L. aurea* populations in NSW by 1996 (White and Pyke, 1996), the distribution of this species in the state is now restricted to the East Coast, and most of the remaining populations occur at sites which are used for industrial purposes, or have been in the past (White and Pyke, 1996, Department of Environment and Conservation, 2005). This change in the distribution of *L. aurea* has led to the suggestion that this species is able to persist in these areas because they are inhospitable to the growth of *B. dendrobatidis*, and therefore act as environmental refuges where *L. aurea* can survive (Threlfall et al., 2008).

The findings of laboratory studies that elevated concentrations of sodium chloride and of a range of trace heavy metals inhibit the growth and survival of *B. dendrobatidis* (Johnson et al., 2003) support this theory that *L. aurea* is able to persist in coastal and industrial areas due to the fungicidal properties of these environments. Threlfall et al. (2008) tested the second part of this theory in a field study that involved measuring and comparing the concentrations of trace metals at a number of *L. aurea* breeding sites across NSW, including one waterbody at Sydney Olympic Park. They found that both zinc and copper

were elevated consistently across these sites, and concluded that pollution from industrial land uses may protect *L. aurea* from infection by *B. dendrobatidis*. The first part of this theory, that sodium chloride may also provide antifungal properties, has not yet been tested in the field. The second aim of this thesis is to test whether the addition of sodium chloride to ponds can be used to reduce *L. aurea* mortality caused by infection by *B. dendrobatidis* in an outdoor field environment.

3.3 Creating a disturbance regime to improve habitat quality

In addition to reducing the impact of causes of mortality on the *L. aurea* population, other management goals at Sydney Olympic Park are to increase its population size and distribution across the site. A number of management actions that are intended to improve habitat quality for each of the three sub-populations have been implemented in an effort to meet this goal. As high-quality habitat is able to support a greater number of individuals (Kawecki, 2008), it is therefore possible to increase the carrying capacity of waterbodies by increasing their habitat value to *L. aurea*, and in turn increase population size. By increasing the habitat quality of ponds which were formerly uninhabitable, so that they begin to meet the requirements of *L. aurea* to an extent to allow individuals to survive there, the distribution of the species can also be expanded through increased pond occupancy. Because increasing population size and distribution of a species are both ways of increasing its ability to survive stochastic events (Shaffer, 1981), the process of improving habitat quality at Sydney Olympic Park has the potential to increase the likelihood of this *L. aurea* population persisting into the future.

Despite being the focus of a number of studies, the habitat requirements of *L. aurea* remain relatively unclear. In their study based at Kooragang Island, Hamer et al. (2002) found that a waterbody was more likely to be occupied by *L. aurea* if it was within 50m of neighbouring waterbodies that were also occupied, and if it contained a high diversity of plant species, including three particular species of freshwater emergents. Pyke and White (1996) found that breeding occurred in a significantly higher percentage of sites across NSW with ephemeral ponds, in comparison to sites with ponds that did not become completely dry for part of the year. Habitat studies were conducted for all three *L. aurea* sub-populations at Sydney Olympic Park, and a different set of habitat features were found to predict the abundance of *L. aurea* for each sub-population (J. Garnham, A. Midson and C. Pollard, unpublished data). The mixed findings of the habitat studies conducted to date both within Sydney Olympic Park and across NSW have not resulted in clear understanding of the habitat requirements of *L. aurea*, and habitat management is therefore challenging.

One of the management actions that has been implemented at Sydney Olympic Park is the thinning of emergent and aquatic plants in ponds when the vegetation becomes particularly dense. The basis for this action is the theory that *L. aurea* is an *r*-selected species (Hamer and Mahony, 2007), meaning it has traits which make it well suited to colonising recently disturbed environments that are an early stage of succession (Brookfield, 1986). Two other actions relating the environmental succession that have also been implemented are the continual creation or rebuilding of ponds, and the removal of trees and tall shrubs adjacent to ponds which cause shading. As the age of a pond increases, the structure and composition of the vegetation that occurs there change, which may make the ponds less suitable for *L.*

aurea. Similarly, vegetation communities that are at an early successional stage do not generally include tall shrubs and trees, and the shading caused by these may make ponds less inhabitable for this suggested colonising species, which has also been observed to bask in the sun (Pyke and White, 2001).

Evaluating the effectiveness of these actions that aim to increase *L. aurea* abundance and pond occupancy by increasing habitat quality is the third goal of this thesis.

3.4 Investigating threats while trialling population supplementation

The reintroduction of *L. aurea* to areas that the species formerly occupied has been attempted at a number of sites throughout NSW, however none have been successful at establishing self-sustaining populations so far. Reasons for this lack of success have been attributed to disease (Stockwell et al. 2008, Klop-Toker et al. 2016, Valdez et al. 2017), predation of tadpoles by exotic fish (Pyke et al. 2008), an inability to breed sufficient numbers for release (Pyke et al. 2008) and potential lack of nutritional resources required for breeding (Klop-Toker et al. 2016). One of the challenges involved in reintroduction projects is that an extremely large number of propagules are generally need to be produced to result in a viable population of adults (Semlitsch, 2002). Finding or creating a release site that provides suitable habitat quality has also been identified as a major cause of reintroduction failure (Germano and Bishop, 2009). Population supplementation, which involves the release of individuals to an area where a wild population of the same species already exists, is a less commonly used alternative to reintroduction, and to date has not been attempted for *L. aurea*. Supplementation requires fewer individuals than reintroduction to produce a population of a

given size, due to the presence of wild individuals. It may also have a higher chance of success, given that it involves the release of individuals to sites that are known to provide high enough habitat quality to support the species.

One of the sub-populations at Sydney Olympic Park was observed to experience declines in abundance and pond occupancy since the habitat was created in 2000, and the cause of these declines are currently unknown. One possibility is that the habitat at a large proportion of ponds has become unsuitable in some way for *L. aurea*. This hypothesis can be tested by releasing captive-bred tadpoles to ponds that are occupied by wild *L. aurea*, as well as ponds that are no longer occupied. If unoccupied ponds do not provide suitable habitat for tadpoles or frogs, then it would be expected that tadpole survival would be higher at ponds occupied by wild tadpoles than unoccupied ponds, or that the abundance of metamorphosed individuals would be higher at breeding ponds than non-breeding ponds, due to higher survival and/or lower dispersal rates.

The final aims of this thesis are to test the effectiveness of population supplementation as a method of reversing declines in abundance and occupancy for a wild *L. aurea* population, while also investigating the cause of the declines.

4 Summary of aims

- To evaluate the effectiveness of pond draining in removing predatory *Gambusia holbrooki* and increasing the reproductive success of *L. aurea* (chapter 1)

- To trial the addition of sodium chloride to ponds as a method of reducing *L. aurea* mortality caused by the amphibian chytrid fungus (chapter 2)
- To evaluate the effectiveness of a disturbance regime as a way of improving habitat quality for *L. aurea* (chapter 3)
- To investigate whether poor habitat quality is the cause of declines in abundance and occupancy in a *L. aurea* population (chapters 4 and 5)
- To trial the use of population supplementation as a method of reversing declines in vulnerable *L. aurea* populations (chapter 5)

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Chapter 1: Removal of an exotic fish influences amphibian breeding site selection.

Declaration of contribution

I, Carla Pollard, am the primary author of this paper entitled “Removal of an Exotic Fish Influences Amphibian Breeding Site Selection”. I developed the concept of the study with assistance from other co-authors, collated existing data collected pre-2008 and undertook fieldwork from 2008 to 2012 to collect data as part of a team. I carried out all analyses and wrote the manuscript, which was then improved through review by my co-authors.

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Research Article

Removal of an Exotic Fish Influences Amphibian Breeding Site Selection

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ABSTRACT For pond-breeding species, the distribution of larvae is a reflection of habitat suitability and adult breeding site selection. Some species preferentially breed in ephemeral ponds, which can provide benefits for larvae. An alternative strategy used by adults to increase offspring survival is to detect aquatic predators and avoid them when selecting breeding sites. We investigated whether either of these types of breeding site selection are contributing to the negative correlation between the distributions of tadpoles of the threatened green and golden bell frog (*Litoria aurea*) and the introduced eastern gambusia (*Gambusia holbrooki*) in Sydney, Australia. From 2003 to 2012 we drained ponds to temporarily remove gambusia and examined the effect of removal on the numbers of male, female, and juvenile frogs, and tadpoles. We found that males preferentially selected fish-free ponds as breeding sites. In addition, the removal of gambusia increased tadpole abundance to over 140 times that of an undrained pond. Pond draining did not influence female or juvenile abundances. The ability to detect and avoid gambusia may be mitigating the effect of predation to a certain extent. We conclude that pond draining to remove exotic fish is an effective strategy that can be used to greatly increase the reproductive success of this and potentially other threatened amphibian species in the presence of exotic predators. © 2017 The Wildlife Society.

KEY WORDS amphibian conservation, exotic fish, *Gambusia holbrooki*, green and golden bell frog, *Litoria aurea*, mosquitofish, pond draining, tadpole predation.

The distribution of a population across a landscape results from a complex set of interactions with competitors, predators, and abiotic conditions (Wellborn et al. 1996). For pond-breeding species with biphasic lifecycles, the location of larvae is a reflection of habitat suitability and breeding site choice by adults (Chesson 1984, Resetarits 1996). Natural selection should favor individuals that maximize offspring survival by identifying and choosing to breed in ponds that provide ample resources for their larvae

(Resetarits 1996, Blaustein 1999). Recently dried and refilled ponds generally contain available nutrients for amphibian and invertebrate larvae and can offer reduced competition and predation in comparison to more permanent breeding sites (Wilbur 1997). For these reasons, many species have adapted to preferentially breed in ephemeral ponds (Wilbur and Collins 1973, Werner 1986, Newman 1992, Griffiths 1997, Blaustein and Schwartz 2001).

Another strategy for increasing offspring success is the ability of adults to detect aquatic predators and avoid breeding in ponds where they are present. This strategy does not offer the increased resources and reduced competition associated with selecting for ephemerality. However, it may be a more reliable way of reducing the risk of predation to

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offspring without increasing risk of offspring mortality due to pond desiccation (Wilbur 1997). Adult females of a range of invertebrate species preferentially select predator-free waterbodies as sites to deposit their eggs (Chesson 1984, Berendonck 1999, Angelon and Petranka 2002, Blaustein et al. 2004). Experiments offering a choice between artificial waterbodies with and without predatory fish have demonstrated fish avoidance in female oviposition site selection in several North American anuran species (Resetarits and Wilbur 1989; Hopey and Petranka 1994; Binckley and Resetarits 2002, 2003), and in male calling site selection in at least one species (Resetarits and Wilbur 1991). The ability of terrestrial adults to identify waterbodies containing aquatic predators appears to rely on the detection of chemicals released into the water by predators (Blaustein et al. 2004, Takahashi 2007, Schulte et al. 2011).

Although predators can have a strong indirect effect on larval distributions by influencing where adults choose to breed, studies examining the relationship between the distributions of amphibian larvae and their predators generally attribute the lack of overlap between the 2 to direct predation (Tyler et al. 1998, Knapp and Matthews 2000, Hamer and Parris 2013). This is particularly the case for studies that are focused on exotic predators because amphibians that lack an evolutionary history with a particular predator have not had the opportunity to develop specific defense mechanisms to evade it (Kats and Ferrer 2003). Predation of larvae by exotic predators, particularly fish, is listed as a major cause of amphibian population declines and extinction events on a global scale (Collins and Storer 2003, Kats and Ferrer 2003). Although direct predation is therefore a logical hypothesis to explain the negative association between amphibian larvae and exotic predators, there are 2 other hypotheses that could potentially contribute to this pattern. One is that adults are selecting ephemeral waterbodies as breeding sites, which are less likely to contain predators. The other is that adults are able to detect predators and avoid breeding in ponds where they occur. If adults do have a preference for ephemeral or predator-free waterbodies, this will have strong implications for the management of amphibian species that are threatened by exotic fish.

For example, one of the major recommendations for the recovery of threatened pond-breeding amphibians is the provision of ephemeral waterbodies that periodically dry (Semlitsch 2002). In cases where existing ponds do not dry naturally, then artificial pond draining can be used as a method of removing exotic fish (Maezono and Miyashita 2004). This type of management strategy would theoretically be beneficial for amphibian species that comply with any of the 3 hypotheses described above, although having a clear understanding of the mechanism responsible for this would allow managers to tailor the regime and increase its effectiveness by optimizing resource allocation. For species that select ephemeral ponds for breeding, pond draining may encourage breeding even if ponds do not contain exotic fish. If adults avoid breeding in ponds with fish, draining a subset of ponds could be a cost effective way to increase reproductive

success and recruitment because frogs would be less likely to breed in undrained ponds where fish remain. The most appropriate management strategy for species where neither type of breeding site selection occurs may be to direct resources to drain as many ponds as possible, to prevent high losses of eggs and tadpoles to fish predation. Other methods of fish removal such as the use of piscicides (Walston and Mullin 2007) and gill nets (Knapp et al. 2007) may provide effective alternative methods of fish removal in some situations but would not encourage breeding in species that select ponds based on ephemerality rather than the absence of fish, as pond draining would.

In addition to exploring the mechanisms underlying the negative relationship between tadpoles and predatory fish, there is a need for experimental field studies that trial pond draining and examine its effectiveness as a method of increasing amphibian reproductive success. We had the opportunity to address both of these areas of uncertainty by examining the effect of draining ponds to temporarily remove the eastern gambusia (*Gambusia holbrooki*) on a population of the green and golden bell frog (*Litoria aurea*). The eggs and larvae of bell frogs are highly susceptible to predation by this exotic fish (Morgan and Buttemer 1996, Pyke and White 2000), which is listed as a key threatening process for this species (Department of Environment and Conservation 2005). Previous research has concluded that bell frog tadpoles are rarely found in ponds containing gambusia largely because of direct predation (Mahony 1993, Pyke and White 2000, Pyke et al. 2008, White and Pyke 2008). However, the lack of co-occurrence could result because adult bell frogs may preferentially breed in ephemeral waterbodies (Pyke and White 1996), which are less likely to contain gambusia. A third hypothesis is that adult bell frogs may be able to detect and avoid breeding in ponds with gambusia.

Our objective was to establish whether direct predation alone is responsible for the frequently observed negative relationship between the distributions of green and golden bell frog tadpoles and gambusia, or if a preference of adult frogs to breed in ephemeral or predator-free ponds may also be playing a role. Secondly, we investigated whether draining ponds to remove gambusia is effective in increasing the reproductive success of green and golden bell frogs.

STUDY AREA

Sydney Olympic Park covers 600 ha of land in New South Wales (NSW), Australia (33°51'S, 151°4'E) that has historically been used for a range of industrial purposes that greatly altered the landscape and underwent an intensive urban renewal program throughout the 1990s (Darcovich and O'Meara 2008, Pickett et al. 2013). Since the green and golden bell frog was confirmed at this site, management objectives have focused on increasing the amount of bell frog habitat, including the construction of 21 large clay-based ponds across a 20-ha area collectively called Narawang Wetland (Fig. 1). Ponds ranged in size from 338 m² to 1,332 m² and were separated by an average distance of 18 m

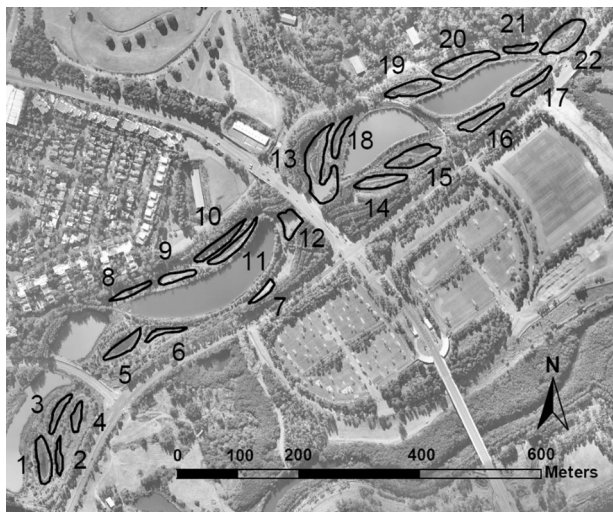


Figure 1. Aerial view of the green and golden bell frog habitat ponds at Narawang Wetland, Sydney Olympic Park, Sydney, Australia. Ponds 12 and 13 are connected under the road and so were treated as a single pond.

(range = 4–76 m). All were fringed with macrophytes, largely composed of 2 dominant species, jointed twig rush (*Baumea articulata*) and river club sedge (*Schoenoplectus validus*). Shortly after its completion in 2000, the wetland was rapidly colonized by bell frogs and gambusia (Darcovich and O'Meara 2008). Gambusia was the most abundant fish species at the site, and was observed at very high densities (up to 698 individuals caught in a single minnow trap). Other species commonly found at Narawang Wetland include 4 other frogs (common eastern froglet [*Crinia signifera*], eastern dwarf tree frog [*Litoria fallax*], Peron's tree frog [*Litoria peronii*], and striped marsh frog [*Limnodynastes peronii*]), reptiles including the eastern long-necked turtle (*Chelodina longicollis*) and a number of lizards, and a wide variety of resident and migratory birds. Long-term daily mean temperatures are lowest in July (Austral winter, max. = 17.4°C, min. = 6.3°C) and highest in January (Austral summer, max. = 28.4°C, min. = 17.6°C). Long-term mean monthly rainfall ranges from 46.2 mm in July to 121.2 mm in February (Bureau of Meteorology 2017). We conducted our study at Narawang Wetland from 2003 to 2012.

METHODS

The bell frog population located at Sydney Olympic Park is one of the largest remaining in NSW and is listed as a key population for the persistence of this species (Department of Environment and Conservation 2005). This once common species began to experience large-scale declines in the 1970s (White and Pyke 2008) and is now listed as endangered in NSW under the Threatened Species Conservation Act 1995 and as vulnerable nationally under the Environmental Protection and Biodiversity Conservation Act 2000. Factors suggested to have played a role in these declines include infection by the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), habitat alteration, and predation by the eastern gambusia, which was introduced to Australia in the 1920s (Mahony et al. 2013).

Pond Draining

The ponds at Narawang Wetland are connected to a water reticulation system and can be drained by pumping water from a sump located at the bottom of each pond. From 2003 to 2011, we drained an average of 8 of the 21 ponds (range = 6–13) annually between August and October. We timed draining so that it occurred just prior to the bell frog peak breeding season, which runs from November to February, to maximize the likelihood of ponds remaining free from gambusia throughout the coming breeding season. Because of constraints of the water reticulation system, we grouped some ponds into small clusters of 2 or 3 ponds that could not be drained independently of each other. This meant that the selection of ponds to be drained could not be randomized; however, we drained a different combination of ponds each year so that we drained each pond an average of 3 times (range = 1–5) over the 9-year study period. The temporal switching of individual ponds between treatment groups was an important aspect of our study design because it allowed for the control of other variables, such as pond size and vegetation composition.

We drained ponds yearly because flooding events allowed gambusia from a nearby creek to recolonize ponds by the following winter at the latest. Once drained, we attempted to keep ponds dry for 4 to 6 weeks so that the mud at the bottom dried out completely and became cracked, to minimize the probability of gambusia remaining. After the drying period, we refilled drained ponds with fish-free water. We considered draining to be successful if there was dry, cracked mud across the entire pond, with no pools of water remaining. We classified ponds into 1 of 4 treatments for analysis: not drained, drained with no fish (if the pond was successfully drained and gambusia had not recolonized the pond by the time of bell frog surveys), drained-then-recolonized (if the pond was successfully drained and gambusia recolonized the pond prior to bell frog surveys), or unsuccessfully drained (if the pond had not completely dried, potentially not removing the gambusia).

Field Surveys

Capture encounter surveys for bell frogs occurred between 2000 and 0400 hours ≥ 3 times/year between November and April, from 2003–2004 to 2011–2012. For the first 5 years of the study, we conducted frog surveys only at a subset of the 21 ponds (11 ponds in 2003–2004, 8 in 2004–2005, 11 in 2005–2006, 11 in 2006–2007, and 15 in 2007–2008). For the last 4 years, we surveyed all 21 ponds. Surveys involved searching the emergent vegetation around the perimeter of each pond and attempting to capture all bell frogs seen. Searchers placed a plastic bag over their hands prior to capture and then inverted the bag around the frog so that it was contained in the bag. After each pond search, we measured the snout-vent length (SVL) of captured frogs and determined their sex. We classified frogs with SVL < 45 mm as juveniles and frogs with SVL ≥ 45 mm as males if they had nuptial pads and as females if they did not. We also recorded the number of frogs seen and total search effort (pond search time \times no. searchers).

We conducted an auditory survey immediately before the commencement of each capture encounter survey. When we arrived at each pond, we listened and counted the number of male bell frogs, and then we imitated the bell frog call and listened again. We recorded the maximum number of frogs calling at any one time before or after call imitation for each pond.

We conducted bell frog tadpole and gambusia surveys concurrently with each frog survey. From the 2003–2004 season to 2005–2006, surveys involved repeatedly sweeping a dip net through the pond and recording the number of tadpoles and gambusia caught. We retained captured individuals in a tub of water until the end of each survey to prevent them from being captured more than once. We also recorded the number of dip-net sweeps at each pond, which was relative to pond size (\bar{x} = 13 sweeps/pond, range = 2–50). From 2006–2007 to 2011–2012, we supplemented dip-net surveys with minnow-trap surveys, in which we tied traps baited with yellow glow sticks to the emergent vegetation in each pond before nightfall. We emptied the traps the following morning and recorded the number of tadpoles and gambusia per trap. We recorded the number of traps set per pond, which was again relative to pond size (\bar{x} = 8 traps/pond, range = 2–30). We classified a pond as containing gambusia at the time of each frog survey if we detected gambusia during the concurrent tadpole and fish survey session. Animal surveys were approved by the University of Newcastle Care and Ethics Committee (ACEC no. A-2008-165) and conducted under a Scientific License from NSW National Parks (SL100092).

Statistical Analysis

We examined 6 response variables: the abundance of juvenile, male, and female bell frogs, the abundance of calling male bell frogs, the abundance of tadpoles, and the abundance of gambusia. Our primary explanatory variable was pond-draining status, which consisted of the 4 treatments described previously. For gambusia and tadpole abundance, dip-netting data were too sparse for analysis because of the low number of individuals caught, so analysis was based solely on trapping data from 2006 to 2012. However, we still used dip-netting data to determine if gambusia were present when bell frog surveys occurred. Because unsuccessful pond draining was relatively rare, a scarcity of data for this treatment group meant that we could not analyze the abundance of gambusia and tadpoles at unsuccessfully drained ponds. We also could not include

drained fish-free ponds in analyses of gambusia abundance because these ponds by definition all had abundances of 0. We used the R software system (version 2.15.1) to fit Poisson regression, negative binomial, zero-inflated Poisson and zero-inflated negative binomial models from the *pscl* package (R Core Development Team 2012) and chose the most appropriate model for each response variable by determining if the response variables were affected by overdispersion or zero inflation.

We identified overdispersion first using the residual deviance divided by the degrees of freedom in the Poisson regression models and we confirmed it with a significant overdispersion term in the negative binomial model. We identified zero inflation by the significance of the terms in the zero portion of zero-inflated models. Because we detected overdispersion for all response variables and zero inflation for some, we used either the negative binomial or zero-inflated negative binomial model for all variables (Zuur et al. 2009). We added the month and year of surveys as additional explanatory variables to control for seasonal effects and differences among years. We used likelihood ratio tests to determine if either of these variables were significant, and if not we excluded them from the final model. We also added the total search time of capture encounter surveys as an explanatory variable into all models for frog abundance (with the exception of calling male abundance, which did not require an offset) and the number of traps set per pond for fish and tadpole abundance (Table 1).

We expressed model effects as rate ratios, which are the ratio of the predicted abundance for the effect of interest (the 3 drained pond treatments) divided by the predicted abundance for the reference treatment (undrained ponds). We controlled the familywise type 1 error rate at α = 0.05 to account for multiple comparisons between the pond-draining treatments by adjusting the significance level using the Bonferroni correction. For the response variables that we were able to evaluate across all 4 draining treatments (i.e., M, calling M, F, juvenile abundance), we set α at 0.05/6 = 0.0083. For tadpole abundance, we were only able to compare 3 draining conditions, so there was no need to adjust the significance level from 0.05. This was because the model output showed that the pond-draining variable had a significant overall effect on this response variable, and therefore there was at most only one null hypothesis that could have been true (Howell 2012). There was also no need to adjust the significance level for gambusia abundance because we could only make 2 comparisons for this response variable.

Table 1. The model selected to investigate the effect of pond draining on green and golden bell frog abundance at multiple life stages, and eastern gambusia abundance, at Narawang Wetland, Sydney Olympic Park, Sydney, Australia, 2003–2012, and covariates and offsets that were included as additional explanatory variables.

Response variable	Model selected	Covariates	Offset
Juvenile frog abundance	Negative binomial	Year and month	Total search time
M frog abundance	Negative binomial	Year	Total search time
F frog abundance	Negative binomial	Year and month	Total search time
Calling male frog abundance	Zero-inflated negative binomial	Year and month	None
Tadpole abundance	Zero-inflated negative binomial	Year and month	Total traps set
Gambusia abundance	Zero-inflated negative binomial	Year and month	Total traps set

RESULTS

Ponds that were drained and then recolonized by fish had lower fish abundance than undrained ponds (rate ratio = 0.60, 95% CI = 0.44–0.81, $P \leq 0.001$). Male bell frog abundance was highest at drained fish-free ponds (5.69 times more than an undrained pond, 95% CI = 3.42–9.46, $P \leq 0.001$; Fig. 2a), followed by drained-then-recolonized ponds (2.18 times more than an undrained pond, 95% CI = 1.47–3.22, $P \leq 0.001$). Male abundance was lowest for undrained and unsuccessfully drained ponds, with no significant difference between these draining conditions ($P = 0.13$). There were more male bell frogs at drained ponds with no fish than there were at drained-then-recolonized ponds ($P = 0.005$), and at drained-then-recolonized ponds ($P \leq 0.001$) and drained fish-free ponds ($P \leq 0.001$) compared with unsuccessfully drained ponds.

The effect of draining and fish on calling male frogs followed a similar pattern (Fig. 2b), with calling male abundance highest at drained fish-free ponds (13.6 times more than an undrained pond, 95% CI = 4.66–39.6, $P \leq 0.001$), followed by drained-then-recolonized ponds (2.45 times more than an undrained pond, 95% CI = 1.38–4.35, $P = 0.002$). Abundance was lowest for undrained and unsuccessfully drained ponds, with no significant difference between these draining conditions ($P = 0.71$). There were more calling male bell frogs at drained ponds with no fish than there were at drained-then-recolonized ponds ($P \leq 0.001$), and at fish-free drained ponds than at unsuccessfully drained ponds ($P \leq 0.001$). The difference

between calling male abundance at drained-then-recolonized ponds compared with unsuccessfully drained ponds did not reach significance ($P = 0.03$).

Of all the life stages studied, draining had the strongest effect on the distribution of bell frog tadpoles, which again followed the same pattern as general male and calling male bell frog abundances (Fig. 2c). Tadpole abundance was 142.5 times higher in ponds that were drained and remained fish-free than in undrained ponds (95% CI = 12.3–1,651, $P \leq 0.001$). Ponds that were drained-then-recolonized had 7.64 times as many tadpoles as undrained ponds (95% CI = 1.19–49.2, $P = 0.03$). Tadpole abundance was higher in drained ponds that remained fish free compared to drained-then-recolonized ponds ($P = 0.02$). Draining did not significantly affect the abundance of juvenile or female bell frogs at the site (Table 2).

DISCUSSION

Our results suggest that male bell frogs are able to detect the presence of gambusia and avoid ponds containing them when selecting breeding sites. The different responses of calling males to each of the 3 conditions where ponds were drained are evidence for this: fish-free ponds were most favored, followed by ponds with reduced fish abundance, and unsuccessfully drained ponds were least favored. Although it is likely that unsuccessfully drained ponds would have had similar fish abundances to undrained ponds because they did not become completely dry to remove gambusia, this could not be confirmed because of a scarcity of data.

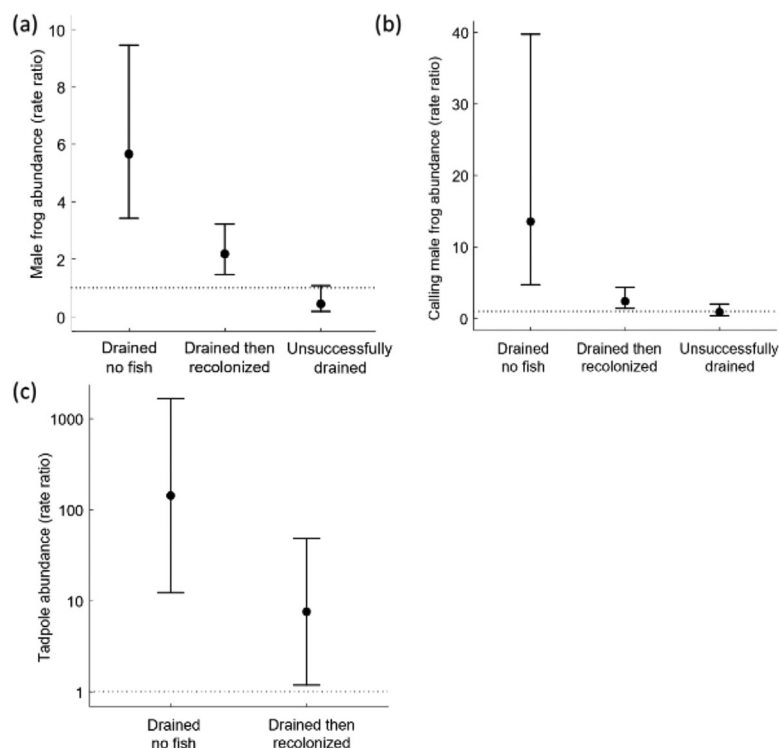


Figure 2. The rate ratios of the abundance of male green and golden bell frogs (a), calling males (b), and tadpoles (c) for each of the 3 treatments where ponds were drained (unsuccessfully drained ponds could not be analyzed for tadpoles) at Narawang Wetland, Sydney Olympic Park, Sydney, Australia, 2003–2012. Undrained ponds are the reference treatment, given a value of 1 (represented by the horizontal line on the y-axis). Error bars show 95% confidence intervals.

Table 2. *P* values for the difference in juvenile and female green and golden bell frog abundance between the 4 draining treatments at Narawang Wetland, Sydney Olympic Park, Sydney, Australia, 2003–2012. The significance level is 0.0083 for these response variables.

Draining condition	Drained fish free	Drained then recolonized	Unsuccessfully drained
Juvenile abundance			
Not drained	0.80	0.95	0.44
Drained fish free		0.83	0.48
Drained then recolonized			0.47
Female abundance			
Not drained	0.26	0.18	0.75
Drained fish free		0.92	0.44
Drained then recolonized			0.45

Male amphibian avoidance of larva predators in the selection of breeding sites has received less attention than that of female amphibians. As far as we are aware, only 2 North American (Resetarits and Wilbur 1991, Petranka et al. 1994, Shulse et al. 2013) and 1 South American (Schulte et al. 2011) anuran species have been demonstrated to have this ability through the experimental exclusion of predators. From an evolutionary perspective, the ability of adults to detect risks to their offspring and to avoid breeding in ponds where risk is high should be strongly selected for because it would increase offspring survival and in turn fitness (Resetarits 1996, Kiesecker and Blaustein 1999). This theory implies the presence of a shared evolutionary past between the predator and prey species, which is true for all 3 frog species referenced above. Our study is novel in that the predator studied was not introduced into Australia until the 1920s, and yet male bell frogs still appear to be able to detect and avoid this exotic fish. A potential explanation is that bell frogs evolved the ability to detect native predatory fish species, using a cue that is also applicable to gambusia (e.g., chemical released as the result of metabolic processes). A negative correlation between calling male spadefoot toads (*Pelobates fuscus*) and the presence of an exotic crayfish (*Pacifastacus leniusculus*) has also been reported in the field (Nystrom et al. 2002), suggesting that other frog species may be able to detect exotic predators. This response was detectable in a complex natural system in that study and the current study, where many other factors must also influence breeding site choice, which suggests that predator avoidance can be a very strong influence of breeding site selection for anurans.

We were unable to confirm whether female bell frogs also avoid gambusia when selecting breeding sites because we never observed ovipositing at the study site. The abundance of previously deposited egg masses or tadpoles cannot be used as an accurate measure of where females have chosen to deposit eggs because their absence may reflect high levels of predation (Ritke and Mumme 1993). To address this, previous studies have used smaller artificial pools, where the predators of interest were restrained by cages and all other predators were excluded (Binckley and Resetarits 2002, 2003). Although the distribution of female bell frogs in this study did not demonstrate fish avoidance, this does not necessarily reflect an inability to detect fish. Females lay

large egg masses with up to 11,000 eggs in a single spawning event (van de Mortel and Goldingay 1998), and therefore would only need to spend a relatively small amount of time occupying potential breeding sites during the breeding season; the rest of the time, they may select ponds that are favorable for general habitat use. By comparison, individual males may call for many nights throughout the breeding season to maximize their chance of finding a mate, which could explain our finding that the distribution of males was heavily influenced by gambusia. Male bell frogs generally call while floating partly submerged within ponds (Pyke and White 2001). Although the movement of amplexant bell frogs over land has been observed (Pyke and White 2001), a previous study that took place within 2 km of our study site observed 25 amplexant pairs of bell frogs for an average of 22.8 hours (± 15.7 SD) and none moved between ponds (Christy 2000). This suggests that there is a strong correlation between male calling and oviposition sites.

One limitation of our study is that the effect of gambusia on bell frog abundance could not be examined in the absence of other larva predators. The ponds also contain native fish species including eels (*Anguilla reinhardtii*, *Anguilla australis*) and several species of gudgeon (*Hypseleotris* spp.), and invertebrates including backswimmers (Notonectidae) and dragonfly and damselfly larvae (Odonata), all of which have been observed to prey on frog eggs or tadpoles (Pyke and White 2001, Werner et al. 2007). However, a laboratory study has previously reported that gudgeon have far lower predation rates than gambusia (Pyke and White 2000), and in addition gudgeon did not reach densities remotely close to that of gambusia at our study site; the highest number of gudgeon trapped in a single pond was 76, in comparison to 867 gambusia. Eels are able to move across land between waterbodies (Tesch 1977) and the invertebrate predators present have mobile life stages, which should allow them to rapidly colonize new ponds (Gooderham and Tsyrlin 2002). Although these factors suggest that an avoidance of gambusia by male bell frogs is the most likely explanation for our findings, they may have been responding to other predators at the study site. Studying male breeding site choice in an artificial, simplified environment where gambusia are the only predators present would help to address this, although it should be noted that small-scale studies do not always provide an accurate representation of natural behavior (Marsh and Borrell 2001).

Pond draining is carried out at Narawang Wetland each year with the goal of increasing the reproductive success of bell frogs by temporarily removing gambusia. We found that draining achieves this goal by increasing tadpole abundance in fish-free ponds to over 140 times that of undrained ponds. However, a small amount of this increase may be attributed to tadpole detectability, which is increased by 4.3 times if gambusia are absent compared to present, using the method of minnow trapping (Sanders et al. 2015). Even in cases where gambusia recolonized drained ponds prior to the beginning of the breeding season, on average the abundance of tadpoles in these ponds was still increased 7-fold. Only in

cases where ponds could not be completely dried because of adverse weather was there no conservation benefit for the funds spent on this management action. Although pond draining did not influence the distribution of juvenile frogs, this is likely to be due to the rapid dispersal of bell frogs away from their home ponds soon after metamorphosis, which has been observed at our study site (Bower et al. 2012) and for other bell frog populations (van de Mortel and Goldingay 1998, Goldingay and Lewis 1999).

Our results can help explain other field studies that have shown that bell frog tadpoles are generally found in fish-free waterbodies (Pyke and White 1996; Pyke et al. 2002, 2008). The dramatic increase in tadpole abundance as the result of gambusia removal demonstrates that the presence of this exotic predator greatly reduces the habitat quality of ponds as bell frog breeding sites. However, our findings also suggest that male selection of fish-free breeding sites may mitigate the severity of egg and tadpole predation by gambusia to a certain degree. The extent to which predation by gambusia can be implicated in bell frog declines currently remains unresolved (Mahony 1999; Hamer et al. 2002a,b; Goldingay 2008; Mahony et al. 2013); perhaps it depends largely on whether bell frogs have access to at least a small number of fish-free ponds where successful breeding and recruitment can occur, which is not the case at some locations (Pyke and White 1996, Lewis and Goldingay 1999). Regardless, gambusia removal is a relatively cost effective way of increasing reproductive success and recruitment, which could assist in making populations more resilient to other threats that are more logistically difficult to mitigate, such as the amphibian chytrid fungus and habitat fragmentation (Mahony et al. 2013). Investigation into whether other Australian frogs that are threatened by gambusia are also able to detect and avoid this exotic species is necessary to accurately assess the extent to which predation by this species has contributed to amphibian declines throughout the continent.

MANAGEMENT IMPLICATIONS

Pond draining to remove gambusia is an effective strategy that can be used to dramatically increase the breeding success of the green and golden bell frog and potentially many other frog species that are threatened by exotic predatory fish. Exotic fish removal can have a beneficial impact even in situations where fish recolonize waterbodies before amphibian breeding has commenced, probably because of a reduction in fish abundance. Even in cases where all available ponds cannot be drained because of limitations of resources or logistical difficulties, draining a subset of ponds could still greatly increase the reproductive success of bell frog populations because male bell frogs can avoid selecting ponds inhabited by gambusia as breeding sites. In cases where artificial pond draining is not viable, the provision of ephemeral waterbodies that dry naturally could be used to provide fish-free breeding sites. Because male bell frogs responded to the absence of fish rather than the process of draining itself, alternative methods of fish removal may also be effective. We suggest that further investigation into whether other amphibians can detect exotic predators would assist in the design of effective recovery plans for threatened species.

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Chapter 2: Effects of pond salinization on survival rate of amphibian hosts infected with the chytrid fungus.

Declaration of contribution

I, Carla Pollard, am a co-author of this paper entitled “Effects of pond salinization on survival rate of amphibian hosts infected with the chytrid fungus”. I carried out a significant proportion of the field and laboratory work required for this paper and contributed to the preparation and review of the manuscript.

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Effects of Pond Salinization on Survival Rate of Amphibian Hosts Infected with the Chytrid Fungus

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Abstract: *The chytrid fungus Batrachochytrium dendrobatidis has been implicated in the decline and extinction of amphibian populations worldwide, but management options are limited. Recent studies show that sodium chloride (NaCl) has fungicidal properties that reduce the mortality rates of infected hosts in captivity. We investigated whether similar results can be obtained by adding salt to water bodies in the field. We increased the salinity of 8 water bodies to 2 or 4 ppt and left an additional 4 water bodies with close to 0 ppt and monitored salinity for 18 months. Captively bred tadpoles of green and golden bell frog (Litoria aurea) were released into each water body and their development, levels of B. dendrobatidis infection, and survival were monitored at 1, 4, and 12 months. The effect of salt on the abundance of nontarget organisms was also investigated in before and after style analyses. Salinities remained constant over time with little intervention. Hosts in water bodies with 4 ppt salt had a significantly lower prevalence of chytrid infection and higher survival, following metamorphosis, than hosts in 0 ppt salt. Tadpoles in the 4 ppt group were smaller in length after 1 month in the release site than those in the 0 and 2 ppt groups, but after metamorphosis body size in all water bodies was similar. In water bodies with 4 ppt salt, the abundance of dwarf tree frogs (Litoria fallax), dragonfly larvae, and damselfly larvae was lower than in water bodies with 0 and 2 ppt salt, which could have knock-on effects for community structure. Based on our results, salt may be an effective field-based B. dendrobatidis mitigation tool for lentic amphibians that could contribute to the conservation of numerous susceptible species. However, as in all conservation efforts, these benefits need to be weighed against negative effects on both target and nontarget organisms.*

Keywords: amphibian decline, *Batrachochytrium dendrobatidis*, chytridiomycosis, disease management, fungicide, habitat manipulation, refuge, sodium chloride

Efectos de la Salinización de Estanques sobre la Tasa de Supervivencia de Hospederos Anfíbios Infectados con Hongos Quitridios

Resumen: *El hongo quitridio Batrachochytrium dendrobatidis ha sido implicado en la declinación y extinción de poblaciones de anfibios a nivel mundial, pero las opciones de manejo son limitadas. Estudios recientes muestran que el cloruro de sodio (NaCl) tiene propiedades fungicidas que reducen las tasas de mortalidad en cautiverio de hospederos infectados. Investigamos si se pueden obtener resultados similares al añadir sal a cuerpos de agua en el campo. Incrementamos la salinidad de ocho cuerpos de agua a 2 ó 4 ppt y dejamos a cuatro cuerpos de agua adicionales con casi 0 ppt y monitoreamos la salinidad durante 18 meses. Renacuajos de rana verde y dorada (Litoria aurea) criados en cautiverio fueron liberados en cada cuerpo de agua y su desarrollo, niveles de infección de B. dendrobatidis y supervivencia fueron monitoreados en el mes 1, 4 y 12. El efecto de la sal sobre la abundancia de organismos no-objetivo también se investigó en análisis de estilo antes y después. Las salinidades permanecieron constantes a lo largo del tiempo sin ser intervenidas. Los hospederos en los cuerpos de agua con sal a 4 ppt tuvieron una prevalencia significativamente más baja a la infección de quitridios y una mayor supervivencia, después de la metamorfosis, que los hospederos en sal a 0 ppt. Los renacuajos en el grupo de 4 ppt fueron más pequeños en longitud después de un mes en el sitio de liberación que aquellos en los de grupos de 0 y 2 ppt, pero después de la metamorfosis el tamaño corporal de todos los grupos fue similar. En los cuerpos de agua con sal a 4 ppt la abundancia de ranas arborícolas enanas*

(*Litoria fallax*), larvas de libélula y de caballitos del diablo fue más baja que en cuerpos de agua con sal a 0 y 2 ppt, lo que podría tener efectos colaterales en la estructura de la comunidad. Con base en nuestros resultados, la sal puede ser una herramienta de mitigación efectiva en el campo de *B. dendrobatidis* para anfibios lénticos que podría contribuir a la conservación de numerosas especies susceptibles. Sin embargo, como en todos los esfuerzos de conservación, estos beneficios necesitan ser sopesados contra los efectos negativos sobre organismos objetivo y no-objetivo.

Palabras Clave: *Batrachochytrium dendrobatidis*, cloruro de sodio, declinación de anfibios, fungicida, manejo de enfermedades, manipulación de hábitat, quitridiomycosis, refugio

Introduction

Infectious diseases of wildlife are increasingly recognized as major drivers of population decline (Smith et al. 2006), but management strategies that are effective, widely applicable, and have minimal impact on nontarget organisms are limited (Wobeser 2007). One strategy for the control of disease is the manipulation of the environment to reduce its suitability for the pathogen. For example, limiting nutrient influx into wetlands has been used to lower mortality rates in nestling wader birds caused by the nematode *Eustrongylides ignotus*, which limits nestling food resources by suppressing the population size of intermediate fish hosts (Spalding et al. 1993). The alteration of water chemistry has been used to reduce the prevalence of anthrax-causing bacteria *Bacillus anthracis* and various mollusc parasites in large mammals (Wobeser 2007). Given that environmental suitability for both host and pathogen varies spatiotemporally, a suitable disease management strategy would be to replicate natural refuge areas (i.e., areas where conditions support host and suppress pathogen population growth [Puschendorf et al. 2011]).

The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) infects the keratin-forming epithelium in the outer epidermal layers of post-metamorphic amphibians and can cause the fatal disease chytridiomycosis in susceptible species (Voyles et al. 2009). Chytridiomycosis has been implicated as the causal agent in the decline and extinction of hundreds of amphibian populations around the world (Skerratt et al. 2007), but few strategies to prevent amphibian declines in the presence of this pathogen are available. Temperature and humidity refuges exist for amphibian hosts (Puschendorf et al. 2011), but would be difficult to create in the field. Recently, it has been discovered that 3 ppt sodium chloride (NaCl) increases the survival rate of infected hosts in captivity (Stockwell et al. 2012), and there is increasing evidence that natural salt refuges exist (Stockwell 2011; Minting 2012; Heard et al. 2013).

The salinity of aquatic habitat could be manipulated for disease management by the direct addition of naturally derived salt from saline groundwater or tidal inundation. However, the ability to create and maintain desired salt concentrations in natural water bodies, the impact of salt

exposure on host growth and survival rates, and effects of salt on the abundance of other species are unknown. Through the use of a field-based experiment, we sought to address these knowledge gaps by adding NaCl to water bodies. For salt to provide a practical and effective mitigation tool, it needs to be shown that the salinity of ponds can be manipulated and maintained at or below desired levels, that hosts use ponds in a manner that has a positive effect on the outcome of infection, and that the addition of salt has minimal negative impacts on target and nontarget organisms.

Methods

The green and golden bell frog (*Litoria aurea*) was once widespread throughout the southeast coast of Australia but has declined in over 90% of its former range in the past 30 years, and chytridiomycosis is implicated as a causal agent (Mahony et al. 2013). Most persisting populations occur within several kilometers of the coastline, and a salt refuge has been suggested as a possible explanation (Stockwell 2011; Mahony et al. 2013).

We conducted our study at the Hunter Wetland Centre Australia, which is in the former range of *L. aurea* and was the site of a reintroduction of this species that failed because of a mass-mortality event caused by *B. dendrobatidis* (Stockwell et al. 2008). The fungus invaded the site naturally with other reservoir host species, and these host species remained at the site for the duration of our study and thus provided the source of infection. The site had 6 created ponds that were 12–16 m in diameter, 1.2–1.7 m deep, and planted with locally endemic vegetation. A 1-m-tall frog-proof fence enclosed the ponds and approximately 6000 m² of terrestrial habitat. We randomly assigned each pond into 1 of 3 treatment groups: 0, 2, or 4 ppt NaCl. These concentrations of salt are within the natural range observed in *L. aurea* habitat (M.P.S., unpublished data). No salt was added to the water bodies in the 0 ppt group. Naturally derived sea salt was added to ponds in the 2 and 4 ppt treatment groups during summer, when water depths were at their lowest, until the desired concentrations were reached. Six months after salt was added, internal fences were constructed to separate ponds into independent replicates.

Six 2000 L troughs were placed at the site as artificial ponds to provide additional replicates. Troughs contained a rocky substrate sloping into water and potted plants that provided shelter. One week prior to releasing tadpoles, water from one of the 0 ppt ponds inhabited by reservoir species was pumped into each trough. Sea salt was added to 4 of the troughs until 2 were 2 ppt and 2 were 4 ppt. Troughs were sealed with a mesh lid so each would be an independent replicate.

We allowed the salt levels in each water body to fluctuate naturally throughout the experiment, except where the measured concentration in the 2 and 4 ppt treatments exceeded these concentrations. In such cases, we added rain water until salts were diluted to the appropriate concentration. Temporal fluctuations in the salinity were measured at the surface and bottom of the water column, from the deepest section of each pond, every 2–4 weeks for 18 months. These salt concentrations were then compared using a univariate general linear model to identify the presence of salt stratification within the water column.

We raised 720 *L. aurea* tadpoles in captivity to Gosner stages 29–33 (Gosner 1960). To ensure tadpoles were not infected prior to release, we randomly selected 120 tadpoles and swabbed their mouthparts in a standardized circular motion 10 times with a fine-tipped sterile swab (Medical Wire and Equipment Company, Wiltshire, UK). We then estimated infection load as the number of genomic equivalents (GEs) detected on each swab following standard protocols for a real-time PCR Taqman assay (Boyle et al. 2004) with a Rotor Gene 6000 real-time DNA amplification system (Corbett Life Science, San Francisco, CA, USA). We included a Taqman exogenous positive control in each sample (Life Technologies, Mulgrave, Australia).

Prior to release, we randomly distributed tadpoles into 12 groups of 60 individuals and measured snout to vent (SVL) length and body weight. A body condition index (BCI) was calculated for each as the residuals produced by regressing weight on SVL (Jakob 1996). The SVL, weight, and BCI of tadpoles in each group were then compared statistically with single-factor analysis of variance (ANOVA). Tadpoles were transported to the study site and released 6 months after the construction of internal fences (12 months after the addition of salt to ponds). The release occurred either directly into the troughs or into baskets submerged in each pond. Triangular baskets (1 × 1 × 2 m), consisting of a steel frame with mesh sides and containing vegetation for shelter, were placed on the bank of each pond so that two-thirds of the basket was submerged. These baskets were designed to hold tadpoles in the pond water and allowed counts to be conducted without having to capture tadpoles in a complex pond environment.

Tadpoles were fed trout pellets weekly, and as metamorphs emerged they were fed crickets. Weekly we caught tadpoles with dip nets in baskets and troughs

and counted the number of tadpoles and frogs to determine survival and metamorphic rate. In frogs, signs commonly associated with chytridiomycosis, including abducted hind legs, red ventral surface and digits, excess shedding of skin, and ulcers, were also monitored. When these signs were observed, we tested the strength of the individual's righting reflex. For individuals with terminal chytridiomycosis, lethargy and a slow righting reflex is indicative that death will occur within 48 hours (Berger et al. 2005). Healthy *L. aurea* juveniles will right themselves in 1–4 s for up to 4 turns (M.P.S., unpublished). Therefore, when an animal was unable to right itself or the time taken to right itself was >8 s, we tested it again 2–4 h later. If no improvement was noted, the animal was swabbed and euthanized by immersion in a buffered (pH 7.0) 0.4% tricaine methanesulphonate (MS-222) solution. For the purposes of analysis, euthanized animals were considered to have died from chytridiomycosis. We compared infection loads of these animals with known disease thresholds for this species (Stockwell et al. 2010) to test this assumption. We determined survival time as the number of days from metamorphosis (emergence of front limbs) to the expression of signs associated with terminal chytridiomycosis and compared survival rate in each group with the log-rank test in Kaplan-Meier survival analysis.

To monitor growth, development, and infection levels over time, we captured, swabbed, weighed, and measured the SVL of each animal 1, 4, and 12 months after release and then calculated BCI for each individual. Gosner stages were also estimated for tadpoles. The prevalence of *B. dendrobatidis* infection in each treatment group, at each time point, was calculated as the number of individuals found to be positive divided by the total number tested and compared statistically with binary logistic regression. We generated 95% confidence intervals with Bayesian methods for proportions to indicate an interval estimate for the true population prevalence. We logarithmically transformed infection load data to normalize its distribution and then compared infection load, weight, SVL, BCI, and Gosner stage among groups for each period with single-factor ANOVAs and Tukey's post hoc tests.

We investigated the effect of elevating the NaCl levels of ponds on the abundance of other amphibian species and aquatic macro-invertebrates because they were the most frequently encountered non-target organisms in the site. We conducted nocturnal auditory surveys at each pond in which we listened for the calls of male frogs for 3 min and recorded the number heard. We then calculated relative abundance as the number of each species calling per minute. We used spotlights to conduct visual encounter surveys. We searched the water body and surrounding vegetation within 5 m of the water's edge for 20 min at each pond, ensuring individuals were not counted twice. We then calculated relative abundance as the number of frogs encountered per minute of search time.

Table 1. Results from binary logistic regression comparing prevalence of *B. dendrobatidis* infection in *L. aurea* at 3 periods among salt (NaCl) treatments of 0, 2, and 4 ppt.

Test	χ^2	<i>p</i>
1 month after release	3.17	0.07
4 months after release	1.92	0.38
12 months after release	7.76	0.02

We surveyed for tadpoles and macro-invertebrates by sweeping a dip net through the middle of the water column 20 times per pond. Sweeps were standardized at 1 m in length at haphazardly selected sites within each pond. We calculated relative abundances as the number captured per sweep. Relative abundance data on frogs, tadpoles, and macro-invertebrates were collected during 5 surveys in each January for 3 years (total of 15 surveys). Year 1 surveys were conducted before the addition of salt, year 2 surveys were conducted 6 months after the addition of salt, immediately before the construction of internal fences, and year 3 surveys were conducted 18 months after the construction of fences. We used generalized linear models to detect differences in relative abundance between years, among salt treatments, and to identify interactions between year and salt treatment, where significant interactions indicated an effect of adding salt or constraining animals to a salt treatment.

Results

Salinity levels did not vary between the top and bottom of the water column ($F = 0.002$, $df = 1$, $p = 0.97$) but did fluctuate over time (ranges: 0.1–1.0 ppt in the 0 ppt group, 0.1–2.6 ppt in 2 ppt, and 0.8–5.3 in the 4 ppt group). Mean concentrations remained significantly different among groups ($F = 75.97$, $df = 2$, $p < 0.001$). There was no significant interaction between water level and treatment group ($F = 0.13$, $df = 2$, $p = 0.89$). On 2 occasions, 2 and 10 months after the release of tadpoles, each of the 2 and 4 ppt troughs exceeded the intended NaCl levels, so water was added. The salinity of the ponds did not exceed desired concentrations.

Prior to release, all tadpoles were negative for *B. dendrobatidis* infection. After 1 month, up to 6% of tadpoles were infected (Fig. 1) and there was no significant difference among treatment groups (Table 1). Infection prevalence increased to 17–27% for tadpoles after 4 months, and, again, there was no significant difference among treatment groups (Table 1). After 12 months in the study site, no tadpoles were detected and all captured individuals were frogs. Infection prevalence differed significantly between salt treatments (Table 1). Tadpole infection loads were 60 GEs after 1 month and 2500 at 4 months. By 12 months, frog

Table 2. Results from single-factor analysis of variances comparing *B. dendrobatidis* infection load, body weight, snout-vent length, and Gosner stage of *L. aurea* at up to 4 periods among salt (NaCl) treatments of 0, 2, and 4 ppt.

Test	<i>F</i>	<i>p</i>
Infection load		
1 month after release	0.72	0.50
4 months after release	0.18	0.84
12 months after release	2.29	0.16
Body weight		
at release	1.41	0.25
1 month after release	1.51	0.22
4 months after release	2.78	0.06
12 months after release	0.30	0.74
Snout-vent length		
at release	0.89	0.41
1 month after release	5.64	0.01
4 months after release	4.20	0.02
12 months after release	0.09	0.92
Body condition index		
at release	0.29	0.75
1 month after release	1.47	0.23
4 months after release	2.74	0.07
12 months after release	0.41	0.67
Gosner stage		
1 month after release	0.38	0.68
4 months after release	0.353	0.70

infection loads were 21 GEs. Infection load did not differ significantly between salt treatment groups at any sampling period (Table 2).

Prior to release, body weight, SVL, and BCI of tadpoles did not differ significantly among groups (Table 2). Body weight, BCI, and Gosner stage did not differ significantly among groups at either 1 or 4 months after release (Table 2). The SVL of tadpoles was significantly larger in 0 and 2 ppt groups than in the 4 ppt group after 1 and 4 months (Table 2). We detected no differences in body size or BCI following metamorphosis after 12 months in the study site.

We found no dead tadpoles in water bodies during weekly surveys, but 65 tadpoles disappeared across all treatment groups. Because there was no means of escape from baskets or troughs, we assumed these animals died without being detected. A total of 245 frogs showed signs of chytridiomycosis following metamorphosis and were euthanized. All but 4 of these animals were infected with the *B. dendrobatidis* fungus, and all but 10 had infection loads greater than the disease causing threshold for *L. aurea* exposed as tadpoles (Stockwell et al. 2010); so, only these individuals were included in analyses. Forty-six percent of *L. aurea* inhabiting 0 ppt water bodies survived to the end of the experiment, significantly less than the 75% and 67% in the 2 and 4 ppt treatment groups respectively ($\chi^2 = 10.38$, $df = 2$, $p = 0.001$; Fig. 2). We found no significant difference in infection load at death between groups ($\chi^2 = 1.70$, $df = 2$, $p = 0.43$).

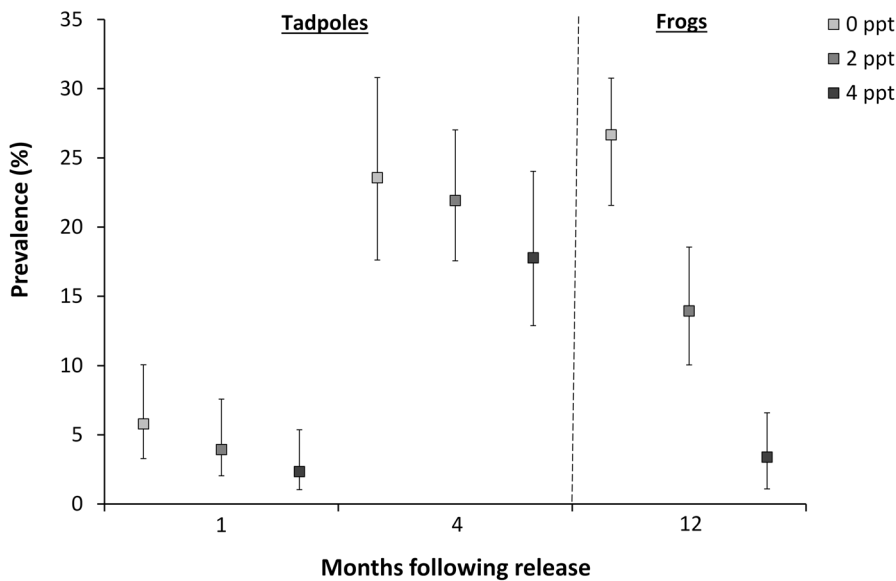


Figure 1. The prevalence of amphibian chytrid fungus *B. dendrobatidis* infection in *L. aurea* after 1, 4, and 12 months in water bodies for 3 salt (NaCl) treatment groups. By 12 months following release all tadpoles had metamorphosed into frogs.

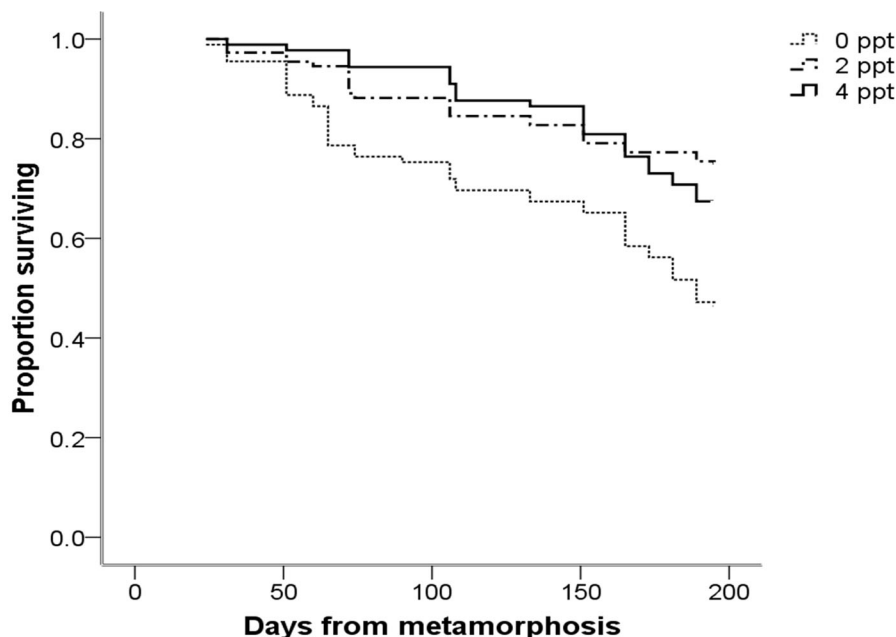


Figure 2. Proportion of frogs surviving over time from metamorphosis for the 3 salt (NaCl) treatment groups.

We identified 4 free-living frog species and 6 macro-invertebrate groups within the study site (Table 3). All 4 of the frog species were heard calling. One was encountered visually in a sufficient abundance for analysis, and 3 species produced tadpoles. The abundance of *Litoria fallax* heard calling, *L. fallax* tadpoles, and back-swimmers differed significantly between the salt treatments, but we found no significant interaction with year (Table 3), indicating abundances were consistent over time and were not due to the addition of salt in year 2. The abundance of *Limnodynastes peronii* tadpoles, *Litoria peronii* tadpoles, mayfly larvae, and diving beetles differed significantly between years, but because we found no significant interaction with salt, these differences occurred consistently across all salt treatment groups. Only

the abundances of *L. fallax* observed, dragonfly larvae, and damselfly larvae had a significant interaction between year and salt treatments (Table 3); abundances in 4 ppt ponds were significantly lower than those in 0 and 2 ppt ponds in years 2 and 3 in all cases.

Discussion

We found that maintaining NaCl concentrations of 2 and 4 ppt in water bodies for at least 18 months was achievable with little to no intervention and increased the survival rate of hosts infected with *B. dendrobatidis*. However, 4 ppt NaCl also resulted in smaller tadpole body size in *L. aurea* and a lower relative abundance of *L. fallax*,

Table 3. Output of generalized linear models of the effect of year, salt (NaCl) treatment, and their interaction on frog, tadpole, and macro-invertebrate relative abundance.

	Predictor variable	χ^2	<i>p</i>
<i>Eastern dwarf tree frogs (Litoria fallax)</i>			
Males heard	year	1.59	0.45
	salt treatment	6.75	0.03
	year*salt treatment	1.07	0.90
Frogs observed	year	7.87	0.02
	salt treatment	12.38	0.002
	year*salt treatment	10.18	0.04
Tadpoles observed	year	2.48	0.29
	salt treatment	6.35	0.04
<i>Peron's tree frog (Litoria peronii)</i>			
Males heard	year	4.38	0.11
	salt treatment	0.63	0.73
	year*salt treatment	2.50	0.65
Tadpoles observed	year	40.63	<0.001
	salt treatment	1.22	0.55
	year*salt treatment	3.14	0.54
<i>Striped marsh frog (Limnodynastes peronii)</i>			
Males heard	year	1.13	0.57
	salt treatment	2.04	0.46
	year*salt treatment	1.13	0.89
Tadpoles observed	year	12.0	0.002
	salt treatment	4.58	0.14
	year*salt treatment	5.06	0.19
Eastern froglet (<i>Crinia signifera</i>) heard	year	0.02	0.99
	salt treatment	4.26	0.17
	year*salt treatment	3.28	0.51
Backswimmers (family Notonectidae)	year	1.61	0.21
	salt treatment	7.35	0.03
	year*salt treatment	0.01	0.99
Dragonfly larvae (suborder Anisoptera)	year	0.18	0.68
	salt treatment	4.38	0.11
	year*salt treatment	13.15	0.001
Water spiders (family Pisauridae)	year	0.07	0.79
	salt treatment	0.95	0.62
	year*salt treatment	0.14	0.93
Mayfly larvae (order Ephemeroptera)	year	6.79	0.009
	salt treatment	2.01	0.37
	year*salt treatment	3.94	0.14
Damselfly larvae (suborder Zygoptera)	year	1.95	0.16
	salt treatment	6.95	0.03
	year*salt treatment	8.41	0.02
Diving beetles (family Dytiscidae)	year	22.24	<0.001
	salt treatment	1.50	0.47
	year*salt treatment	0.07	0.97

dragonfly larvae, and damselfly larvae. These results suggest that the creation of salt refuges may be an effective management tool for the *B. dendrobatidis* fungus but that the benefits for host survival need to be weighed against the potentially negative effects on tadpole fitness, nontarget species abundance, and community dynamics.

The salt added to water bodies dissolved evenly and did not stratify within the water column. Although stratification can occur naturally, most frequently through the inflow of saline groundwater or the overlay of rainwater (Turner & Erskine 2005), it can result in portions of a water body becoming uninhabitable (Hart et al. 1990). Salt was added to ponds when water depths were at their

lowest, and these small volumes may be responsible for the even dissolution of salt through the water column. Larger water bodies may require agitation, and additions may need to be made in stages and with frequent monitoring to avoid adding too much salt, which can be permanent and irreversible in wetland systems (Hart et al. 1990). Salt concentrations fluctuated between ponds and over time but did not increase beyond the predetermined maximums. Despite these fluctuations, the salinity levels of each treatment group remained significantly different throughout the study.

The addition of 2 and 4 ppt NaCl to water bodies significantly increased the survival rate of *L. aurea* relative

to its survival in water bodies where NaCl was not added. Survival rate of infected hosts can be determined by the rate at which individuals become infected and the rate at which infection load increases over time to reach a level that impairs biological function (Stockwell et al. 2010; Vredenburg et al. 2010). In infection experiments, higher survival rates in hosts exposed to NaCl have been associated with lower infection loads (Stockwell et al. 2012), but we did not observe this. This may be a reflection of higher variability in the timing of infection establishment in a more natural setting. Instead, the lower survival rates we observed were associated with a lower prevalence of infection in animals exposed to NaCl for 12 months.

The prevalence of infection depends on the transmission rate between hosts and the duration that infected individuals remain in the population (Begon et al. 2002). Therefore, the lower prevalence in the 2 and 4 ppt water bodies may have been due to lower growth rates and infective capacity of the *B. dendrobatidis* fungus at these salt concentrations resulting in a lower probability of acquiring an infection; a positive effect of salt exposure on host immunity, physiology, or behaviour that reduces susceptibility to infection; higher mortality rates in the 2 and 4 ppt groups resulting in a shorter duration of infected individuals remaining in the population; fewer individuals in the higher salt treatments resulting in lower zoospore loads and probability of acquiring an infection; or increased rates of clearing infection in the higher salt concentrations.

The susceptibility of amphibian hosts to *B. dendrobatidis* depends on the innate immune system, which for epidermal infections consists of antimicrobial peptides and mutualistic bacteria (Rollins-Smith et al. 2002; Harris et al. 2006). Factors such as frequency of sloughing (Davidson et al. 2003) and behavior (Rowley & Alford 2007; Hossack et al. 2013) can also alter infection dynamics. However, nothing is currently known about whether these factors are affected by exposure to salt. In order for interactions between host susceptibility and salt to explain the lower prevalence observed in 2 and 4 ppt, as suggested above, the effect of salt on host immunity, physiology, and behavior would need to completely prevent the establishment of the pathogen so that hosts remain uninfected. Alternatively, multiple factors related to host and pathogen could act synergistically in the presence of salt to reduce host susceptibility, but neither of these options seems likely.

The comparison among treatment groups showed that animals in the 2 and 4 ppt groups did not have higher mortality rates than animals in the 0 ppt group, which means the removal of infected animals from the population in salt groups was not the cause of lower prevalences. Animals in 4 ppt groups also did not occur in lower densities than those in the 0 ppt group, but they were significantly smaller, which could have a similar effect on prevalence as density. Smaller individuals would release

fewer zoospores and be smaller targets for zoospores to locate, which could in turn reduce the probability of acquiring an infection. However, the effect of body size on *B. dendrobatidis* prevalence varies; some studies show larger individuals are more likely to be infected (Kriger & Hero 2006; Voordouw et al. 2010) and other studies show the reverse (Beard & O'Neill 2005; Pearl et al. 2009). These differences have generally been attributed to varying habitat use patterns and immunocompetence. Therefore, the idea that fewer individuals in the higher salt treatments results in lower zoospore loads and probability of acquiring an infection also appears to be an unlikely explanation for the lower prevalence in 4 ppt groups.

Litoria aurea can clear themselves of infection in the wild (M.P.S., unpublished data), but this is usually associated with high summer temperatures that kill the fungus (Woodhams et al. 2003). Given that the salt concentrations we used are not fatal to *B. dendrobatidis* (Berger et al. 2009), it is unlikely that the lower prevalence in 2 and 4 ppt groups is the result of an increased probability of clearing infections with high salt concentrations. Therefore, the most likely cause of prevalence differences is reduced probabilities of acquiring infection. Growth rates of *B. dendrobatidis* are significantly lower in NaCl concentrations of 4 ppt than in 0 ppt, a result that could lead to relatively fewer zoospores in the environment (Stockwell et al. 2012). These salt concentrations also significantly reduce the motility of zoospores (Stockwell et al. 2012), which would further reduce transmission rates. Our results confirm that in a field setting, the NaCl concentration of water bodies can be manipulated to inhibit *B. dendrobatidis* infection levels in *L. aurea* and promote survival.

Despite these positive effects, exposure to salt concentrations above an individual's tolerance can alter growth, development, and fitness (Kearney et al. 2012), which in turn can affect population size and persistence (Halse et al. 2003; McKenzie et al. 2003). Although *L. aurea* tadpoles have been observed in up to 5 ppt NaCl in the field (Christy & Dickman 2002), tadpoles in the 4 ppt treatment group were significantly shorter in length than those inhabiting water bodies with lower salinities. Smaller body size in the presence of salt could be the result of investing energy in osmoregulation instead of growth or could be due to the effect of salt on food availability. This impact of salt on body size could in turn delay the time taken to reach sexual maturity, reduce competitive fitness, increase vulnerability to predation, or cause other long-term sublethal effects (Duellman & Trueb 1994). However, lower body length was not associated with lower body weight, BCI, or earlier Gosner stage, which suggests it had no effect on structural size, energy reserves, or development (Jakob 1996). In addition, the response of *L. aurea* tadpoles to salt varies dramatically across studies. One study found

significantly lower survival and growth rates at 1.87 ppt and above relative to lower salinities (Christy & Dickman 2002), and another found higher survival rates and longer individuals at 5.44 ppt relative to lower salinities (Kearney et al. 2012). Therefore, impacts may not be predictable within a species, but because we found no difference in body size following metamorphosis, these negative impacts may not be long lasting.

In addition to potential negative effects on target species, increasing salinity also poses risks to the survival and persistence of nontarget organisms (Hart et al. 1990). However, negative effects depend on the salt levels involved and the salt sensitivity of the environment. In our study, ponds elevated to 4 ppt had lower relative abundances of *L. fallax*, dragonfly larvae, and damselfly larvae. Given that frogs and macro-invertebrates play an important role in trophic food webs, acting as both predators and prey at different stages in their life cycles (Blaustein & Wake 1990; Wallace & Webster 1996), the impact of adding NaCl to ponds on these organisms may have extended effects on entire ecosystems. Altering salt levels in ponds may also negatively impact vegetation structure and diversity, as well as the abundances of numerous other organisms that co-occur with *L. aurea*. However, because the environment at our study site was highly managed via planting and weeding programs and because fences limited animal movements in and out of the site, these effects could not be determined.

The applicability of using salt as a mitigation tool will rely primarily on the salt tolerance of the target system. Amphibians as a group are relatively tolerant of osmotic imbalances. In the presence of salt most post-metamorphic amphibians accumulate electrolytes and urea in body tissue and plasma to maintain a higher osmotic pressure than their surroundings. This, in combination with decreased skin sodium transport and reduced urine flow, prevents desiccation (Shoemaker & Nagy 1977). However, some amphibian species are unable to adapt to changing osmotic gradients, and the inability of larval amphibians to produce urea makes them the most salt-sensitive life stage (Shoemaker & Nagy 1977; Shpun et al. 1992). Generally, those species that can tolerate salt and that have xeric terrestrial habitats will be the most tolerant of salt exposure (Hart et al. 1990; Shpun et al. 1992) and will benefit from the creation of salt refuges.

Ultimately, the adoption of habitat manipulation as a mitigation strategy for disease will require a trade-off between the positive effects for host populations and negative effects on the more salt-sensitive components of the environment. For *L. aurea*, the ongoing decline of this species (Mahony et al. 2013), its susceptibility to chytridiomycosis (Stockwell et al. 2010), the lack of success in establishing populations in the presence of the *B. dendrobatidis* fungus (Stockwell et al. 2008), and its post-decline occupancy of coastal areas with a saline influence (Mahony et al. 2013) means that the creation

of salt refuges to extend this species distribution back into its former range could be a viable option. Negative effects could also be reduced through the creation of mosaic habitats, where some water bodies are made into salt refuges and others are not.

As with any conservation effort that involves altering the environment, an understanding of likely outcomes and caution in implementation are required (Wobeser 2007). This can be achieved through the use of site- and species-specific trials, detailed monitoring of environmental response, and the capacity to respond adaptively over time. As a management tool, increasing the NaCl concentration of water bodies would be both time- and cost-effective because it can be applied widely and to areas where both host and pathogen aggregate.

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Chapter 3: Using adaptive management to evaluate the effectiveness of a disturbance regime in the conservation of a threatened amphibian.

Declaration of contribution

I, Carla Pollard, am the primary author of this paper entitled “Using adaptive management to evaluate the effectiveness of a disturbance regime in the conservation of a threatened amphibian”. I developed the concept of the study with assistance from other co-authors, collated existing data collected pre-2008 and undertook fieldwork from 2008 to 2014 to collect data as part of a team. I carried out all analyses and wrote the manuscript, which was then improved through review by my co-authors.

Signed:	Carla J. Pollard	Date: 11/08/2017
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Endorsement by Co-authors

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Signed: Jenny O'Meara Date: 11/08/17

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Signed: A. Prof. Frances Martin Date: 17/08/2017

Using adaptive management to evaluate the effectiveness of a disturbance regime in the conservation of a threatened amphibian

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ABSTRACT

Ecological disturbance drives the evolution of life history traits. Some species are adapted to high levels of disturbance and have traits that make them successful at colonising newly created landscapes, while at the other end of the continuum there are species with traits that are necessary to successfully compete for limited resources in environments where disturbance is infrequent. While anthropogenic disturbance alters landscapes in a way that reduces habitat quality for many species globally, it also benefits a number of species that are early-successional specialists and depend on a high frequency of disturbance events to create suitable habitats. We investigated whether the threatened green and golden bell frog (*Litoria aurea*) is one such species by examining data collected as part of an adaptive management regime which was implemented from 2000 to 2016 at Sydney Olympic Park, Australia. This regime created a mosaic of habitat ranging in degree of disturbance, via the

construction of new ponds, and the removal of macrophytes and shade trees from a subset of existing ponds. The abundance of male, female, juvenile and calling male bell frogs, as well as the number of breeding events were recorded annually. Bell frogs used ponds of all ages and peak abundance varied by life stage and sex, suggesting a mosaic of ponds at different ages may be beneficial for habitat management. Although variability in abundance increased with pond age, we found no evidence of a gradual decline in bell frog abundance or breeding frequency as ponds aged. In addition, bell frog abundance was reduced by macrophyte removal, and was not affected by shade tree removal. Our findings suggest that the green and golden bell frog may not be as restricted to early-successional landscapes as was previously believed based on its life history characteristics, although management of ponds will still be required to prevent ponds reaching a stage of late succession, and to prevent the loss of pond function and ensure connectivity. This study provides a rare example of the iterative phase of adaptive management being successfully applied to provide suitable habitat for a threatened species while simultaneously providing the opportunity to learn more about their habitat requirements to improve future management.

INTRODUCTION

Ecological disturbance influences the composition of ecosystems across landscapes and acts as a selective pressure that drives the evolution of life-history traits (Southwood, 1988). Species that are adapted to high levels of disturbance tend to possess traits and abilities that make them successful at colonising newly created landscapes, such as dispersal over large distances, rapid development and high fecundity (Pianka, 1970, Petraitis et al., 1989). At the other end of the continuum, there are species that have traits to maximise their ability to compete with other species, which are necessary to be successful in landscapes where disturbance is low and resources are therefore more limited (Pianka, 1970). Some species are generalists and can persist in both frequently and infrequently disturbed environments, and others are specialists and limited to either early-successional environments created by frequent disturbance events or late-successional environments that form in the absence of frequent disturbance (Russell et al., 2002, Russell et al., 2004, Werner and Glennemeier, 1999, Canessa et al., 2013, Semlitsch et al., 2009, Brawn et al., 2001, Swanson et al., 2011).

Habitat alteration caused by anthropogenic sources of disturbance such as agriculture, urbanisation and logging often has a negative impact on biodiversity (Bionda et al., 2011, Cano and Leynaud, 2010), and has been identified as a causal agent in the decline of many species (Hamer and McDonnell, 2008, McKinney and Lockwood, 1999).

Amphibians are particularly susceptible to habitat alteration (Alford and Richards, 1999), which is one of the factors that has contributed to them being the most threatened taxonomic group on a global scale (Stuart et al., 2004). However, studies focused on the response of amphibian populations to anthropogenic disturbance (Clawson et al., 1997, Denton et al.,

1997, Russell et al., 2002, Russell et al., 2004, Canessa et al., 2013, Warren and Büttner, 2008, Skelly et al., 2002), in addition to natural events such as fire (Hossack and Corn, 2007), hurricanes (Woolbright, 1996), and beaver herbivory (Stevens et al., 2006), have demonstrated that disturbance can benefit a range of amphibian species. These events reduce pond or stream shading, which in turn increases water temperatures, dissolved oxygen levels and the growth of periphyton and other food sources for herbivorous larvae; all of which can benefit larvae survival, growth and development (Werner and Glennemeier, 1999, Skelly et al., 2002). They can also alter the composition of vegetation in a way that provides more suitable breeding (Woolbright, 1996) or foraging (Warren and Büttner, 2008, Greene et al., 2008) habitat for adults. Some amphibian species are adapted to early-successional habitats, and are only able to colonise waterbodies after a disturbance event has occurred and persist there if subsequent disturbance is frequent enough to maintain early-successional vegetation communities (Denton et al., 1997, Canessa et al., 2013, Hossack and Corn, 2007).

Given that disturbance frequency plays such a large role in habitat suitability, determining whether a species is adapted to early- or late-successional communities is a critical step in implementing successful conservation programs. Protecting habitat from disturbance (particularly disturbance caused by human activities) is a common philosophy in endangered species management, however a lack of disturbance may itself be a threatening process if the species is an early-successional specialist. For example, Apennine yellow bellied toads (*Bombina pachypus*) abandon agricultural ponds as breeding sites when farmers stop draining the ponds and clearing out vegetation within and adjacent to ponds (Canessa et al., 2013). Two other endangered anurans, the natterjack toad (*Bufo*

calamita) and yellow-bellied toads (*Bombina variegata*), prefer heavily disturbed sites, where vehicular traffic creates patches of bare soil and significantly reduces riparian vegetation, over undisturbed sites (Warren and Büttner, 2008). Clearing trees and reintroducing livestock to graze at sites that were historically used by natterjack toads can facilitate successful reintroduction projects for this species (Denton et al., 1997).

The green and golden bell frog (*Litoria aurea*, hereafter bell frog) shares many of the life history characteristics with the species discussed above, which allows it to colonise and persist in early successional habitats. These include the ability to disperse over relatively long distances (Pyke and White, 2001), a short tadpole development time (Hamer et al., 2002), fast development to maturity (Hamer and Mahony, 2007) and high fecundity (Van De Mortel and Buttemer, 1996). In addition, bell frogs have previously been associated with ephemeral, unshaded ponds (Pyke and White, 1996), and the majority of sites where they persist since largescale declines began in the 1970s (White and Pyke, 1996) are located in urban or industrial landscapes with some level of anthropogenic disturbance (Mahony et al., 2013). Based on this evidence, the managers of a bell frog population located in Sydney Olympic Park, Australia, consulted with herpetologists to design and carry out an adaptive management regime which incorporated disturbance, aimed at improving habitat quality for this threatened species.

One of the key advantages of adaptive management is that it facilitates learning about a species or system at the same time as managing it, by using management actions as a series of experiments to test different hypotheses about the way that the species or system functions (Williams, 2011). At the time that the management regime commenced at Sydney Olympic Park, the method of incorporating disturbance events into the management of bell

frog populations had not been tested. However, by following available knowledge about the life history characteristics of bell frogs and general guidelines for the conservation of pond-breeding amphibians (Semlitsch, 2002), the managers and herpetologists designed a habitat management program which included removing shade trees and macrophytes from some ponds, as well as constructing new ponds to replace those that failed. This provided a mosaic of habitat ranging in degree of disturbance and pond age, to ensure that suitable habitat was available. An annual monitoring program collected data on bell frog abundance, distribution and breeding activity to inform this management program. Using this data, we aimed to determine whether the bell frog is an early-successional specialist, and hypothesised that if this is the case, then the abundance of bell frogs should increase at ponds where macrophytes or shade trees were removed, and that bell frog abundance should gradually decrease over time as ponds age and late-successional communities develop. Because disturbance can affect different sexes and life stages of amphibian species in different ways (Semlitsch et al., 2009) and bell frog pond occupancy differs among life stages (Bower et al., 2013), we examined the response of female, male, calling male and juvenile bell frogs to these changes to habitat, as well as the frequency of breeding events.

METHODS

Study site and species

The bell frog was once a common species on the east coast of Australia, but began to experience large-scale declines in the 1970s (White and Pyke, 1996). In New South Wales (NSW) over 80% of populations have gone extinct (White and Pyke, 1996, White and

Pyke, 2008), leading to the species being listed as endangered in this state under the *Biodiversity Conservation Act 2016* and as vulnerable nationally under the *Environmental Protection and Biodiversity Conservation Act 2000*. Infection by the amphibian chytrid fungus *Batrachochytrium dendrobatidis*, habitat alteration, and predation by the introduced eastern gambusia (*Gambusia holbrooki*) have been suggested as contributing factors to population declines, and continue to threaten the persistence of remaining populations (Mahony et al., 2013). The bell frog population located at Sydney Olympic Park is one of the largest remaining in NSW and is listed as a key population for the persistence of this species (Department of Environment and Conservation, 2005) and Sydney Olympic Park as a priority site for its management

Sydney Olympic Park covers 600 ha of land in south-eastern Australia that has historically been used for a range of industrial purposes that greatly altered the landscape and underwent an intensive urban renewal program throughout the 1990s (Darcovich and O'Meara, 2008, Pickett et al., 2013). Since the bell frog was found at this site in 1992, management objectives have focused heavily on improving the quality of habitat ponds, and well as constructing new ponds to increase the amount of available habitat. There are three primary habitat precincts at the site, which are separated by roads and estuarine waterways but all within 2 km of each other, called the Brickpit, Narawang Wetland and Kronos Hill/Wentworth Common (Figure 1). The Brickpit is a 16 ha disused quarry which consists of some ponds that formed in the 1990s as the result of past quarrying processes, as well as ponds constructed from 1998 to 2010, totalling 66 waterbodies ranging in surface area from 3 m² to 6,991 m². Kronos Hill/Wentworth Common is a 40 ha corridor of land which was remediated to address leachate contamination caused by industrial activities and

converted to bell frog habitat with the construction of 42 ponds between 1993 and 2009. Ponds in this precinct range from 5 m² to 22,085 m². Narawang Wetland was constructed from 1998 to 2000 across a 20 ha area and consists of 21 large clay-based ponds ranging in surface area from 338 m² to 1,332 m². Pond hydrology and the composition of aquatic and emergent plant species vary greatly across the site.

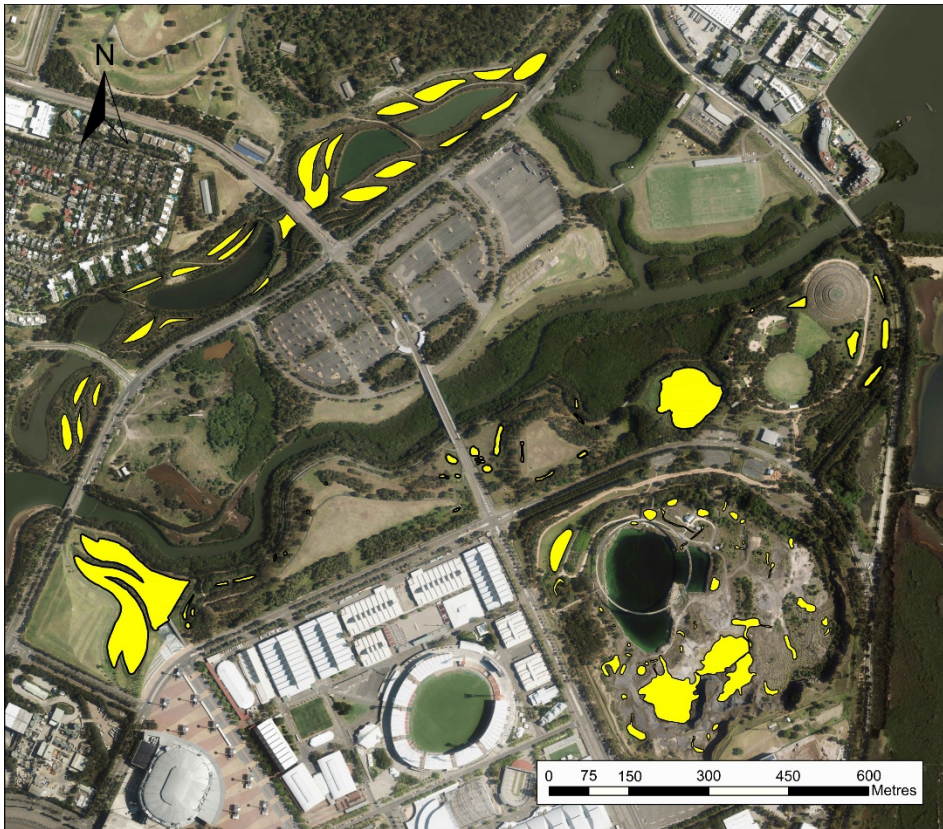


Figure 1. Map of the primary bell frog habitat at Sydney Olympic Park, Australia. Habitat ponds are shaded yellow.

Pond treatments

Habitat inspections were carried out yearly from 2000 onwards. These involved visual inspections of the ponds at the three primary bell frog habitat precincts by a team comprised of Sydney Olympic Park Authority (SOPA) staff and consultant herpetologists.

The goal of the inspections was to identify ponds that no longer met a set of habitat standards, so that actions could be taken to improve habitat quality based on expert opinion available at the time. When ponds became overgrown by macrophytes (consisting mostly of the species *Baumea articulata* and *Schoenoplectus validus*) and approached 100% cover, a recommendation was made to remove vegetation from some of the ponds so that at least half of the pond surface area was comprised of open water. Macrophytes were removed from 16 ponds in 2003, 4 ponds in 2005, 6 ponds in 2007, 11 ponds in 2008 and 4 ponds in 2011. Macrophyte removal was undertaken using hand tools (pruning saws, secateurs and McLeod tools) and occurred from August to October (austral late winter and spring), so that it occurred prior to the bell frog peak breeding season, which extends from November to February. Macrophyte growth rates generally decline at the end of summer and stop during autumn, before beginning again in late spring (Gorme et al., 2012). It can therefore be concluded that ponds that were overgrown by macrophytes in late winter/early spring were also overgrown during in the previous bell frog breeding season.

During habitat inspections, the presence of trees adjacent to ponds was also examined. When trees were close enough to shade ponds, it was recommended that they be removed to allow more sunlight to reach the surface of the water. Trees (mostly *Casuarina* and *Eucalyptus* species) were removed from the edge of 1 pond in 2002, 3 ponds in 2004, 9 ponds in 2008, 8 ponds in 2009, 8 ponds in 2010, 4 ponds in 2011, 3 ponds in 2012, 1 pond in 2013 and 1 pond in 2014. The timing of tree removal was largely dependent on the growth rate of trees, and in some cases was staggered across different years due to the logistical challenges involved. It was carried out from March to July, again avoiding the peak bell frog breeding season.

Ponds that had ceased holding water during inspections and were no longer believed to provide suitable habitat were sometimes reconstructed by using heavy machinery to excavate the pond, installing a new pond liner and refilling the pond. In cases where this occurred, the age of that pond was recorded as 0 during the year of reconstruction (so for example a pond that was built in 1995 then reconstructed in 2000 would be recorded as 4 years old in 1999, 0 years old in 2000, 1 year old in 2001, and so on).

Bell frog surveys

Bell frog surveys began in 1997 at Kronos Hill/Wentworth Common, in 1998 at the Brickpit, and in 1999 at Narawang Wetland, and continued until 2016 in all precincts. Two methods were used to detect and examine the abundance of bell frogs: capture encounter (CE) surveys and auditory surveys (Bower et al., 2014). Capture encounter surveys occurred between 20:00 h and 04:00 h an average of 4 (range 1 to 10) times per year between October and June. Surveys involved searching the emergent vegetation around the perimeter of each pond and attempting to capture all bell frogs seen. Searchers placed a plastic bag over their hands prior to capture and then inverted the bag around the frog so that it was contained in the bag. After each pond search, the snout-vent length (SVL) of captured frogs was measured and their sex determined. Frogs with SVL <45 mm were classed as metamorphs if they had a tail or tail stub, and as juveniles if they lacked these characteristics. Frogs with SVL \geq 45mm were determined to be males if they had nuptial pads and as females if they did not. The total number of frogs seen and total search effort (pond search time multiplied by number of searchers) were recorded.

Auditory surveys involved listening and counting the number of male bell frogs that were calling upon arrival at a pond, after which we imitated the bell frog call and then listened again. The maximum number of frogs calling at any one time either before or after call imitation was recorded for each pond. From 2008/09 onwards, an auditory survey was carried out immediately before the commencement of each CE survey. This was also the case for most of the earlier seasons, however auditory surveys were sometimes conducted at a pond without a CE taking place and vice versa during this period of time. Ponds were surveyed an average of 4 (range 1 to 9) times each year, from September to May.

The two methods that were used to survey bell frog tadpoles were dip-netting and minnow-trapping. Dip-netting surveys were carried out from 1997/98 to 2012/13, and involved repeatedly sweeping a dip net through the pond and recording the total number of tadpoles caught without replacement. From 2008/09 onwards, dip-netting surveys were conducted concurrently with each frog survey. Prior to this, they were conducted independently of frog surveys in some seasons. On average, ponds were dip-netted 6 (range 1 to 13) times per season, from September to June. Minnow-trapping was carried out from 2006/07 to 2012/13. Traps baited with yellow glow sticks (Glowsticks Ltd) to attract tadpoles (Grayson and Roe, 2007) were tied to the emergent vegetation in each pond before nightfall. The traps were emptied the following morning and the number of bell frog tadpoles was recorded. Trapping surveys were repeated an average of 3 times per season (range 1 to 10), from October to February. The number of dip net sweeps and the number of traps set were both relative to pond size (average of 5 dip net sweeps per pond, range 1 to 50; average of 6 traps per pond, range 1 to 50). Tadpole surveys were not conducted during seasons 2013/14 to 2015/16.

From 2008/09 onwards, all ponds in the primary habitat precincts were included during yearly surveys. Prior to this, some yearly surveys were restricted to a subset of the total ponds in some precincts due to budgetary constraints.

Statistical analysis

The six response variables that we examined were total bell frog abundance (which included all bell frogs detected in CE surveys), the abundance of female, male and juvenile bell frogs, the abundance of calling male bell frogs, and the proportion of surveys when breeding was detected. The latter variable was determined by adding the number of surveys when breeding was detected at a particular pond in a given season, by the presence of metamorphs in CE surveys or the presence of tadpoles and/or metamorphs in dip-netting or minnow-trapping surveys, and dividing this by the total number of surveys carried out at that pond during that season. This measure was used instead of tadpole abundance because the different methods used to detect tadpoles over the course of the study period prevented us from quantifying tadpole abundance in a way that could provide a reliable comparison between different breeding seasons. The primary explanatory variables were pond age, macrophyte removal and shade tree removal.

Pond age

To examine the effect of pond age on the five response variables that were measures of abundance, we used the R software system (version 3.3.0) to fit Poisson regression, negative binomial, zero-inflated Poisson and zero-inflated negative binomial models from the 'pscl' package (R Core Development Team, 2012) and chose the most appropriate model for each response variable by determining if the response variables were affected by

overdispersion or zero inflation. Overdispersion was identified firstly using the residual deviance divided by the degrees of freedom in the Poisson regression models and it was confirmed by a significant overdispersion term in the negative binomial model. We identified zero inflation by the significance of the terms in the zero portion of zero-inflated models. Because we detected overdispersion for all response variables, but no more zero inflation than could be accounted for by the negative binomial model, we used the negative binomial for all five response variables (Zuur et al., 2009) (Table 1). The sixth response variable, the proportion of surveys in which breeding was detected, was fitted with a logistic regression model (also using R), which is the appropriate model for binary response variables (Zuur et al., 2009).

In addition to pond age, the year and month when surveys were carried out were included in all models (with the exception of the model for breeding events, to which only the year was added because the results of multiple surveys within the year were summarised to give a single value for that year). This accounted for differences in the number of bell frogs detected between surveys caused by seasonal and yearly variability in the population size, which has been observed at our study site (Pickett et al., 2014). Total search time was also added to the models for the four response variables that were based on CE surveys (total, female, male, and juvenile frog abundance), to account for differences in the length of surveys that may have occurred due to different people conducting fieldwork.

The dataset that was used to examine pond age included all breeding seasons surveyed, with some exceptions: in 2005/06, the sex of frogs was not determined, so this year was excluded from the models for female and male abundance. In addition, all surveys at Kronos Hill/Wentworth Common after December 2011 were excluded from all models,

as captive bred bell frog tadpoles were released at some ponds within the precinct at that time. Finally, seasons 2013/14 to 2015/16 for the Brickpit and Narawang Wetland were excluded from models for pond age, due to a scarcity of data caused partly by the other primary precinct being removed and partly due to a reduced number of surveys occurring during these years.

Macrophyte and shade tree removal

To examine the effect of macrophyte removal on the six response variables, ponds were divided into a treatment group or a control group, on the basis of whether or not they had undergone macrophyte removal. For both ponds types, analysis was restricted to surveys from the breeding season directly prior to macrophyte removal and the breeding season directly following macrophyte removal. Four groups were therefore compared using the before-after control-impact (BACI) study design (Green, 1979): treated ponds one season pre-treatment, treated ponds one season post-treatment, control ponds one season pre-treatment and control ponds one season post-treatment. Control ponds consisted of all non-treatment ponds in the same precinct that the treatment occurred in a given year; for example, if macrophyte removal was only carried out in the Brickpit and Narawang Wetland in 2005, then control ponds for that year would be restricted to all non-treatment ponds in those two precincts, in the breeding seasons 2004/05 and 2005/06. Because all ponds at the sites were not always surveyed using all four survey methods prior to 2008/09, some treatment ponds could not be included in analysis. Table 2 shows the total number of treatment ponds that were included in analysis for each response variable.

Program R was used to carry out the same process described above of fitting four different model types to the five response variables that were measures of abundance. Because we detected overdispersion for all response variables and zero-inflation for some, we used the negative binomial or the zero-inflated negative binomial model for all five response variables (Table 2). Logistic regression was again used for the frequency of breeding events. The explanatory variables that were added to each model were pond treatment (treated or control), survey context (pre or post treatment), and the interaction of pond treatment and survey context. This interaction was used to determine if the treatment had a significant effect on bell frogs, by comparing the difference in abundance and breeding event frequency over time at treatment ponds with the difference in abundance and breeding event frequency over time at control ponds. The use of the BACI design accounts for variability in the response variable that may be caused by factors other than the predictor variable of interest (Green, 1979). For example, if treatment ponds happened to have physical characteristics such as size, hydrological regime or distance to neighbouring waterbodies that made them either less or more appealing to bell frogs than control ponds, then abundance could be consistently higher or lower at treatment ponds, but would not increase or decrease over time. It is also possible that variability in climatic conditions could cause bell frog abundance and breeding event frequency to be higher at ponds during the pre-treatment breeding seasons than post-treatment breeding seasons, or vice versa. The inclusion of control ponds enables the uncoupling of temporal variability that is unrelated to the predictor variable of interest from the response to that predictor variable.

Survey month and year and total search effort for CE surveys were also added to some models using the same process as described for above for pond age (Table 2). The effect of shade tree removal on the six response variables was analysed using the same process as described for macrophyte removal (Table 2).

RESULTS

The mean number of total bell frogs, females, males, juveniles, calling males and the frequency of breeding events detected varied significantly in ponds of different ages (Table 1). However, there was no increasing or decreasing trend in abundance or frequency of breeding events as pond age increased (Figure 2). The amount of variability in mean abundance and frequency of breeding events generally increased as ponds became older (Figure 2). The mean highest abundance for total bell frogs was at ponds aged 19 years (\bar{x} = 2.0 frogs, 95% CI 1.2 to 3.3), and the lowest was at 6 year old ponds (\bar{x} = 0.8 frogs, 95% CI 0.63 to 1.0, Figure 2.a). Mean female abundance was highest at 5 year old ponds (\bar{x} = 0.43, 95% CI 0.32 to 0.58) and lowest at 18 year old ponds (\bar{x} = 0.12, 95% CI 0.06 to 0.26, Figure 2.b). Mean male abundance was highest at 15 year old ponds (\bar{x} = 0.50 frogs, 95% CI 0.31 to 0.80) and lowest at ponds that were less than one year old (\bar{x} = 0.15, 95% CI 0.10 to 0.21, Figure 2.c). Mean juvenile abundance was highest at 18 year old ponds (\bar{x} = 1.66, 95% CI 0.75 to 3.66) and lowest at 6 year old ponds (\bar{x} = 0.11, 95% CI 0.07 to 0.17, Figure 2.d). Mean calling male abundance was highest at 18 year old ponds (\bar{x} = 1.3, 0.4 to 4.0) and lowest at ponds aged 2 years and less than one year (\bar{x} = 0.05, 95% CI 0.03 to 0.10 for both, Figure 2.e). The mean frequency of detected breeding events was highest at

19 year old ponds (\bar{x} = 0.43, 95% CI 0.23 to 0.65) and lowest at 6 and 16 year old ponds (both \bar{x} = 0.02, 95% CI 0.01 to 0.03 and 0 to 0.13 respectively, Figure 2.f). Bell frogs from all life stages studied occurred in ponds that were less than one year old (Figure 2).

Table 1: The GLMs (generalised linear models) used to determine the relationship between pond age and bell frog abundances and breeding events, with p-values for pond age. NB = negative binomial, tst = total search time.

Response variable	Model	Pond age P-value
Total bell frog abundance	NB with year, month and tst	<0.001
Female abundance	NB with year, month and tst	<0.001
Male abundance	NB with year, month and tst	<0.001
Juveniles abundance	NB with year, month and tst	<0.001
Calling male abundance	NB with year and month	<0.001
Breeding events	Logistic regression with year	<0.001

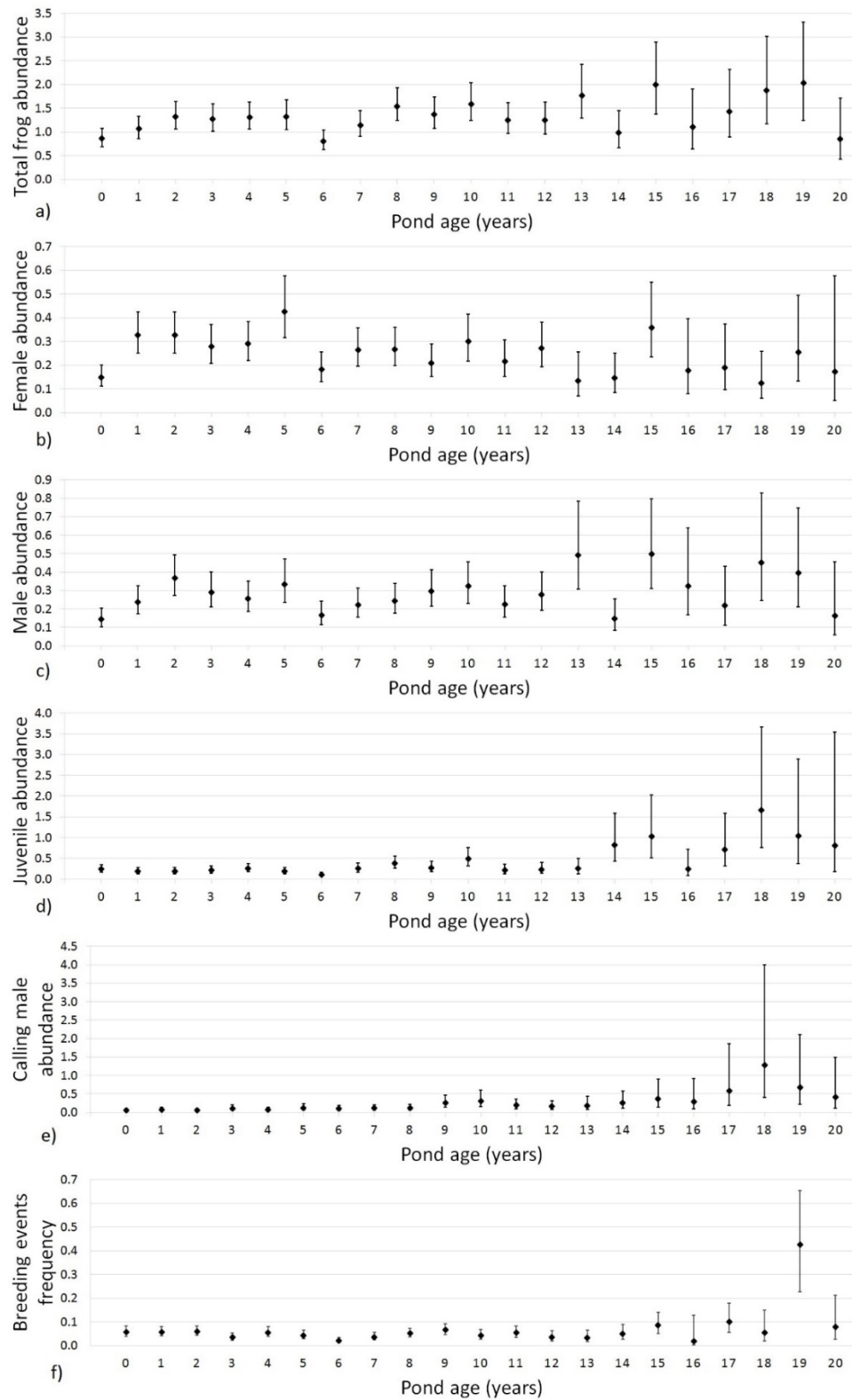


Figure 2. Mean total bell frog abundance (a), female (b), male (c), juvenile (d) and calling male abundance (e), and frequency of detected breeding events (f) at ponds aged 0 to 20 years old. Error bars show 95% confidence intervals.

The removal of macrophytes had a significant negative effect on total bell frog abundance (Table 2). The mean number of total bell frogs dropped from 1.45 (95% CI 1.0 to 2.1) to 0.9 (95% CI 0.6 to 1.3) frogs per survey at treatment ponds, and increased from 0.8 (95% CI 0.7 to 1.0) to 0.9 frogs per survey (95% CI 0.7 to 1.1) at control ponds (Figure 3.a). Female, male, juvenile and calling male abundance all decreased post-treatment at ponds with macrophytes removed, and increased at control ponds (figures 3.b, 3.c, 3.d and 3.e), however the differences in abundance between the two pond types for these subgroups did not reach significance (Table 2). The frequency of breeding events decreased post-treatment at both treated and untreated ponds (Figure 3.f), but the difference between treatment types was not significant (Table 2).

Table 2: The GLMs (generalised linear models) used to investigate the effect of two different pond treatments on bell frog abundances and breeding events, the number of treatment and control occasions, and the p-value for the interaction between pond type (control and treatment) and context (pre and post treatment).NB = negative binomial, ZINB = zero-inflated negative binomial, tst = total search time.

Treatment	Response variable	Model	N treatment occasions	N control occasions	Interaction P-value
Macrophyte removal	Total bell frog abundance	NB with year, month and tst	35	247	0.033
	Female abundance	NB with year, month and tst	31	219	0.544
	Male abundance	NB with year, month and tst	31	219	0.770
	Juvenile abundance	NB with year, month and tst	35	247	0.711
	Calling male abundance	ZINB with year and month	32	228	0.292
	Breeding events	Logistic regression with year	37	296	0.681
Shade tree removal	Total bell frog abundance	ZINB with year, month and tst	30	255	0.926
	Female abundance	NB with year, month and tst	30	255	0.239
	Male abundance	ZINB with year, month and tst	30	255	0.875
	Juvenile abundance	NB with year, month and tst	30	255	0.541
	Calling male abundance	ZINB with year and month	30	270	0.517
	Breeding events	Logistic regression with year	30	280	0.667

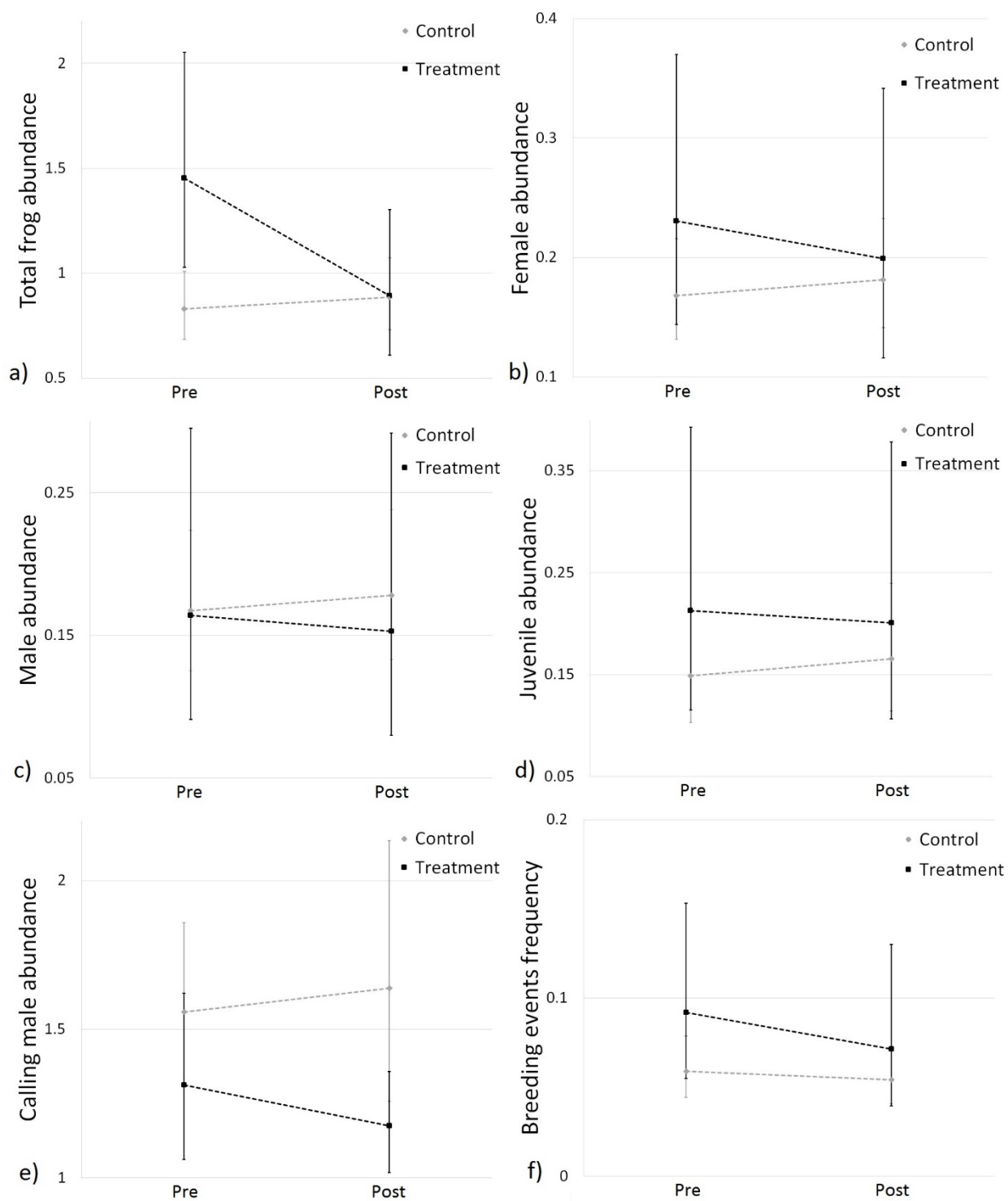


Figure 3. Mean total bell frog abundance (a), female (b), male (c), juvenile (d) and calling male abundance (e), and frequency of detected breeding events (f) at ponds where macrophytes were removed and control ponds, one breeding season pre- and post-treatment. Error bars show 95% confidence intervals.

Shade tree removal did not significantly affect any of the response variables (Table 2). Mean total bell frog abundance, as well as the abundance of male and juvenile frogs, decreased slightly at both treatment and control ponds post-treatment (Figures 4.a, 4.c and 4.d). Female abundance increased at treatment ponds and decreased at control ponds post-treatment (Figure 4.b). Calling male abundance decreased at treatment ponds and increased at control ponds post-treatment (Figure 4.e). The frequency of breeding events increased slightly at both treatment and control ponds post-treatment (Figure 4.f).

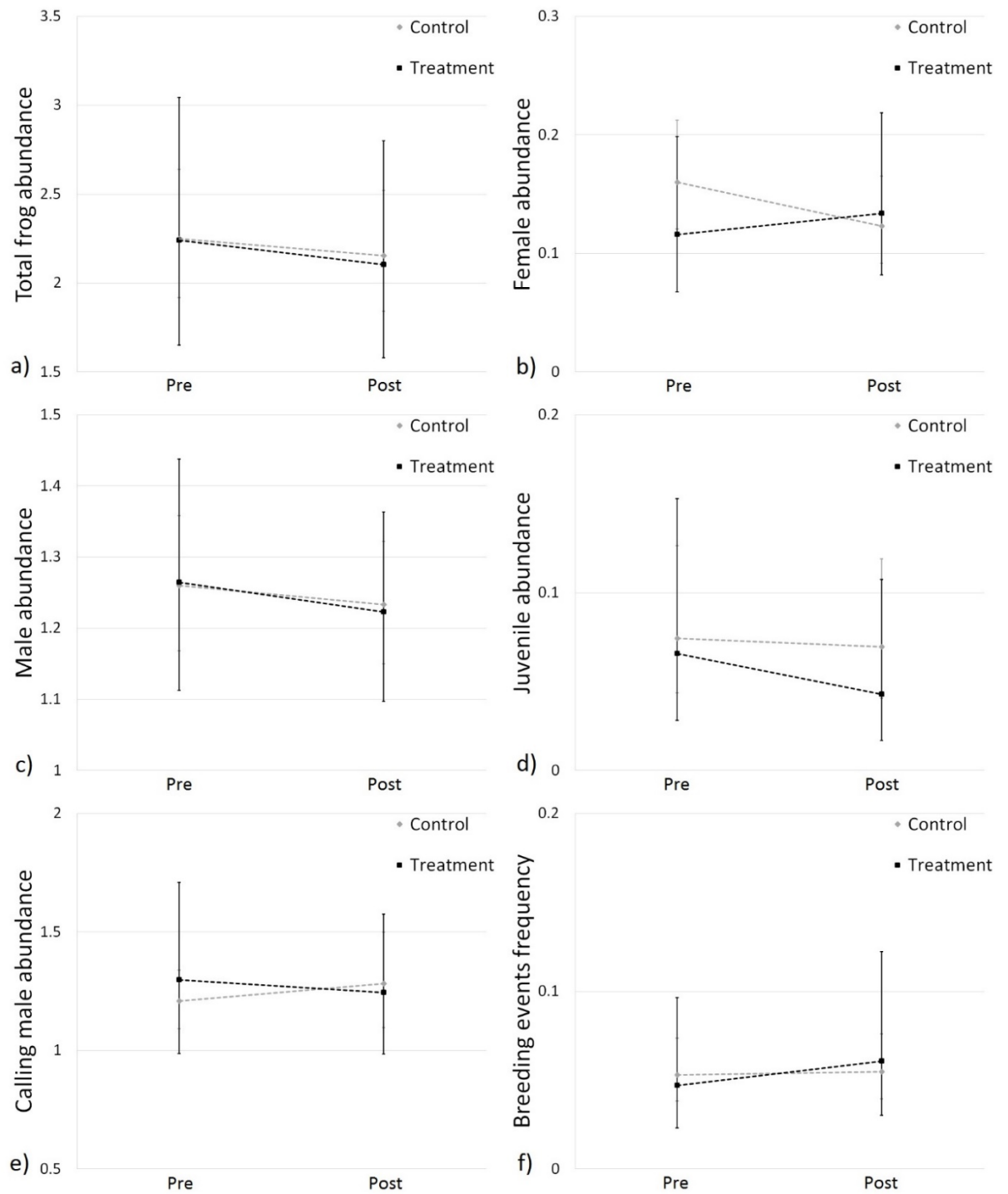


Figure 4. Mean total bell frog abundance (a), female (b), male (c), juvenile (d) and calling male abundance (e), and frequency of detected breeding events (f) at ponds where shade trees were removed and control ponds, one breeding season pre- and post-treatment. Error bars show 95% confidence intervals.

DISCUSSION

Bell frogs were able to rapidly colonise newly formed ponds that were less than one year old, as was expected based on their life history characteristics. However, there was no evidence of a decline in bell frog abundance or breeding frequency as ponds aged. Aging can make ponds unsuitable for early-successional specialist amphibians in a number of ways: the development of a canopy cover as trees surrounding the pond grow can reduce water temperatures, dissolved oxygen levels and food resources for larvae (Werner and Glennemeier, 1999, Skelly et al., 2002), and the replacement of bare ground with vegetation can reduce foraging opportunities for adults (Warren and Büttner, 2008). Additionally, as time passes and other amphibians eventually colonise the pond, competition for resources increases and may exclude the early colonising species (Denton et al., 1997). Our results suggest that none of these processes are reducing habitat suitability for bell frogs at Sydney Olympic Park. The increase in variability in mean frog abundance as ponds age suggests that younger ponds are more similar in terms of habitat suitability, and as ponds age some become less suitable and others more suitable. Investigating what features contribute to differences in suitability between older ponds would be valuable in informing future pond management.

Shade tree removal did not affect the abundance of bell frogs at any life stage, which further questions the hypothesis that later successional ponds do not provide suitable habitat for bell frogs. Tree removal via both natural and artificial methods has been shown to increase the abundance of many pond-breeding anuran species (Stevens et al., 2006, Woolbright, 1996, Clawson et al., 1997, Perison et al., 1997). Some anuran species cannot occupy ponds with closed canopies (Skelly et al., 1999), largely due to a lack of sunlight

inhibiting periphyton growth and therefore restricting available food resources for larvae (Skelly et al., 2002, Werner and Glennemeier, 1999). Because none of the ponds at Sydney Olympic Park had 100% canopy cover pre-treatment, we cannot conclude that ponds within closed forest systems would provide suitable habitat. Previous surveys of forested sites throughout north-eastern NSW failed to detect bell frogs (Lemckert, 1996), suggesting that if completely closed canopies were allowed to develop over ponds, this would reduce habitat quality and bell frogs may cease to persist there. Complete canopy cover may also reduce water temperatures and make conditions more favourable for the amphibian chytrid fungus (Berger et al., 2004), which is a cause of bell frog mortality at Sydney Olympic Park (Penman et al., 2008) and one of the major threats to the persistence of the species (Mahony et al., 2013). However, our findings suggest that bell frogs do not avoid partially shaded ponds, despite the findings of a correlative study of occupied and unoccupied ponds showing most ponds occupied by bell frogs were entirely unshaded (Pyke and White, 1996).

Bell frogs have been observed to bask in the sun (Pyke and White, 2001), a process which is postulated to increase their body temperature potentially allowing for better digestion and maximal growth (Hamer et al., 2003). It was therefore expected that bell frogs would preferentially inhabit ponds where macrophytes were partially removed, to provide areas of open water and reduce shading. Instead, we found that total bell frog abundance decreased significantly after macrophyte removal. Male, female and juvenile and metamorph bell frogs at another site have been found to favour aquatic vegetation over open water in the selection of microhabitats (Valdez et al., 2016). These findings combined with ours suggest that the presence of open water to provide basking opportunities may not

be as important in the provision of suitable habitat as was previously believed (Pyke and White, 1996). It should also be noted that our study did not take into account frog detectability, which is likely to be higher post-macrophyte-removal due to a decrease in the structural complexity of the vegetation. It is therefore possible that the trend of bell frogs to avoid ponds after macrophyte removal is even more pronounced than our results indicate.

We found no evidence that the development of vegetation communities driven by succession are reducing habitat quality for bell frogs at Sydney Olympic Park. Our findings suggest that bell frogs may not be as disturbance-dependent as is currently believed, and may not be as restricted to early-successional habitats as other anurans with similar life-history characteristics (Canessa et al., 2013, Denton et al., 1997). It should also be noted, however, that other aspects of land use at the site act as barriers to succession which may be benefiting bell frogs. At Kronos Hill/Wentworth Common, for example, many of the ponds are adjacent to areas of grass that are regularly mown as they are also used for human recreation. Because the Brickpit is a disused quarry, the majority of the landscape is covered by shale, which hinders plant growth. These factors reduce the structural complexity of terrestrial vegetation, providing open areas for foraging which may benefit bell frogs (Valdez et al., 2016) as well as other anurans (Heard et al., 2008, Werner and Glennemeier, 1999). Although we did not find evidence that disturbance events are necessary to improve habitat in the short term, maintenance such as the periodic removal of sediment and vegetation from within ponds is still essential for ponds to continue to hold water and function as bell frog habitat in the long term. In addition, the provision of ponds that range in age may be beneficial, as peak abundance occurred at a range of pond ages for different life stages.

Our study provides a relatively rare example of the effective application of adaptive management to simultaneously achieve conservation goals while providing the opportunity to learn more about the ecology of the species being managed. Some components of the set-up phase of adaptive management, such as the use of predictive models to assist in the prioritisation of management alternatives, are relatively well represented in the published literature (Allen et al., 2011). However, there is a lack of reporting on the iterative phase of adaptive management (Keith et al., 2011), where actions are trialled, monitored and evaluated, providing an opportunity to learn more about the species or system being managed which then leads to improved future management (Williams, 2011). Common barriers to the implementation and reporting of the iterative phase include the cost of monitoring regimes that are comprehensive enough to detect responses of the species or system (McLain and Lee, 1996), and hesitation to report findings if actions were unsuccessful in achieving management goals (Keith et al., 2011). If these barriers can be overcome, adaptive management can in the long term save resources and improve management outcomes, as actions which do not meet goals can be identified and discontinued, and managers can gain a greater understanding of the requirements of the species or system being managed (Williams, 2011).

Our findings suggest that the use of frequent disturbance events to create early-successional habitats is not necessary to encourage bell frogs to continue to use existing habitat, and actions that involve pond disturbance can be reserved for situations when they are required to prevent the loss of pond function. In cases where conservation funds are limited, bell frog populations would be more likely to benefit from the removal of the predatory eastern gambusia, which is another action implemented at Sydney Olympic Park

and increased bell frog tadpole abundance to over 140 times that of ponds where gambusia was present (Pollard et al., 2017). The environmental managers at Sydney Olympic Park created a mosaic of habitats on a successional continuum that ensured suitable habitat was provided for the persistence of the bell frog population while simultaneously facilitating the opportunity to learn more about the species' requirements in relation to habitat succession to improve future management. We found that this was an effective method of investigating the disturbance requirements of an endangered frog, which could not be predicted based on its life history characteristics alone.

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Chapter 4: Combining ex situ and in situ methods to improve water quality testing for the conservation of aquatic species.

Declaration of contribution

I, Carla Pollard, am the primary author of this paper entitled “Combining ex situ and in situ methods to improve water quality testing for the conservation of aquatic species”. I developed the concept of the study with assistance from other co-authors, managed the captive breeding program and undertook laboratory and field work to collect data. I carried out all analyses and wrote the manuscript, which was then improved through review by my co-authors.

Signed:	Carla J. Pollard	Date: 11/08/2017
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Endorsement by Co-authors

Signed:	Michelle P. Stockwell	Date: 25/07/2017
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Signed:	Deborah S. Bower	Date: 25/07/2017
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Signed:	John Clulow	Date: 27/07/2017
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Signed:	Michael J. Mahony	Date: 15/08/2017
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Endorsement by the Faculty Assistant Dean Research Training

Signed:

A. Prof. Frances Martin

Date: 17/08/2017

Combining ex situ and in situ methods to improve water quality testing for the conservation of aquatic species

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ABSTRACT

1. Determining whether water quality is suitable is an important part of managing aquatic species for conservation, although it is often challenging to achieve. Past approaches have largely consisted of tests exposing individuals to artificial solutions, or field studies that examine the effect of a subset of water quality parameters on the distribution or abundance of a species.

2. Owing to the complex nature of water chemistry in natural systems, which is difficult to replicate using laboratory studies or to capture entirely with correlational field studies, these types of study may not be suitable for determining accurately whether or not water quality at a particular site is suitable for a target species. In situations where conservation outcomes rely heavily on achieving this, an alternative approach is therefore needed.

3. Embryos of the threatened green and golden bell frog *Litoria aurea* were placed in water collected from ponds that were used by this species for breeding and ponds where breeding was not detected at Sydney Olympic Park, Australia. After 19 days, the tadpoles were placed in enclosures in the same breeding and non-breeding ponds, and monitored until they metamorphosed.

4. There was no difference in tadpole survival, time to metamorphosis or body condition between the two treatments, indicating that poor water quality was not a cause of low pond occupancy by tadpoles at the site and resources should be directed towards investigating other potential causes.

5. We suggest that this method of an *ex situ* followed by an *in situ* exposure study is an effective approach to eliminating or confirming poor water quality as a cause of population declines and reduced occupancy, for species that are aquatic for at least part of their life cycle. Other applications include establishing that artificially created habitat provides suitable water chemistry, or identifying a potential location for a reintroduction project.

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KEY WORDS: green and golden bell frog; habitat suitability; *Litoria aurea*; tadpole survival; water chemistry; water quality

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INTRODUCTION

The composition of species in aquatic ecosystems is heavily influenced by water chemistry. Each species is able to tolerate a particular range of conditions along a continuum of water quality parameters, and cannot persist in environments where one or more aspect of water quality lies outside this range. Human land-use in the catchment areas of streams and other water bodies can heavily affect water quality, and in turn alter species composition. Urban development and agricultural practices such as crop farming and cattle grazing alter water chemistry by increasing water conductivity, turbidity and nutrient loads, and decreasing dissolved oxygen (Bishop *et al.*, 1999; Ehrenfeld, 2000; Knutson *et al.*, 2004; Schmutzer *et al.*, 2008; Hamer and Parris, 2011). Determining whether water quality is suitable is an essential part of understanding the abundance and distribution of aquatic species, and identifying causes of population declines or range contractions. The success of habitat creation initiatives and the translocation or reintroduction of species to new or previously occupied landscapes all rely on the provision of water bodies with water quality values that meet the needs of that species. If water quality is unsuitable for any stage of the life cycle of the species, successful recruitment cannot occur and the population will not persist.

However, the process of determining conclusively whether water quality is suitable at a given site for a particular species is challenging. One approach has been to expose individuals to artificially made solutions that increase in extremity along a gradient of a single parameter of water chemistry, and examining the effects on behaviour, development and survival (Freda and Dunson, 1985; Marco *et al.*, 1999; Miaud *et al.*, 2011). These types of studies provide information on the tolerance range of individual species for a particular aspect of water chemistry, which can be used to compare with values in the field to determine if poor water quality is likely to be limiting the distribution of that species (Rouse *et al.*, 1999). However, because interactions among different aspects of water chemistry in natural systems can alter their impacts on aquatic species,

testing the impact of one water quality parameter in isolation may not reflect accurately the impact that it is having on wild populations. Even in slightly more natural conditions, such as mesocosm experimental design using pools inoculated with water from a natural source, manipulating water quality parameters can have unexpected effects on a study species which conflict with trends observed in natural systems (Egea-Serrano and Tejedo, 2014). A more common approach which addresses this problem is to measure a range of water quality parameters in the field, then look for correlations between these and occupancy, abundance or breeding site use of aquatic species (Glooschenko *et al.*, 1992; Healey *et al.*, 1997; Gustafson *et al.*, 2009; Keitzer *et al.*, 2013; Grant *et al.*, 2014). While these studies provide a way of identifying aspects of water chemistry that may be unsuitable for aquatic species, they are less suitable for eliminating poor water quality as a potential cause of population declines and range contractions. This is because of the complex nature of water chemistry; it is not possible to measure all aspects, so a subset of parameters is selected. If no correlations between occupancy or abundance of a species and the water quality variables measured are found, this does not conclusively show that water chemistry is not restricting the distribution of that species; perhaps there is a water quality problem that has not been captured by the variables measured in the study.

While these approaches have merit in developing an understanding of the effect of different aspects of water chemistry on aquatic species, an alternative approach is needed in cases where conservation outcomes rely heavily on determining whether or not water quality at a particular site is suitable for the species being managed. The technique of using aquatic species as bioindicators, by exposing embryos or larvae to water collected from the field and monitoring the effect on development and growth, is commonly used in the area of water quality monitoring for environmental and human health (Herkovits *et al.*, 1996; Hallare *et al.*, 2005). We suggest that this method could also be highly applicable to the management of threatened aquatic species for conservation, and provide a robust way of determining whether water quality

is suitable at specific locations. We also propose that adding a second component could further enhance this approach: following a conventional *ex situ* component, in which individuals can be closely observed during the earlier, more vulnerable life stages, with an *in situ* experiment in which individuals are placed in enclosures in the water bodies of interest would allow indirect effects of poor water quality to be incorporated. These include interactions between water quality parameters and other organisms at the site, which can have a range of effects such as influencing the prevalence of pathogenic diseases (Stockwell *et al.*, 2015) and abundances of parasites (McKenzie, 2007; King *et al.*, 2010). Through these processes and others, these interactions can affect the development, survival and distribution of wild populations.

The effectiveness of this approach was tested as a method of determining whether poor water quality is a cause of the low pond occupancy by tadpoles observed in a population of the threatened green and golden bell frog *Litoria aurea* in Sydney, Australia. This species began experiencing large-scale declines in the 1970s (Mahony, 1996; White and Pyke, 2008) and as a result it is now listed as endangered in NSW under the *Threatened Species Conservation Act 1995* and as vulnerable nationally under the *Environmental Protection and Biodiversity Conservation Act 2000*. Factors suggested to have played a role in these declines include infection by the amphibian chytrid fungus *Batrachochytrium dendrobatidis*, habitat alteration and predation by the eastern mosquitofish which was introduced to Australia in the 1920s (Mahony *et al.*, 2013), but little is known about the water quality requirements of this species.

Survival of embryos and tadpoles has been identified as a factor strongly influencing population viability of *L. aurea* (Pickett *et al.*, 2016), making the identification of factors that reduce survival at this life stage particularly important for its conservation. Captive-bred embryos were first exposed in captivity to water collected either from ponds that were occupied by tadpoles or metamorphs during the past four years, or ponds where no tadpoles and metamorphs had been detected during this time. Tadpoles were then

placed in enclosures within the ponds themselves. It was hypothesized that if poor water quality is restricting tadpole distribution and pond occupancy, then survival and body condition would both be lower, and development slower, in individuals that develop in water where successful breeding does not naturally occur, while similarities between the two pond types would suggest that recruitment is not being constrained by water chemistry at the site.

METHODS

Study site

This study took place at one of the three primary bell frog habitat precincts at Sydney Olympic Park, located in south-eastern Australia. Referred to as Kronos Hill/Wentworth Common, the site consists of 37 ponds across a 40 ha corridor of land (Figure 1), most of which were constructed specifically as bell frog habitat during the 1990s (Darcovich and O'Meara, 2008; Pickett *et al.*, 2013). Before this, the land was used for a range of industrial purposes and landfilling, which resulted in leachate contamination that was addressed with a remediation programme (Darcovich and O'Meara, 2008). While bell frogs occupied most of the ponds shortly after their construction (27 of 33 available ponds in the 2000/2001 breeding season), occupancy has gradually declined, and by 2011/12 breeding season only seven of 37 available ponds were occupied. The number of ponds that are used for breeding varies greatly each year and is generally low (range 0 to 11 ponds). The factors that are currently restricting pond occupancy and breeding to a small subset of available water bodies are unknown.

Determining breeding and non-breeding ponds

All 37 ponds at the study site were surveyed for 4 years before the captive breed and release project started, to examine the distribution of bell frogs at different life stages across the site. Visual encounter surveys for frogs were conducted three times per year between November and February (Austral summer) from 2008 to 2012. This involved searching the emergent vegetation around

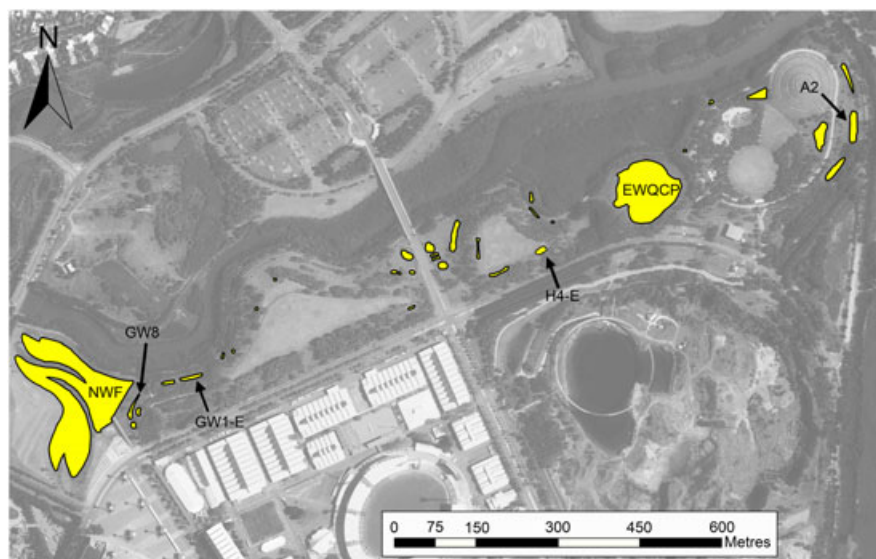


Figure 1. Aerial map of the study site, Kronos Hill/Wentworth Common, at Sydney Olympic Park, Australia. Bell frog habitat ponds are outlined in black and shaded yellow, and the six ponds that water was collected from and tadpoles were placed in are labelled. Ponds NWF, GW8 and EWQCP were classified as breeding ponds, and GW1-E, H4-E and A2 as non-breeding ponds.

the perimeter of each pond and recording the number of metamorphs (determined visually by the presence of at least one forelimb and a tail or tail stub) or fully developed bell frogs seen. Tadpole surveys were also conducted concurrently with each frog survey. These involved tying minnow traps baited with yellow glow sticks to the emergent vegetation in each pond before nightfall. The traps were checked for bell frog tadpoles the following morning. In addition, a dip net was swept repeatedly through each pond as another method of detecting bell frog tadpoles. The number of dip net sweeps and the number of traps set were both relative to pond size (range of 1–50 traps and 2–20 dip net sweeps per pond).

Using the data collected in bell frog and tadpole surveys, ponds at the study site were classified as breeding ponds if either tadpoles or metamorphs were detected at least once during the 4 years of sampling. Ponds where the species was not detected at all, or where only fully developed frogs (not tadpoles or metamorphs) were found, were classified as non-breeding ponds. Three breeding ponds (named NWF, GW8 and EWQCP) and three non-breeding ponds (named GW1-E, H4-E and A2) were then selected as treatments in this study (Figure 1). This choice was guided by the need to include ponds that were distributed

relatively evenly across the site, did not become dry during periods of drought, and were not adjacent to public paths so that equipment was less likely to be moved or vandalized.

Vegetation communities were similar across the six study ponds and included sedges (*Schoenoplectus validus*, *Baumea articulata* and *Bolboschoenus fluviatilis*), rushes (*Juncus* sp.), common reed (*Phragmites australis*) and water-plantain (*Alisma plantago-aquatica*). All ponds had a mixture of open water and emergent vegetation, with the proportion of vegetation cover ranging from approximately 20% to 90%. Ponds ranged in surface area from 109 to 22 085 m² (Figure 1). The shallowest pond had a maximum depth of 65 cm, and the deepest ponds reached depths of more than 150 cm in some places.

Captive breeding

Five male and five female bell frogs were captured in September and October 2012 from a pond at the study site and taken back to the University of Newcastle. They were placed in a breeding tank containing tap water that had been aged for one week before the frogs were introduced. Breeding occurred on one occasion in late October (day 0), with the production of at least 5900 eggs that may have been from multiple pairs of parents (a single

clutch usually contains from 2000 to 6000 eggs (Pyke and White, 2001)).

Ex situ study

After the eggs were laid, water was collected from the six ponds (three breeding, three non-breeding) at Kronos Hill/Wentworth Common. Four white plastic tubs (28 × 42 × 15 cm) were filled with 10 L of water from each pond; this was repeated with water from the breeding tub to act as a control, so 28 tubs in total were used. Approximately 50 eggs (range 42–61, maximum density of six individuals per litre; variation was caused by the thick gelatinous substance that holds bell frog egg masses together, making it difficult to separate the eggs without damaging them) were added to each tub within 24 hours of being deposited. The room in which tadpoles were held was lit with fluorescent ceiling lights between the hours of 7 am and 7 pm, and also received natural daylight owing to the presence of many skylights and windows. Air temperature was maintained between 20 and 30 °C for the duration of the study. The position of tubs in the room was randomized.

Embryos were observed daily and eggs that did not show any signs of development by day 4 were counted and removed. Tadpoles were fed approximately one quarter of a teaspoon of *Spirulina* powder (Morlife Pty Ltd, Arundel, Australia) per tub every 1 to 2 days, beginning on day 4, and the water in all tubs was replaced only once during the 18-day laboratory experiment, on day 12, using water from the same sources as when they were initially filled. Live embryos/tadpoles were counted on days 2, 4, 6, 9, 12 and 18. The discrepancies in numbers between counts were treated as deaths. The presence of algae that came from the ponds in some of the tubs made counting difficult, especially when the tadpoles were young, causing tadpoles to be missed on some occasions. In cases where more tadpoles were counted than had been detected in earlier surveys, the previous counts were increased to match later ones.

In situ study

On day 19, tadpoles were transported to Kronos Hill/Wentworth Common at Sydney Olympic

Park. Before release, hand-held callipers were used to measure the snout-vent length (SVL) and lateral width at the widest part of the abdomen of 10 tadpoles that were to be placed in each of the six ponds, and their gosner stage was also recorded. An average of 284 tadpoles (range 232–297) were released into 1 m³ baskets (aluminium frames with 2 mm gauge mesh on all six surfaces) partially submerged within each of the six ponds that water had been collected from for the *ex situ* study. These numbers were made up of half of the tadpoles remaining from the *ex situ* study that had previously been held in water from the same pond, with additional tadpoles that were raised in the breeding tank. The baskets were positioned so that one side rested on the bottom of the pond, allowing the sediment to enter through the mesh. They were placed at a depth of approximately 50 cm, so that the top half of the baskets protruded above the surface of the water. This accounted for potential increased water levels following rain, to ensure that tadpoles always had access to the surface to gulp air.

Once each week, half a cup of trout pellets (Ridley Aquafeed, Ridley Corporation, Melbourne, Australia) was added to each basket (this was done to supplement the tadpoles' diet, which consisted mainly of algae that grew inside the baskets). Tadpoles were captured using a hand net and counted every week, and the SVL and abdomen width of at least 40 tadpoles (sampled randomly from each basket, range 40–50) were measured. Once they had metamorphosed (determined by the presence of at least one front leg, stage 42 on Gosner's table (Gosner, 1960)), the metamorphs were released from the baskets into the ponds. The field study ended after 7 weeks, at which point any remaining tadpoles were released. Water temperature, dissolved oxygen, conductivity and salinity of the six ponds were measured at least every 2 weeks during the *in situ* study (on a total of six occasions), using a hand-held water quality meter (Model 556, YSI Incorporated, Ohio). Measurements were collected from three points within each pond.

Statistical analysis: *ex situ* study

A Cox proportional hazards model (all analysis was conducted in SAS /STAT version 12.3) was used to

examine whether tadpole survival differed between the three treatment conditions (raised in water collected from breeding or non-breeding ponds, or aged tap water). Variability between individual tubs was accounted for by including tub number in the model as a random effect. The ponds from which water was collected were nested within treatment type (breeding or non-breeding), which was included as a fixed effect in the model. This was done to test whether there was significant variability in the survival of tadpoles raised in water from different ponds within the same treatment, and would also have accounted for this if necessary.

Eggs that did not show any signs of development were labelled as 'unviable eggs', and analysed separately from eggs that did develop. To test whether there was a significant difference in the proportion of unviable eggs found in each of the three treatments, a mixed effects logistic regression model was used. Again, the pond that water was collected from was nested within treatment type, which was tested as a fixed effect, and tub number was included as a random effect.

Statistical analysis: in-situ study

To test whether survival differed for tadpoles placed in baskets within breeding and non-breeding ponds, a Cox proportional hazards model was again used. Pond was included as a random effect to account for variability between individual ponds, and treatment (breeding or non-breeding) was used as a fixed effect. Tadpoles that metamorphosed and were released before the conclusion of the study were removed for the analysis. Tadpole development was assessed using the same Cox proportional hazards model described above. Rather than measuring time to death, it was used to

measure time to metamorphosis by changing the event of interest, and tadpoles that died were removed. Tadpole body condition was examined by comparing abdomen width between tadpoles raised in breeding and non-breeding ponds using an ANCOVA, with treatment as a fixed effect. Body size variability was accounted for by including SVL as a second fixed effect, as was pond variability by including pond as a random effect.

RESULTS

Ex situ study

An average of $80.5\% \pm 1.3$ SE of the eggs that were added to tubs as part of the *ex situ* study showed signs of development, and were considered to be viable. The proportion of viable eggs was similar across all three treatments ($79.5\% \pm 1.7$ SE in water from breeding ponds, $81.8\% \pm 2.1$ in water from non-breeding ponds and $79.8\% \pm 3.8$ in aged tap water, Table 1), and no significant differences were found ($F(2, 20.6) = 0.47$, $P = 0.63$).

An average of $93.0\% \pm 3.8$ SE tadpoles survived to the end of the 18-day *ex situ* study. Average survival was also similar across the three treatments ($88.4\% \pm 8.5$ SE in water from breeding ponds, $96.2\% \pm 2.4$ in water from non-breeding ponds and $96.7\% \pm 2.4$ SE in aged tap water, Table 1). No significant differences in tadpole survival were found between any of the three types of water that tadpoles were raised in ($\chi^2(2) = 0.12$, $P = 0.94$).

In situ study

Tadpole survival was high for the duration of the *in situ* study, with an average of $94.7\% \pm 1.6$ SE of the

Table 1. The number of eggs added to water collected from ponds at the start of the *ex situ* experiment, eggs that showed signs of development and were considered viable, and tadpoles that survived to the end of the 18-day *ex situ* experiment

Treatment	Water source	Total number of eggs added	Number of eggs determined viable	Number of surviving tadpoles	Number of replicate tubs
Breeding pond water	Pond EWQCP	203	158	156	4
Breeding pond water	Pond GW8	199	155	107	4
Breeding pond water	Pond NWF	196	162	160	4
Non-breeding pond water	Pond A2	201	155	150	4
Non-breeding pond water	Pond GW1-E	194	166	165	4
Non-breeding pond water	Pond H4-E	213	177	166	4
Control	Aged tap water	195	156	151	4

Table 2. The number of tadpoles that were added to enclosures placed within ponds at the beginning of the *in situ* experiment and individuals that survived to the end of the *in situ* experiment

Treatment	Pond name	Total number of tadpoles added	Number of surviving individuals
Breeding pond	EWQCP	297	289
Breeding pond	GW8	232	216
Breeding pond	NWF	293	260
Non-breeding pond	A2	290	286
Non-breeding pond	GW1-E	295	272
Non-breeding pond	H4-E	295	290

tadpoles that were placed into baskets when they were 19 days old surviving to metamorphosis. Survival was similar for both treatments, at $93.0\% \pm 2.5$ SE for tadpoles held in breeding ponds, and $96.4\% \pm 2.1$ SE for those in non-breeding ponds (Table 2), and no significant difference was found ($\chi^2(1) = 2.5$, $P = 0.12$). There was also no significant difference in the time it took tadpoles to reach metamorphosis between those placed in breeding and non-breeding ponds ($\chi^2(1) = 0.45$, $P = 0.50$). All of the tadpoles that were examined to determine Gosner stage (10 from each basket) were at stage 25 when placed into the baskets. Metamorphs were first detected at two breeding and one non-breeding pond when the tadpoles were 46 days old (individuals may have begun to metamorphose from as early as 40 days, as no metamorphs were detected on the previous survey, which took place at 39 days). By 68 days, all the tadpoles held in baskets had metamorphosed, with the exception of 39 individuals that were released into the pond as tadpoles at that point (23 in GW1-E, 13 in H4-E and 3 in GW8). Body

condition did not differ between tadpoles held in breeding and non-breeding ponds ($t(4) = 0.68$, $P = 0.53$). The mean water temperature, dissolved oxygen, conductivity and salinity at each pond are shown in Table 3.

DISCUSSION

The purpose of this study was to test the approach of exposing embryos and tadpoles to water collected from breeding and non-breeding ponds and then placing them in enclosures within those ponds as a way of determining water quality suitability at a given site. This method was used successfully to establish that poor water quality was not restricting embryo and tadpole survival at the study site, and that this was not the cause of low pond occupancy by tadpoles. This has implications for habitat management at Kronos Hill/Wentworth Common; because the findings suggest that water quality is equally suitable between breeding and non-breeding ponds, resources can be directed to exploring other potential causes for the limited number of ponds in which successful breeding occurs, such as elevated numbers of tadpole predators, or adults choosing not to breed there (Bower *et al.*, 2013).

If this study had detected a problem with water quality at non-breeding ponds, an appropriate course of action would involve directing resources at further studies to determine which aspect of water chemistry was unsuitable, then at altering the management of existing ponds to attempt to improve water quality. If this method were to be used before a full-scale translocation or reintroduction project and found that water quality was not suitable, this would alert managers of the

Table 3. Mean water quality parameters (temperature, dissolved oxygen, conductivity and salinity) for the six ponds where water was collected for the *ex situ* study and that tadpoles were placed in for the *in situ* study, with standard error

Treatment	Pond	Water temperature ($^{\circ}\text{C}$)	Dissolved oxygen (mg L^{-1})	Conductivity ($\mu\text{S cm}^{-1}$)	Salinity (ppt)
Breeding pond	EWQCP	25.1 ± 0.5	4.28 ± 0.56	826.6 ± 12.2	0.4 ± 0
Breeding pond	GW8	22.5 ± 0.4	4.03 ± 0.61	984.3 ± 8	0.5 ± 0
Breeding pond	NWF	24.9 ± 0.4	4.66 ± 0.49	1031.4 ± 23.2	0.52 ± 0.01
Non-breeding pond	A2	24.9 ± 0.5	3.80 ± 0.57	889.6 ± 18.6	0.45 ± 0.01
Non-breeding pond	GW1-E	21.3 ± 0.4	2.32 ± 0.29	988.8 ± 33.9	0.51 ± 0.02
Non-breeding pond	H4-E	21.7 ± 0.3	2.23 ± 0.34	1011.9 ± 19.7	0.53 ± 0.01

need to select a different site or attempt to improve the water quality before large numbers were released. We suggest that this *ex situ* then *in situ* exposure method is effective in situations such as these, where the primary goal of research is to establish with as much certainty as possible that all aspects of water quality are suitable, and where the outcome of this can inform decision making for management. Although this study only involved one anuran species, this methodology could also be applied to any aquatic-breeding amphibian, as well as many fish.

The conclusion that the water chemistry at non-breeding ponds is suitable for bell frog embryos and tadpoles is based on the finding that mean survival was not lower at any life stage for individuals exposed to water from non-breeding ponds compared with breeding ponds, in both the *ex situ* and *in situ* components of the study. This suggests that the lack of successful recruitment at non-breeding ponds is not caused by embryos or tadpoles being unable to tolerate any aspect of water chemistry, and therefore dying before being detected in surveys. The highest rates of mortality occurred during the egg to early embryo stage, as some eggs did not show any signs of development, and eventually began to decompose. Unsuitable water chemistry can halt the development of amphibian embryos and prevent them from hatching successfully (Freda and Dunson, 1985). It is very unlikely, however, that this occurred in the current study, as the number of eggs that failed to develop was not significantly higher for water collected from non-breeding ponds than for water from ponds where successful breeding does occur, or aged tap water. It is more likely that the eggs were not fertilized; further studies are needed to determine if this is due to the breeding taking place in captivity, or if it is natural for a relatively large proportion (approximately 20%) of bell frog eggs to remain unfertilized.

The similarity between mean body condition and time to metamorphosis in breeding and non-breeding ponds strengthens the hypothesis that water quality at non-breeding ponds is suitable for bell frog tadpoles. Unsuitable water quality conditions that are not harmful enough to cause death can still have damaging effects on the health

of aquatic species, and prevent them from functioning normally (Hallare *et al.*, 2005; Miaud *et al.*, 2011). Delayed time to metamorphosis is a recognized response of amphibian larvae to poor water quality (Griffis-Kyle, 2007), as is reduced feeding activity (Marco *et al.*, 1999), which should cause affected individuals to have a lower body condition than those raised in suitable conditions. Another way that poor water quality could reduce the body condition and growth rate of tadpoles is by preventing the growth of algae, which is an important food source for tadpoles (although a small amount of trout pellets were fed to the tadpoles once a week in the field experiment, their diet consisted mostly of algae growing inside the enclosures). The finding that body condition and development were similar at breeding and non-breeding ponds suggests that neither process is occurring at the study site, adding further evidence that non-breeding ponds provide conditions that are suitable for the development of healthy bell frog tadpoles.

Previous approaches to determining if the water quality at a particular site is suitable for target species have largely involved measuring a subset of water quality parameters and comparing these either with the tolerance range of the same or similar species determined in laboratory experiments, or the presence or abundance of that species. If this method had instead been used, then a lack of any correlation would have had ambiguous implications. One possible explanation could be that no aspect of water quality was limiting tadpole survival. However, it would also be possible that a water quality parameter that was not measured was reducing tadpole survival, or that it was measured, but at a point in time when it was not at the extreme edge of its range, as many water quality parameters fluctuate greatly over time (Driscoll *et al.*, 1987; Vitt *et al.*, 1995). For this reason, the use of the method tested in this study is recommended in situations where the success of conservation efforts relies heavily on knowing that the water quality at a particular site is suitable for the species being managed, such as selecting potential sites for reintroduction, or creating new habitat ponds.

Although the cost of this type of study may be comparatively higher, this must be balanced against the risks associated with incorrectly believing water quality to be suitable. If the cost of captive breeding is too high, or the species being managed is difficult to breed in captivity, another option could be to collect eggs or larvae from the field, as Egea-Serrano and Tejedo (2014) did in a similar type of study where tadpoles were placed in enclosures at a number of field sites to examine the effects of pollution. In addition, if the water quality of non-breeding ponds had been found to be unsuitable, it would not have been possible to determine which aspect of water chemistry caused this without carrying out further studies. However, we argue that because of the large number of water quality parameters that exist, it is more cost-effective to establish first that there is a water quality problem, before looking for its cause. It should also be noted that fertilization and the early stages of cleavage were not included in the experiment, and that to include these stages it would be necessary for adults to be housed in enclosures with water collected from the sources of interest. This was not possible in the present study, which was limited by the number of adults available for captive breeding and the need to house all adults in a single enclosure to maximize the likelihood of a breeding event occurring.

Overall, the two-part exposure method was found to be an effective way of establishing that the water quality at the study site is suitable for bell frog embryos and tadpoles. The initial *ex situ* component allowed individuals to be closely observed during their most vulnerable life stage, and if any treatments were found to have adverse effects it would have been possible to intervene and remove them quickly. This is an important feature of study design for cases where threatened species are being bred for conservation purposes. Once no negative effects were found at this stage, the *in situ* component allowed a more comprehensive investigation of water quality, which ensured that interactions between water quality parameters and other organisms at the site did not cause unsuitable conditions for the study species. We believe that this method may be useful in understanding and managing other partly or

completely aquatic species, as a reliable way of establishing whether the water quality at a given site will allow them to survive and successfully complete their life cycle.

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Chapter 5: Trialling captive breeding and release methods for reversing declines in a wild frog population.

Declaration of contribution

I, Carla Pollard, am the primary author of this paper entitled “Trialling captive breed and release methods for reversing declines in a wild frog population”. I developed the concept of the study with assistance from other co-authors, managed the captive breeding program, collated existing data collected pre-2008 and undertook fieldwork to collect data from 2008 to 2014 as part of a team. I carried out all analyses and wrote the manuscript, which was then improved through review by my co-authors.

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Trialling captive breeding and release methods for reversing declines in a wild frog population

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ABSTRACT

The reintroduction of a species to formerly occupied sites is commonly used as a tool in conservation programs for a wide range of taxa, although it is expensive and has relatively low rates of success. Population supplementation, in which individuals are added to an existing vulnerable population to increase its viability, is an alternative strategy with benefits including fewer difficulties in building a large enough population to overcome problems associated with Allee effects and low genetic diversity, and less chance of failure due to unsuitable habitat quality. We trialled the use of population supplementation as a method of reversing declines in abundance and occupancy for a wild population of the threatened green and golden bell frog (*Litoria aurea*) in Sydney, Australia. We released over 13,000 tadpoles that were bred in captivity from adults that we temporarily removed from the site. Tadpoles were released into six ponds, three which were used as breeding sites by the wild population, and three that were not. The addition of these tadpoles increased bell frog abundance for approximately two years post release and naïve pond occupancy for at least three years, but did not increase population size, though it may have

slowed its decline. Modelled pond occupancy was stable and did not decline directly prior to the tadpole release unlike the other measures of population health, nor did it increase after the release. Our results indicate that poor habitat quality is unlikely to be responsible for the declines observed prior to the supplementation program, as increases in abundance were not higher at breeding than non-breeding ponds, and dispersal rates were high for all release ponds, regardless of their use by the wild population. This study shows that population supplementation can temporarily reverse or at least slow declines in wild populations of aquatic breeding amphibians, but is unlikely to provide long term benefits until the cause of declines can be determined and mitigated.

INRODUCTION

As human activities have threatened the persistence of an increasing number of species across the world in recent decades, management programs aimed at conserving them have become more frequently used (Balmford et al., 2005, Scott et al., 2010, Scott et al., 2005). The reintroduction of species that have undergone population declines into areas that they previously occupied is a widely used conservation tool that has been used across a range of taxa (Fischer and Lindenmayer, 2000, Griffith et al., 1989, Schultz et al., 2008, Germano and Bishop, 2009). Despite its frequent use, the value of reintroduction is widely debated, due largely to its relatively high expense in comparison to in situ management options (Snyder et al., 1996, Balmford et al., 2005) and estimated success rates of around 25 to 45% (Fischer and Lindenmayer, 2000, Griffith et al., 1989, Germano and Bishop, 2009). Impediments to success that have been identified include poor habitat quality at reintroduction sites, difficulty in obtaining enough individuals to form a functioning population and failure to address the threats that were responsible for the species becoming locally extinct in the first place (Germano and Bishop, 2009, Griffiths and Pavajeau, 2008, Fischer and Lindenmayer, 2000, Dodd and Seigel, 1991).

Population supplementation, in which individuals are released to an area where a wild population of the same species already exists, is a less commonly used alternative tool for managing threatened species (Fischer and Lindenmayer, 2000, Germano and Bishop, 2009). The goal of conservation-based supplementation projects is generally to increase the viability of vulnerable populations by increasing their size, the proportion of available habitat that is occupied, or enhancing genetic diversity (Champagnon et al., 2012). Due to its infrequent use compared with reintroductions, the success rate of population

supplementation in achieving these goals is less well understood (Champagnon et al., 2012, Fischer and Lindenmayer, 2000), however there is evidence that supplementation may be more beneficial in some regards. For example, while it can be challenging to obtain enough individuals with enough genetic diversity to successfully create a new self-sustaining population (Germano and Bishop, 2009), the presence of an existing wild population means that fewer individuals are required to create a large enough population to overcome obstacles such as Allee effects and low genetic diversity that can threaten population viability (Champagnon et al., 2012). In addition, the persistence of the species at the site of supplementation provides evidence that habitat quality is adequate for survival and decreases the likelihood of released animals failing to survive due to poor habitat quality (Wilson et al., 2011). Unlike reintroduction projects, the opportunity to take a proportion of the remaining population and use the offspring as the source of released individuals is available in supplementation projects, which removes the potential problems associated with using stock from a population that has been in captivity for multiple generations or from another wild population that is not locally adapted to the site (Allendorf et al., 2001, Robert, 2009, Wisely et al., 2008).

Despite these advantages, it has been well documented that population supplementation and other forms of ex situ conservation are unlikely to have long-term success if the threatening processes that led to population declines and extinctions are not determined and addressed (Griffiths and Pavaiseau, 2008, Seigel and Dodd, 2002, Scott et al., 2005). Ideally, supplementation should occur simultaneously with active in situ management aimed at improving the habitat quality at the release site, such as changing vegetation to improve foraging opportunities (Adamski and Witkowski, 2007). In cases

where the cause of population declines or extinctions remains unknown, the release of animals should be carried out in an experimental manner that allows this to be investigated, so that management can be improved in the future (Griffiths and Pavajeau, 2008). In this way, supplementation programs can potentially be used as a way of reducing the risk of extinction of vulnerable populations in the short term, while gathering information that can hopefully then be used to improve its viability in the long term (Zippel and Mendelson III, 2008).

We trialled this approach for a wild population of the threatened green and golden bell frog (*Litoria aurea*, hereafter bell frog) located in Sydney, Australia. Amphibians are generally regarded as good candidates for ex situ conservation as they often have high fecundity, offspring that don't require parental care, hard-wired physiology and behaviour and therefore do not require pre-release training, and small body size in comparison to other taxa (Bloxam and Tonge, 1995, Balmford et al., 1996). Although the bell frog conforms to these traits, all efforts to reintroduce the species to its former range have so far been unsuccessful at establishing self-sustaining populations. Reasons for this have been attributed to disease (Stockwell et al., 2008, Klop-Toker et al., 2016, Valdez et al., 2017), predation of tadpoles by exotic fish (Pyke et al., 2008), an inability to breed sufficient numbers for release (Pyke et al., 2008) and potential lack of nutritional resources required for breeding (Klop-Toker et al., 2016). No supplementation of existing bell frog populations has been reported to date.

We aimed to determine whether population supplementation could be used to successfully increase the size and distribution of a vulnerable bell frog population that had experienced recent declines in abundance and occupancy, and could therefore potentially

provide a more effective alternative conservation strategy than attempting to reintroduce extinct populations. We also aimed to determine if a reduction in habitat quality was the cause of these declines, by releasing individuals to both ponds that were used as breeding sites by the wild population and ponds that were not. We hypothesised that if this was the case, then there would be a greater increase in abundance at breeding ponds after the release of individuals than at non-breeding ponds. In addition, it would be expected that a greater portion of individuals released at non-breeding ponds would move away from the pond of release to seek more suitable habitat in comparison to those released at breeding ponds.

MATERIALS AND METHODS

Study species and site

The bell frog population at Sydney Olympic Park is one of the largest remaining in New South Wales (Department of Environment and Conservation, 2005), since widespread declines beginning in the 1970s lead to the extinction of the over 80% of populations in the state (White and Pyke, 1996, White and Pyke, 2008). This study took place at one of the three primary habitat precincts within Sydney Olympic Park, referred to as Haslam's Creek/Wentworth Common. It is a 40 ha corridor of land comprised largely of remediated grassland and open woodland, interspersed with 37 ponds that were mostly constructed specifically to provide bell frog habitat during the 1990s (Darcovich and O'Meara, 2008, Pickett et al., 2013). The number of ponds that were occupied by bell frogs was found to decline over time since standardised annual monitoring began in the 1999/2000 breeding

season, until the 2011/12 breeding season when naive occupancy reached its lowest point (Pollard et al., 2017a).

Captive breeding and release

Tadpoles were bred in captivity at the University of Newcastle, using adult frogs (9 females and 7 males) that were captured from the study site in September and October 2012.

Breeding occurred on two occasions, once in late October and once in late November 2012.

It is likely that each breeding event consisted of clutches from multiple pairs of parents, as five female and five male frogs were housed together in the breeding tank at a time.

Tadpoles were housed indoors and fed Spirulina powder (Morlife Pty Ltd, Arundel, Australia) (see Pollard and colleagues (2017a) for more details). When the first cohort of tadpoles was 19 days old, they were transported to the study site and released into six ponds: three where bell frog breeding had been detected at least once during the previous four breeding seasons (named NWF, GW8 and EWQCP), and three where breeding had not been detected during the previous four breeding seasons (named GW1-E, H4-E and A2). More details about pond selection are provided by Pollard and colleagues (2017a). A basket (1 m³, aluminium frame with 2 mm gauge mesh on all 6 surfaces) was placed partially submerged into each of the six ponds, and a proportion of the tadpoles (range 232 to 297) were placed into each basket. The remaining tadpoles were released freely into the six ponds (range 684 to 713 per pond). The total number of tadpoles from the first cohort released to the site was 5,960.

Tadpoles that were in the baskets were fed half a cup of trout pellets (Ridley Aquafeed, Ridley Corporation, Melbourne, Australia) per basket once a week to

supplement their diet, which consisted mainly of algae growing inside the baskets. Once they metamorphosed (determined by the presence of at least one front leg, stage 42 on Gosner's table (Gosner, 1960)), the metamorphs were marked using fluorescent visible implant elastomer (VIE) tags (Northwest Marine Technology, Shaw Island, USA), and then released into the pond. VIE tags were injected on the right side of the lower lateral ventral surface of the abdomen, following the method described by Bainbridge and colleagues (2015). A unique combination of colours was used for metamorphs from each of the six ponds.

The second cohort of tadpoles were released to same six ponds when they were 20 days old. All were released freely into ponds (range 1203 to 1204 per pond). The total number of tadpoles released from the second cohort was 7,223, bringing the total number of tadpoles released at Haslams Creek/Wentworth Common to 13,184. Adults that were captured for the breeding program were released by early January 2013.

Field surveys

Capture encounter surveys for bell frogs were carried out an average of 4 times per breeding season (range 2 to 10) between October and May (Austral Spring to Autumn) from 1999-2000 to 2015-2016. Each survey spanned a period of up to three consecutive nights. For the first two and last eight breeding seasons of the study period, all ponds at the site were surveyed. For the remaining seven breeding seasons (2001-2002 to 2007-2008), at least half of the total number of ponds were surveyed. Capture encounter surveys followed the method described by Pollard and colleagues (2017b). In the 2012-2013 breeding season, a UV flashlight (Northwest Marine Technology) was shone on the abdomen of captured

frogs, to determine if a VIE tag was present. The presence of VIE tags was not examined in subsequent breeding seasons because VIE tag retention rates for bell frogs are known to be low over a year post-marking (Bainbridge et al., 2015).

Mark-recapture surveys were carried out at four of the ponds at the site (including two release ponds, NWF and GW8) in six consecutive breeding seasons from 2009-2010 to 2015-2016. These ponds were selected because they generally support higher number of bell frogs compared with other ponds at the site, providing enough individuals to be captured to allow for population size to be estimated. One primary sampling event consisting of three to six secondary events on consecutive nights (each night represents an individual secondary sampling event) took place once each breeding season, in January (the first three seasons), February (the fourth and fifth seasons) or December (the sixth season). This sampling methodology conforms to the Pollock's robust design model, which allows for deaths, migration and recruitment to occur between primary sampling events (open population), but assumes that populations are closed to these processes between secondary sampling events within the same primary sampling event (Pollock, 1982, Kendall, 2001).

Mark-recapture surveys followed the same procedure as CE surveys. A Trovan LID-560ISO pocket reader was used to detect the presence of previously implanted passive integrated transponder (PIT) tags in captured frogs, and if none was present a PIT tag was implanted subcutaneously in the dorsal or dorso-lateral regions (Christy, 1996). In the 2009-2010 survey, only frogs that had a snout-vent length (SVL) of at least 45 mm were tagged. After this smaller PIT tags became available, which allowed frogs with an SVL of at least 35 mm to be tagged in the following five seasons.

Statistical analyses

To investigate how bell frog abundance changed over time at Haslams Creek/Wentworth Common, we used the R software system (version 3.3.0) to fit a negative binomial model with frog abundance (based on CE surveys) as the response variable and survey year as the primary predictor variable. The total search time of surveys and survey month were also included as additional predictor variables. The model selection process followed the method described by Pollard and colleagues (2017b).

Multi-season robust design occupancy modelling was carried out using program Mark (version 8.1) to investigate differences in pond occupancy over time. This design is similar to Pollock's robust design for population size estimation as it incorporates primary sampling events, between which the occupancy status of sites may change, and secondary sampling events, which are assumed to be closed to colonisation and extinction (MacKenzie et al., 2003). In the current study, each CE survey that took place during a single breeding season constituted a secondary survey, which together formed one primary sampling event per breeding season. In all candidate models occupancy (Ψ) was estimated directly for the first breeding season, and derived through the dynamic processes for subsequent breeding seasons. The colonisation (γ) and extinction (ϵ) probabilities, as well as detection probability (p), were held constant in some models and allowed to vary with time in others. Candidate models were ranked according to the Akaike information criterion corrected for small sample sizes (AICc), and the candidate model with the smallest AICc value was determined to be the best supported model (Burnham and Anderson, 2003).

The combined population size for the four ponds that had mark-recapture surveys was estimated using Pollock's robust design in program Mark. Parameters that were included in the models were apparent survival (ϕ), temporary emigration (γ), capture probability (p), recapture probability (c) and population size (N). The candidate model set included combinations where apparent survival, capture probability and recapture probability were constant and time varying. In some models, capture probability was made to equal recapture probability. Population size was time varying in all models, as the primary research focus was to investigate temporal changes in population size. Two types of temporary emigration were included in the models: γ'' , which is the probability that an individual will be away from the study site during a primary survey session, given that it was present during the previous primary survey session, and γ' , the probability that an individual will be away from the study site during a primary survey session, given that it was also absent during the previous primary survey session. Three treatments of the emigration parameters were included in the candidate models: $\gamma'' \neq \gamma'$ (representative of Markovian movement), $\gamma'' = \gamma'$ (representative of random movement) and $\gamma'' = \gamma' = 0$ (representative of no temporary emigration) (Kendall, 2001). Either or both types of temporary emigration were held constant in some models and time varying in others. The best supported model was once again determined using AICc values.

A before and after control impact (BACI) analysis was attempted using generalized linear models in Program R, to compare relative bell frog abundance at breeding and non-breeding ponds before and after the tadpole release. Due to the low numbers of frogs detected at the release ponds in CE surveys, data was too sparse to allow these models to

run successfully. The mean number of frogs detected in CE surveys at each release pond (\pm SE) is included in the results section, although no statistical comparison was possible.

RESULTS

Mean bell frog abundance varied significantly throughout the seventeen year study period ($P \leq 0.001$, Figure 1), and was highest at 1.6 frogs per pond (95% CI = 1.0-2.4) in the 2007-2008 breeding season. During the thirteen seasons before the tadpole release, mean abundance fluctuated continually with no apparent overall trend of increasing or decreasing, although it was lowest in the breeding season directly before the tadpole release (2011-2012), when it dropped to 0.2 frogs per pond (95% CI 0.1-0.3). In the first breeding season after the release, mean abundance increased to 0.8 frogs per pond (95% CI 0.6-1.1), and then steadily declined until it reached 0.2 frogs (95% CI 0.1-0.4) again in the final season of the study.

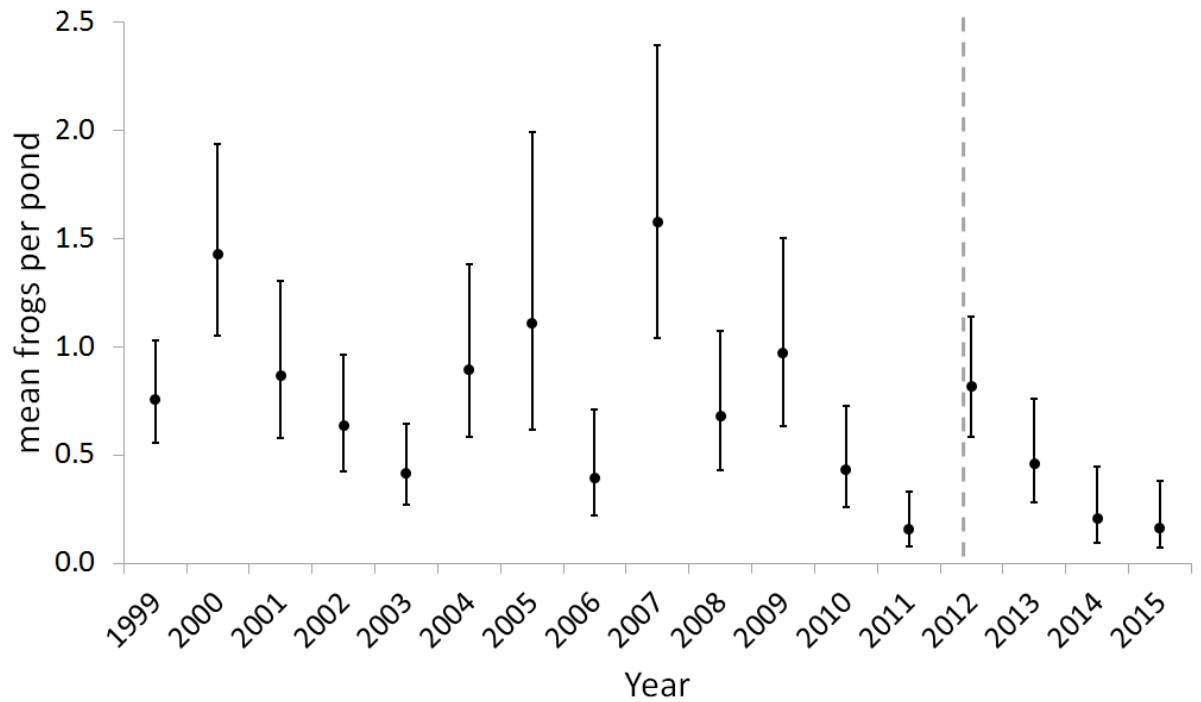


Figure 1. Mean number of bell frogs detected per pond during CE surveys, from the 1999/2000 breeding season (written as 1999) to the 2015/2016 breeding season (written as 2015). Error bars show 95% confidence intervals, the dotted grey line represents the tadpole release.

Occupancy estimates were obtained from the best fitting model which had extinction and colonisation probabilities constant, and detection probability varying between breeding seasons ($\Psi(t) \epsilon(.) \gamma(.) p(\text{season})$) and an AICc weight of 95%. Estimated occupancy was highest in the first breeding season of the study (1999-2000) with 86% of ponds occupied (95% CI 62-98%, Figure 2). Occupancy was estimated to gradually decrease from then, until it plateaued and remained between 53% (95% CI 43-64%) in 2008-2009 and 52% (95% CI 40-63%) in 2015-2016. The tadpole release had no apparent effect on modelled occupancy, which remained at 52% in the breeding seasons immediately before and after it was carried out. Naïve occupancy also shows an overall downward trend over time prior to the tadpole release, with the exception of increases that took place

between 2003-2004 (57%) and 2004-2005 (82%), and between 2006-2007 (46%) and 2007-2008(63%, Figure 2). In contrast with modelled occupancy, naïve occupancy increased from 13% in the season prior to the tadpole release to 54% in the season following it.

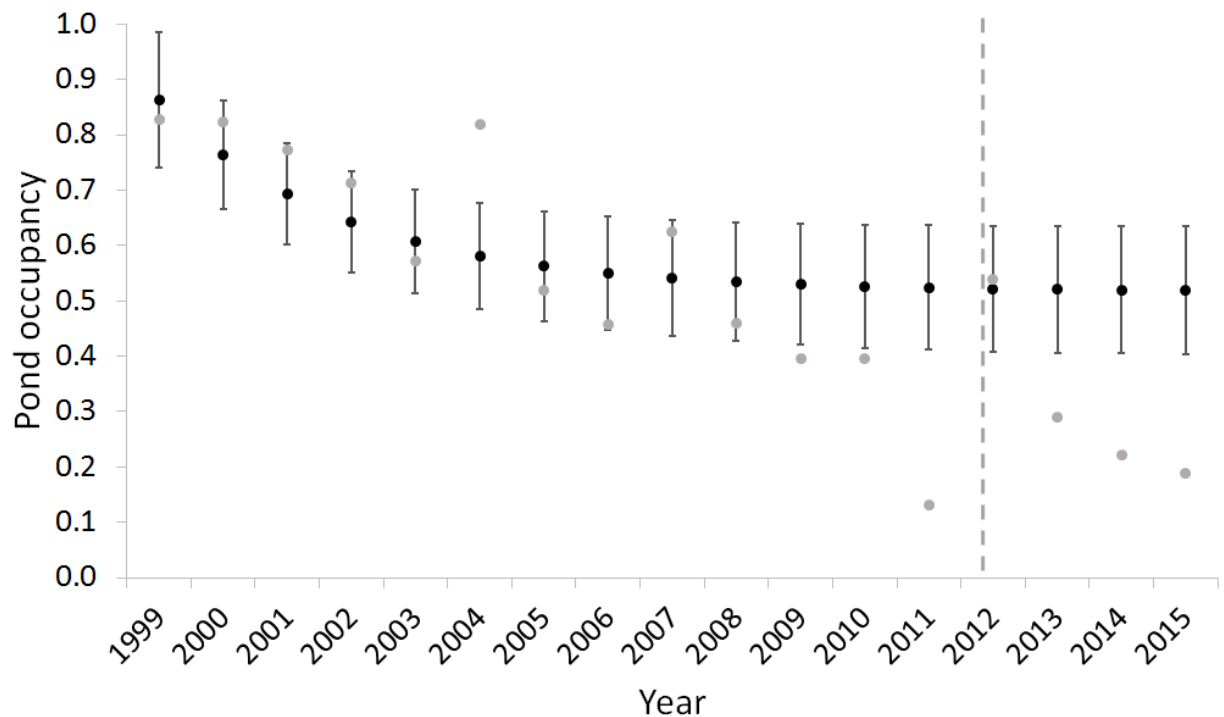


Figure 2. Modeled pond occupancy estimates (black) and naïve occupancy (grey) from the 1999/2000 breeding season (written as 1999) to the 2015/2016 breeding season (written as 2015). Error bars show 95% confidence intervals, the dotted grey line represents the tadpole release.

Population size estimates were obtained from the best fitting model which had apparent survival time varying, no temporary emigration, and capture and recapture probabilities equal and time varying ($\phi(t) \gamma'' = \gamma' = 0 \ c = p(t) \ N(t)$). The population was estimated to be 270 (95% CI 229-345) in the 2009-2010 breeding season, and then rose to peak at 361 frogs (95% CI 351-377) in 2010-2011 (Figure 3). In the season before the

tadpole release, it dropped to 166 frogs (95% CI 152-195), and it remained similar in the season following the release (178 frogs, 95% CI 165-204). It declined from that point to 107 (95% CI 97 to 129) in the next season and finally to 51 (95% CI 50 to 59) in the last season of monitoring.

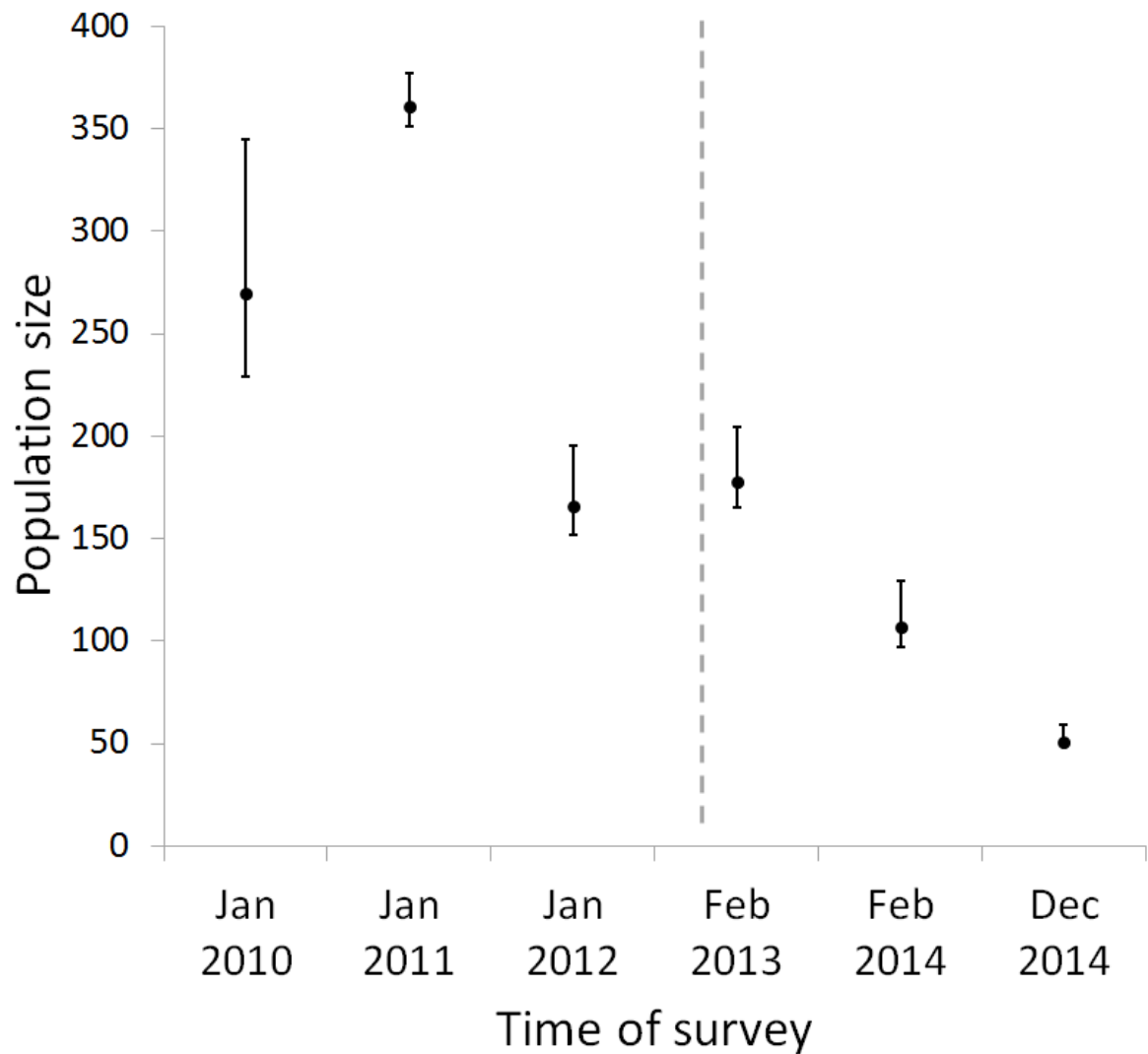


Figure 3. Estimated bell frog population size over 6 consecutive breeding seasons at the Northern Water Feature (NWF), Kronos Hill/Wentworth Common. Error bars show 95% confidence intervals, the dotted grey line represents the tadpole release.

Mean bell frog abundance was higher one season post-release than one season pre-release at one breeding pond (GW8) and all three non-breeding ponds (Figure 4). Of the other two breeding ponds, no frogs were found either pre- or post-release at one (EWQCP), and fewer frogs were found post-release than pre-release at the other (NWF). No frogs were found at any of the non-breeding ponds pre-release. Post-release mean abundance was relatively low across all six release ponds, ranging from 0 to 5.9 (± 2.2 SE) frogs per pond. The percentage of frogs with VIE tags that were found away from their release pond ranged from 31 to 63% at breeding ponds and 50 to 100% at non-breeding ponds (Table 1). When data was combined for all six release ponds, 57% of VIE tagged frogs were found away from their release pond. The post-release movement of VIE tagged frog across the site in the breeding season following the release is shown in Figure 5.

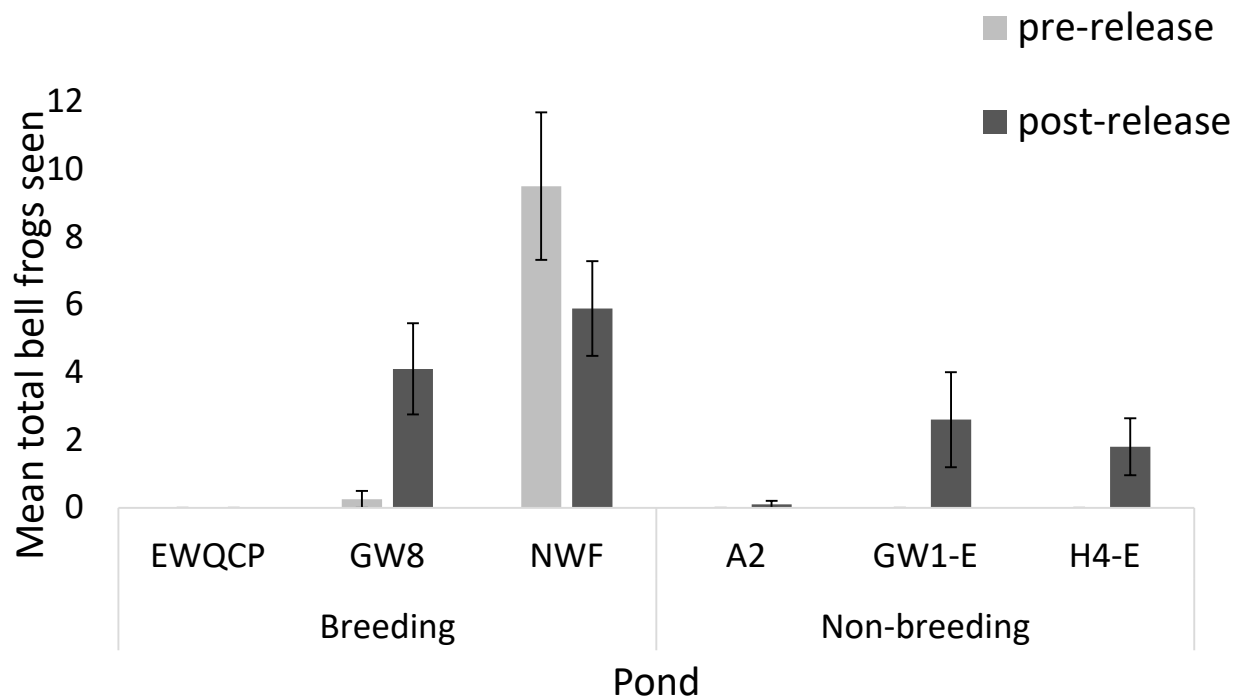


Figure 4. The mean number of total bell frogs detected during CE surveys one breeding season before (2011-2012) and one season after (2012-2013) the tadpole release, at the six release ponds. Error bars show standard error.

Table 1: The total number of captive bred frogs with VIE tags that were detected in the 2012-2013 breeding season either at their pond of release or at any other pond at Haslams Creek/Wentworth Common (away) during CE surveys.

Treatment	Pond	At release pond	Away	Proportion away	Total
breeding	EWQCP	0	0	-	0
	GW8	29	13	31%	42
	NWF	7	12	63%	19
non-breeding	A2	1	1	50%	2
	GW1-E	10	17	63%	27
	H4-E	0	20	100%	20

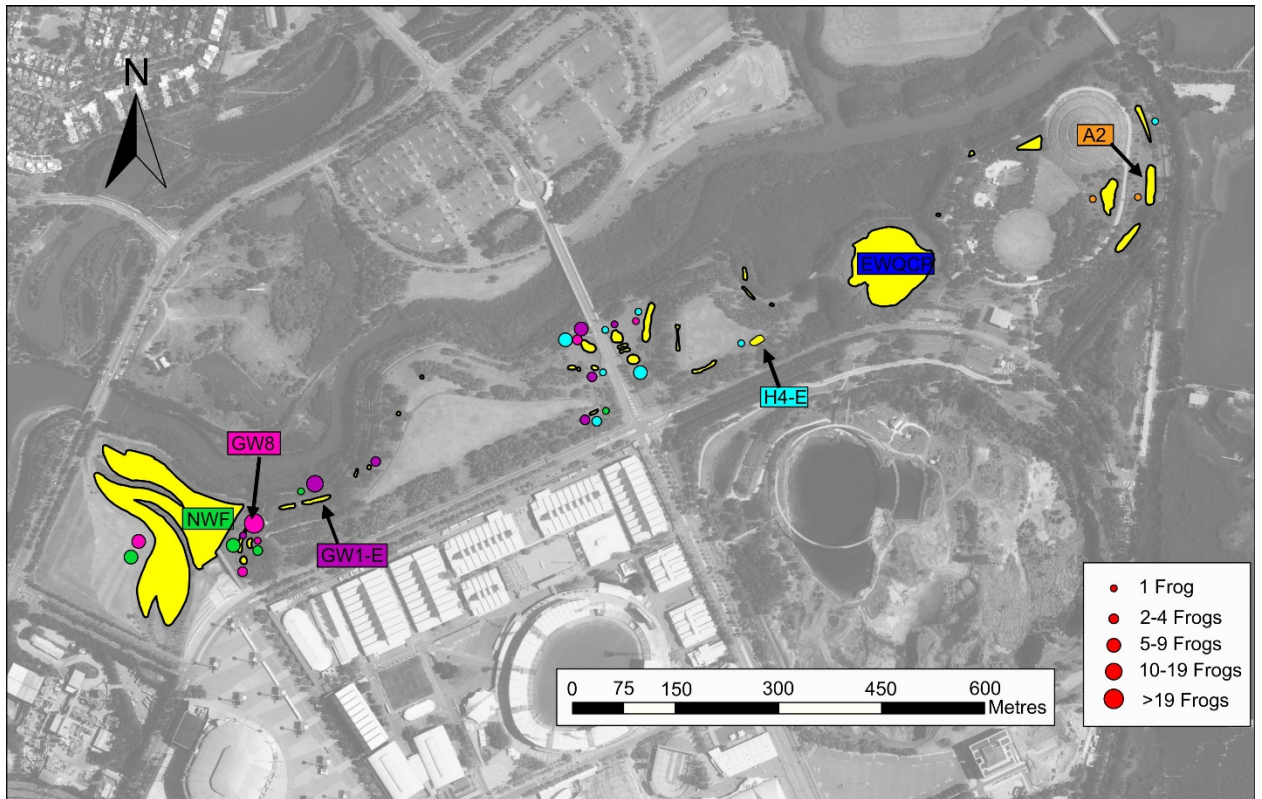


Figure 5. Map of the release site, Haslams Creek/Wentworth Common, showing the movement of released VIE tagged frogs in the breeding season immediately following the release. The six release ponds are labelled (breeding ponds: NWF, GW8 and EWQCP; non-breeding ponds: GW1-E, H4-E and A2), and each label has a different colour. The coloured circles adjacent to ponds depict the number of VIE tagged frogs from the release pond of the corresponding colour that were found there.

DISCUSSION

The action of releasing over 13,000 tadpoles to the site appeared to have a positive effect on bell frog abundance which continued to approximately 12 months (two breeding seasons) after the release. By approximately two years post release, abundance had declined and was similar to pre-release levels. It is however difficult to determine the extent to which changes in abundance can be attributed to the release, as abundance fluctuated greatly on an annual bases during the 13 seasons prior to the release. Modelled occupancy

initially declined at the site, but then plateaued and remained stable from around the 2008-2009 breeding season. This was in contrast with naïve occupancy, which generally declined gradually over time until it dropped rapidly from 39% of available ponds in 2010-2011 to 13% in 2011-2012, prompting the decision to undertake the supplementation project.

The tadpole release appears to have had a positive effect on naïve occupancy, which continued until the conclusion of this study, approximately three years post release. The difference in findings between modelled and naïve occupancy may be explained by changes in detection probability of the species over time, as seasons with low naïve occupancy corresponded with those with low abundance, suggesting bell frogs were still present at ponds during those seasons but may not have been detected due to low numbers. The tadpole release did not have a strong effect on population size at the group of four ponds that supports the highest number of bell frogs at the site. As was the case with abundance and naïve occupancy, population size dropped between the 2010-2011 and 2011-2012 breeding seasons. In the season immediately after the release, it remained similar, before dropping significantly the following two breeding seasons (1 and 2 years post release). It is however possible that the release may have slowed the decline in population size that was observed to have begun before it took place.

Our finding that releasing a large number of individuals can lead to short-term increases in the size and distribution of populations is in line with the outcomes of supplementation projects for other species. Adamski and Witkowski (2007) succeeded in increasing a population of Apollo butterflies (*Parnassius Apollo*) from 30 individuals to over 1,200, however this required annual release of additional individuals over an eleven year period. This process was still continuing at the conclusion of their study, and they

suggested that the population would begin to decline if releases ceased, based on its rate of reproduction. Similarly, Westemeier and colleagues (1998) reported that supplementing a small population of greater prairie chickens (*Tympanuchus cupido pinnatus*) increased reproductive success and in turn population size, but also stipulated that the ongoing addition of individuals from other populations would be necessary to maintain these benefits in the long term.

A number of factors could have contributed to our finding that most of the benefits associated with the supplementation of the bell frog population at Haslams Creek/Wentworth Common were not be maintained in the long term. High rates of dispersal away from the release site has been reported for relocated mammals (Maguire and Servheen, 1992, Fischer and Lindenmayer, 2000), birds (Skjelseth et al., 2007) and invertebrates (Adamski and Witkowski, 2007), and is identified as one of the most common causes of translocation failure in amphibians and reptiles (Germano and Bishop, 2009). We released individuals at the tadpole stage rather than post-metamorphosis to try to limit the drive to disperse (Germano and Bishop, 2009), however high dispersal rates of bell frogs that were released as tadpoles at another site has also been reported (Valdez et al., 2016). Keeping a portion of individuals within baskets at the release site until after they had metamorphosed did not prevent high rates of dispersal. However, a tendency for wild bred juvenile bell frogs to disperse from their natal ponds has also been detected previously at our study site (Bower et al., 2013). Further research is required to determine whether the levels of dispersal we observed were the result of being relocated as tadpoles, or a natural function of the bell frog's biology.

The number of individuals that are released also strongly affects the success of supplementation and reintroduction projects (Germano and Bishop, 2009). Semlitsch (2002) has suggested that 10,000 to 50,000 eggs should be released if the goal is to reach an adult population of 100 individuals for aquatic-breeding amphibians. The 13,000 tadpoles released in our study falls within this range, and is the largest release of bell frog tadpoles reported so far. Despite this, it is possible that larger numbers may be required to achieve greater increases in abundance and occupancy. Steffensen and colleagues (2010) found that of over 70,000 captive bred pallid sturgeon released into a wild population, only 1% were recaptured, demonstrating that releasing a very large number of an aquatic species can make a relatively small contribution to the population.

Conversely, releasing too many individuals can be detrimental if it increases the population to a point where it reaches the carrying capacity of the release site and resources become limiting, or the increase in density leads to increased transmission rates of disease. It is unlikely that the former occurred in the case of this study, as most of the release ponds had no or few bell frogs prior to the release, particularly in comparison to the numbers they supported historically. The latter is a more likely explanation for the decline in abundance and population observed one year after the release; the amphibian chytrid fungus is present at the release site (Penman et al., 2008) and has caused the decline or extinction of reintroduced bell frog populations (Stockwell et al., 2008, Klop-Toker et al., 2016). Although exotic fish are not present at the site, large numbers of native eels (*Anguilla australis* and *A. reinhardtii*) were observed at some of the ponds (particularly NWF and EWQCP). High rates of tadpole predation by eels may explain why no or very few metamorphs or juveniles were observed at some of the release ponds.

Our findings suggest that poor habitat quality is not the cause of declines in bell frog abundance, naïve occupancy and population size observed at the site prior to the supplementation project. If it was the cause, there should have been a larger increase in abundance at breeding than non-breeding ponds after the release, due to released individuals at those ponds having higher survival rates and lower dispersal rates. Instead, abundance only increased at one of the breeding ponds (GW8) in comparison to all three non-breeding ponds, and dispersal rates from all ponds were high. In addition, water quality at non-breeding ponds was found to be suitable for bell frog tadpole growth, development and survival (Pollard et al., 2017a). These combined findings suggest that another factor besides habitat quality is driving bell frog distribution at the site. Some amphibian species are known to base habitat selection on the presence of conspecifics rather than habitat attributes, which can decrease the costs of searching for suitable habitat (Rudolf and Rödel, 2005, Swanson et al., 2007). In a laboratory study, juvenile bell frogs were shown to be attracted to conspecifics (Pizzatto et al., 2016), which may explain the aggregated distribution observed in wild populations, where seemingly suitable ponds remain unoccupied. If this was the case, however, released frogs should have been more likely to remain at ponds that had high numbers of bell frogs prior to the release. Instead, the pond with the highest pre-release bell frog abundance, NWF, had the equal second highest rate of dispersal observed.

We found that breeding tadpoles in captivity and releasing them to a wild population was relatively successful in reversing or at least stalling declines in the short term (less than two years). The process of temporarily removing a small proportion (<20) of adults from the site to breed in captivity was not only ecologically beneficial as the

offspring were locally adapted to the site, but also cost effective as the captive colony was only held for four months. The costs of upkeep for a continually running a captive bell frog colony for reintroduction or supplementation projects are far higher than this, and to date have produced less offspring despite containing larger numbers of adults (Daly et al., 2008, Pyke et al., 2008). In order for the benefits of supplementation to remain on a long-term basis after release, it will be necessary to build a greater understanding of the causes of declines in remaining bell frog populations so that they can be mitigated. Our findings suggest that poor habitat quality is not responsible, and other potential causes should be the focus of future research. In the meantime, population supplementation is a relatively cost effective way of buying more time for vulnerable populations, although it is unlikely to offer a permanent solution.

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Conclusions

The combined findings of this thesis provide a rare example of the way that adaptive management can be used to simultaneously manage a threatened species using available information, while simultaneously using management actions as a series of experiments to test different hypotheses about its ecology to improve future management. Although only some actions tested were found to be effective in terms of meeting their conservation goals, all are valuable as they add to our understanding of important ecological traits such as habitat requirements, the factors driving habitat selection and the impact of threatening processes. The removal of gambusia had a strong positive effect for *L. aurea* conservation, as it greatly increased reproductive success. This was found to be due at least in part to an avoidance of ponds containing gambusia by calling males, which was surprising given that gambusia is an exotic species and therefore does not share an evolutionary past with *L. aurea*. These results suggest that gambusia removal is highly beneficial and should be prioritised in the management of remaining *L. aurea* populations that coexist with this invasive predator.

The addition of salt to ponds to raise salinity to 4 ppt was also found to be a beneficial action for *L. aurea* conservation, as it lowered the prevalence of infection by the amphibian chytrid fungus post metamorphosis and increased survival. It would therefore be a valuable method to include in management regimes for the majority of remaining populations which inhabit locations where chytrid is present, including Sydney Olympic Park. It could also potentially be used to improve the success of future reintroduction projects, as high levels of mortality caused by chytrid has been identified as the cause of failure of some previously attempted reintroductions. In contrast, the use of vegetation clearing and shade tree removal to imitate early successional habitat did not have a positive effect on bell frog abundance.

This suggests that it is not required to maintain habitat quality, at least in landscapes that are dominated by urban or industrial land uses that provide incidental disturbance, which applies to most of the *L. aurea* populations remaining in NSW. It would be more effective for management plans for these populations to focus on other strategies, such as gambusia control or chytrid mitigation, though vegetation removal may still be necessary in some cases to maintain pond function and prevent completely closed-canopy habitats from developing.

The release of over 13,000 tadpoles was moderately successful in reversing declines in naïve occupancy and abundance experienced at one of the three primary habitat precincts at Sydney Olympic Park, though these benefits were relatively short lived. Increases in abundance lasted less than two years post release, although increases in naïve occupancy remained to the end of the study period (at least three years). Population size declined within approximately one year. This action also allowed us to establish that unoccupied ponds provide conditions that support normal survival, growth and development of *L. aurea* tadpoles, and that poor water quality is not the cause of low pond occupancy by wild tadpoles at the site. In addition, we also found no evidence that ponds that are not used for breeding at the site do not provide suitable conditions for *L. aurea* after metamorphosis. Further research is required to determine the cause of declines in occupancy and abundance of the species at the site, and to explain its current distribution. Until this occurs, attempts to increase abundance and occupancy by population supplementation are unlikely to be successful in the long term.

The research presented in this thesis has applications for the management of *L. aurea* at Sydney Olympic Park and at other sites which face similar threats. It will hopefully provide useful information for those involved in managing these populations, that can be used to

assist in the prioritisation of actions for conservation. It also provides a rare example of the practical application of adaptive management to meet both conservation and research goals in the management of a threatened species. Previous studies exploring the effectiveness of adaptive management in threatened species conservation have focused mainly on structured decision making using predictive models. The findings of this thesis demonstrate that the other components of adaptive management, particularly the iterative phase, can also be successfully applied to this purpose, and provide a framework for doing so that could be informative for a wide range of threatened species.

Appendix: List of publications related to thesis published during candidature

Bower, D. S., Pickett, E. J., Stockwell, M. P., Pollard, C. J., Garnham, J. I., Sanders, M. R., Clulow, J. & Mahony, M. J. (2014). Evaluating monitoring methods to guide adaptive management of a threatened amphibian (*Litoria aurea*). *Ecology and evolution*, 4(8), 1361-1368.

Bower, D. S., Stockwell, M. P., Pollard, C. J., Pickett, E. J., Garnham, J. I., Clulow, J., & Mahony, M. J. (2013). Life stage specific variation in the occupancy of ponds by *Litoria aurea*, a threatened amphibian. *Austral Ecology*, 38(5), 543-547.

Garnham, J. I., Stockwell, M. P., Pollard, C. J., Pickett, E. J., Bower, D. S., Clulow, J., & Mahony, M. J. (2015). Winter microhabitat selection of a threatened pond amphibian in constructed urban wetlands. *Austral ecology*, 40(7), 816-826.

Mahony, M. J., Hamer, A. J., Pickett, E. J., McKenzie, D. J., Stockwell, M. P., Garnham, J. I., Keely, C. C., Deboo, M. L., O'Meara, J., Pollard, C. J., Clulow, S., Lemckert, F. L., Bower, D. S., Clulow, J. (2013). Identifying conservation and research priorities in the face of uncertainty: a review of the threatened bell frog complex in eastern Australia. *Herpetological Conservation and Biology*, 8(3), 519-538.

Pickett, E. J., Stockwell, M. P., Bower, D. S., Garnham, J. I., Pollard, C. J., Clulow, J., & Mahony, M. J. (2013). Achieving no net loss in habitat offset of a threatened frog required high offset ratio and intensive monitoring. *Biological Conservation*, 157, 156-162.

Pickett, E. J., Stockwell, M. P., Bower, D. S., Pollard, C. J., Garnham, J. I., Clulow, J., & Mahony, M. J. (2014). Six-year demographic study reveals threat of stochastic extinction for remnant populations of a threatened amphibian. *Austral Ecology*, 39(2), 244-253.

Pickett, E. J., Stockwell, M. P., Pollard, C. J., Garnham, J. I., Clulow, J., & Mahony, M. J. (2012). Estimates of sex ratio require the incorporation of unequal catchability between sexes. *Wildlife Research*, 39(4), 350-354.