Investigating and integrating animal behaviour in the conservation and management of an endangered amphibian

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Statement of Originality

I hereby certify that the work embodied in this thesis is my own work, conducted under normal supervision. This thesis contains no material that has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution, and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

Signed:

Melanie Sandra James

Date 26/3/2018

And last but by no means least, thank you to the frogs. You were great.

Sometimes a little cranky, but great.

Contents

After frog calls were recorded, we used Raven Pro Interactive Sound Analysis Software (CLO 2014) to edit the calls. The aim of call editing was to make a mp3 file with 10 minutes of calling which represented different call types. Using Ford (1989) definitions of call types; A) grunts and B) drone call types, we used the cut function ensured different call types were represented in a single recording. The final file had 10 minutes of consistent calling. This calling was repeated 5 times to form a 50-minute sound file. A 10 minute stretch of silence was then introduced after the 50 minutes of looped calling to prevent habituation to calling. A small mp3 player with an USB charging port and audio jack (female) input was loaded with the 50 minute call file and was programmed to repeat.

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Chapter 1.   Overview

1.1 Summary

The Earth is experiencing a period of mass extinction due to human development and expansion (Wake & Vredenburg 2008). It has been estimated that 866 animal, plant, fungi and protist species have become extinct in recent history, and 25,821 species were declared either Critically Endangered, Endangered or Vulnerable in 2017 (IUCN 2017). Causal agents of population declines and biodiversity loss include climate change, land clearing, habitat modification and the introduction of exotic competitor or predator species (Vitousek *et al.* 1997) and disease (Skerratt *et al.* 2007) which affect species from global to local scales. The magnitude of species loss and threat of further extinctions has caused worldwide attention, instigating efforts to identify and conserve species at risk (Redford & Richter 1999). Species management programs typically aim to identify causal agents of decline, assess species requirements for survival and reproduction and understand population processes so that informed decisions can be made to reverse population declines. An important step in this process is gaining an understanding of the factors which affect species distribution (Guisan *et al.* 2013; Noss *et al.* 1997).

Conservation programs often aim to understand an animal's distribution by identifying what constitutes habitat. Factors commonly examined include abiotic and biotic attributes of the landscape including available shelter and food, as well as an animal's interaction with heterospecifics (Campomizzi *et al.* 2008). In the instance that these factors or interactions correlate with species presence or abundance either positively or negatively, it is assumed that these factors are actively selected for or avoided (Batt 1992). However, additional behavioural factors can affect distribution, such as attraction to (Ahlering *et al.* 2010) or avoidance of conspecifics (same species) (Keren-Rotem *et al.* 2006; Stamps 1983), causing strong aggregations or segregation of animal distribution over a landscape, respectively. Despite the influence of these factors on distribution, conspecific attraction and avoidance are not commonly considered by conservation programs when attempting to understand, predict and alter species distributions (Campomizzi *et al.* 2008). As animals experiencing conspecific attraction or avoidance may deviate from the correlation model assumed by habitat selection, research programs aimed at assisting endangered species cannot afford to ignore conspecific interactions (Manly *et al.* 2009).

A last resort for conservation initiatives is breeding animals in captivity, creating or restoring habitat and translocating animals back into populations that are experiencing population decline or have become locally extinct. Current research in conservation biology has focused on identifying and assessing animal behaviour which can limit the success of conservation initiatives such as; multi-spatial-level habitat selection (McGarigal *et al.* 2016), conspecific attraction (Campomizzi *et al.* 2008) and mate selection within captive breeding (Chargé *et al.* 2014a; Chargé *et al.* 2014b). As these factors influence species distribution and survival, they therefore affect the success of habitat construction programmes and the persistence of naturally occurring or translocated populations.

Amphibians are a globally threatened taxon with 33 extinct species and 2,100 species declared either critically endangered, endangered or vulnerable (IUCN 2017). Factors causing amphibian decline include the human facilitated spread of chytrid fungus (*Batrachochytrium dendrobatidis*) (Skerratt *et al.* 2007), global climate change, introduced species as well as habitat loss and modification (Brown *et al.* 2012; Stuart *et al.* 2004). Considerable research has been undertaken on causal agents of decline, along with understanding population processes and habitat requirements that affect the persistence of populations (Wake & Vredenburg 2008). Despite the fact that many amphibian species show signs of conspecific attraction and/or avoidance, the influence of conspecific interactions on spatial distribution and subsequent declines of amphibians is under-investigated.

This current research project explores the potential for particular behaviours which may influence species distribution and the success of habitat creation and translocation programmes for the green and golden bell frog (*Litoria aurea*).

For the first research paper, I assessed conspecific call attraction in *L. aurea*. Over a landscape, animal distributions can be skewed as a result of conspecific attraction and aggregation. This can hinder habitat restoration and creation programmes as species may fail to colonise available habitat, despite its suitability. It has been noted from past research that *L. aurea* uses particular habitat and has distributional traits which suggest the presence of conspecific attraction, and using speakers playing calls can successfully attracted *L. aurea* at short distances, forming new aggregations (James *et al.* 2015: Attachment 1). In the first research chapter, I aimed to use speaker systems playing calls to manipulate the landscape distribution of *L. aurea*. I placed a stand with a speaker playing call broadcast in a treatment waterbody (T), a stand with no calls broadcasted as a manipulative control (MC) and no stand

or speakers as a control (C). This design was replicated in five areas on Kooragang Island, Australia, and waterbodies were surveyed to measure changes in abundance and calling over two and a half breeding seasons. We found that speaker introduction did not increase abundance or calling at T relative to MC and C. We did, however, find that the length of time males called was longer at T, compared to MC and C. As the length of calling time may be extended using conspecific call broadcast , provision of conspecific stimulation at translocation sites may improve breeding activity and retention of the population post-release by reducing dispersal.

For the second research chapter, I assessed habitat selection of *L. aurea*. The site selection of breeding individuals is a crucial component of a species habitat selection and can help to direct conservation programmes. However, very little is known about the microhabitat selection of calling male *L. aurea*. This study aimed to distinguish if male aggregations are associated with specific habitat features within a waterbody and describe their use of available habitat structures. Within waterbodies we compared calling locations relative to non-calling locations for water variables (temperature, salinity, dissolved oxygen), microclimate (temperature, humidity, average and maximum wind speed) and habitat (percentage coverage of water, ground, emergent vegetation and floating vegetation). Overall, males were associated with lower salinity and higher dissolved oxygen, higher percentage coverage of emergent vegetation and bare ground, and low percentage coverage of open water. Males were most commonly found in the water floating between or beside emergent vegetation or perched on emergent vegetation above water level. This suggests that males may select habitat to protect themselves from predators, or for breeding; providing appropriate vegetation, dissolved oxygen and salinity for embryo and tadpole development. This provides supportive information for previous studies on habitat selection, indicating what habitat is preferred by breeding males to improve monitoring, habitat creation and rehabilitation.

For the third research chapter, I assess a habitat construction programme. Habitat creation programmes are often used to compensate for the loss of habitat for endangered species, with varying results. I describe an early stage wetland construction programme implemented for *L. aurea* on Ash Island, NSW Australia. Seven ephemeral (flooding) and two permanent waterbodies were constructed near an existing population. The wetland was designed to increase landscape aquatic habitat, based on adaptive management learnings from past

research. In this study, I assess the initial use of this habitat by *L. aurea*, and initial findings on the design suitability. Surveys in constructed wetlands and in the broader Kooragang area showed that *L. aurea* rapidly colonised and called at constructed ephemeral wetlands but not permanent wetlands. The chorus size in constructed wetlands was large in comparison to other populations in coastal NSW, and a range of other frog species also bred onsite. Female *L. aurea* used a nearby remnant wetland (adjacent to the constructed wetlands), and used different habitat to males. Similar habitat use variation between sexes was reflected in the broader population. Most male and female *L. aurea* captured on Ash Island were under 12 months of age, and body condition in the constructed wetlands was higher than in the broader population. Waterbody design successfully protected waterbodies from overland flooding, and ephemeral waterbodies dried, which suggests the drying regime may protect the constructed habitat long-term from infestation of predatory fish. Elevated salinity from ground water in permanent waterbodies (intended to ameliorate chytrid disease in the landscape) was higher than anticipated and requires further monitoring. It is hoped that this programme may help guide other conservation projects creating habitat for amphibians under threat.

For the fourth research paper, I assess sexual selection in *L. aurea*. As a conservation strategy for *L. aurea*, captive breeding programmes supplement at-risk populations and translocate individuals to their former ranges. However, breeding programmes are undertaken with very little information on sexual selection and its exclusion can reduce the fitness of released animals. The aim of the fourth study was to assess whether forms of sexual selection occur for *L. aurea* to inform captive breeding programmes. In the wild I studied mate selection. Firstly, we aimed to assess if the size and body condition of amplexing individuals (grasping to breed), differed from other individuals in the population as an indication of female sexual selection or male-male competition. Secondly, we investigated if male and female amplexing pairs were size correlated as an indicator of size assortative mating, and thirdly we made observations on behavioural interactions in the breeding waterbody to complement the analysis. In Whangarei, New Zealand, we captured *L. aurea* over 4 survey nights, undertaking capture-mark-recapture and measuring morphometrics of snout vent length (SVL), right tibia length (RTL) and weight, calculated body condition. We compared the SVL, RTL and weight of breeding individuals to non-breeding individuals and found that amplexing males were larger with better body condition, however, female size did not differ. Male-female pairs were not size assortative and aggressive interactions were recorded

between males. Larger male size may be an indicator of either female selectivity or larger-male mating advantage through aggressive interactions. As removal of sexual selection in captive breeding programmes can reduce fitness and place conservation initiatives at risk, I recommend incorporating sexual selection by placing multiple males of varying sizes in breeding tanks with females to facilitate female selectivity or larger-male mating advantage.

Based on the results of the current studies, I have identified possible constraints on the use of conspecific attraction for this species, and also recognised its potential use in translocations programmes to improve project outcomes. As a result of microhabitat assessment, habitat creation and management programmes can use specific parameters to design, maintain and monitor habitat for calling males. Assessment of a habitat construction project designed from previous research recommendations shows initial project success and provides information to refine future habitat construction programmes. Finally, assessment of sexual selection in *L. aurea* provides vital information to conservation programmes breeding animals for translocation to work toward improving the fitness of released individuals. Overall, the current study provides key aspects of *L. aurea*'s biology and ecology that have not been clearly addressed in the literature and aims to improve conservation efforts. In light of recent extinctions and increasing pressures on wildlife, continued research on key threatening processes and behavioural ecology is crucial to help guide conservation.

1.2 Preamble

This thesis is presented as a series of chapters, targeting key gaps in knowledge for *L. aurea* conservation programmes.

Brief description of the thesis chapters:

1. **Overview**

A brief synthesis of the thesis

2. **Introduction**

The introduction chapter is a critical review, synthesising key topics pertinent to the research chapters. The introduction discuses factors which influence species distribution and the role animal behaviour plays in distribution and reproduction. The introduction then addresses potential hazards when neglecting behavioural traits in conservation programmes and possible ways that behavioural traits can be utilised for the management and conservation of endangered species. This thesis aims to provide information to the management and conservation of an endangered amphibian, the green and golden bell frog (*Litoria aurea*). Gaps in knowledge are discussed and topics for the focus of the thesis are then described, including; conspecific attraction, micro-habitat selection of calling males, a habitat construction programme and mating patterns.

3. **Can conspecific call playback change amphibian distribution? Building from localised distribution manipulation to a larger scale: a continuing case**

Species distribution is defined by both habitat and the social/competitive interactions between animals. For *L. aurea*, distribution is not solely defined by habitat, but is also affected by conspecific attraction, where landscape distribution is skewed as animal's aggregate. The calls of conspecifics played from speakers have been used to redistribute within-waterbody distribution of *L. aurea*. Chapter 3 attempts to build up from within-waterbody redistribution to a landscape scale redistribution. If this is possible, species managers may use speakers to redistribute animals to waterbodies which may improve survival and reproductive success for conservation.

4. **Microhabitat selection and temporal changes in calling behaviour of male green and golden bell frogs (*Litoria aurea*) in N.S.W. Australia**

Although species distribution is defined by both habitat and the social/competitive interactions between animals, these are not mutually exclusive. Conservation programs often focus on large scale habitat selection, however, large scale occupancy studies can miss key components of habitat if microhabitat is not addressed adequately. We assess the microhabitat use of calling male *L. aurea* aggregations, to determine if chorus locations formed by conspecific attraction correlate with particular habitat variables such as water quality, climate and vegetation. Finding key selected habitat components within breeding habitat can help refine occupancy studies and habitat creation programmes.

5. **Rapid colonisation of constructed waterbodies by the endangered green and golden bell frog (*Litoria aurea*) and five other frog species**

Litoria aurea has been the focus of numerous habitat creation programmes with limited success. Based on extensive research and conservation efforts in the last 45 years, a considerable amount of information has been obtained on the habitat requirements and potential novel protection methods for *L. aurea*. This chapter details the design process for a habitat creation programme based off this research and details the first year of monitoring, assessing colonisation success.

6. **Larger males get the girls: mating patterns help direct captive breeding strategies for the endangered green and golden bell frog (*Litoria aurea*)**

Captive breeding programmes are used to breed animals for translocation programmes, where they supplement small populations with new individuals or reintroduce the species into its former range or created habitat. Many of these programmes operate with little understanding of key information on breeding behaviour that can limit programme quality through reduced animal fitness, such as sexual selection. This study assesses the mating patterns of *L. aurea* in a rarely studied breeding event to inform captive breeding programs contributing to supplementation, reintroduction and habitat creation programmes.

7. **Research synthesis and conclusions**

This chapter synthesises the findings of the four papers to assess the impact of the thesis in the overall context of endangered species management, with recognition of the research needed to continue behavioural investigations for conservation.

Chapter 2.   Introduction

2.1 Factors influencing distribution and abundance and ecological theories

2.1.1 Habitat and species distribution

The distribution of a species is determined by a complex variety of factors, which interact to drive spatial occupancy and abundance. Spatially, organisms live in areas that will support their growth, survival and reproduction, where the sum of all tolerable conditions and resources forms their fundamental niche (Krebs 2009). Suitable habitat is often patchy, for example, the occurrence of discrete patches of wetlands or other water repositories constrain the distribution of many aquatic species (Wassens *et al.* 2010). Within such areas, distribution can be further constrained by specific conditions such as water chemistry (Bridges & Semlitsch 2000; Haramura 2008; Rudolf & Rodel 2005), shelter (Smith *et al.* 2003) vegetation complexity and wetland hydrology (Healey *et al.* 1997; Heard *et al.* 2008; Parris & McCarthy 1999; Wassens *et al.* 2010). Additionally, oviposition locations with appropriate characteristics of water body depth, vegetation type (Crump 1991; Rudolf & Rodel 2005), sediment depth (Rudolf & Rodel 2005) and predation pressure (Rieger *et al.* 2004) may be required for reproduction.

Aggregation (or patchiness) of resources results in a mosaic of suitable and semi-suitable sites, often separated by areas that are unsuitable for permanent habitation sites in a landscape (Figure 1A) (Wiens 1976). High quality habitat can support higher density (number of animals per unit of space) or abundance (number of animals per habitat) (Hixon & Johnson 2001) than semi-suitable sites (Figure 1B). Consequently, animals may be restricted to and live in higher abundance in suitable areas compared to unsuitable areas and disperse accordingly through available habitats (Fretwell & Lucas 1970; Nocera *et al.* 2009). Alternatively, territorial animals may space themselves and their territories at the edge of conspecific territories (Stamps, 1988), which may decrease density as a result of the configuration and spacing of home ranges. In this instance, dominant individuals often monopolise high quality habitat and less dominant individuals are forced into low quality habitat (Hache *et al.* 2013).

The primary vital processes of habitat driven distribution include survival and reproduction. An individual's capacity to contribute genes to the next generation is known as their fitness (Krebs 2009), which is directly affected by the presence or absence of suitable resources. Thus, there is a strong interaction between habitat characteristics and fitness. In general, animals that encounter high quality habitat survive and reproduce more successfully, and animals that encounter low quality habitat have higher mortality and lower rates of reproduction. For example, the development and survival of tree frog hatchlings (*Hyla chrysoscelis*) depend on the suitability of oviposition sites. If animals oviposit in unsuitable areas there is decreased growth and survivorship resulting in a low parental fitness (Resetarits & Wilbur 1989). This contributes to a heterogeneous distribution and abundance of a species over a landscape.
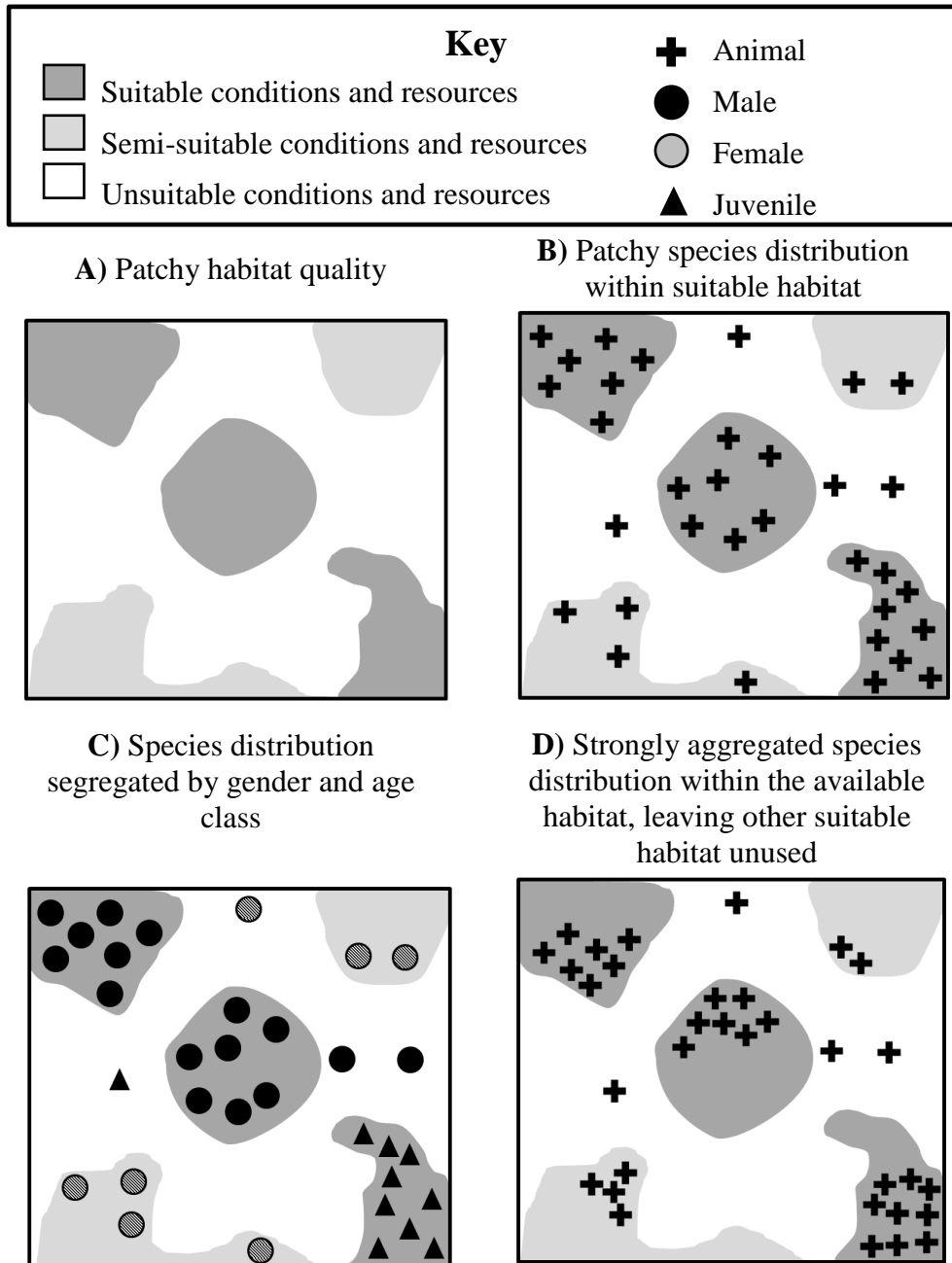
Figure 1: Hypothetical distribution of species across a landscape affected by habitat quality, and differential spatial partitioning of the landscape determined by age and sex as well as physical attributes of the habitat.

Some species require different habitat types during different stages of their lives, for example, the multiphasic life cycle of amphibians. Amphibians require different habitats and have different movement and dispersal patterns through their life cycle due to a multiple phase lifecycle (biphasic) life cycle through the occurrence of aquatic tadpoles and terrestrial juveniles and adults. For example, until metamorphosis tadpole movement is restricted to the habitat in which the parent oviposits. After metamorphosis movement between patches of

habitat is possible and in many species, juveniles disperse away from natal waterbodies and toward breeding waterbodies (Gamble *et al.* 2007; Gamble *et al.* 2006). Adults may migrate seasonally between breeding waterbodies and over-wintering habitat (Semlitsch 2008a) or disperse to newly formed flooded habitat to breed (Hamer *et al.* 2002b). This terrestrial movement between suitable patches (and often through unsuitable habitat) can be facilitated by favourable weather such as high humidity and rain. This allows amphibians to move over the terrestrial environment with less chance of desiccation (Wassens *et al.* 2007). Consequently, species distribution fluctuates between patches of habitat due to the movement processes of migration and dispersal.

Researchers who seek to explain species distributions often assess habitat as the explanatory factor through spatial modelling and statistics (Guisan & Zimmermann 2000), that correlates habitat characteristics with occurrence and occupancy patterns. This field of theory has three generalised hierarchies commonly used to determine habitat requirements: the geographical range (landscape occupancy), home range (broad habitat) and local features within a home range (micro-habitat) (MacKenzie 2006). Landscape and home range models often use habitat traits to predict occupancy on a broad scale, attempting to understand what general features are favourable. Alternatively, finer scale sampling can focus on the activity of an animal in their home range and their use of micro-habitat. Microhabitat studies provide biological meaning to distribution and an understanding on how the animal uses a resource at different life stages or at different times of the year. For example, *Litoria raniformis* occurs in areas which are seasonally flooded and within permanent water bodies consisting of high vegetation complexity of fringing and emergent vegetation (Wassens *et al.* 2010), but in a microhabitat level, they particularly use rocks, bare soil, leaf litter and aquatic vegetation (Heard *et al.* 2008). Thus, the study of habitat features in different scales can be complimentary to understand a species' distribution.

2.1.2 Heterospecific and conspecific interactions

2.1.2.1 Competition and predation

Despite the importance of habitat quality in determining species distribution, biotic factors, such as heterospecific and conspecific interactions may also affect species distributions, via competition and predation. Although there is ample evidence that those interactions influence both distribution and abundance, distribution models often neglect their inclusion as intrinsic

features of the habitat (Amarasekare & Possingham 2001; Campomizzi *et al.* 2008). In the instance that a species shares a common limiting resource and experiences interspecific competition (Amarasekare 2003) they must develop different resource requirements (MacArthur & Levins 1967) or use the same resources in a different location or at a different time of day (Chesson 2000a, b). In addition to resource and spatial competition, the distributions of predator and prey species have a complex pattern of temporal and spatial movements (Amarasekare 2003). In response to predator presence, prey may change behaviour (e.g. change the time in which activity takes place) (Pizzatto *et al.* 2008), change micro-habitat use (e.g. forage in areas with more protection) (Colley *et al.* 1989; Garcia *et al.* 2004), change their site of occupancy (e.g. actively move away from predators) (Gonzalo *et al.* 2006) or change the location of oviposition (Resetarits & Wilbur 1989; Spieler & Linsenmair 1997). This indicates that animals actively respond to changes in competition and predation over time and space and that distribution and niche are influenced by conditions, resources and interactions with other species.

Conspecific presence may also influence distribution through competition and predation, differentially affecting gender and life stage. In mammals, males and females of the same species may use different resources and/or different locations (Figure 1 C). This may be due to competition for resources, different resource requirements and specialisations (Bowers & Smith 1979; Bowyer 1984; Litvaitis 1990). For many anuran species, males spend a considerable amount of time at breeding sites, cycling between calling and non-calling states due to changes in weather (Brooke *et al.* 2000) and/or due to fluctuations in hormone levels as a result of high energy intensive calling (Leary *et al.* 2004). However, females may avoid breeding areas and males to decrease sexual harassment or females may mature later or breed less frequently and thus have no need for breeding habitat (Berven 1990; Gamble *et al.* 2007). Early life stage (tadpoles, metamorphs and juveniles) may also be affected by competition and predation. Juveniles often show an ontogenetic habitat shift, which is a shift in habitat use or spatial distribution between adults and juveniles. This may be due to competitive exclusion where juveniles are less able to compete for resources and move away from competitive adults or that they may use different resources and/or different locations (Figure 1C) (Stamps 1983). Alternatively, cannibalistic pressure may result in a similar shift in habitat use to avoid predation, where juveniles may disperse (Colley *et al.* 1989; Rudolf *et al.* 2010). Thus, an animal's niche is a result of species' reaction to, and influence on,

conditions and resources as well as competition and predation within a habitat, which forms their realised niche.

2.1.2.2 Negative density dependence

In addition to the influence of environmental conditions, resources and heterospecific interactions on spatial arrangement, conspecific density may also determine species distribution. A population's demographic rate is determined by mortality (loss of animals) and recruitment (gaining animals), as well as the behavioural processes of emigration (loss of animals) and immigration (gaining animals). Density dependence occurs when there is a positive or negative relationship between a population's density, and a population's demographic growth rate (Hixon & Johnson 2001) via an effect on individual fitness (Courchamp *et al.* 2008).

A population with negative density dependence (NDD) experiences a decreasing growth rate as density increases. This occurs because animals living in higher densities must compete for resources such as food, shelter or predator-free space (Finstad *et al.* 2009; Hixon & Johnson 2001; Hixon & Jones 2005). In this instance, there is a decrease in birth and immigration Figure 2 A) and an increase in deathrate and emigration Figure 2 B). This results in a decreasing growth rate as population density increases. Additionally, due to low reproductive output, the fitness of an individual decreases as the density of conspecific competitors increases Figure 2 C) (Fretwell & Lucas 1970; Rosenzweig 1991). Consequently, for organisms experiencing NDD, behaviour may adjust. For example, animals at high densities may immigrate to other suitable habitats (density dependent dispersal) and avoid high density locations when dispersing (Matthysen 2005). In the instance that there is no competition for resources or other density dependant processes, a species may be considered density independent (DI), where growth rate does not fluctuate in response to changing population density (Figure 2 A,B).

Population demographic processes
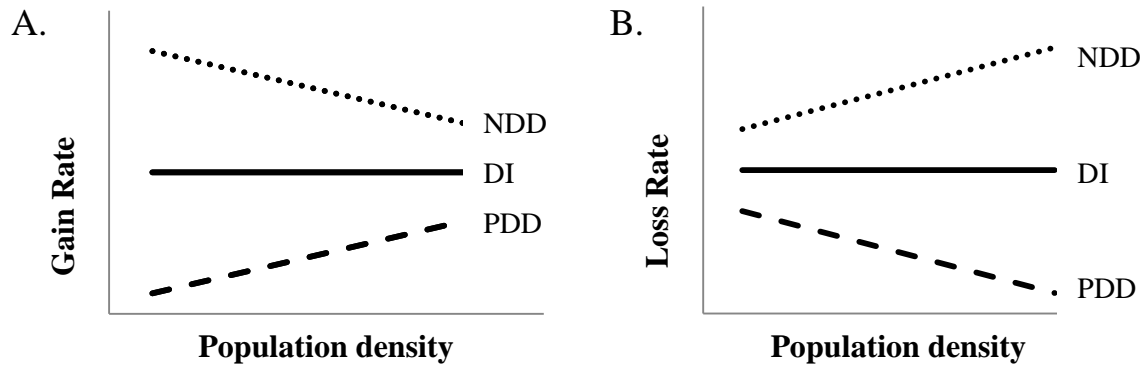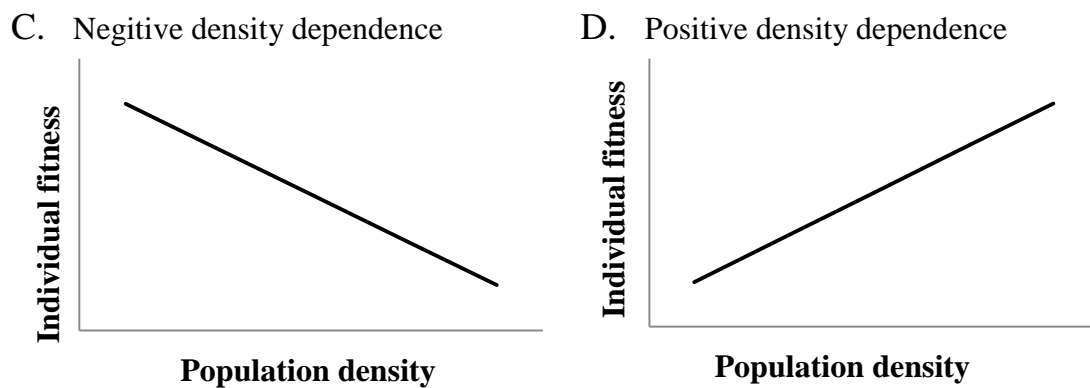
Figure 2: The potential influence of density on the gain (A) or loss (B) of organisms (the per capita demographic rate). Gain is due to the rate of immigration and births, whereas loss is due to death and emigration. Three scenarios are displayed: negative density dependence (NDD), density independence (DI) in grey and positive density dependence (PDD) in black.

2.1.2.3 Conspecific aggregations

Within suitable habitat, many species tend to aggregate despite NDD effects such as resource competition. Allee (1927) noticed that many animal species, including a wide variety of organisms both invertebrates and vertebrates, aggregate at some stage in their lives. Resulting species distribution is therefore not only aggregated within suitable areas, but may be strongly aggregated within the available habitat, leaving other suitable habitat unused (Figure 1D). This challenged the accepted paradigm, that animals space themselves due to resource availability. Research since these initial observations have found that aggregation operates in a wide range of non-territorial taxa such as colonial and flocking birds (Gardner 2004; Gilbert et al. 2006), mammals (Hoeck 1982, 1989; Weddell 1991), schooling fish (McLennan &

Ryan 1997, 1999) and insects (Stamps *et al.* 2005). Additionally, a number of competitive and/or territorial animals aggregate, such as crabs (Stamps *et al.* 2005), lizards (Stamps 1988), birds (Ahlering *et al.* 2010) and amphibians (Bee 2007). To form an aggregation, animals must be able to recognise stimulus from conspecifics to move towards them. Stimulus which may assist in the identification of a conspecific can include visual spotting, olfactory cues (hormones and smell) and auditory calling (Courchamp *et al.* 2008; Fretwell & Lucas 1970). Considerable research has assessed the processes which instigate aggregation in birds and lizards which have formed much of theoretical ideas and practical knowledge on conspecific attraction.

2.1.2.4 Positive density dependence

In addition to conspecific attraction facilitating habitat selection, some animals may benefit from aggregating at higher densities (Ward 2005). A species with *positive* density dependence (PDD) experiences an increasing population growth rate and fitness as density increases. At high density there is an increase in birth and immigration Figure 2 A) and a decrease in deathrate and emigration (Figure 2 B). Additionally, as population density increases, there is an increase in fitness Figure 2 D). For example, social animals living at higher densities may protect against harsh weather and facilitate survival and reproduction (increase births and fitness) through huddling (Gilbert *et al.* 2006) or increase predator vigilance while foraging, reducing predation risk and increasing foraging efficiency (decrease death) (Gardner 2004). Due to the biphasic nature of amphibian lifecycles, conspecific attraction can occur in each life stage for different reasons. For example, adults may choose to communally oviposit to increase egg survival or development (Berven 1982). Tadpoles may choose to school to protect from predation through a decreased risk of predation (dilution effect), or by confusing predators in large groups (swamping effect) (Hamilton 1971; Morgan & Godin 1985; Schwartz & Wells 1985; Watson *et al.* 2006). Alternatively, tadpoles may experience an increase in feeding efficiency or improved thermoregulation in larger groups (Waldman & Adler 1979).

Conspecific attraction may offer multiple advantages to breeding individuals. Acoustic communication assists anurans in coordinating the reproductive behaviour of males and females. For many species of anurans, males aggregate and chorus during favourable environmental and seasonal conditions to attract females and deter competition from other males. However, females use calling to guide movement towards reproductively ready mates

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and may assist in choosing a mate (Arch & Narins 2009). An 'explosive breeding' strategy (Wells 1977) involves reproduction over a short period of time (e.g. < 24 hrs) in unpredictable and sometimes temporary environments such as flooded swales. In this breeding strategy, conspecific attraction would provide a particular advantage, by allowing males to evesdrop on other males which have found suitable breeding habitat (Bee 2007). A 'prolonged breeding' strategy operates over longer period of time (sometimes months) and is undertaken in predictable environments, such as permanent waterbodies.

Both explosive breeding and prolonged breeding males may benefit from aggregation. Hypothesised benefits include that larger choruses of males may and increase a females choice of mates, increase female visitation (Gamble *et al.* 2007) or more social stimulation may increase reproductive performance (Darling 1952). If these factors benefit an individual, an organism may increase immigration into higher density areas and increase emigration away from areas with smaller densities. For example, marbled salamanders (*Ambystoma opacum*) show high levels of dispersal from breeding waterbodies with low conspecific abundance (< 10 individuals), and low dispersal for waterbodies with higher abundance. This may suggest this species chooses to abandon waterbodies with lower abundance levels and prefer to occupy waterbodies with higher conspecific density (Gamble *et al.* 2007), and that animals are unlikely to settle in a location without the presence of conspecifics (Stamps 1988). Consequently, species distribution and abundance can vary not only with habitat quality, but also the levels of competition, predation and the density of conspecifics within a habitat.

2.1.2.5 Using conspecifics to find suitable habitat

A number of researchers have suggested that conspecific presence facilitates habitat selection by providing information about habitat quality (Betts *et al.* 2008; Betts *et al.* 2010; Harrison *et al.* 2009; Nocera *et al.* 2009; Virzi *et al.* 2012). In this instance, individuals may choose to occupy habitat based on the presence and possibly abundance of conspecifics as occupancy indicates habitat suitability. Stamps *et al.* (2005) studied how ghost crabs (*Ocypode rotundata)* choose foraging patches on a beach. She placed varied quantities of food in quadrats and viewing the visitation of each location. This species showed a preference for quadrats where conspecifics were present, despite the presence of other food quadrats close by and despite the hindrance to foraging caused by competition in high density areas. Consequently, it has been suggested that the presence of conspecifics indicate that a location

has a high quality or quantity of food. Ultimately, it is argued that an animal's fitness is increased through a reduction of energy spent searching for suitable habitat and reducing time exposed to unsuitable conditions, inadequate resources and predators (Semlitsch 2008a; Stamps 1988). Another example showing that conspecifics can aggregate in particular areas, despite suitable resources elsewhere is suggested for an anuran: the Guinea River Frog (*Phrynobatrachus guineensis*). This species is a communally nesting tree-hole breeder which use the presence of conspecifics as an indicator of low predation in oviposition sites (Rudolf & Rodel 2005).

2.2 Threats to small populations and colonisation capacity

Species which experience conspecific attraction are vulnerable to two major behavioural effects which threaten population persistence; small population numbers (the Allee effect) and limited colonising capacity. For species' that benefit from close living, decreases in population density can threaten persistence through reduced survival and reproduction. The *per capita* growth rate of a population is the number of deaths relative to the number of births. This measure indicates if a population is growing, is stable or decreasing in abundance (Krebs 2009). In the same sense that positive density dependent effects provide benefits to populations at high densities, low population densities may cause detrimental effects. Where species density is low *per capita* growth rate is low or reduced (Figure 3), they may experience a low probability of finding mates, decreasing reproductive output. Additionally, survival may be reduced through loss of positive density effects, such as predator swamping and the dilution effect (Cheetham *et al.* 2011; Stamps 1988). This loss of individual fitness and decrease in population growth rate is known as the Allee effect. There can be weak or strong Allee effects. Weak Allee effects occur when there is a decrease in population *per capita* growth rate as density decreases, but population growth rate remains at or above zero (Figure 3). However, with a strong Allee effect, the per capita growth rate becomes negative, or even strongly negative, at low densities (Figure 3). In the instance that population experiences strong Allee effects and *per capita* growth is negative, a population is in decline and at risk of becoming locally extinct (Courchamp *et al.* 2008).
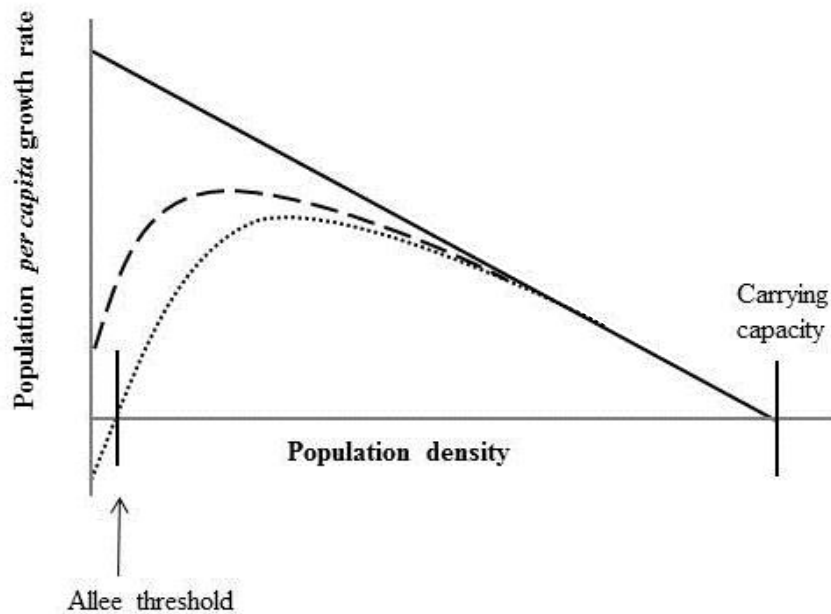
Figure 3: The influence of population density on per capita growth rate for negative density dependent model (solid line) compared to weak Allee effects (dash line) and strong Allee effects (dotted line). The carrying capacity indicates maximum population density where per capita growth rate becomes 0 (loss = growth in a stable population) and the Allee threshold indicates the point where per capita growth rate becomes negative (Courchamp et al. 2008).

Species that experience positive density effects often choose to settle near conspecifics when dispersing or migrating through the landscape (Ahlering et al. 2010). Amphibians both migrate and disperse throughout the environment at different stages of their life, moving from aquatic to terrestrial habitat and between areas of aquatic habitat. Specifically, migration is a round trip between locations (movement to and from) such as migration from breeding habitat to overwintering habitat. Dispersal is a geographical movement towards a new location without returning to the original location, such as between natal waterbodies and breeding waterbodies (Semlitsch 2008a). However, species which experience positive density dependent effects may fail to settle at a location without the presence of conspecific stimulus (Stamps 1988). In the management program for an endangered territorial forest song bird black capped vireo (*Vireo atricapilla*), the invasive brown-headed crowbird (*Molothrus ater*) excluded *V. atricapilla* from parts of its former range. Management programs removed the *M. ater* from parts of the forest, but *V. atricapilla* did not move back into their former range. An experiment was designed, where speakers were placed in some uninhabited areas playing conspecific call playback to simulate conspecific presence (treatments) and other areas had no speakers (controls). It was found that *V. atricapilla* respond to the calls of conspecifics and settled in areas with speakers, leaving controls uninhabited (Ward & Schlossberg 2004).

More specifically, in a laboratory setting male juvenile lizards (*Anolis aeneus*) choose to form territories adjacent to conspecifics. Stamps (Stamps 1988) has also shown that without the presence of competitors, the lizard *Anolis aeneus* fails to establish territories, a behavioural trait which extends into adulthood. Consequently, species' that experience conspecific attraction may fail to colonise areas without conspecific presence, and the use of artificial cues can act as a novel management tool to assist conservation programs.

For amphibians, failure to colonise areas without conspecifics poses a number of issues for individual survival and for population distribution. As population distribution is isolated to patchy wetlands, amphibians that disperse or migrate may require conspecific presence to settle. This may increase time spent finding conspecifics and ultimately increases time exposed to predation and desiccation while searching (Semlitsch 2008a). Additionally, conspecific attraction may affect the pattern of waterbody occupancy via the propensity of an individual animal or species to disperse, the level of habitat selection exhibited by the individual animal or species (Gamble *et al.* 2007), barriers to dispersal (e.g. roads) (Vos & Chardon 1998) and the distance between and isolation of habitat patches (Ficetola & De Bernardi 2004). However, conspecific attraction is rarely cited as an influence and has the potential to decreases the chance that a waterbody will become inhabited. For example there may be a decrease in dispersal (e.g. through incentive to stay such as competitive interactions) and decreased colonisation of habitat without conspecific presence (Gamble *et al.* 2007).

Small population sizes and reduced colonising ability pose major problems for wildlife managers and conservation biologists. Amphibian conservation programs often involve 1) research to define a species habitat (Schmidt & Karch 2005) 2) create habitat which tries to reflect the habitat used by a population (Brown *et al.* 2012) and 3) animal reintroduction (translocation) programs (Denton *et al.* 1997). Habitat selection models assume that an animal's position is independent of other animal's position, and they do not attract or repel each other. Thus, habitat models may not capture the relationships between animals, which limits the predictive ability of habitat modelling as conspecific attraction or avoidance may cause strong aggregation within available habitat (Campomizzi *et al.* 2008). Additionally, conspecific attraction may prevent dispersal and colonisation of habitat created for the target species, or avoidance may cause dispersal at early life stages (Ahlering *et al.* 2010). This skewing of distribution causes major issues in modelling distribution to environmental

parameters, as spatial autocorrelation can lead to biased estimates of habitat use, reducing the performance of models (Wintle & Bardos 2006). Consequently, conservation research should first of all determine if a species exhibits conspecific attraction or density dependence, incorporate behavioural components into habitat modelling and attempt to utilise conspecific attraction as a novel management tool.

Much of what is known of amphibian conspecific attraction has been undertaken in a laboratory setting. Conspecific attraction has been found between adult amphibians through a range of stimulus types. Research on conspecific attraction has strongly focused on sexual pheromones (Müller-Schwarze 2006). For example, adult great barred frogs (*Mixophes fasciolatus*) preferentially choose to settle near the odours of conspecifics (Hamer *et al.* 2011). A number of species also use visual cues, such as hand waving (Criswell 2008) and throat inflation (Narins *et al.* 2003) which often assists in forming territories and instigating competition between males. Considerable research has been undertaken on auditory calling, but very little has been undertaken on the influence of auditory calling on distribution. Non-distribution studies have focused on speciation, breeding strategies, the response of gravid (egg bearing) females to different sounds and to a males call and the biological properties of auditory reception and production (Gerhardt & Huber 2002). The limited research to assess distribution and the influence of conspecific call attraction has shown that in laboratory settings, males (Bee 2007) and gravid females (Gerhardt & Klump 1988) approach the sound of a male chorus, not only singular male callers as was previously reported (Gerhardt & Huber 2002).

Three recent studies (Buxton *et al.* 2018; Buxton *et al.* 2015; James *et al.* 2015) found that the production of conspecific chorus playback from speakers can manipulate the short distance distributions of a frog species, drawing callers to previously unused habitat. These studies detected conspecific attraction, but study design did not substantiate the effectiveness of conspecific call playback in realistic circumstances. One study installed artificial waterbodies with conspecific call playback, however, they removed the competing influence of local choruses (Buxton *et al.* 2018; Buxton *et al.* 2015). The other assessed how call playback affected natural distributions within waterbodies with the presence of competing conspecific attraction (James *et al.* 2015). However, despite the overwhelming evidence that amphibians exhibit conspecific attraction, it has not yet been extended into a population without artificial waterbodies or exclusion of competing conspecific calls.

Due to worldwide amphibian declines, and its potential significance for conservation programs, habitat selection and the effect of conspecific stimuli on amphibian behaviour needs considerable attention within amphibian literature. Ultimately, understanding more about attraction may improve management by informing habitat assessment and creation.

2.3 The endangered green and golden bell frog (*Litoria aurea*)

2.3.1 Information on Litoria aurea

In response to range contractions and population declines, the endangered green and golden bell frog (*Litoria aurea*) has been the focus of considerable research and conservation effort. The species exhibits a number of behavioural and distributional traits that can be targeted to assist conservation. *L. aurea* is native to the east coast of N.S.W. Australia Figure 4:A) and was once described as a common species (Courtice & Grigg 1975). However, abundance has dramatically declined and the species distribution has geographically contracted to approximately 10 % of its former range Figure 4:B) (Pyke & White 2001b). Remaining populations are small, fragmented and under threat. Consequently, this species is listed as endangered under the *NSW National Park and Wildlife Act 1974 (Endangered species (Intérm Protection) Act 1991)*. A number of major threatening processes have been implicated as causal agents of decline, including predation from the introduced mosquito fish *Gambusia holbrooki* (Morgan & Buttemer 1996) and habitat loss, modification and fragmentation (DEC 2005). However, more recently, the effect of the disease chytridiomycosis caused by the fungal pathogen *Batrachcochytrium dendrobatidis* has been identified as the primary causal agent of decline (DEC 2005; Penman *et al.* 2008).
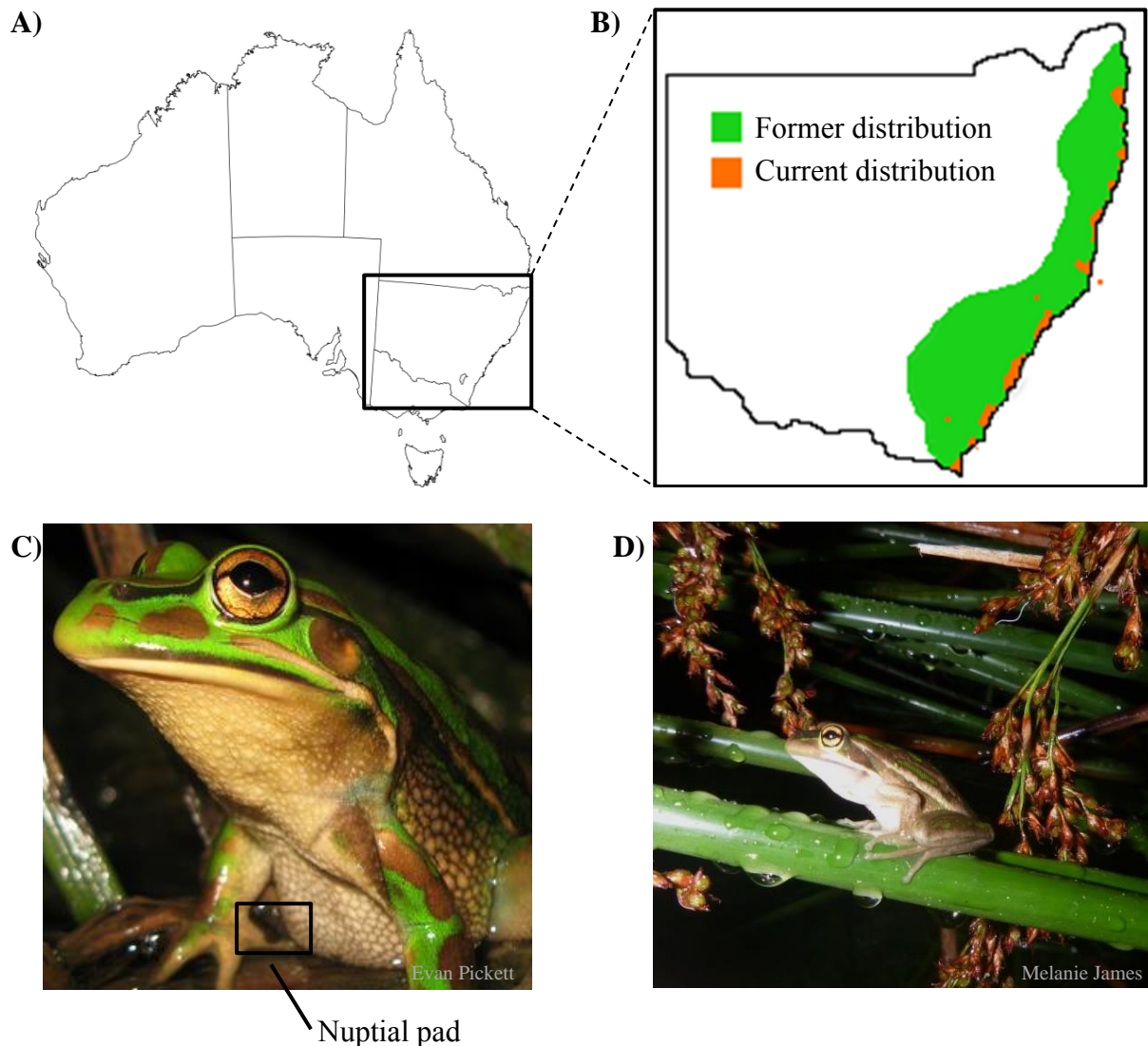
Figure 4: The distribution of the green and golden bell frog (*Litoria aurea*) across maps of A) Australia and B) New South Wales and photographs of C) an adult male with a raised black nuptial pad on the forefinger used for grasping females and D) a juvenile *L. aurea*.

Litoria aurea is semi-aquatic, generally non-arboreal and use a wide variety of water bodies, from wetlands, flooded ditches, marshes, swamps and a number of man-made water bodies (Courtice & Grigg 1975; Pyke & White 2001b). Males aggregate and chorus within waterbodies to attract females from October to February. Throughout the breeding season, females approach callers and males grasp the female in amplexus, however, the behavioural processes behind mate selection are unknown. After amplexus is commenced eggs are deposited in water where they become tangled around vegetation (Pyke & White 2001b). However, it has not been reported whether females and/or males play a role in oviposition site selection. There are five phases to their lifecycle; egg, tadpole, metamorph, juvenile and

adult frog, where adults can be distinguished from juveniles through snout vent length (SVL), where an adult SVL is considered to be ≥ 40 mm (Figure 4:D). Males can be distinguished from females in the adult phase through the presence of a nuptial pad (a raised grey or black pad) on the forefinger (Figure 4:C) which is assumed to assist in grasping females throughout amplexus (Pyke & White 2001b).

2.3.2 Gaps in knowledge

2.3.2.1 Conspecific attraction

A number of behavioural traits suggest that *L. aurea* exhibits conspecific call attraction. Male *L. aurea* have a loud and conspicuous call, which can be heard from long distances. This species is known to disperse to ephemeral waterbodies after rain events and flooding (Hamer 2002; Hamer *et al.* 2008), where loud conspicuous calling may assist animals searching for habitat, acting as a locator mechanism for spatially and temporally unpredictable breeding habitats (Bee 2007). As dispersal poses a number of risks to survival, using conspecific calls as a locating mechanism may reduce the time and therefore the risks taken to locate suitable habitat (Semlitsch 2008a). Additionally, despite the presence of apparently widely available and suitable waterbodies nearby, *L. aurea* often form chorusing aggregations in particular waterbodies, leaving other suitable waterbodies uninhabited (Hamer & Mahony 2009; Stockwell 2009).

A number of studies have noted that *L. aurea* fail to colonise all available and apparently suitable habitat (Pickett *et al.* 2013; Stockwell 2009). Failure to colonise all suitable waterbodies may suggest poor colonising capacity, however, extensive investigation of their colonising capabilities indicates that when conditions are favourable individuals may disperse short distances such as hundreds of metres (Hamer 2008; Mahony *et al.* 2013; Pyke & White 2001a) while populations can rapidly disperse and expand over distances of up to 10 km in a season (Daly 2014). In addition to landscape aggregation, this species clusters in small sections of larger waterbodies (James *et al.* 2015). Within these waterbodies, conspecific call broadcast can manipulate adult distribution, showing the first direct evidence of conspecific attraction for adult *L. aurea* (James *et al.* 2015). Additionally, in experimental conditions, juveniles actively choose to aggregate (Pizzatto *et al.* 2015). Thus, *L. aurea* may act as a model species to assess the presence of conspecific attraction and habitat selection. However, the extent to which conspecific attraction occurs over landscapes is currently unknown and

has not been adequately addressed in order to be considered as a potential management strategy.

2.3.2.1 Microhabitat

Another factor relatively unexplored for *L. aurea* is the microhabitat selection of calling males in the context of ovipositing and conspecific attraction. For some amphibian species, male frogs call at sites where females prefer to oviposit based on particular habitat variables, such as water depth (Rudolf & Rodel 2005). Currently, very little is known about the specific habitat selection of *L. aurea*, with information being restricted to landscape based selection (Garnham 2009; Garnham *et al.* 2015; Hamer *et al.* 2002b; Midson 2009; Pollard 2009; Pyke & White 1996; Valdez *et al.* 2016; Valdez *et al.* 2015), observational notes (reviewed in Pyke & White 2001a) and assessment of direct habitat structure use (Garnham *et al.* 2015; Valdez *et al.* 2016). However, habitat use of calling males has not yet assessed direct selection of structures (e.g. vegetation, rocks) alongside depth, water quality and climate variables. Considering that *L. aurea* aggregate within some vegetation patches and not others (James *et al.* 2015), clumped distribution may be a factor of microhabitat selection as well as conspecific attraction. Specifically, some males may make choices on a place to occupy within a waterbody based on particular habitat features. After a male settles and starts to call, other males may then eavesdrop on their calls, and settle next to the settled callers (Ahlering *et al.* 2010). Thus, assessment of microhabitat of calling males can decouple if calling location is solely due to conspecific attraction, or if habitat plays a role in settlement.

2.3.2.3 Habitat construction

Based on the current literature and an understanding of the habitat of *L. aurea*, a habitat construction programme has been undertaken in the Hunter region, NSW Australia. In attempts to develop successful management strategies research has focused on how key threatening processes affect population decline (DEC 2005) and what landscape and microhabitat is used by adults and juveniles (Bower *et al.* 2012; Garnham 2009; Garnham *et al.* 2015; Hamer *et al.* 2002b; Midson 2009; Pollard 2009; Pyke & White 1996; Valdez *et al.* 2016; Valdez *et al.* 2015). Additionally, assessments of water quality in occupied waterbodies have provided information on adult and tadpole tolerance and preference thresholds (Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman 1998; Pyke *et al.* 2002; Stockwell 2011; Werkman 1999). Previous plans to recover this species

have focused on the creation of breeding habitat (Darcovich & O'Meara 2008; Goldingay & Lewis 1999; Pyke *et al.* 2008; White & Pyke 2008a; White & Pyke 2008c) and reintroducing (translocating) animals to areas where they have become locally extinct (Pyke *et al.* 2008; Stockwell *et al.* 2008). However, these initiatives have showed varying success, frequently failing to establish self-sustaining populations, with a few exceptions (Bower *et al.* 2011; Stockwell 2009). However, relatively few habitat creation programmes manage to incorporate designs based on research that aims to improve conservation outcomes and remove key threatening processes.

2.3.2.4 Breeding behaviour

In addition to limited information on the conspecific attraction and microhabitat selection of calling males, the reproductive strategies of *L. aurea* remain relatively understudied. It is known that *L. aurea* displays no parental care, and that females often live in terrestrial areas resulting in a disproportionate sex ratio (Pickett 2009; Valdez *et al.* 2016). Males call from within water or on branches of vegetation, where there is competition between males in the form of auditory responses and aggressive fights (Ford 1986; Ford 1989). It is known that females directly oviposit in the water around vegetation (Hamer *et al.* 2002b), and some observational notes have stated that some females have been seen moving short distances while in amplexus (Pyke & White 2001a). However, it is yet unreported whether females choose a mate amongst males or whether males choose a location suitable for oviposition. As *L. aurea* has a loud call, and some aggressive behaviour has been recorded (Bower *et al.* 2012; Ford 1986; Ford 1989; James *et al.* 2015), the mating system may include female attraction to call qualities, or male-male competition to achieve amplexus. However, the dynamics of *L. aurea* aggregative choruses and details of their breeding ecology have not been intensely studied.

2.3.2.5 Summary of gaps in knowledge

Based on this critical review, there are several major areas of uncertainty and questions that need further examination and clarification. As the reproductive ecology of *L. aurea* has not been explored in great detail, the current study investigated four key questions; (1) the concept of landscape scale conspecific attraction (2) breeding site selection (3) *L. aurea's* response to newly available habitat and (4) sexual selection. Specifically, I explored whether calls from speakers can manipulate site occupancy of calling males to alter their spatial distribution and increase their site specific spatial density for conservation purposes. I also investigated whether calling male distribution is linked to particular habitats and habitat

components to further inform conspecific attraction research and habitat selection studies with the intention of providing practical insights for habitat. Additionally, the effectiveness of a new habitat construction programme and the breeding behaviour of *L. aurea* associated with that programme was studied to provide information for both the specific program and to inform other conservation programmes for this species.

Chapter 3. Can conspecific call playback change amphibian distribution? Building from localised distribution manipulation to a larger scale: a continuing case

James, M. S. Stockwell M. P., Pizzatto L., Clulow J., Mahony M. J.

3.1 Abstract

Animal aggregation can confound landscape-scale management by skewing distribution and preventing dispersal to unoccupied areas. This can hinder habitat restoration and creation programmes as species may fail to colonise available habitat, despite its suitability. Conspecific (same species) call broadcast (CCB) from speakers can manipulate species distribution by attracting animals into new aggregations. Consequently, we may be able to attract threatened species to high quality, poorly occupied habitats to improve conservation efforts. For the endangered green and golden bell frog (*Litoria aurea*) we aimed to assess their response to playback calls on a landscape scale, attempting to increase occupancy of five treatment (T) waterbodies with CCB. Each treatment was paired with a manipulative control (MC - had speaker stands and no CCB), and a control (C - no stands or CCB). To assess response to broadcasts, in T, MC and C we compared *L. aurea* abundance and calling prior to call broadcast to during the broadcasting period. Analysis between T, MC and C took into account temporal effects, assessed climate and rainfall to abundance and calling. We also assessed if the introduction of speakers lengthened the breeding season by encouraging calling by reviewing length of treatment abundance and calling compared to MC, C and a reference waterbody. Results indicated no statistically significant increase in abundance or calling in T relative to MC and C. Higher rainfall, medium temperatures (16 °C - 26 °C) and higher relative humidity (>70 %) were associated with increased calling, but measures of abundance (male, female and juvenile attendance) were only related to higher rainfall. Waterbody attendance and maximum chorus size were higher and lasted longer after chorus formation in response to call broadcast. As calling at breeding waterbodies may be improved by calling stimulation, translocation programmes may be able to use call broadcast to increase breeding activity in already populated waterbodies. Despite a limited response to CCB, future research into CCB's role in species distribution should consider the strength of

conspecific attraction in response to distance, competing conspecific cues and habitat preference.

3.2 Introduction

With many species experiencing population decline from human impact, understanding animal movement patterns is key to managing populations in structurally complicated landscapes. A species' distribution is commonly described as dependent on physiological requirements (Fretwell & Lucas 1970; Huey 1991) and availability of limited resources such as shelter, food and breeding habitat (Amarasekare & Possingham 2001; Batzli 1968; Fox 1982; Lim & Giles 1987; Mabry & Stamps 2008). Low availability and high competition for these limiting resources influence the survival and reproductive potential of individuals (fitness). In response, many animals disperse to reduce density and competition (Matthysen 2005). However, in some cases there is positive density dependence (PDD) in which fitness increases with population density, and animals may actively seek populated areas, creating aggregations (Allee 1927). PDD is common throughout the animal kingdom, in both non-territorial and territorial animals such as birds (Ahlering *et al.* 2010; Gardner 2004; Gilbert *et al.* 2006), mammals (Hoeck 1982, 1989; Weddell 1991), lizards (Stamps 1988, 1991), fish (McLennan & Ryan 1997, 1999), invertebrates (Stamps *et al.* 2005) and amphibians (Bee 2007). Consequently, behavioural traits exhibited by aggregative species can influence landscape movements, affecting the way managers need to approach population and landscape management.

Aggregating can provide a number of benefits. For example, some species group for protection from weather or predation (Gardner 2004; Gilbert *et al.* 2006; Morgan & Godin 1985; Schwartz & Wells 1985; Waldman 1982). Grouping may also improve mate finding and mate choice, where aggregation can increase availability and variety of mates, facilitating sexual selection and increasing offspring fitness (better genes theory) (Gamble *et al.* 2007; Murphy *et al.* 2017). Increased reproductive competition can also improve hormone and sperm production (Brzoska & Obert 1980; Burmeister & Wilczynski 2001; Chu & Wilczynski 2001; Wilczynski & Lynch 2011). Additionally, evesdropping on a conspecific's (same species) location through visual spotting, olfactory cues or auditory communication can quickly help individuals to identify high quality habitat (Betts *et al.* 2008; Betts *et al.* 2010; Harrison *et al.* 2009; Nocera *et al.* 2009; Virzi *et al.* 2012). This quick identification of habitat could reduce the time spent searching and the associated risks, such as exposure to

predators or desiccation (Bee 2007; Semlitsch 2008a). Thus, aggregation may improve an individual's fitness by improving survival and reproductive success.

A major concern in modern species management is that PDD further threatens small populations through decreased population density (Courchamp *et al.* 2008). Species that rely on aggregation are vulnerable to low population density effects, as a population decline removes benefits from aggregation (Courchamp *et al.* 2008; Courchamp *et al.* 1999). This causes reduced survival and/or reproductive opportunity which reduces *per capita* growth rate (Allee effect), placing further pressure on already declining populations (Courchamp *et al.* 2008; Gascoigne *et al.* 2009). Another effect of low density includes a reduced capacity to disperse and colonise (Gamble *et al.* 2007). Species that experience PDD often settle near conspecifics when dispersing or migrating through the landscape (Ahlering et al. 2010). Consequently, they may fail to disperse away from a populated area or, while dispersing individuals may fail to settle at a location without the presence of conspecifics (Hoeck 1982; Stamps 1988; Ward 2005; Ward & Schlossberg 2004). These declining and isolated populations have reduced genetic flow and are vulnerable to inbreeding depression and stochastic events such as disease or weather, which may render small populations extinct (Shaffer 1981). Consequently, conservation efforts need to focus on increasing per capita growth rate and connecting populations (Courchamp et al. 2008).

A number of studies have shown that the spatial distributions of animals exhibiting PDD can be manipulated using artificial cues. For species which aggregate, artificial conspecific cues such as visual, olfactory and auditory calling (Courchamp *et al.* 2008; Fretwell & Lucas 1970) can affect species' habitat selection and occupancy. Using physical models of conspecifics, mirrors or placing speaker systems playing conspecific calls distribution can be manipulated in a laboratory and in the wild, redistributing local and landscape occupancy (Ahlering *et al.* 2010; Betts *et al.* 2008; Betts *et al.* 2010; Harrison *et al.* 2009; James *et al.* 2015; Kress 1997; Narins *et al.* 2003; Nocera *et al.* 2009). Some of these studies have also used conspecific attraction for conservation, redistributing populations to areas with low predation from invasive species or recolonising islands (Ahlering *et al.* 2010). This offers a phenomenal opportunity for land managers looking to improve landscape connectivity, redirect species to high quality habitat or away from development and other conflicting land uses to improve survival and reproductive output.

Conspecific attraction and the use of calls to manipulate landscape distribution has been well established in birds, but not for amphibians, despite amphibians having shown a strong propensity for aggregation (Gerhardt & Huber 2002). Currently, three studies have addressed this in the wild for three species, with varying levels of success. For *Hyla chrysoscelis, Spea multiplicata* and *Litoria aurea*, new chorus locations can be established using call broadcast (Buxton *et al.* 2018; Buxton *et al.* 2015; James *et al.* 2015) but five species did not form new chorus locations (Buxton *et al.* 2018; Buxton *et al.* 2015). As amphibians are broadly experiencing population declines, local extinctions and isolation from human development and land use change, if present, conspecific attraction manipulation may offer some opportunities for successful population management. In addition to manipulating spatial distribution, Buxton *et al.* (2018) found that calling was extended temporally, suggesting the breeding season may be extended. Although extension of the breeding season may be seen as a way to encourage more reproduction, caution is needed to ensure that prolonging breeding season does not affect body condition prior to winter and possibly increase susceptibility to chytrid (Garner *et al.* 2009).

A species which aggregates and exhibits short distance conspecific attraction is the green and golden bell frog (*Litoria aurea*) (James *et al.* 2015). *Litoria aurea* once inhabited the majority of the state of NSW Australia, however, distribution is now constrained and populations are declining (Mahony *et al.* 2013) and is listed as endangered in NSW (*Threatened Species Conservation Act 1995*) and vulnerable in Australia (*Environment Protection and Biodiversity Conservation Act 1999*). Being an obligate wetland breeder, its distribution is further constrained by the presence of water (Semlitsch 2008a; Valdez *et al.* 2015), which varies spatially due to land modification (Albrecht 2000; NCIG 2013), invasive predators (Hamer *et al.* 2002a) and disease (Stockwell *et al.* 2015a), and temporally as a result of rainfall and flooding (Hamer *et al.* 2008). Thus, the use of conspecific cues on a landscape scale may help to a) locate suitable habitat over a varied landscape or b) provide PDD effects. However, the effectiveness of redistribution has not been assessed across a landscape.

The main aim of this study was to assess if call broadcast can attract conspecifics on a landscape scale and affect waterbody abundance. Based on previous successful manipulation at short distance within waterbodies (James *et al.* 2015), we predicted that frog abundance (the number of individuals seen) and the number of individuals calling would increase in waterbodies with CCB, whereas in wetlands without playback calling would remain

unchanged. Secondary aims of this study were to assess whether call broadcast incited calling outside of natural chorusing periods or if broadcast extended the length of the breeding season. We predicted that waterbodies with speakers broadcasting calls would maintain *L. aurea* calling at different times to waterbodies without broadcast and would retain calling later in the season compared to controls. We also aimed to measure if temporal changes in temperature, humidity, wind and rainfall influenced abundance and calling between survey nights. We predicted that rainfall and climate would influence abundance of *L. aurea* within wetlands, with wetter weather and warmer, more humid and less windy nights increasing abundance.

3.3 Method

3.3.1 Breeding and habitat selection behaviour of L. aurea

Litoria aurea are obligate waterbody breeders with a spring to summer breeding season, *L. aurea* has been described as a prolonged breeder in permanent water, with explosive breeding tendencies in response to heavy rain and availability of ephemeral waterbodies (Courtice & Grigg 1975; Hamer *et al.* 2008; Hamer & Mahony 2010; Mahoney 1999). Population distribution is generally constrained to areas with freshwater (Hamer *et al.* 2002b; Hamer *et al.* 2008; Valdez *et al.* 2015). However, individuals are known to move long distances, dispersing > 200 m to nearby waterbodies (Hamer *et al.* 2008; Hamer & Mahony 2010) and 10 km within breeding season, showing large scale dispersal and colonisation, sometimes followed by local extinction of newly colonised areas (Daly 2014). Male *L. aurea* call and aggregate, females do not call, and the distribution of adults within a waterbody is aggregated based on calling (James *et al.* 2015). Calling males generally use waterbodies with water with salinity < 3.3 ppt, dissolved oxygen between 0.17 - 16.5 mg/L, pH between 5.3 - 9.9 and temperatures between 15.7 - 30°C (Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman 1998; Pyke *et al.* 2002; Stockwell 2011; Werkman 1999). However this does not necessarily determine distribution (Hamer *et al.* 2002b). Research has described the microhabitat (physical substrate used) for this species, showing use of aquatic vegetation by adults and juveniles, and use of terrestrial habitat by females more than is expected based on availability (Valdez *et al.* 2016 and Chapter 5). While calling, males generally float in the water or perch on vegetation (Chapter 2 and 3). Over winter, frogs regularly used reeds and rock structures within 5 m of water (Garnham *et al.* 2015).

3.3.2 Study site and species distribution

The current study was undertaken in one of the largest remaining populations of *L. aurea* in N.S.W. Australia, in the Hunter River on the deltic northern Ash Island and southern Kooragang Island, Newcastle (-32.862863 S, 151.728355 E) (Figure 5:A). Ash Island currently forms the Hunter Wetlands National Park (HWNP), consisting of freshwater wetlands, coastal saltmarsh and mangroves listed under the Ramsar Convention. The landscape has a complicated history of agricultural grazing, road and industry infrastructure which has affected vegetation communities, wetland shape and hydrology (OEH 2014). This activity on both islands has resulted in a number of man-made swales and large water filled ditches, some of which are permanent (hold water during severe drought), semi-permanent (drying during severe drought) or ephemeral (filling after short periods of heavy rain) (NCIG 2007, 2013; Williams *et al.* 2000). The distribution of *L. aurea* on the islands is patchy, where some wetlands are highly occupied and others remain irregularly used (Hamer 2002), which occurs in another *L. aurea* population (Pickett *et al.* 2013), and high abundance areas are generally constrained within a kilometre of permanent water (Valdez *et al.* 2015). Heavy summer rains are considered a precursor for dispersal to breed in ephemeral areas surrounding permanent water (Goldingay & Newell 2005; Hamer 2008). Such landscape history has led to a structurally complicated mosaic of wetlands across the landscape with complex hydrology and ecology.

Within the islands wetland mosaic, threatening processes are unevenly distributed. The introduced predatory fish *Gambusia holbrooki* exists in many permanent wetlands and spreads during flooding between infested and previously non-infested wetlands (Klop-Toker *et al.* 2017). Additionally, pressure from the disease-causing fungus chytrid *Batrachochytrium dendrobatidis* is unevenly distributed across the island (Stockwell 2011), possibly influenced by salt presence as an inhibitor of the fungus (Stockwell *et al.* 2015c) and with host species (Johnson & Speare 2003; McMahon *et al.* 2013).
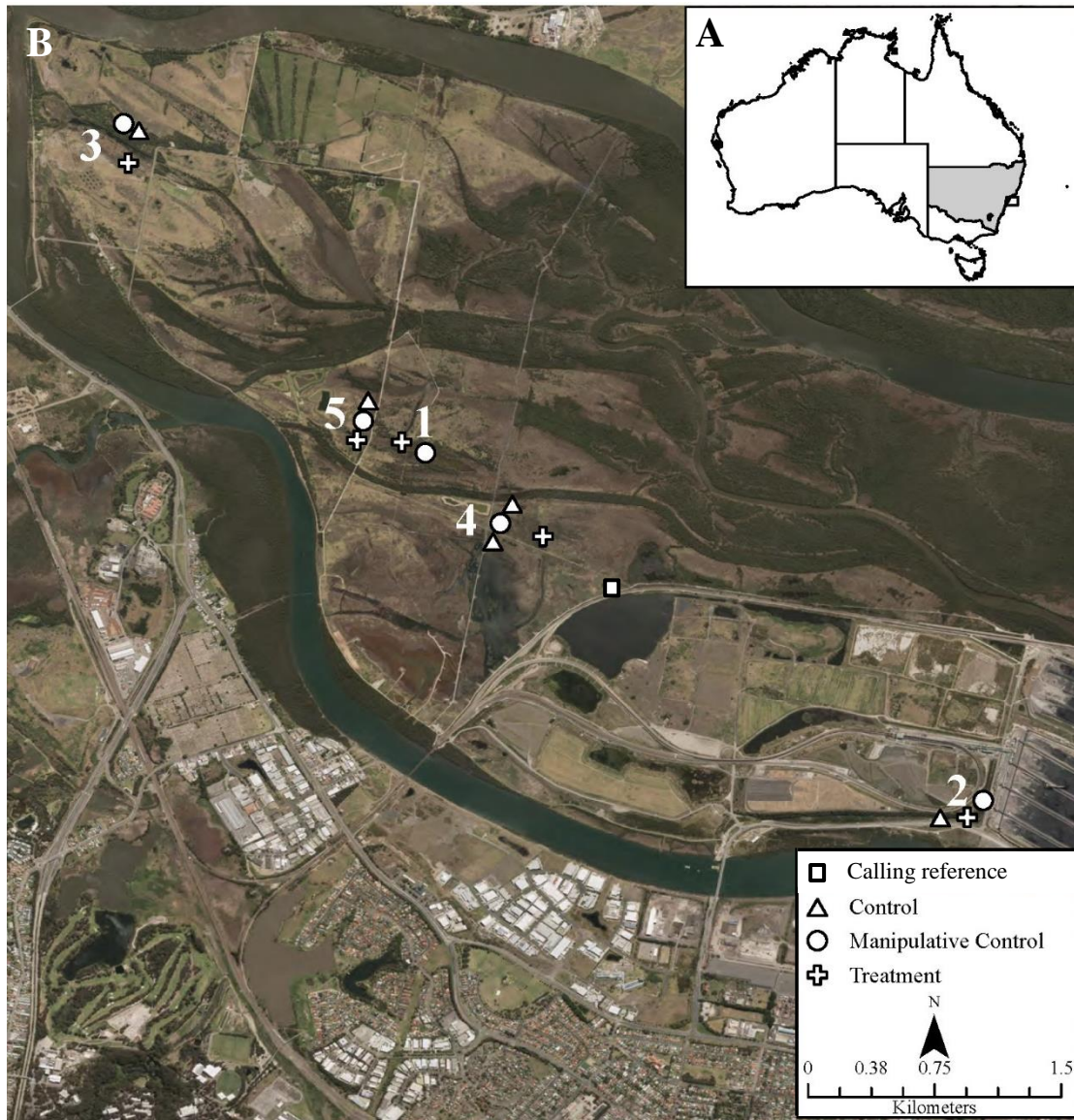
Figure 5: A) Map of Australia with Ash and Kooragang Islands (square) (-32.862863 S, 151.728355 E). On the map, 15 waterbodies are identified by symbols. Groups of waterbodies formed five replicate clusters (1-5), each replicate cluster contained a Treatment, in which speakers broadcasted *L. aurea* calls and a Manipulative Control in which speaker was set up without broadcasting sound. For the replicate clusters, additional shallow 'scrapes/swales' acted as Controls with no speakers or stands (excluding replicate cluster 1). Replicate clusters two and three are considered low abundance.

3.3.3 Experimental waterbodies: treatments, manipulative controls and controls

To manipulate the distribution of *L. aurea* to unoccupied areas, we identified five replicate locations with three clustered wetlands within 200 m of each other, which had records of low *L. aurea* abundance (Klop-Toker, K. and Valdez, J. unpublished data). Waterbodies chosen were semi-permanent. Waterbodies were required to have a minimum of 10 m^2 of mostly open water with fringing and emergent vegetation to reflect the habitat *L. aurea* frequents and

breeds on Ash Island and Kooragang Island (Valdez *et al.* 2015). To ensure water quality was suitable, during the breeding season we took water quality readings using a YSI Professional Plus water quality metre to measure temperature (^{o}C), salinity (ppt), pH and dissolved oxygen (mg/L). Calibration was done before water quality survey nights. Water quality measuring was done three times per waterbody, for the surface (epilimnion) and close to the wetland base (hypolimnion), or on the surface for shallow waterbodies and then averaged. Results were compared to tadpole and adult tolerance thresholds in the literature (Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman 1998; Pyke *et al.* 2002; Stockwell 2011; Werkman 1999). Water quality needed to be within these thresholds during the sampling period for the waterbodies to be used in the experiment. As a result, five independent replicate areas with multiple (clustered) waterbodies were available for the study.

The five replicate clusters were chosen randomly from the available semi-permanent waterbodies. For each cluster, a waterbody was randomly allocated as a treatment (T), in which speakers would broadcasted *L. aurea* calls and a manipulative control (MC) in which speaker would be set up without broadcasting sound. As the HWNP is a publicly accessible area, semi-permanent waterbodies provided protection for the speaker systems from vandalism through water presence as a deterrent and vegetation as camouflage from visual spotting. For each replicate cluster, a number of 'scrapes/swales' surrounding the treatment and manipulative controls were assigned as general controls (C - no speaker box or sound). Recording *L. aurea* abundance and calling in ephemeral swales can track the movements of abundance and choruses in the experimental zones and detect explosive response to large rain events (Hamer *et al.* 2008). With five replicate clusters on the island, this resulted in a total of 15 waterbodies, 5 treatments, 5 manipulative controls 5 controls This number of waterbodies was dependent on availability within the landscape.

3.3.4 Pre-broadcast surveys

We undertook survey nights in 2012-2013 visiting each waterbody 11-13 times to establish baseline abundance data in the experimental waterbodies. Surveys were undertaken from September to March (approximately 153 days) to coincide with the spring-summer *L. aurea* breeding season and consisted of both auditory and visual encounter methods. Auditory surveys (AUD) were used to determine the total abundance of calling male *L. aurea* which involved approaching a waterbody after dark and listening for frog calls. *L. aurea* calls were

then imitated for one minute to elicit a response from males. The number of calling males prior to and post call imitation were recorded, following the procedure in Bower *et al.* (2012).

Following auditory surveys, visual encounter surveys (VES) were conducted. These surveys involved a thorough search of the waterbody, emergent and fringing vegetation as well as terrestrial habitat surrounding each waterbody. On sighting a frog, the sex of *L. aurea* was determined using secondary sexual characteristic (males having a nuptial pad on the forefinger, females without a nuptial pad). Surveyors were trained in estimating frog snout vent length (SVL), where frogs smaller than 45 mm were considered as juvenile (if the surveyor was uncertain the frog was labelled as a sub adult). Frogs that were positioned in a way that nuptial pads could not be seen were classified as an unsexed adult. The total time spent surveying and number surveyors was recorded to assess survey effort and determine relative abundance of *L. aurea* (the number of minutes spent searching multiplied by the number of searchers). Climate variables of temperature (^{o}C), relative humidity (%), barometric pressure (hPa) and the maximum and average wind (km/hr) were measured using a Kestrel 2000. Once a month, based on probe availability, we used an YSI Professional Plus to record water quality as previously described. This monthly monitoring was to ensure water quality remained suitable for the frogs. Results were compared to tadpole and adult tolerance thresholds in the literature (Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman 1998; Pyke *et al.* 2002; Stockwell 2011; Werkman 1999).

3.3.5 Litoria aurea call recordings and speaker design

On Ash Island, we recorded *L. aurea* calls from males that called consistently and without prompting. We used a compact flash recorder (Marantz: PMD 660 and Rode: NGT-2 microphone), which recorded a stereomp3 file at 44,000MHz. Microphones were placed 1.5m equidistant from the two calling males. Four background males were also present in the final sound recording.

After frog calls were recorded, we used Raven Pro Interactive Sound Analysis Software (CLO 2014) to edit the calls. The aim of call editing was to make a mp3 file with 10 minutes of calling which represented different call types. Using Ford (1989) definitions of call types; A) grunts and B) drone call types, we used the cut function ensured different call types were represented in a single recording. The final file had 10 minutes of consistent calling. This calling was repeated 5 times to form a 50-minute sound file. A 10 minute stretch of silence was then introduced after the 50 minutes of looped calling to prevent habituation to calling. A small mp3 player with an USB charging port and audio jack (female) input was loaded with the 50 minute call file and was programmed to repeat.

To create a speaker system to broadcast calls from in the experiment, the speaker systems were made from two separate charging structures. The first structure was the speaker system, which consisted of a 20-watt 12-volt solar panel connected to a 12-volt battery connected to an amplifier (13.5 watts); a twilight switch and then a single portable boat speaker designed to withstand outdoor conditions (4-Ω, 100 Hz−15kHz). The second structure was the mp3 system, which consisted of a 10-watt 12-volt solar panel connected to a 12-volt battery and then connected to a USB car-charger that permanently charged the mp3 player. The amplifier was then connected via a male audio jack to the mp3 player. The amplifier and mp3 were connected and maintained playing 24 hr/day, but the separation of the speaker from the amplifier using the twilight switch cut off power to the speaker and therefore ceased call broadcast during the day. Call broadcast occurred between ~ 8 pm and 5 am eastern daylight savings time (EDST).

In the field, the natural call amplitude of *L. aurea* was assessed using a digital sound level metre (Dicksmith EM646). A total of 23 individual male frogs had call amplitude measured and an average of all amplitudes (71 dB from 1 m distance) was considered an average and appropriate sound level for call broadcast.. To place speakers in the wetlands, we constructed a two-shelved platform, which had four stake style legs, the lowest shelf was wooden and 150 cm from the sharp leg bases, where the speaker system was placed. The second shelf was 30 cm above the first shelf and was made from a clear plastic to form a large awning. This awning protected the speaker system, allowed light through to the twilight switch, and made a flat place for the solar panels to be fixed using duct tape. The wooden stands were secured in the wetlands by pushing the spikes ~20 cm into the mud beside emergent vegetation and tying the stand to the vegetation with rope for extra stability. A stand and box were

introduced to one manipulative control waterbody per cluster to ensure animals were not attracted to the presence of a stand and box. When speakers malfunctioned, they were replaced the next survey night.

3.3.6 During-broadcast surveys

Manipulation was attempted during spring and summer when *L. aurea* breeds, with calls broadcasted from August to March in the 2013 – 2014 and 2014 – 2015 summer breeding season, and from August to November in 2015. In the 2013 – 2014 season, call broadcast was instigated in 5 treatments for 14 nights per month with VES and AUD surveys undertaken in each waterbody twice a week (6 wetlands one night, 9 wetlands the next night, then repeated to survey each waterbody twice per week). Water quality was recorded once a month to assess the changes in water characteristics and ensure wetlands remained suitable. Surveys were also undertaken in manipulative controls and controls. At the end of each survey night, a reference waterbody known as permanently occupied and a history of reproduction (Hamer 2008) had an AUD survey to act as an indication of natural calling behaviour on the island.

Due to seasonal climatic changes and their interactions with *L. aurea* dispersal and waterbody water quality, some changes were made to method and replicate clusters in the final two seasons; 2014 – 2015 and 2015 half season. Firstly, in the first year of surveys (2013 – 2014) two weeks of call broadcast per month failed to coincide with large rain events. As *L. aurea* disperse in response to rain events (Hamer *et al.* 2008) in the 2014 - 2015 and 2015 survey seasons, calls were broadcast permanently to ensure speaker systems were broadcasting during rain events. During permanent broadcast, VES and AUD surveys for each waterbody was undertaken a minimum of four times per month. Secondly, in the 2014 - 2015 and 2015 survey seasons, a large change in pH made one replicate cluster unsuitable, and it was removed from the study. One other replicate cluster posed a threat to a neighbouring experiment assessing habitat and distribution, as conspecific attraction could change habitat use and confound results. This replicate cluster was also removed from the study (Alex Callen, *personal communication*). This removal of two replicates resulted in three clusters for the last two years of the study (3 treatments, 3 manipulative controls, 3 controls).

3.3.7 Tadpole surveys

We surveyed for tadpoles in all wetlands once a month to assess if *L. aurea* had bred, and as a secondary detection method for adult presence on non-survey nights. To survey we used a

60 cm^2 dipnet sweeping in 5 m lengths, twice in open water and twice in vegetated areas as well as on the surface and underwater to account for microhabitat use (Klop-Toker *et al.* 2017). We also undertook tadpole trapping twice a year in November and March to detect if *L. aurea* had bred in the studied waterbodies. We tied funnel fish traps to emergent vegetation and baited each trap using trout pellets and a yellow glow stick from 5 pm and 8 am. The number of traps set was relative to wetland size, which resulted in a range of 5 – 40 traps per wetland. *L. aurea* can spend from 1.5 to 11 months as tadpoles (Anstis 2002; Browne *et al.* 2003). Consequently, trapping twice over the summer and dip netting monthly was seen as adequate to detect breeding events.

3.3.8 Statistical analysis

3.3.8.1 Effect of call broadcast

Data was checked for over-dispersion using a mix of generalised linear models (GLM) and zero inflated models, using the Akaike information criterion (AIC) to compare the goodness of fit for the various models in R Studio V. 3.4.2 (RStudioTeam 2015). The sequence of models was; GLM with Poisson distribution and log link function, zero-inflated Poisson, negative binomial distribution and zero-inflated negative binomial distribution. Models confirmed over-dispersion was present and the AIC of the four model types compared. To choose the best model, a reduction in the AIC value was seen as an improvement and best described the over-dispersion (Burnham & Anderson 2004) (Table 1). As baseline surveys produced considerable number of zeros in the data, baseline surveys were excluded from the analysis. From AIC comparisons, calling data was zero inflated and negative binomial, which cannot currently be accounted for in the statistical package. Consequently, baseline surveys and calling data was instead depicted visually, described and discussed.

Table 1: Akaike information criterion (AIC) and degrees of freedom for abundance and calling data using various methods in generalised linear models to assess over-dispersion. Differences of 10 or more reflect strong support for the model with the lower AIC. Differences 4 to 7 are of intermediate support. Difference < 4 have little support (Burnham & Anderson 2004). Bold values indicate the most supported model.

| | All waterbodies | | | | High abundance waterbodies | | | |
| | *L. aurea* abundance | | *L. aurea* calling males | | *L. aurea* abundance | | *L. aurea* calling males | |
	df	AIC	df	AIC	df	AIC	df	AIC
Poisson regression	18	1336.13	18	625.58	18	1040.21	18	468.40
Zero inflated Poisson regression	19	1043.76	19	391.71	19	874.57	19	279.57
Negative binomial regression	19	**908.84***	19	367.79	19	**762.81***	19	279.16
Zero inflated negative binomial regression	20	909.33	20	**363.63***	20	763.52	20	**271.55***

To factor in temporal nature of the experiment, we undertook further analysis. The data for effect of artificial call broadcast on abundance and calling males compared treatments to manipulative controls and controls, using a generalised linear mixed model (GLMM) with the Poisson distribution and log link function and the likelihood ratio test (LRT) in R Studio V. 3.4.2 (RStudioTeam 2015). Fixed effects were: treatment (treatment, manipulative control and control), time (pre-broadcast surveys and during broadcast surveys) and the interaction between treatment and time. The search effort (the number of minutes spent searching multiplied by the number of searchers) was included as an offset for the abundance VES analysis. To account for temporal changes in abundance over the breeding season with weather and reproductive activity, random-effects were tested for waterbody, replicate cluster, year and month; however, waterbody was not a contributor to variability in the data and was excluded from the final analysis. To check the appropriateness of the GLMM model in light of previous checks for over-dispersion, a negative binomial GLMM was undertaken. However, the model failed to converge suggesting the standard deviation was close to zero, which indicates that the random effects had adequately accounted for the main cause of over-dispersion.

3.3.8.2 Subset of the data – high abundance waterbodies

In order to determine if low abundance waterbodies affected statistical output, we reanalysed a subset of the data. The main dataset was split to remove wetlands with low abundance, determined by waterbodies with more than 50 % of waterbodies uninhabited in each cluster.

This reduced dataset was checked for over-dispersion using the same methods for the main dataset. A GLMM was then fit using the same methods for the main dataset, which showed that random effects of waterbody, year and month adequately accounted for over-dispersion by comparing with the negative binomial distribution test for over-dispersion.

3.3.8.3 Effect of speakers on length of breeding season

To assess if the introduction of speakers lengthened the breeding season by encouraging calling, calls from the 2015 breeding season from January to March were graphed for T, MC and C.

3.3.8.4 Rainfall and climate

To assess the influence of climate and rain variables on abundance and calling, air temperature, humidity, average wind and maximum wind, total rain on the day of survey, the day prior, three days prior and an accumulation of 11 days of rain prior to surveys were included in the model. Auditory survey data from the reference waterbody was also incorporated in order to determine the effectiveness of a reference waterbody at predicting general calling patterns in other waterbodies across Kooragang Island.

3.4 Results

3.4.1 Observations of waterbody use

There were 104 nights of surveys over the two and a half spring-summer seasons (1 baseline abundance and 2.5 call playback seasons). During the three-year survey period 9 of 15 surveyed waterbodies had a chorus at least once. Throughout this time, within the ephemeral waterbodies there were eight chorusing periods, 3 in the baseline surveys and 5 during speaker placement. We found that there were 2 major chorusing events per year. There was one chorusing event at the beginning of each breeding season, and a second occurred after waterbody drying when heavy rains caused ephemeral flooding.

3.4.2 Pre-broadcast and during-broadcast abundance and calling

Baseline surveys for waterbody abundance indicated that three replicate clusters (1, 2 and 3) had low abundance and calling, with 6 of 8 and 7 of 8 waterbodies having zero records within the survey days, respectively (Figure 6: A,C). Two replicate clusters (4 and 5) had higher

abundance and calling, with *L. aurea* absent in only 1 of 7 waterbodies prior to call broadcast. During call broadcast, low abundance replicate clusters 1-3 maintained low abundances with minor increases in presence in some waterbodies (Figure 6:A). Two low abundance treatment waterbodies showed slight increase in calling during call broadcast (Figure 6:D), however, from field observations, this was only one to two individuals appearing in treatment waterbodies and calling for 2-3 nights. Both males positioned themselves within 3 m of the speaker system.

In high abundance replicate clusters during broadcast surveys, graphs indicated a higher mean abundance of frogs. Replicate cluster 4 did not dry completely throughout the study, and showed highest abundance in the control prior to call broadcast (Figure 6:A), however, this trend switched after call broadcast started, with treatment waterbody increasing abundance significantly above the control (Figure 6:B). Appearance of a large chorus of up to 15 chorusing males and 26 *L. aurea* sighted appeared after one rain event in the replicate 4 treatment waterbody. A female was found with a 2 mm diameter bipolar black and white coloured egg on her body, and post-capture inspection showed she was not gravid and had no signs of egg development. The chorus remained within the waterbody from the end of January to late February 2014. Replicate cluster 5 showed small increases in abundance and smaller chorusing events in the treatment waterbody compared to pre-broadcast surveys (Figure 6:A).
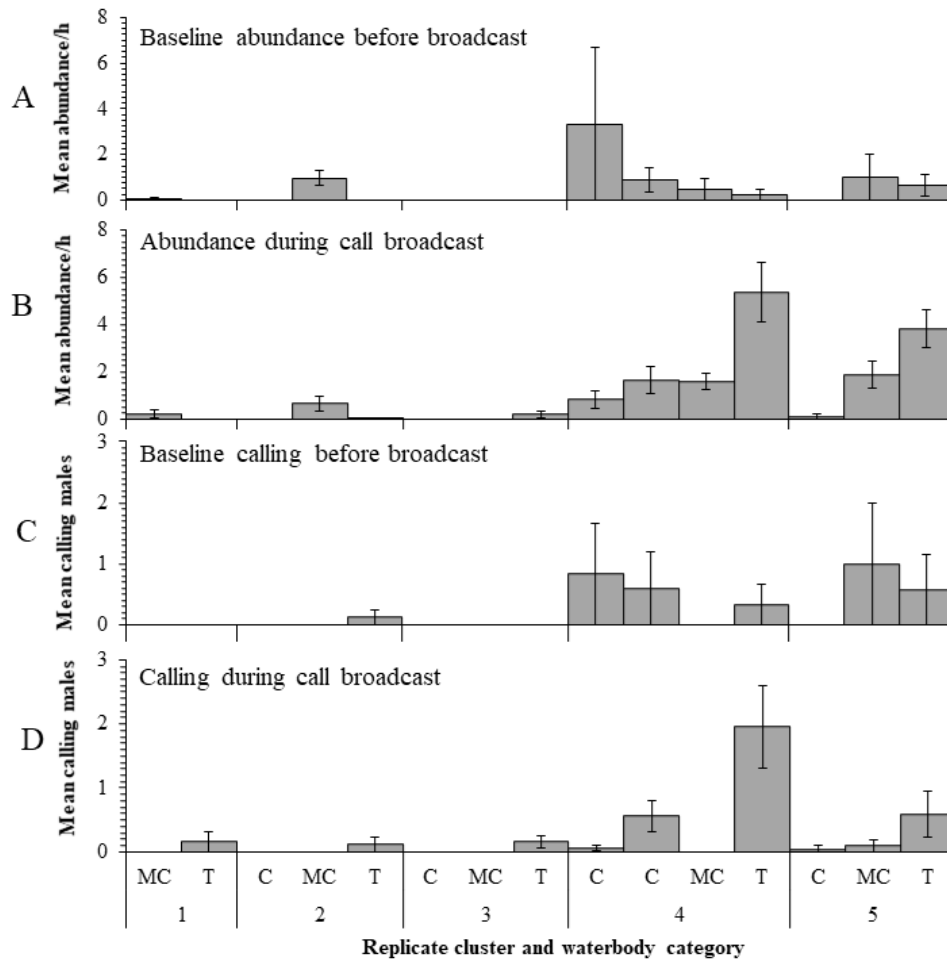
Figure 6: Mean waterbody abundance per hour (A) and total calling males (C) prior to speaker broadcast and mean waterbody abundance per hour (B) and total calling males (D) during *Litoria aurea* call broadcast, within each treatment cluster for treatment (T), manipulative control (MC) and control (C).

Visual comparison of survey data for all replicate clusters indicated that both abundance and calling males (Figure 7: A, B) in MC and C had low counts with minor variability between baseline, pre-broadcast and during broadcast surveys. Abundance and calling in T waterbodies visually indicates a minor increase in abundance and calling during-broadcast (Figure 7: A, B).
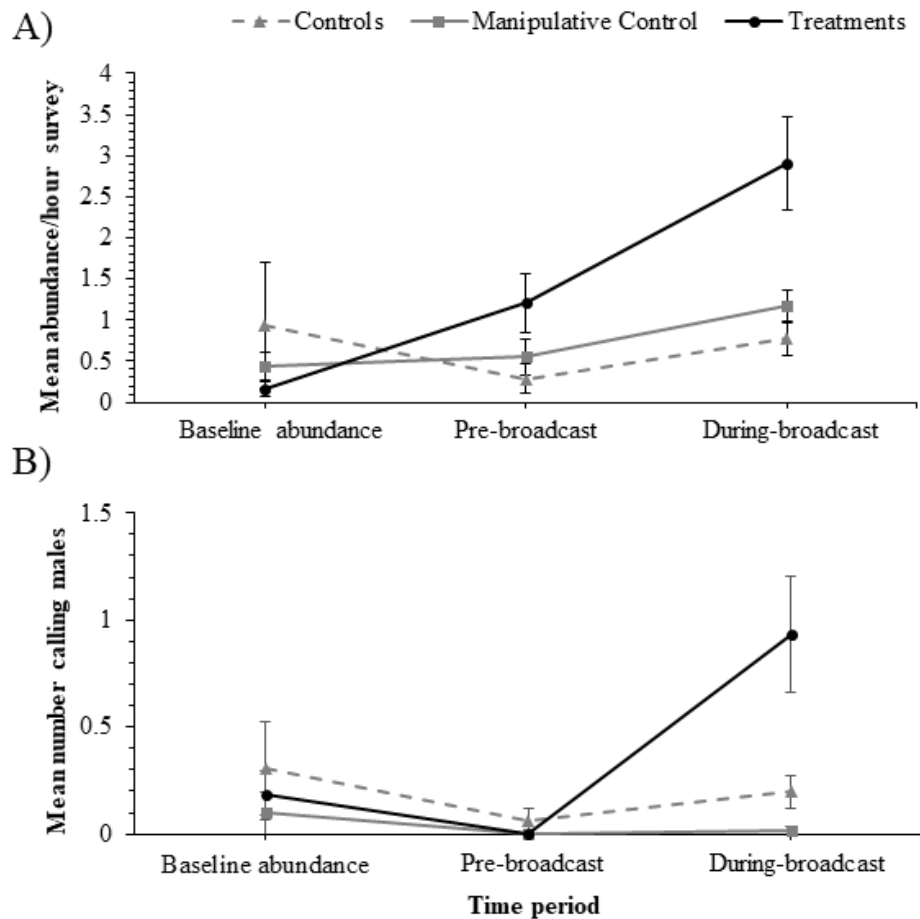
Figure 7: Mean abundance over a 40 minute survey of *Litoria aurea* from raw data displaying pre-call broadcast and during-call broadcast surveys for A) Mean abundance and B) Mean number of calling males (±SE).

3.4.3 Effect of speakers on length of breeding season

Over the 2014-2015 breeding season, two high abundance and calling periods occurred, once at the beginning of September and a second in late January, and very few *L. aurea* were seen or heard in December and early January (Figure 8: A and B). From mid-January, abundance and calling was greatest in T, followed by lower abundance in calling in C, and no calling in MC (Figure 8). Trends of calling males between January to February 2015 indicated that calling reduced by the beginning of February and ceased by mid-February. At the end of the breeding season in February, T calling did not extend past calling in C. The calling reference waterbody had similar visual trends to T, MC and C, showing similar times of chorus peaks in September and January. Calling in the reference waterbody had started prior to speaker placement in September, had low calling through December and January, and calling recommenced at a similar time to T

and C. Peaks of calling in T waterbodies continued after calling in the reference waterbody and C had reduced. All waterbodies ceased calling in the same time period.
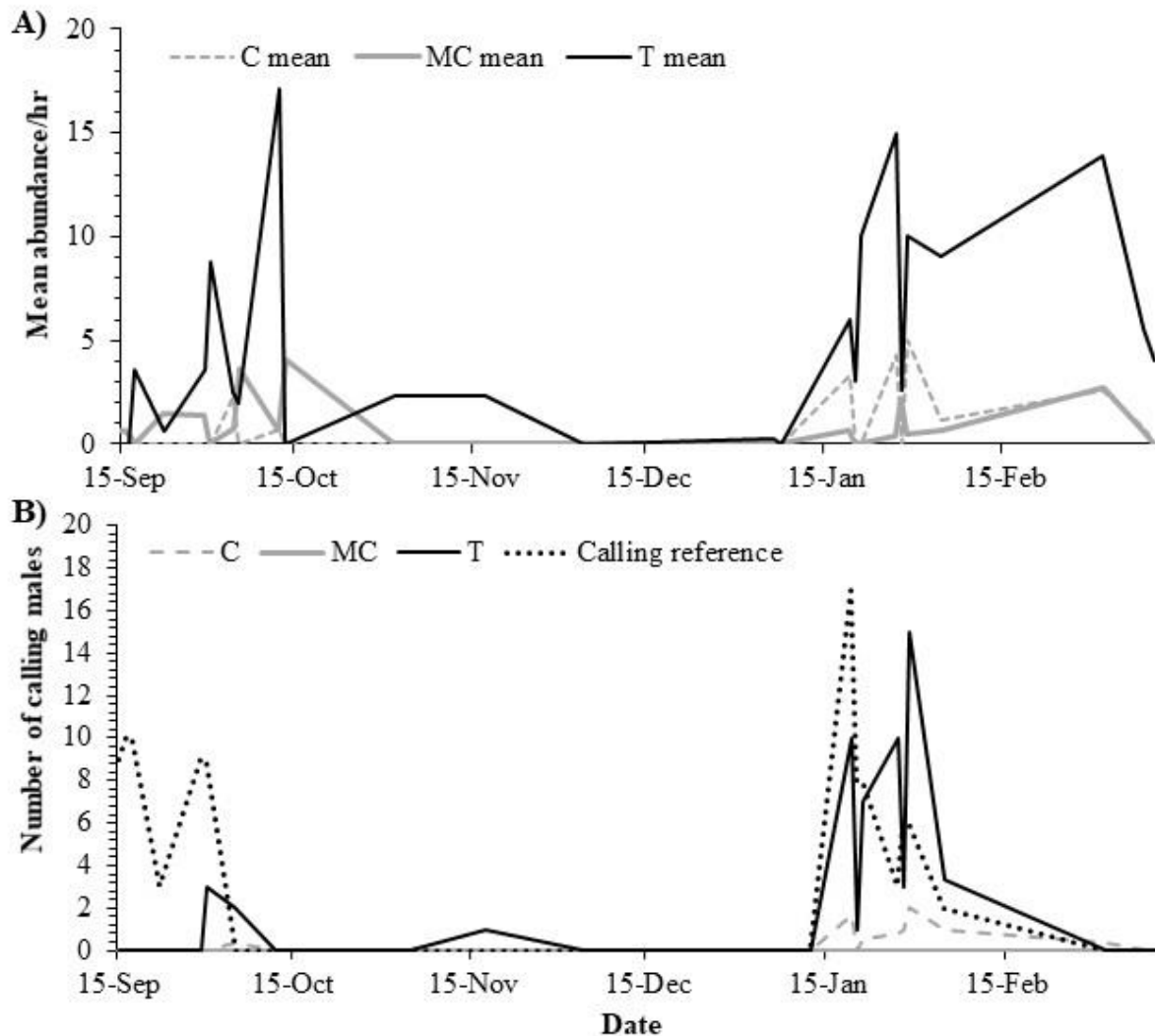
Figure 8: A) Mean abundance of *Litoria aurea* (±SE) and B) mean number of calling male *L. aurea* (±SE) from speaker placement on the 15th September 2014 to the end of the breeding season in March 2015 for waterbodies; treatment (T) broadcasting *L. aurea* calls, manipulative control (MC) in which speaker was set up without broadcasting sound and 'scrapes/swales' as general controls (C) with no speaker box or sound. Calling reference waterbody shows the total number of calling males on a survey night.

3.4.4 Analysis of calling broadcast effect

Visual trends of experimental waterbodies showed slight increase in C, MC and T between pre-broadcast and during-broadcast surveys (Figure 9). The MC and C waterbodies showed minimal increase in abundance and calling and T increased slightly compared to MC and C Figure 9). Despite visual trends which appear to show increases in T relative to MC and C between pre and during broadcast surveys, overall changes in treatment abundance were not

significant ($\chi^2 = 2.617$, df = 2, p = 0.270). Further analysis using emmeans indicated that comparisons between T and MC (z ratio = -0.832, p = 0.093) and T and C (z ratio = -0.761, P = 0.072) and MC to C (z ratio = -0.832, p = 0.406) were not significantly different. Within the T waterbodies, confidence intervals were considerably wider then MC and C. Overall, results remained unchanged when analyses were restricted only to high abundance waterbodies (so results are not masked by extreme low abundance in some replicate clusters) ($\chi^2 = 2.457$, df = 2, p = 0.2927).
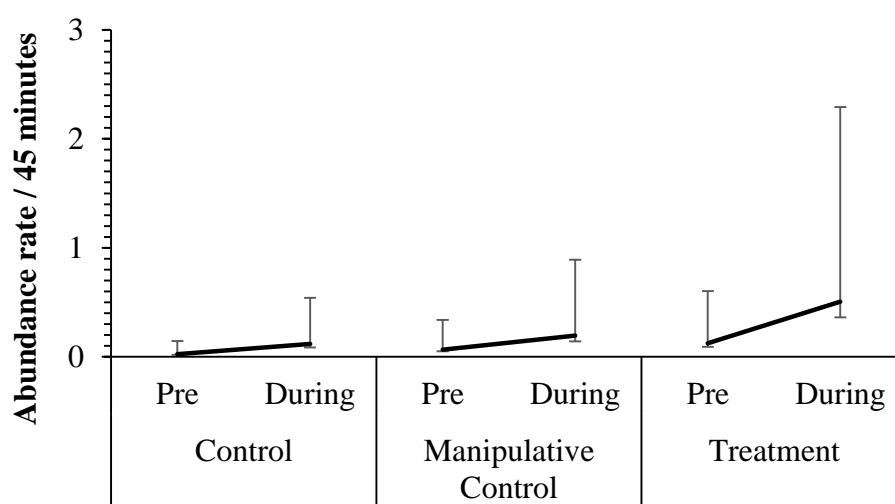
Figure 9: Estimated mean abundance over a 40 minute survey of *Litoria aurea* ± CI determined from Generalised Linear Model in R (RStudioTeam 2015) accounting for seasonal effects and repeated measures.

3.4.5 Abundance and calling in relation to rain and climatic variables

The abundance of *L. aurea* and calling males was positively related to rainfall on the survey day, the day before and total rainfall 11 days prior to the survey but was not related to rainfall 3 days prior to surveys (Table 2). *L. aurea* abundance was not related to humidity, temperature or barometric pressure (Table 2). Calling male abundance showed a positive relationship with temperature and relative humidity but not barometric pressure (Table 2). Calling occurred above 16 °C and below 26 °C, with above 70 % and below 95 % relative humidity.

Table 2: Significance output for climatic variables in a Generalised Linear Mixed Model analysing the response variable number of *Litoria aurea* per hour and number of calling males compared to fixed effects of rainfall and climate. Statistical significance indicated by asterisks (*).

Variable	Pr (>|z|)	
	Abundance	Calling
Rain on survey day	0.029*	0.009*
Rain the day before	0.001*	0.015*
Rain 3 days prior	0.09	0.29
Rain 11 days prior	<0.001*	0.009*
Humidity	0.41	0.025*
Temperature	0.85	0.02*
Barometric pressure	0.15	0.14

3.4.6 Dip-netting surveys

Over the seasons, five breeding events were detected in the experimental waterbodies. Two breeding events showed successful metamorphosis in 5MC and 4T. Surveys in the large waterbody 5T failed to detect tadpoles after finding an egg on a female's body, *G. holbrooki* were detected within the waterbody. Two clutches died due to waterbody drying, once in 4C (Gosner 23 to 25) and once in 5C (Gosner 23 to 25). Another breeding event was detected outside the experimental zone in a shallow swale which died due to waterbody drying (Gosner 34 to 39).

3.4.7 Water quality

Water quality throughout the experimental period indicated that all waterbodies throughout the pre and during broadcast surveys were within optimal thresholds for temperature, salinity and pH (Figure 10: A,B,D). However, in replicate cluster 1 salinity breached optimal tadpole thresholds (Figure 10: B). In replicate cluster 1, the pH standard error indicated that readings spanned outside the optimal thresholds for tadpoles, and salinity was high, with both mean and standard error spanning outside tadpole tolerance thresholds. Salinity and pH, however, remained within adult thresholds. Dissolved oxygen in replicate cluster 5 treatment waterbody was highly variable (Figure 10: C).
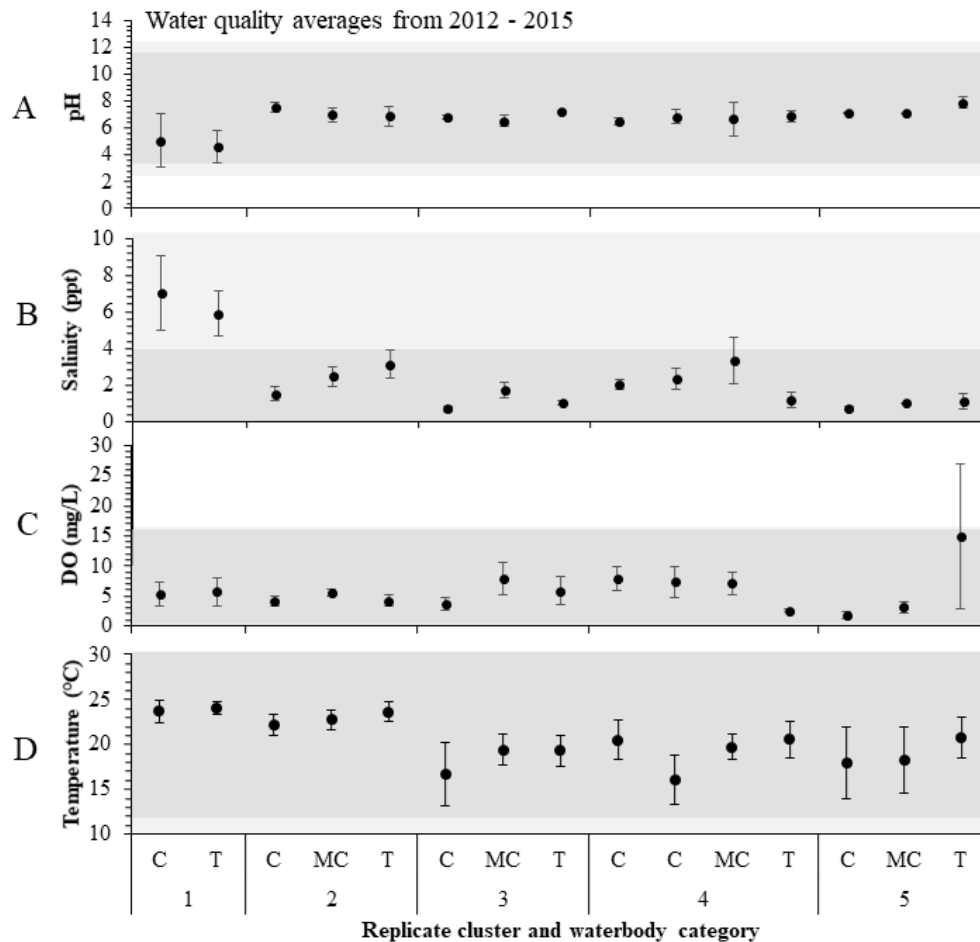
Figure 10: Mean water quality per waterbody (±SE) for pH (A), salinity (B), dissolved oxygen (C) and temperature (D). Light grey: Adult tolerance range which is broader than tadpole tolerance, Dark grey: tadpole tolerance range overlapping adult tolerance (Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman 1998; Pyke *et al.* 2002; Stockwell 2011; Werkman 1999).

3.5 Discussion

The use of call broadcast has wide implications for conservation initiatives, as many amphibian species exhibit conspecific attraction behaviours such as chorusing and aggregation (Gamble *et al.* 2007; Gaston *et al.* 2010). For example, conspecific attraction could be used to encourage breeding in high quality habitat or increase landscape connectivity in fragmented landscapes to reduce impacts that threaten population persistence such as disease, predation and population isolation. The current study aimed to investigate whether this strategy could be applied to *L. aurea.*

3.5.1 General findings

Despite past evidence that localised distribution of *L. aurea* can be manipulated by call broadcast (James *et al.* 2015: Attachment 1) and that colonising behaviour appears as a part of their breeding behaviour (Daly 2014; Hamer *et al.* 2002a), the current study did not produce statistically significant evidence of an increase in abundance and calling in response to call broadcast. Despite no overall statistically significant response, in one T waterbody, a large chorusing formed with over 20 chorusing frogs and 26 individual frogs observed. In this chorus, one female was found with a single mature egg, displaying the characteristic black and white animal and vegetal poles (eggs as described in Pyke & White 2001a) attached to her body, indicating recent oviposition in the waterbody. In the two low abundance cluster replicates, single calling males appeared in the treatment waterbodies for 2-3 days, calling from next to the speaker system. In an additional observations while carrying out this study, we observed that *L. aurea* bred once in the presence of *G. holbrooki,* an event that has been reported in other studies (Hamer *et al.* 2002b; Klop-Toker *et al.* 2017; Mortel & Goldingay 1998), and three times in waterbodies with short hydroperiods that dried before tadpoles could metamorphose (resulting in reproductive failure).

With respect to the secondary aims of this study, we found some minor temporal variation in call behaviour in treatments and correlation of calling behaviour with weather. However, by the end of the breeding season, calling in treatment waterbodies ceased at a similar time to controls and the reference waterbody, indicating in this study, that unlike some other frog species (Buxton *et al.* 2018; Martínez-Rivera 2008), *L. aurea* did not extend the length of their breeding season in response to call broadcast. Nevertheless, waterbody attendance and maximum chorus size were higher and lasted longer after chorus formation in response to call broadcast. We also found that higher rainfall, medium temperatures (16 °C - 26 °C) and higher relative humidity (>70 %) were associated with increased calling, but measures of abundance (male attendance) were only related to rainfall.

3.5.2 Correlation between rainfall and climate with calling and abundance

The current study found that abundance and calling was positively correlated with rain on the survey day, the day before and with the total cumulative rain for 11 days prior to survey. Calling was also related to higher levels of humidity and temperature. Associations between rainfall, climate and *L. aurea* abundance and calling has been reported in some studies

(Hamer *et al.* 2008) and likely indicates that rainfall and climatic factors provide good conditions for dispersal and provide ephemeral breeding habitat. As calling and abundance was not related to rainfall within 3 days of a survey this may indicate that weather three days prior to a survey is not a good predictor of *L. aurea* activity. The lack of correlation between calling 3 days after rain provides a greater insight into *L. aurea's* relationship with rainfall. This shows that *L. aurea* immediately disperse and call 2 days after rain, similar to explosive breeding frogs as a result of newly available habitat (Wells 1977). The reduction of abundance and calling with weather three days prior to survey may indicate that calling and breeding events are over quickly, which places considerable time constraints on studies attempting to detect or study breeding events.

Despite the relationship between weather and calling, not all studies investigating this phenomenon found a correlation (Valdez *et al.* 2015). A possible explanation for disparity between studies is differential survey effort. Hamer *et al.* (2008) undertook 14 surveys per waterbody per year over 2 years including opportunistic surveys of choruses in ephemeral habitat. The current study also undertook surveys per waterbody 11-13 times in 2013-14 season, 14-18 in 2014-15 season and 13 in the 2015 half season. However, Valdez *et al.* (2015) surveyed 4 times per waterbody per year for three years. Considering high fluctuations in rain, drought and the explosive breeding behaviour of *L. aurea*, relationships between chorusing events and rainfall may not be detectable at low sampling rates (4 days in a 153 day season). Consequently, serious consideration should be given to survey design to ensure surveys are undertaken at a high enough frequency for the detection and capture of rare calling events (Edwards *et al.* 2005; Edwards *et al.* 2004; Green & Young 1993) when correlating these to climatic variables (Hill 1992).

3.5.3 Failed breeding events

One of the most important findings of this study was that three separate breeding events failed as ephemeral waterbodies dried before tadpoles could metamorphose. In these events, since *L. aurea* exhibit no phenotypic plasticity to accelerate metamorphosis to avoid waterbody drying (Hamer *et al.* 2002a), it can be suggested that *L. aurea* is not well-adapted for rapid changes in hydroperiods or sudden weather events that dry waterbodies. It has been proposed that variable timing of metamorphosis, large clutch size and variable use of permanent and ephemeral waterbodies have evolved to maintain breeding success in the presence of environmental unpredictability and variability. It is apparent that flooded areas

chosen for oviposition were too shallow and had too short hydroperiods in the events recorded in the current study, indicating that even though this bet-hedging strategy of ovipositing in waterbodies of varying hydroperiod seems to have evolved as a part of the reproductive strategy of *L. aurea*, it is a strategy that may fail and result in reproductive loss; nevertheless, the presence of such a strategy suggests that there is a net adaptive benefit overall to the species. Although it is tempting to suggest the modification of landscapes to rescue clutches that will die, or to increase water depth in very shallow areas, altering survival of wild individuals can exacerbate a negative selection for maladaptive traits (Massaro *et al.* 2013), such as tilting towards breeding in shallow waterbodies at a frequency that is too high.

3.5.4 Possible confounding factors influencing conspecific attraction response

Although previous research demonstrated that *L. aurea* exhibits conspecific attraction, application of call broadcast in a landscape scale was not effective for manipulating distribution, based on the experimental design and level of replication undertaken in this study. The current study was undertaken in more realistic circumstances than laboratory and field-based assessment studies (Bee 2007; Buxton *et al.* 2015; James *et al.* 2015; Swanson *et al.* 2007), such as low population density, variable and in some cases, long distances between waterbodies, fluctuating abundance in the landscape and highly variable environmental conditions and pressures (Hamer *et al.* 2002b; Hamer *et al.* 2008; Hamer & Mahony 2010). As a result, there are a number of possible confounding factors reducing the effectiveness of conspecific call broadcast for conservation.

3.5.4.1 Distance

A possible factor influencing the effectiveness of conspecific attraction is detection by frogs of calls broadcast from speakers. Previous research has indicated that call attraction can operate between 40 m - 100 m under laboratory conditions (Christie *et al.* 2010; Swanson *et al.* 2007) and at greater distances when deployed with artificial ponds (Buxton *et al.* 2015). In the current study, speakers playing calls at normal call sound levels emitted by *L. aurea* (~71 dB) could be heard by researchers up to 200 m away in appropriate environmental conditions, in areas known to contain *L. aurea*. Considering the long distances travelled by *L. aurea* during the breeding season and the aggregatory behaviour over landscapes, it is likely that conspecific attraction plays a role in aggregatory behaviour. Consequently, it is unlikely that

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a failure to detect calls across the landscape was a limiting factor, and that calls from speakers could be heard at distance sufficient to be detected across the study landscape, and that other confounding factors may have limited response to broadcast.

3.5.4.2 Differential habitat selection

It is possible that behavioural mechanisms drive differential habitat selection, confounding the outcomes of this study. For the current study, the waterbodies chosen were considered suitable, with appropriate vegetation characteristics and within water quality thresholds. However, another form of habitat selection that may influence dispersal decisions is preference for permanent or ephemeral waterbodies. It is possible that behavioural phenotypes (personality) vary within the population (Brodin et al. 2013; Caspers et al. 2015). Personality is defined as behavioural differences within a population that are stable and consistent between contexts, for example, animals consistently displaying bold or shy behaviour when; feeding, dispersing, mating and responding to predation (Sih *et al.* 2004a; Sih *et al.* 2004b). This variation in behaviour may occur as a result of natural selection or due to stochastic evolutionary processes such as drift or founder effects, where variety in behaviour favours population survival and variable personality becomes stable and hereditable within a population (Brodin *et al.* 2013; Caspers *et al.* 2015; Dingemanse *et al.* 2003; Dingemanse *et al.* 2007). It is known that within a population some *L. aurea* exhibit site fidelity at permanent waterbodies (Hamer 2008) and some individuals disperse, sometimes to great distances (Hamer 2008; Pyke & White 2001a). Consequently, it is possible that dispersing *L. aurea* display variable behaviour compared to non-dispersers.

As it is possible for differential behaviours to occur within a population, it is important to hypothesise why varying behaviour may benefit *L. aurea* in the spatial context of this experiment. Throughout Kooragang Island, the landscape is spatially and temporally variable in wetland distribution, structure and hydrology (OEH 2014). It has been suggested that *L. aurea* employ a reproductive bet-hedging strategy; spreading reproductive risk between permanent and ephemeral habitat. For example, permanent waterbodies may generate higher risk of predation from *G. holbrooki* and other aquatic predators, but less risk of pond drying, whereas ephemeral waterbodies are at risk of drying but have reduced exposure to predation (Hamer *et al.* 2002a). This form of bet-hedging strategy spreads reproduction over different habitats to avoid asymmetrically distributed risks (Caspers *et al.* 2015; Hopey & Petranka 1994; Lips 2001; Segev *et al*. 2011). Establishment of this form of bet-hedging could be

driven through phenotypic behaviours and natal learning (Camacho *et al.* 2016). As the current study was undertaken in the ephemeral flooding belt, and the T and MC waterbodies were semi-permanent, it is possible that individuals in the ephemeral flooding belt show phenotypic bet-hedging preference for ephemeral waterbodies. For example, individuals that remain at permanent water may show preference for permanent water, and dispersing individuals may show preference for ephemeral habitat. However, very little is known about the variability in behavioural strategies driving dispersal and colonisation for *L. aurea*. As population persistence is influenced by a species' ability to adapt to changing climatic conditions in light of climate change (Merilä & Hendry 2014), this gap opens an important area for further research. Irrespective, in the context of the current study, it could be hypothesized that variation in behavioural traits that push certain individuals towards aggregation in certain waterbody types could result in such behaviours overriding impulses to respond simply to broadcast calls, buffering any effect in the landscape in response to the call broadcasts.

3.5.4.3 Other forms of conspecific attraction

Another factor that could have influenced the results of this study is competitive conspecific attraction. Currently the only study that has assessed conspecific attraction with the presence of competitive influence is James *et al.* (2015), where animals were attracted to *close proximity* chorus sounds regardless of competitive choruses. Other studies on *L. aurea* and other frog species did not test for conspecific interference either by not including that in laboratory experiments (Christie *et al.* 2010; Swanson *et al.* 2007) or by removing competitive influence in the field (Buxton *et al.* 2018; Buxton *et al.* 2015). Although it is generally considered that dispersal is triggered by rain, little is known about the choices *L. aurea* make while dispersing and the strength of attraction between conspecifics. It is also not known how far frogs will travel to a chorus, whether they will leave an existing chorus, preferentially travel to smaller or larger choruses, or establish their own chorus regardless of cues from other waterbodies. In this instance, competitive conspecific attraction may have retained animals at their waterbody or attracted them to other waterbodies, diluting the number of frogs responding to the application of conspecific calls from speakers.

3.5.4.4 Timing of speaker placement

Another potential influence on the effectiveness of the current study is timing of call broadcast. For birds, previous research has noted that the best response to call broadcast occurs prior to the onset of natural calling, before individuals settle (Andrews *et al.* 2015). For *L. aurea*, calling often occurs immediately after heavy rain, and thus it is likely that conspecific attraction occurs over a 24-hour period in which where frogs move and settle at a chorus. At the beginning of the 2014 - 2015 breeding season, there was limited response in T, possibly due to delays in speaker placement at the beginning of the breeding season. Consequently, placing speakers as pre-season cues is likely the most effective deployment period, prior to warming in spring and heavy rain in summer, and placing them later may have resulted in a greater resistance to movement in response to broadcast calls.

3.5.5 Revised implications for habitat selection and amphibian conservation

Although conspecific attraction assists formation of chorusing for *L. aurea* at short distance (James *et al.* 2015) and may increase the length of time that maximum chorus size is maintained, we were not able to manipulate distribution on a landscape scale. Conspecific attraction manipulation for a number of bird species has managed to redistribute populations (Ahlering *et al.* 2010; Ahlering & Faaborg 2006; Ward *et al.* 2011). However, for amphibians, the concept of conspecific attraction has only appeared to work at small scales in the laboratory (Christie *et al.* 2010; Swanson *et al.* 2007) and over uncertain distances (Buxton *et al.* 2015; James *et al.* 2015) but likely less than 250 m in the field. The current study also had no way to determine where animals in the large aggregation and single callers came from, and consequently distance over which animals are dispersing to aggregate in response to specific rain events is still an unknown factor that needs to be further explored in laboratory experiments (Christie *et al.* 2010; Swanson *et al.* 2007). As no strong response was found in the current study for *L. aurea*, this suggests that the applicability of call broadcast for conservation may be more limited for amphibians than has been found for birds. For example, conspecific attraction in practice, may not be able to increase connectivity between waterbodies, meta-populations, re-establish populations to their former range or decrease the risk of haphazard dispersal by providing directions to suitable habitat. Consequently, larger scale landscape conservation management of this and similar species, may not greatly benefit from the use of call broadcast for manipulating abundance.

Although abundance and calling did not significantly increase in response to call broadcast it may promote calling and reproduction at populated wetlands and provide learning tools for newly translocated animals. For some species of amphibians hearing conspecific calls can stimulate release of breeding hormones, egg production and encourage breeding activity (Wilczynski & Lynch 2011). As a number of translocation programmes have had limited success achieving reproduction (Callen Unpublished; Klop-Toker *et al.* 2016; Pyke *et al.* 2008), placement of speakers may encourage breeding activity. Additionally, call broadcast may provide learning tools for newly translocated animals that are naïve to habitat quality at the site of translocation (Scillitani *et al.* 2013). A number of *L. aurea* translocation programmes have failed to retain individuals at translocation sites, possibly due to dispersal (Pyke *et al.* 2008; Stockwell *et al.* 2008; White & Pyke 2008b) as a result of maladaptive habitat selection or lack of conspecific attraction. Placement of call broadcast at translocation sites may thus contribute to retaining individuals and increase reproductive behaviour at specific sites. This provision of call broadcast at translocation sites may also provide newly released individuals with information on high quality habitat that may adapt behaviour post-release. Additionally, call broadcast could be very valuable to first time breeders. Adults that have bred several times may become fixed in their waterbody preference and use one or two regularly, assessing waterbody quality on past breeding performance. However, inexperienced first-time breeders only have natal learning and experience from where they metamorphosed. Thus, call broadcasts could be very important to first time breeders, and conspecific cues may help to direct their breeding site, as has been found for birds (Ahlering *et al.* 2010; Ahlering & Faaborg 2006). However, considerable care should be taken to determine what constitutes high quality habitat. If conspecific attraction can encourage breeding at populated waterbodies and provide information to naïve translocated animals, call broadcast could promote waterbodies with adequate depth/hydroperiods to support development to metamorphosis and particular salinities that provide optimal protection against chytrid (Stockwell *et al.* 2015b). Consequently, further investigation is needed to determine if conspecific attraction through call broadcast can benefit translocations.

3.5.6 Improvements for future studies

From the current study, some challenges are worth noting to improve future research. Waterbody availability was a restrictive factor, as Ash Island has a patchy distribution of water with highly changeable salinity, where *L. aurea* distribution is restricted by availability

of freshwater (Valdez *et al.* 2015). This resulted in a lack of opportunity to replicate conspecific attraction experiments within a population's natural distribution. Additionally, the shifts in distribution of *L. aurea* seen during the study indicate how variable habitat use is, and it was noted that different waterbodies in the ephemeral belt were used each year for chorusing and breeding. Based on the speed at which choruses formed immediately after large rain events, call broadcasts need to be playing prior to and during the rain event. This is likely the reason for a lack of any response in the first year. This has implications for laboratory experiments, as there is a need for appropriate weather conditions to trigger behaviour and observations may be limited to short periods of time. Other issues to be managed for future research includes speaker system malfunction, which in the current study likely came about due to the complexity of the equipment and weaknesses in the electrical connections. It is strongly suggested that when attempting to manipulate distribution, manufactured systems should be used with minimal modification required, such as Farrell and Campomizzi (2011).

3.6 Conclusions

It is apparent that conspecific attraction is a factor in *L. aurea* management, however the effectiveness of call broadcast as a management tool may be more limited than originally envisioned. Speakers did not significantly increase abundance in treatments, particularly in comparison to ephemeral waterbodies. However, calling at treatment waterbodies increased and may be used to encourage short distance movement and increased breeding behaviour in high quality waterbodies, although this needs considerably more future research to verify.

3.7 Acknowledgements

Chapter 4. Microhabitat selection of calling male green and golden bell frogs (*Litoria aurea*) in N.S.W. Australia

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4.1 Abstract

The site selection of breeding individuals is a crucial component of a species habitat selection and can help to direct conservation programmes. However, habitat assessment often focuses on landscape and microhabitat scale without assessing breeding habitat in detail. The habitat of the endangered green and golden bell frog (*Litoria aurea*) has been studied extensively at both broad and microhabitat levels and has been used to direct conservation programmes aimed at restoring and creating habitat. However, reproductively ready males aggregate spatially, which could suggest males (1) select habitat for reproduction or protection, (2) aggregate to compete, chorus and attract mates. This study aimed to distinguish if male aggregation is associated with habitat features as an indicator of habitat selection.

Over the spring and summer breeding season, we compared calling locations relative to non-calling locations for water parameters (temperature, salinity, dissolved oxygen) and microclimate (temperature, humidity, average and maximum wind speed). We also assessed habitat as; open water, bare ground, emergent vegetation and floating vegetation. Using a Linear Mixed Model controlling for spatial and temporal effects, we found that males were associated with emergent vegetation and bare ground, and negatively associated with open water. Calling males were also associated with lower salinity and higher dissolved oxygen. This suggests that males may select habitat for breeding or protection from predators, providing appropriate vegetation, dissolved oxygen and salinity for embryo and tadpole development, and shelter from predators. These results may assist land managers to create and rehabilitate habitat within a waterbody when the priority is to promote the abundance of calling males.

4.2 Introduction

An important step in establishing the process to halt and reverse population decline is understanding factors affecting species distribution, however, research on key habitat can be

limited (McGarigal *et al.* 2016; Noss *et al.* 1997). When choosing habitat, animals assess habitat quality to support growth, survival and reproduction, and their ability to choose appropriate habitat determines their fitness (Krebs 2009). Conservation programs often try to understand the factors affecting species distribution through large scale habitat selection correlations. This can include correlation with large-scale topography and microclimate (Wiens 1976), or at a landscape or home range scale, with water quality, shelter, food, predator pressure and competition for resources (Compton *et al.* 2002; Heard *et al.* 2008; MacKenzie 2006; Wassens *et al.* 2010). This research can help identify habitat conservation strategies (Murphy & Noon 1992), however, the habitat chosen for breeding and direct placement of eggs (oviposition) can markedly affect reproductive success in heterogeneous environments (Refsnider & Janzen 2010; Resetarits & Wilbur 1989; Rudolf & Rodel 2005). Thus, fine-scale information on habitat selection may provide further information for conservation programmes undertaking rehabilitation or habitat construction (Campomizzi *et al.* 2010; Marsh & Borrell 2001; Mayor *et al.* 2009; Mcclure *et al.* 2012).

Assessment of breeding habitat selection can be particularly important for taxa experiencing worldwide population declines and extinctions, such as amphibians (Hayes *et al.* 2010). Factors contributing to declines in amphibian populations include the human facilitated spread of the disease causing chytrid fungus (*Batrachochytrium dendrobatidis*) (Skerratt *et al.* 2007), climate change, introduced species, and habitat loss, modification and population isolation (Brown *et al.* 2012; Ficetola & De Bernardi 2004; Stuart *et al.* 2004).

Adult amphibians exhibit habitat selection on landscape, home range and oviposition scales. For example, at the waterbody level, animals may select for habitat with particular temperature, salinity, pH, dissolved oxygen, waterbody size, depth, hydroperiod, vegetation type and structure and pressures from competition and predation and the influence of conspecific attraction (Alvarez & Nicieza 2002; Alvarez *et al.* 2013; Anderson *et al.* 1999; Blomquist & Hunter Jr. 2009; Crump 1991; Egan & Paton 2004; Freda & Dunson 1986; Haramura 2007, 2008; Kopp *et al.* 2006; Laurila 1998; Plénet *et al.* 2000; Refsnider & Janzen 2010; Resetarits & Wilbur 1989; Rieger *et al.* 2004; Rudolf & Rodel 2005; Seale 1982; Smith & Van Buskirk 1995). On a finer microhabitat scale, specific locations within waterbodies may be selected. For example, adults may choose to breed in shallow, sunny/warm regions of waterbodies (Dougherty *et al.* 2005; Egan & Paton 2004), in emergent vegetation (Cary 2010) or in deep water and denser submergent vegetation (Kern *et al.* 2013) or in lower

temperatures (Howard 1978b). This selection can be driven by female oviposition choice (Silva 2008), however, in some species males also choose resources for offspring survival or mate attraction, and defend those resources from rival males (Howard 1978a, b; Kelly 2008). Thus, breeding habitat selection influences both male and female distribution (Bergman 1999; Copp *et al.* 2010).

Oviposition selection in taxa such as fish and amphibians is crucial as they often leave eggs unattended and survival and development of offspring is reliant on the oviposition habitat (for a review see Refsnider & Janzen 2010). Placing eggs in high quality sites can (1) maximise embryo survival and optimise development, (2) improve the survival of breeding individuals, (3) adjust the phenotype of offspring to improve fitness and/or stabilise demographics or (4) provide high quality habitat for offspring (Refsnider & Janzen 2010). For example, placing eggs in vegetation may protect from predation (Orizaola & Florentino 2003) and development may be improved from optimal water temperatures (Seale 1982) and dissolved oxygen (Mills & Barnhart 1999; Mills *et al.* 2001), but outside optimal conditions may cause developmental abnormalities or reduced survival.

In addition to direct assessment of habitat based on quality, both natal learning and conspecific attraction can influence breeding habitat selection. The conditions experienced during early life stages may result in preferential choices for habitat reflecting the conditions experienced at early development stages (Stamps *et al.* 2009) such as habitat structures (Aubret & Shine 2008; Wiens 1970) or chemical cues (Belden *et al.* 2000; Ferrari & Chivers 2009; Hepper & Waldman 1992), and can happen at egg or tadpole stage. However this does not necessarily occur for all species (Ousterhout *et al.* 2014). Additionally, inadvertent cues i.e. acoustic communication or olfactory cues from a settled conspecific may be used by dispersing individuals as a sign of good quality habitat and stimulate settlement and thus aggregation (Betts *et al.* 2008). Consequently, decisions on habitat quality, natal learning and conspecific attraction may influence breeding habitat selection and ultimately fitness.

One species that would benefit from an understanding of breeding habitat selection is the endangered green and golden bell frog (*Litoria aurea*). This species is native to the east coast of Australia and listed as endangered in NSW (*Threatened Species Conservation Act 1995*) and vulnerable nationally (*Environment Protection and Biodiversity Conservation Act 1999*). Large scale habitat selection has been assessed (Mahony *et al.* 2013), however, distribution of calling males is aggregated despite the presence of other suitable habitat in the landscape

(Hamer & Mahony 2010; Stockwell 2009). Additionally, *L. aurea* actively choose to aggregate often within patches of emergent vegetation, in which conspecific attraction facilitates chorus formation (James *et al.* 2015).

There are a number of possible explanations for *L. aurea's* aggregation, which have not been fully explored for *L. aurea*. (1) It is possible that males choose particular habitat within a waterbody to defend as a resource/site/territory for oviposition (de Oliveira & Arturo Navas 2004; Wells 1978). From this, exploring *L. aurea* may use calling as public information on habitat quality, where calling stimulates aggregation for males to quickly identify high quality habitat and reducing time spent undertaking risky dispersal (Betts *et al.* 2008; Betts *et al.* 2010; Harrison *et al.* 2009; Nocera *et al.* 2009; Virzi *et al.* 2012). (2) Males may choose to aggregate in particular habitat for survival, where vegetation and access to water may provide escape from predators. (3) Alternatively, aggregation may not be related to breeding habitat, and instead offer protection from predators (e.g. the dilution effect or predator confusion Cheetham *et al.* 2011; Stamps 1988), provide better habitat for foraging (Stamps *et al.* 2005) improve mate finding, mate choice and competition (which may improve offspring fitness Gamble *et al.* 2007; Murphy *et al.* 2017), stimulate reproductive readiness and behaviour (Brzoska & Obert 1980; Burmeister & Wilczynski 2001; Chu & Wilczynski 2001; Wilczynski & Lynch 2011) or facilitate communal nesting (Berven 1982). None of these scenarios have been studied for *L. aurea* and in light of population declines, information on breeding behaviour may be crucial in order to direct conservation efforts.

The aim of this study was to determine if the calling location of males within waterbodies was related to particular vegetation, water quality or microclimate and describe their use of these habitats. This information can provide more information on *L. aurea's* breeding strategy and guide management and conservation programmes on appropriate breeding habitat.

4.3 Method

4.3.1 Available information on Litoria aurea calling behaviour

From previous studies, a range of waterbody conditions and characteristics describe the commonly used habitat which *L. aurea* use. While overwintering, *L. aurea* use vegetation and rock shelters (Garnham *et al.* 2015), and over the spring-summer breeding season, they use waterbodies with particular water quality, and directly using rocks, bare ground, and

aquatic vegetation (Garnham 2009; Hamer *et al.* 2002b; Midson 2009; Pollard 2009; Valdez *et al.* 2016). Calling males have been noted as specifically using open water, vegetation matts and open water amongst debris, overhanging and emergent aquatic vegetation, sometimes in patches of vegetation (Courtice & Grigg 1975; Humphries 1979; James *et al.* 2015; Osborne & McElhinney 1996; Thomson *et al.* 1996; Valdez *et al.* 2016). Due to the current rarity of the species, breeding is not regularly observed, however, past research has indicated that females move on the surface of the water while in amplexus to tangle eggs in vegetation (Courtice & Grigg 1975; Ford 1986).

4.3.2 Study site

An assessment of microhabitat selection in calling male *L. aurea* was undertaken in NSW Australia, on Kooragang Island (-32.860727 S, 151.723370 E), in Sydney Olympic Park (-33.841743 S, 151.073256 E) and at Avoca North (-33.462165 S, 151.434301 E) (Figure 11: A). Eight permanent and one ephemeral waterbodies were chosen as survey sites as they are known to have calling from previous research (Darcovich & O'Meara 2008; Hamer & Mahony 2010; Pyke & White 2008). This included three semi-permanent/permanent waterbodies at Kooragang Island (Figure 11: B). As *L. aurea* often colonise flooded (ephemeral) areas after rain (Hamer *et al.* 2008), three additional survey sites were added to opportunistically survey as new choruses appeared in ephemeral habitat (Figure 11: B), one ephemeral waterbody from Avoca Lagoon (Figure 11: C) and one ephemeral and five semi-permanent/permanent waterbodies from Sydney Olympic Park (Figure 11: D).

Figure 11: A) Australia with survey sites (square) and map of the east coast of NSW (grey). Map of survey waterbodies on B) Kooragang Island (-32.860727 S, 151.723370 E), C) Avoca Lagoon (-33.462165 S, 151.434301 D) and E) Sydney Olympic Park (-33.841743 S, 151.073256 E), NSW Australia.

4.3.3 Study design and quadrat placement

This study was undertaken from 2012 – 2015 throughout the spring and summer months (September- March) when *L. aurea* are known to call and reproduce. As male *L. aurea* are known to call from both aquatic vegetation from the surface of open water (Courtice & Grigg 1975; Humphries 1979; James *et al.* 2015; Osborne & McElhinney 1996; Thomson *et al.* 1996; Valdez *et al.* 2016) and within vegetation patches both fringing the waterbody or in islands of vegetation (James *et al.* 2015), each waterbody was surveyed by walking a strip transect around the aquatic zone. The edge of the waterbody was defined as the point at which water ceased, either due to dense vegetation or land. *Litoria aurea* most commonly call from within waterbodies (Valdez *et al.* 2015: James M. S. personal observation). From the edge, 1 m of land and 5 m of open water was surveyed. Islands of vegetation were also surveyed in the same method. At one waterbody, an open expanse of water was fringed with dense bull rush (*Phragmites australis*) and water continuing for > 100 m into an ephemeral

field. For this waterbody, the edge of *P. australis* and the water defined the edge of the waterbody.

Surveyed waterbodies had high variability in size, and to keep the number of quadrats to a reasonable number for a survey, different sized random number tables were used. To determine the size of the random number table, we measured waterbody perimeter using GoogleEarth (2012). If waterbody perimeter was < 300 m perimeter we used a 1 - 25 m random number table, and if waterbodies were > 300 m in perimeter we used a 1 - 50 m random number table. In one permanent waterbody, the perimeter was > 1400 m and thus a 1 − 100 m random table was used. Different sized random number tables dependent on pond size made surveying large ponds viable within a survey night.

Surveys were undertaken within waterbodies between 9 pm and 3 am (ESDT). A standardised starting location was chosen for each waterbody based on ease of access. On approaching the standardised starting location, three minutes of listening for calls was followed by call imitation for one minute, and has proven as a successful method to illicit call response for *L. aurea* (Bower et al. 2012; Hamer et al. 2008; Hamer & Mahony 2010). Following call imitation, researchers listen for a further three minutes to identify the presence or absence of calling in the waterbody and the location of calling males within the waterbody. Researchers then entered the waterbody and randomly chose to walk left or right along the vegetation which fringed the waterbody (walking route) in order to place calling male quadrats and random quadrats. Calling males were located by call and by visually spotting their throat movement, aided by eye shine. If a calling male was sighted within 10 m of the entry point, the male was targeted for a calling male quadrat. If calling males were not visible, two researchers approached quietly and used triangulation to sight the male. Researchers recorded what substrate the frog used as: (i) floating on open water; (ii) perching on a floating matt of algae, submergent vegetation or dead plant matter or floating grass; (iii) floating on water in emergent vegetation; (iv) perching on the ground; (v) perching on ground vegetation; (vi) perching on a rock; (vii) perching on emergent vegetation. The male was then approached quietly and a 1 m^2 frame quadrat made from PVC piping was placed over the male, with the male in the centre of the quadrat. If the male moved away, the location that they moved was noted to ensure the frog could not be surveyed a second time.

After the quadrat was placed down the searchers recorded; the date, waterbody name, and Global Positioning System (GPS) location, water parameters, microclimate and substrate

coverage were recorded. Vegetation and substrate coverage was measured as the percentage basal coverage of substrate (e.g. rocks or ground) and of each plant genus (using Sainty & Jacobs 2003) within the 1 m^2 quadrat. Water quality was assessed at the position the male sat in the water or under where the male perched, at approximately 15 cm depth or less if the water was shallower, using a *YSI Professional Plus* recording water temperature (°C), dissolved oxygen (mg/L) and salinity (ppt). Microclimate conditions were recorded using a portable weather meter (Kestrel 4000), and were taken from within the quadrat close to the individual (< 10 cm), which included temperature, average wind speed, max wind speed and humidity. Care was taken to avoid moving the vegetation and disturbing the microclimate around the calling male. After the first calling male quadrat was sampled, if other calling males were within a 10 m radius of the sampled male, surveyors continued to place quadrats on each calling male until no calling males remained within a 10 m radius.

If at the survey start, no calling males were detected within 10 m of entry, a random number table was used to measure the distance from the entry point to the first random quadrats. When the surveyor reached the random site, the quadrat was haphazardly placed in the edge and centre. As waterbodies generally contained islands of vegetation surrounded by open water, islands were treated as edges, where the edge of vegetation was randomly placed in the edge and the centre. The microhabitat variables were then sampled. Random and calling male quadrats were continued until the full waterbody was surveyed.

As researchers used triangulation to locate calling males in vegetation, we have assumed that the detectability of frogs within waterbodies with different structural complexity is equal.

4.3.4 Statistical methods

4.3.4.1 Substrate use description

To characterise the habitat used by *L. aurea*, the substrate in which individuals sat use was described (e.g. floating on open water) and the species of vegetation used was described. To indicate if particular plant species were used at different heights for calling position or posturing, we compared the height at which calling males sat compared to the substrate used, using a Linear Mixed Model in the program SAS in JMP v.11 (SAS 2016).

4.3.4.2 Analysis of calling presence/absence to substrate, water parameters and microclimate

Due to considerable presence of zeros in vegetation data, prior to analysis vegetation and physical structures were merged together to create subsets of substrate; water, floating vegetation, emergent vegetation and ground (rock, dirt).

To detect habitat selection, we compared locations selected by males (calling location) compared to sites without calling males (non-calling location). As conspecific attraction may cause aggregation of calling male *L. aurea* within a waterbody (James *et al.* 2015), prior to analysis the spatial pattern of quadrats was checked for clumped or dispersed data using mapped UTM GPS coordinates in JMP v.11 (SAS 2016). Visually, distribution of calling quadrats was aggregated compared to distribution of randomised non-calling quadrats which were spread out. As calling and non-calling quadrats had different distributions, the quadrat locations were spatially autocorrelated which must be accounted for in the analysis stage (Valcu & Kempenaers 2010). The nature of spatial correlation was assessed prior to the main modelling analysis, for each habitat measure (all water quality, microclimate and substrate). A variogram of each habitat measure was produced and three different models were tested for the spatial relationship: Exponential, Matérn or Gaussian. The goodness of fit for each curve was assessed and chosen visually and with the Akaike information criterion. Consequently, the relationship between distance and each habitat measure was determined prior to analysis by choosing the most appropriate curve for each habitat measure.

To assess if sites chosen by calling males correlate with particular habitat measures, we modelled the outcome variables (water quality, microclimate and substrate), using linear mixed models (LMM) in SAS Studio University Edition (SAS 2016). The categorical variable of location (calling or non-calling) was the primary explanatory variable. Further covariates were included if they were likely to affect the habitat measure being analysed. For example, dissolved oxygen may be affected by temperature, time of night and vegetation (Rehman *et al.* 2017). The waterbody location (Avoca Lagoon, Sydney Olympic Park and Kooragang Island) was also added to the model to assess any general differences in habitat as a result of geographical segregation. These variables were treated as fixed effects. Random effects in the model were waterbody (to control for potential correlations due to differences between waterbodies) and a 'waterbody by day' term to account for likely correlation due to repeated measurements each day within a waterbody. Spatial autocorrelation (including a

nugget effect term was added to the model using the repeated statement) based on the most appropriate spatial model (Exponential, Matérn or Gaussian) from the results of the variagraphic investigation.

4.4 Results

4.4.1 Substrate use descriptions

In total, 977 quadrats were assessed which included 176 calling locations and 801 non-calling locations over 47 survey nights. The maximum number of calling males detected within a waterbody was 14 and the minimum was 1 male, with a mean of 4.1 (±SE 0.48). A total of 15 males were found that did not call, however, they sat within 10 m of an existing chorus, and no calls were heard from these males while surveying the chorus. All waterbodies contained a large proportion of open water with fringing and patches of emergent vegetation. Males used *Schoenoplectus, Phragmites, Baumea*, *Lepironia* and occasionally *Typha,* where 61 % of males were most commonly found in the water floating between or beside stems of emergent vegetation (Figure 12). The second highest habitat used was perching on emergent vegetation above water level, males occasionally used floating vegetation masses, rarely floated in open water or used rocks and were not found calling on the ground (Figure 12). When males floated in emergent vegetation, they often held the emergent vegetation or submerged or floating plant debris, sat on algae or floated in open water holding algae or plant debris. The height from water or ground that males sat ranged from 0 – 150 cm, but overall, the height at which *L. aurea* called from was not dependent on the predominantly available vegetation type (F = 1.57, df = 12, p = 0.11).
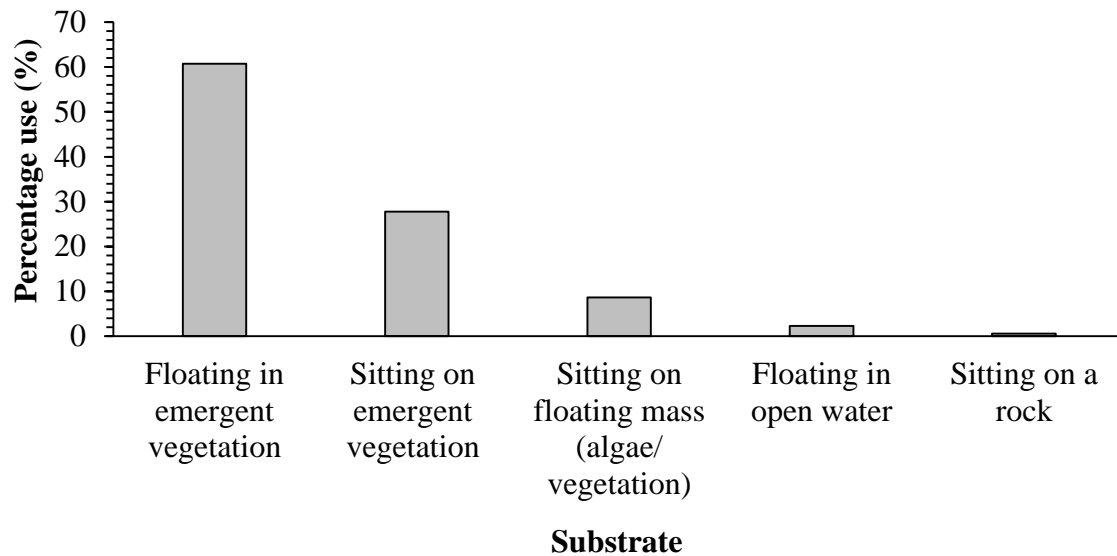
Figure 12: Percentage substrate use for calling males from August to March, on Ash Island (2013-2014, 2014-2015), Avoca Lagoon (2014-2015) and Sydney Olympic Park (2012-2013).

4.4.2 Analysis of calling presence/absence to substrate, water parameters and microclimate

Analysis of substrate use indicated that males were positively associated with emergent vegetation and ground, and negatively associated with open water (Figure 13:Table 3). No relationship was seen between calling and non-calling locations for floating vegetation. Males used vegetation with a range of 0 % to 100 % density, with an average of 40.5 % and open ground from 0 % to 90 % and an average of 1.7 %. Calling males sites were associated with higher dissolved oxygen, lower salinity and shallower depths (Figure 14: Table 3), however were not associated with particular humidity, maximum or average wind, air and water temperature (Figure 14: Table 3). Males were generally found in water depth ranging from 0 cm to 100 cm, and modelling indicated males used an average of 18.4 cm depth (Figure 14: Table 3). Males were found in water with dissolved oxygen ranging from 0.54 mg/L to 26 mg/L, with an average of 3.7 mg/L, and in areas with salinity ranging from 0 ppt – 4.65 ppt with an average of 1.9 ppt (Figure 14: Table 3).

Covariates within the model showed a relationship between depth and: temperature, dissolved oxygen (Table 3) and emergent vegetation (Table 4). Dissolved oxygen was not affected by emergent or floating vegetation (Table 3). Salinity showed no relationship with depth (Table 3), and the density of emergent vegetation had no influence on analysis of humidity, maximum and average wind, water or air temperature (Table 3).
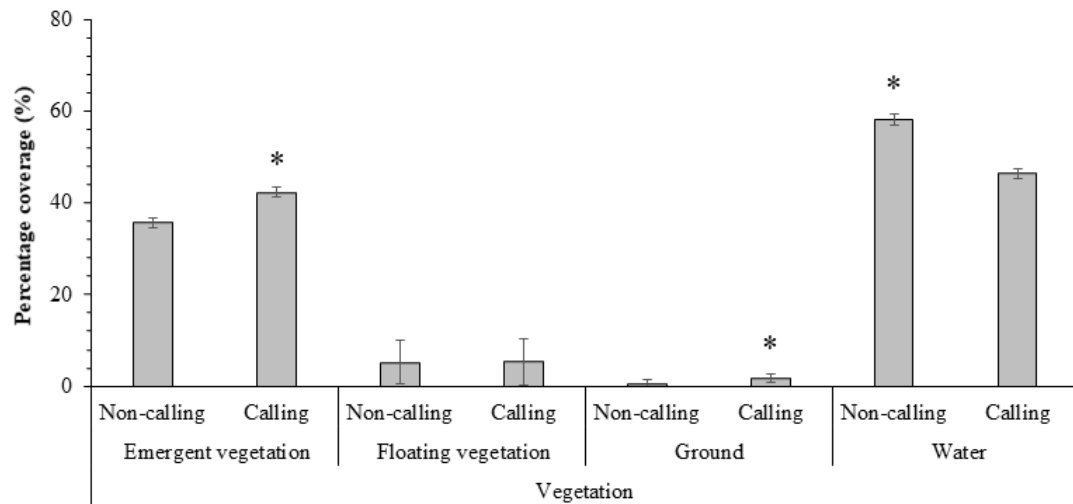
Figure 13: Comparison between mean substrate availability at calling and non-calling quadrats. Substrate availability is displayed as a percentage coverage for each substrate: emergent vegetation, ground, floating vegetation and water, based on 1m^2 quadrat with substrates measured at basal level. Linear Mixed Models (SAS 2016) were used to determine estimated marginal means ± 95% CI adjusted for covariates with statistical significance between calling and non-calling sites indicated by asterisks (*).
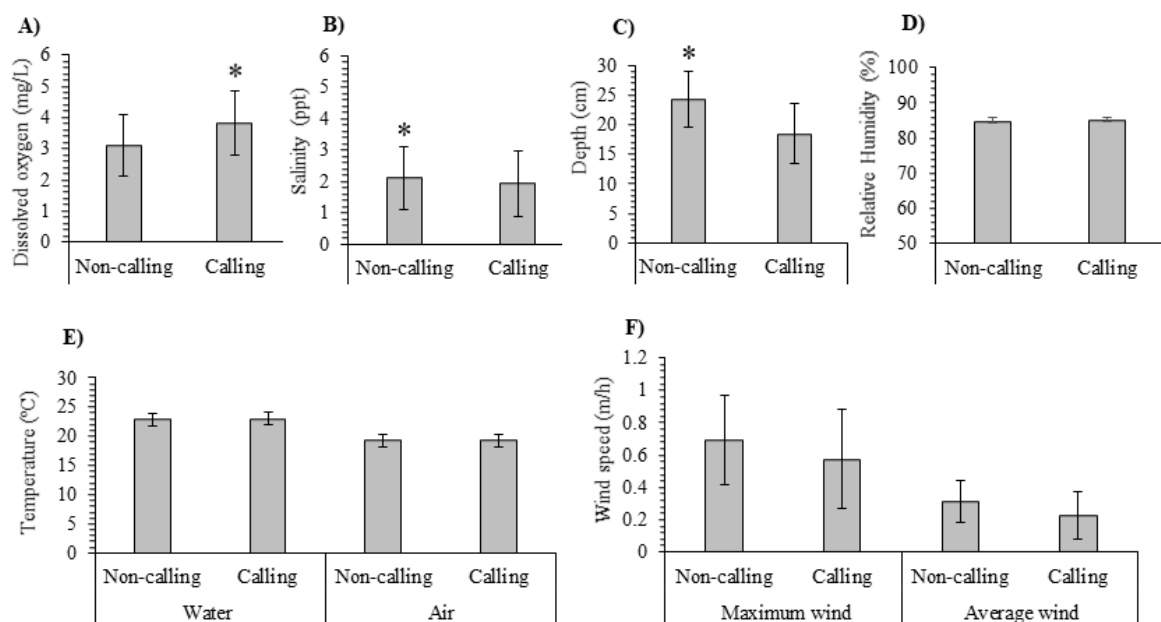
Figure 14: Mean water quality and microclimate at *Litoria aurea* calling locations and non-calling locations from a 1m^2 quadrat. Estimated means ± CI with statistical significance indicated by asterisks (*) determined from Generalised Linear Mixed Model in SAS (SAS 2016) accounting for spatial autocorrelation and random effects.

Table 3: Linear Mixed Models for calling male *Litoria aurea* location compared to non-calling location for water quality and microclimate in SAS (SAS 2016). Analysis accounted for spatial autocorrelation using Exponential, Matérn or Gaussian curves dependent on measure of fit from the Akaike information criterion and visual assessment. Covariates included within each model are listed from A – G, statistical significance for main effects indicated by bold and asterisks (*) and significant covariates in bold.

		Main effect and covariates	DF	Den DF	F Value	Pr > F
Water variables	**Water temperature (Exponential)**	Calling/non-calling quadrats	1	514	0.65	0.4219
		A) Region (Sydney/Newcastle/Central Coast)	3	514	2.37	0.0697
		B) Month	6	519	5.19	**<0.0001**
		C) Depth	1	514	35.15	**<0.0001**
		D) depth*depth	1	514	15.15	**0.0001**
		E) Time	1	514	2.48	0.1158
		F) Time*time	1	514	1.52	0.2187
		G) Floating vegetation	1	514	3.09	0.0796
		H) Emergent vegetation	1	514	1.34	0.2482
	Dissolved Oxygen (mg/L) (No spatial component required)	Calling/non-calling quadrats	1	508	16.17	**<0.0001***
		A) Region (Sydney/Newcastle/Central Coast)	3	508	0.69	0.5595
		B) Month	6	509	0.16	0.983
		C) Depth	1	508	5.19	**0.0232**
		D) Depth*depth	1	508	0.66	0.4164
		E) Time	1	508	19.43	**<0.0001**
		F) Time*time	1	509	17.48	**<0.0001**
		G) Water temperature	1	508	3.70	0.0551
		H) Emergent vegetation	1	509	0.24	0.6236
		I) Floating vegetation	1	509	0.61	0.4343
		J) Salinity	1	508	1.16	0.2811
	Salinity (Exponential)	Calling/non-calling quadrats	1	519	8.91	**0.0030***
		A) Region (Sydney/Newcastle/Central Coast)	3	519	3.25	**0.0215**
		B) Month	6	519	3.43	**0.0025**
		C) Depth	1	519	0.01	0.9391
	Depth (Exponential)	Calling/non-calling quadrats	1	526	11.50	**0.0007***
		A) Precinct	3	526	8.42	**<0.0001**
		B) Month	6	526	1.35	0.2333
Micro-climate	**Air temperature (Gausian)**	Calling/non-calling quadrats	1	519	0.03	0.8725
		A) Region (Sydney/Newcastle/Central Coast)	3	519	0.57	0.6377
		B) Month	6	519	8.18	**<.0001**
		C) Time	1	519	8.19	**0.0044**
		D) Time*time	1	519	4.72	**0.0303**
		E) Emergent vegetation	1	509	0.26	0.6548
	Humidity (Exponential)	Calling/non-calling quadrats	1	514	0.11	0.7446
		A) Region (Sydney/Newcastle/Central Coast)	3	514	4.35	**0.0049**
		B) Month	6	514	4.34	**0.0003**
		C) Time*time	1	514	122.01	**<0.0001**
		D) Emergent vegetation	1	514	0.15	0.6950
	Maximum wind (Exponential)	Calling/non-calling quadrats	1	518	2.12	0.1462
		A) Region (Sydney/Newcastle/Central Coast)	3	518	1.12	0.3416
		B) Month	6	518	2.22	**0.0404**
		C) Emergent vegetation	1	519	2.42	0.1201
		D) Time	1	518	0.51	0.4739
		E) Time*time	1	518	0.41	0.5239
		F) Emergent vegetation	1	518	0.07	0.7869
	Average wind (Exponential)	Calling/non-calling quadrats	1	495	3.29	0.0702
		A) Region (Sydney/Newcastle/Central Coast)	3	495	0.55	0.6468
		B) Month	6	495	3.20	**0.0043**
		C) Time	1	495	0.31	0.5755
		D) Time*time	1	495	0.26	0.6089
		E) Emergent vegetation	24	495	1.46	0.0732

Table 4: Linear Mixed Models for calling male *Litoria aurea* location compared to non-calling location for vegetation, water and ground coverage in SAS (SAS 2016). Analysis accounted for spatial autocorrelation using Exponential, Matérn or Gaussian curves dependent on measure of fit from the Akaike information criterion and visual assessment. Covariates included within each model are listed from A – G, statistical significance for main effects indicated by bold and asterisks (*) and significant covariates in bold.

		Main effect and covariates	DF	Den DF	F Value	Pr > F
Physical features	**Emergent vegetation (Exponential)**	Calling/non-calling quadrats	1	549	3.88	**0.0495***
		A) Region (Sydney/Newcastle /Central Coast)	3	549	7.95	**<0.0001**
		B) Depth	1	549	7.95	**0.0050**
		C) Salinity	1	549	0.20	0.6540
	Floating vegetation (Exponential)	Calling/non-calling quadrats	1	549	0.02	0.8938
		A) Region (Sydney/Newcastle /Central Coast)	3	549	0.84	0.4699
		B) Depth	1	549	1.79	0.1811
		A) Salinity	1	549	0.10	0.7498
	Ground (No spatial component required)	Calling/non-calling quadrats	1	559	4.85	**0.0280***
		A) Region (Sydney/Newcastle /Central Coast)	3	559	0.15	0.9292
		B) Emergent vegetation	1	549	6.54	0.4190
	Water (Exponential)	Calling/non-calling quadrats	1	550	17.71	**<0.0001***
		A) Region (Sydney/Newcastle /Central Coast)	3	550	5.34	**0.0013**
		B) Salinity	1	550	0.11	0.7384

4.5 Discussion

Many habitat studies aimed at contributing to the conservation of threatened taxa focus on habitat at a landscape scale or home range, without assessing key elements of breeding habitat. The current study is the first to quantify the physical microhabitat used by calling male *L. aurea* and the relationship between water quality, microclimate and vegetation for breeding behaviour. This understanding builds on the existing knowledge for *L. aurea* and can be used to further refine habitat rehabilitation and construction programmes.

In the current study, some males called independently without the presence of other calling males within the pond. Where males called together, there was a range of 2 – 14 males per pond, not including counts of potentially silent males that attended the chorus. These non-calling males may be satellite males, employing alternative breeding strategies such as intercepting females before they reach the chorus (Halliday & Tejedo 1995) or may remain silent to avoid predation (Green 1990).

We found that males generally called within ranges of salinity and other water quality thresholds that support the development and survival of tadpoles, except in the case of some instances of calling where salinity was above optimal or survival thresholds for tadpoles. Of the major environmental variables investigated, we found that calling males were positively associated with low water salinity, high dissolved oxygen, shallower water, emergent vegetation and ground. Calling males showed no selection for sites on the basis of maximum or average wind speed, air or water temperature, or floating vegetation. Males called from shallower water with an average of 18.4 cm depth, with salinity ranging from 0 ppt – 4.65 ppt. Based on thresholds developed from previous research, salinity values in this study were similar to that detected for optimal conditions for calling males and tadpoles in other studies, only being higher in salinity at the maximum threshold measurement by 0.65 ppt (Pyke & White 2001a; Stockwell 2011; Werkman 1999). However, in one of those other studies, tadpole survivorship was reduced at 2 ppt (Christy & Dickman 2002), a lower value than reported in separate studies. In the current study, males called from sites at an average of 1.9 ppt, and thus calling males on average called from locations with appropriate salinity for tadpole development and survival. In terms of dissolved oxygen, males called from 0.54 mg/L to 26 mg/L which fits within the minimum measurement range reported for previous research. However, dissolved oxygen was considerably greater in the current study compared to past work which indicated optimal levels for tadpoles from 0.14 - 16 mg/L (Pyke & White 2001a). Despite this, it is unlikely that increased oxygen levels would be detrimental to embryo and tadpole survival. Measures of water temperature indicated that males called from 12.1°C - 30°C which was within temperature ranges previously described (Browne & Edwards 2003; Hamer 1998; Penman 1998).

4.5.1 Substrate use

Aquatic vegetation has been identified as an important component of *L. aurea*'s habitat for calling males and for direct oviposition (Hamer 2002; Heard *et al.* 2008; Pyke & White 1996), this was corroborated in the current study, where calling males showed a strong preference for higher emergent vegetation density than availability (i.e. non-random association), and directly floating in water amongst emergent vegetation, sitting on emergent vegetation and mats of floating vegetation, but rarely floated in open water or perched on rock. This strong preference for vegetation differs from the habitat use of a similar species, the growling grass frog (*Litoria raniformis)* that instead preferentially uses: rocks, bare soil

and leaf litter near the water's edge and floating vegetation mats, which all have low vertical structural complexity and occasionally perched on horizontal stems of emergent vegetation (Heard *et al.* 2008). Instead, male *L. aurea* used high levels of vertical complexity preferring emergent vegetation to float in or perch on, and only occasionally used vegetation mats. Disparity between these sibling species' habitat use highlights the importance of species specific research.

Preference for vegetation may be occurring for various reasons. Males may have selected vegetated areas for oviposition, to protect eggs from predators (Formanowicz & Bobka 1989) and provide protection for tadpoles after hatching (Sanders *et al.* 2015). Alternatively, *L. aurea* may lay eggs in vegetation to protect from sunlight and UV-B radiation that reduces hatchling success (Van de Mortel & Buttemer 1998). Vegetation may also help anchor eggs (Pyke & White 2001a). Alternative to oviposition choice, physical structures such as vegetation may provide calling males protection for a number of reasons. Prolonged exposure to wind can cause desiccation, which can be prevented through sheltering behaviour (Schwardzkopf & Alford 1996). However, accounting for vegetation density as a covariate indicated that both maximum and average wind speeds were not significantly reduced by the vegetation, and humidity was not increased by vegetation density. Additionally, all males sat in the water or directly next to water, providing access to water to rehydrate, or to warmer temperatures in the water. Instead, vegetation may protect calling males from predation, or vegetation may provide particular posturing locations to enhance call amplitude (Gerhardt & Huber 2002; Wells 1977), or may provide higher abundance of prey items (Almany 2004; Hornung & Foote 2006; Kremen *et al.* 1993).

Despite male *L. aurea's* selection for emergent vegetation, males have been found calling (Courtice & Grigg 1975; Thomson *et al.* 1996) and breeding in sparsely vegetated water, such as watering troughs, baths and laundry tubs (Pyke & White 2001a) newly constructed wetlands (Chapter 5) and effluent waterbodies (Chapter 6). However, the presence of breeding and calling in sparsely vegetated habitat shows that very little vegetation is actually required for calling and oviposition. Conversely, vegetation has been repeatedly identified as preferred, in the current study and other studies for adults, juveniles (Garnham 2009; Garnham *et al.* 2015; Pollard 2009; Pyke *et al.* 2002; Valdez *et al.* 2016; Valdez *et al.* 2017a), tadpoles (Morgan & Buttemer 1996; Mortel & Goldingay 1998) and for oviposition (Morgan 1995; Pyke & White 2001a). Thus, as *L. aurea* use both dense and sparsely

vegetated habitat, higher vegetation density for oviposition is not likely the main driver determining calling location. This suggests that vegetation likely operates as protection for males from predation, and that unless sparsely vegetated waterbodies are opportunistically used and are suboptimal habitat, i.e. without adequate vegetation to provide shelter from predation. Regardless, when provided with particular conditions males prefer dense vegetation.

In the current study, males were found calling in shallower water with a small portion of ground compared to random sites within a waterbody. On average, the percentage coverage of ground in calling male locations was 1.7 %, and thus, proportions of use are generally low. As males were often found in fringing vegetation or vegetation islands, the consistent presence of small amounts of ground are likely due to males occurring near the edges of waterbodies in shallower water. Additionally, males did not call from open ground. Thus, it is more likely that males position themselves around the edges of waterbodies in shallower water and in vegetation, and do not directly select for small portions of ground.

4.5.2 Water parameters

In the current study, males were found at locations with higher levels of dissolved oxygen than randomly selected sites. Correlation of male presence may be a result of selection for higher oxygen or other variables that affect oxygen. It is possible high dissolved oxygen sites were chosen, as eggs are dependent on available dissolved oxygen at the oviposition site (being absorbed by the embryo) (Seymour & Bradford 1995) and hatchling success and tadpole development is linked to available oxygen (Kern *et al.* 2013; Moore & Townsend 1998; Sacerdote & King 2009). However, whether adults are capable of detecting dissolved oxygen is uncertain, and dissolved oxygen may instead auto-correlate with other variables that were directly selected. For example, submerged vascular vegetation is known to release oxygen through roots and rhizomes where oxygen assists degradation and nitrification processes (Rehman *et al.* 2017). Despite addition of oxygen to water by vegetation, factoring vegetation into the dissolved oxygen analysis showed no significant interactions. Alternatively, decomposition of vegetative biomass can reduce oxygen levels in water (Tang *et al.* 2013) and different areas within a waterbody may experience different levels of decomposition due to varying amounts of decomposing biomass. Consequently, males may have been in locations with a high level of living emergent plant biomass, but low decomposing plant biomass. A closer assessment of the chemical processes within

waterbodies may determine if and how vegetation and decomposition play a role in varying dissolved oxygen within waterbodies and how this may affect embryo development and survival.

The saline conditions that males call from may be of great interest to researches using salt to treat chytrid fungus (Hossack 2011; Hossack *et al.* 2013; Stockwell 2011) and the factors influencing salinity thus deserves further consideration. In the current study, calling males were found in locations with lower salinity. Considering the propensity of *L. aurea* to disperse and call after heavy rain (Hamer *et al.* 2008; Valdez *et al.* 2015), it could be considered possible for chorusing to occur in shallow water which is (1) less connected to groundwater and (2) exposed to dilution from rainfall. However, within the modelling there was no relationship between depth and salinity, indicating that shallow water did not contain less saline water. Consequently, it is not yet understood whether calling males directly select for lower salinity. Although males selected for lower salinity, males called from an average of 1.9 ppt which fits well with tadpole survival and chytrid treatment. The optimal tadpole tolerance for salinity is < 2 ppt (Christy & Dickman 2002), indicating calling male average salinity use sits within the optimal range. Considering that more saline water has been linked to treatment of chytrid (2 ppt – 6 ppt) (Hossack 2011; Hossack *et al.* 2013; Stockwell 2011), calling male sites on average were also close to being appropriate for chytrid treatment. To treat chytrid, conservation programmes have started to apply salt to waterbodies (Callen Unpublished) and connect constructed waterbodies to groundwater (Chapter 5). The ranges of salinity that males call from have implications for these salt-based conservation programmes, indicating which ranges of salinity males choose to call at in a free moving population and that some exposure occurs to chytrid treating salinity levels.

4.5.3 Implications for future research and conservation

4.5.3.1 Habitat creation programmes

Based on the identification of highly used habitat characteristics, habitat creation and reintroduction programmes can incorporate these features. We recommend that conservation efforts should focus on providing emergent vegetation within waterbodies around 40.5 % density as this represents the mean use for calling males (minimum of 33.4 % and maximum of 47.6% based on model CI's). However, much of the vegetation used in the current study was fringing, in patches or islands with large expanses of open water, where open water may

be an important aspect of waterbody selection. Within waterbodies, there should be shallower areas available, as males used an average of 18.4 cm depth (ranging from 0 cm to 100 cm). Aims for water chemistry should target dissolved oxygen at 3.7 mg/L (ranging 0.54 mg/L to 26 mg/L), and salinity around 1.9 ppt (ranging from 0 ppt – 4.65 ppt). Incorporation of these features should provide appropriate conditions for chorusing, alongside adherence to previous research on water quality thresholds (Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman 1998; Pyke *et al.* 2002; Stockwell 2011; Werkman 1999), which should provide a framework for water quality to improve embryo development and survival.

In order to facilitate these conditions within waterbodies, conservation managers can design and manage waterbodies to create suitable calling conditions. In design, managers can control depth profiles to provide shallower areas for males, which we recommend providing shallow waterbody fringes and islands within the waterbody. Both passive management and active management of vegetation is possible when designing and managing waterbodies. Within the modelling, vegetation density decreased with depth. Thus, provision of some deeper areas may passively control vegetation long term and maintain open water as a general waterbody characteristic. In order to actively manage vegetation density, a vegetation harvesting regime could maintain open areas and where vegetation exists in fringes and islands, maintain ~ 40% density in vegetated shallow areas to replicate the conditions used by males. When constructing waterbodies, salinity can be controlled by avoiding saline ground water, or providing a mosaic of salinities to increase the chances of some being optimal at any given time. Additionally, the size of the catchment can be increased to dilute any naturally occurring salinity. Oxygen may be maintained by promoting vegetation which supports dissolved oxygen in water and monitored to ensure decomposing vegetation does not reduce dissolved oxygen.

Although we suggest providing waterbodies with these suitable conditions for calling as an indicator of a waterbodies breeding potential, there are many other aspects of *L. aurea*'s life cycle that require different habitat features. For example, provision of mosaics of waterbodies with differing features may facilitate male/female segregation (Valdez *et al.* 2016) and juvenile dispersal after metamorphosis (Bower *et al.* 2012), which is key to providing habitat which supports sex and age-based habitat partitioning. Additionally, it is important to provide wetlands with a mosaic of salt concentrations to passively treat frogs for chytrid (approximately 2 ppt) (Hossack 2011; Hossack *et al.* 2013; Stockwell 2011) while retaining

salinity within tadpole tolerance thresholds (Christy & Dickman 2002; Stockwell *et al.* 2015c; Werkman 1999). Keeping these factors in mind, for management of landscapes where calling and breeding are the limiting factor, more emphasis can be placed on providing calling habitat features.

4.5.3.2 Male distribution and conspecific attraction

From the current study and previous research on conspecific attraction (James *et al.* 2015), it is apparent that both habitat within a waterbody and conspecific cues influence the distribution of calling male *L. aurea*. This indicates that *L. aurea* most likely use a multimodal method to choose habitat, where chorus location within a waterbody is likely determined by the habitat choice of the first male, and the subsequent aggregation forms around initial calling settlers as a result of conspecific attraction. This provides important information to conservation managers looking to use conspecific attraction in wild populations as a management method. Research on birds and conspecific attraction has shown that animals can be drawn to poor quality habitat by poorly placed conspecific cues i.e. speakers playing calls (Ahlering *et al.* 2010; Betts *et al.* 2008; Betts *et al.* 2010). As *L. aurea* employs both habitat selection and conspecific attraction to choose chorus locations, the use of conspecific call broadcast should carefully consider the results of the current study when choosing (1) waterbodies and (2) sections of waterbodies in which to play calls, noting the recommended parameters in *Section 4.5.3.1*.

4.5.3.3 Monitoring Litoria aurea habitat

In addition to providing information for the creation of habitat, studies such as this one can also refine habitat assessment and monitoring programmes. Landscape occupancy studies and monitoring for *L. aurea* often take single or several samples at the centre of the waterbody (Callen Unpublished; Valdez *et al.* 2015) several locations in the same distance from shore (Garnham 2009; Pollard 2009) or do not specify how readings were obtained (Klop-Toker *et al.* 2016; Valdez *et al.* 2017b). From the current study, high levels of variability were seen within waterbodies between samples (e.g. significance for oxygen and salinity), future research should take samples from specific locations within a waterbody. These specific locations should include a range of depths from shallow too deep, both within varying densities of vegetation and open water, targeting locations with healthy vegetation and also signs of decomposition. From this data, application of more detailed microhabitat sampling

alongside accompanying variables within an analysis of occupancy could refine models assessing occupancy patterns (McGarigal *et al.* 2016).

4.5.4 Limitations of the study

A major assumption in the current study, is that all calling males are capable of assessing the quality of habitat and making good decisions for settlement. It has been shown that amphibians sometimes select poor quality habitat, for example waterbodies with short hydroperiods (Chapter 3), poor temperature (Seale 1982), with parasites and disease (Kiesecker & Skelly 2000) and predator/competitor presence (Hamer *et al.* 2002b; Klop-Toker *et al.* 2017). Additionally, for species with conspecific attraction, social or territorial attraction can override habitat selection, leading to settlement in poor quality habitat (Betts *et al.* 2008; Howard 1980). *Litoria aurea* has been noted as exhibiting conspecific attraction (James et al. 2015) and sometimes breed in poor habitat with short hydroperiods which cause the desiccation of whole clutches (*James M. S. personal observation see Chapter 3).* Poor decisions may be exacerbated by conspecific attraction as an individual making poor decisions habitat decisions, as frogs may be drawn to the poor-quality habitat due to social/territorial attraction.

Another assumption in the current study, is that we assessed calling male site selection as a proxy for oviposition, but it cannot be assumed that oviposition occurs within the chorus. Published literature on breeding behaviour does not adequately describe oviposition to assess if males or females choose the egg deposition site. Oviposition at the site of amplexus has been observed four times, once in a small chorus at the edge of a waterbody in *Juncus*, once in a patch of emergent vegetation in SOP (*James M. S. personal observation*) and, twice in New Zealand in sparse grass vegetation (Chapter 6). It has been reported that amplexing pairs directed by females have moved 100 m (Pyke & White 2001a unpublished observations). However, details of the observations were unclear in that report; it was not noted where females moved from or to, and there were no observations of prevailing conditions, waterbody type e.g. ephemeral/permanent or oviposition selection. As already noted with respect to calling sites being potentially a flawed proxy, males may select habitat for protection from predators while calling rather than as optimal spawning sites. However, sites protecting adult males from predators may provide indirect benefits to offspring. Thus, it cannot be assumed that females within a waterbody choose oviposition site or that males

directly select habitat for oviposition highlighting the need for further data obtained through careful observation and study.

Assessment of the sampling technique used in the current study is also crucial to understand true habitat selection independent of biases resulting from non-random sampling (Sanders *et al.* 2015). In the current study, 100% of detected males were surveyed and none were inaccessible due to deep/open water or other constraints. Nevertheless, using a strip transect for non-calling locations as applied in the current study would have biased sampling towards highly vegetated shallow areas, reducing the number of samples taken in deep open water. This may have falsely resulted in failure to detect correlations, for example, there may have been: (1) no positive correlation with emergent vegetation and (2) shallow water and (3) no negative correlation with open water. Despite this bias within the sampling technique, a positive correlation was, nevertheless, detected for shallower water, higher vegetation density, and negative association with open water. If this study was fully randomised, positive associations for these variables would be far more extreme and would more strongly support the conclusions made in this paper.

One variable which should be considered very cautiously is water temperature (given that it was not identified as a significant correlate with calling). As previously discussed, reduced sampling at the centre of deeper waterbodies likely reduced sampling rates at deeper and cooler temperature locations. This would be exacerbated by the sampling method, where water quality samples were taken from ~ 15 cm depth to represent the surface temperatures experienced by the calling males. As this did not sample the bottom of the water, this does not represent the full temperature profile that embryos may experience. As egg and tadpole development can be improved in warmer waters with optimal temperatures (Bachman 1969; McLaren & Cooley 1972; Moore & Townsend 1998; Zweifel 1977), and breeding individuals may directly select for temperature (Kurdíková *et al.* 2011), water temperature may still be an important habitat feature and needs to be assessed further.

4.5.5 Future research on direct habitat selection and natal learning

Identification of specific variables in this study used by calling *L. aurea*, indicates that there is a gap in knowledge on direct selective behaviour. Generally, the mechanisms that drive habitat selection by individuals within populations can include: (1) animals directly assessing habitat quality from experience or using conspecifics as an indicator of good quality habitat,

(2) natal learning where individuals show preference for habitat similar to that experienced in development stages, (3) returning to their place of origin, and (4) indirect selection of oviposition by selecting a mate (for a review see Refsnider & Janzen 2010). These options directly affect distribution through dispersal and settlement patterns. As we found that males choose emergent vegetation, shallower water with high dissolved oxygen and lower salinity, future research should assess if males or females directly oviposit in these conditions in choice experiments (Iwai *et al.* 2007), and if egg clutches gain advantages from ovipositing in these conditions (Mills & Barnhart 1999; Mills *et al.* 2001; Orizaola & Florentino 2003; Seale 1982). Furthermore, rearing of these individuals and subsequent choice experiments at juvenile and adult stages would directly assess if natal learning occurs for *L. aurea* (Benard & McCauley 2008; Davis & Stamps 2004; Stamps *et al.* 2009). This would provide key information on direct oviposition selection and natal learning which would assist explaining dispersal and colonisation behaviour.

4.6 Conclusion

The current study and recent research have provided insights into *L. aurea* habitat requirements, providing opportunities to guide habitat construction and monitoring programmes. Overall, we found that chorus size varied greatly, that possible satellite males sat within chorus space, and that males preferentially use emergent vegetation to float in water or perch on vegetation. The sites males called from were characterised by lower salinity, shallower water and higher dissolved oxygen. To inform monitoring, habitat creation or rehabilitation programmes, research that investigates the link between waterbody attributes and breeding activity is crucial to frame larger questions on the development and survival of endangered species.

4.7 Acknowledgements

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Chapter 5. Rapid colonisation of constructed wetlands by the endangered green and golden bell frog (*Litoria aurea*) and five other frog species

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5.1 Abstract

Habitat creation programmes are often used to compensate for the loss of habitat for endangered species, with varying results. We describe an early successful wetland construction programme implemented to determine if artificially constructed wetlands could provide additional breeding habitat for green and golden bell frog (*Litoria aurea*), on Ash Island, NSW Australia. Seven ephemeral (flooding) and two permanent waterbodies were constructed near an existing population. The wetland was designed to increase landscape aquatic habitat, based on adaptive management learnings from past research, and reduce disease and predator impacts. Soil bunding was constructed around individual wetlands to prevent overland flooding and spread of invasive, predatory fish through the system, and ephemeral wetlands were built into the system to allow waterbody drying in case of such invasive fish infestation. Wetlands were dug to an appropriate elevation to connect to groundwater to increase waterbody salinity for chytrid disease management. In this study, we assess the initial, successful use of this habitat by this species, and initial findings on the design suitability.

Surveys conducted across Ash Island gathered baseline data on adult, juvenile and tadpole body condition, sex ratios, estimated age and habitat use. Surveys were undertaken at coastal populations other than Ash Island to assess chorus sizes, before similar surveys were repeated at the constructed wetlands on Ash Island. *L. aurea* rapidly colonised constructed ephemeral wetlands on Ash Island, with chorusing males and breeding present. The chorus size in constructed wetlands was large in comparison to other populations in coastal NSW, and a range of other frog species also bred onsite. Female *L. aurea* used a nearby remnant wetland (adjacent to the constructed wetlands), and different habitat to males that was reflected in the broader population. Most male and female *L. aurea* captured on Ash Island were under 12 months of age, and body condition in the constructed wetlands was higher than in the broader population. Bunding and ephemerality to reduce predation showed early signs of success as a

wetland habitat strategy for *L. aurea*, however elevated salinity from ground water (intended to ameliorate chytrid disease in the landscape) was higher than anticipated and requires further monitoring. It is hoped that this programme may help guide other conservation projects creating habitat for species under threat.

5.2 Introduction

In the current era of development and environmental management, new habitat is often constructed for a threatened species to offset habitat loss, however, stories of successful habitat creation can be rare. For many programmes which offset habitat loss with created habitat (compensation), results are often considered poor as the habitat does not meet species requirements or mitigation standards (Burgin 2010; Kihslinger 2008; NRC 2001; Semlitsch 2008b; Sudol & Ambrose 2002). This may be for a number of reasons, including: the habitat may not exclude key threatening processes (KTP), is too far from or has limited connectivity to the site of habitat loss (Ruhl & Salzam 2006) and rehabilitation science and information on the threatened species' may not be thoroughly established (Germano & Bishop 2009; Germano *et al.* 2015). However, after considerable research effort and trial and error, some programmes can incorporate past experience and show early signs of success.

The Australian green and golden bell frog (*Litoria aurea*) has undergone an extensive decline in range and abundance since the 1960s when it was considered common along the coast and eastern slopes and tablelands of NSW. The species is now restricted to small, isolated and declining populations (Mahony *et al.* 2013). Various Key Threatening Processes (KTP) (OEH 2017) have been associated with its decline. Of these, the pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*) that dramatically increases mortality and population decline over winter (An & Waldman 2016; Klop-Toker *et al.* 2016; Mahony *et al.* 2013; Scheele *et al.* 2016; Skerratt *et al.* 2007) is probably the most significant; in its absence other threatening processes may have had little impact on this species. Adding to the primary impact of chytridiomycosis, the invasive fish (*Gambusia holbrooki*) is considered likely to have increased the decline by acting as a predator and competitor of *L. aurea*. *G. holbrooki* readily disperses across landscapes between wetland waterbodies during flooding events, and is suggested to decrease reproductive success and population recruitment of *L. aurea* (Klop-Toker *et al.* 2017; Pollard *et al.* In Prep) when it is present. Other suggested KTP to the persistence of *L. aurea* include climate change, habitat loss and modification, loss of habitat connectivity and the synergistic effects of the various KTP together (Brown *et al.* 2012; Muir

2008; Stuart *et al.* 2004). As a consequence of its decline in recent decades, *L. aurea* is classified as vulnerable, nationally and globally (*EPBC Act 1999*)(IUCN 2015), and endangered in the state of NSW (*TSC Act 1995*).

L. aurea has been the focus of extensive conservation efforts, including habitat construction and reintroductions, as a result of population declines and continued loss of habitat (Goldingay 2008). The results of such efforts have been mixed. Some constructed wetlands have been considered a success (at least in the short-medium term), with evidence of breeding and the presence of all stages of the life cycle from tadpoles to juvenile and adult frogs (Darcovich & O'Meara 2008; Pickett *et al.* 2013; Pyke *et al.* 2008; Stockwell 2009; White & Pyke 2008b). However, other attempts at habitat construction and/or reintroduction have not been so successful (Daly *et al.* 2008; Klop-Toker *et al.* 2016; Stockwell 2009; Stockwell *et al.* 2008; White & Pyke 2008b). Many factors may have contributed to sub-optimal or failed attempts. A reoccurring issue is the continued action of the primary KTP, chytridiomycosis, amplified by the influence of other processes such as poor-quality habitat, failure to achieve a critical population density and absence of connectivity to existing metapopulations (Daly *et al.* 2008; James *et al.* 2015; Pyke *et al.* 2008; Stockwell *et al.* 2008; White & Pyke 2008b). These are common and reoccurring issues for wetland conservation programmes generally (Kihslinger 2008). The variable and often sub-optimal outcomes of *L. aurea* management programmes emphasise the need to understand what constitutes high quality habitat (especially where habitat is to be constructed) and to understand how occupation of such habitat is affected and limited by KTP.

Research that has characterised key ecological and biological traits pertinent to *L. aurea* conservation may assist conservation programmes targeting both in situ management (reducing impacts of KTP) and in establishment of additional constructed habitat where the frogs occur (or where they may be reintroduced). *L. aurea* males chorus in permanent waterbodies, but also disperse during heavy rain to chorus in ephemeral (flooded) depressions (Hamer *et al.* 2008; Valdez *et al.* 2015). However, the method by which *L. aurea* locate habitat is not fully understood, for example, it is unknown whether directional migration occurs, or if *L. aurea* disperse widely and opportunistically use available habitat (Semlitsch 2008a). Some evidence suggests that *L. aurea* may not succeed in avoiding dispersal to and breeding in wetlands that have low reproductive potential due to high predation risk (Klop-Toker *et al.* 2017). During dry climatic periods, permanent water can act as drought refuges,

and vegetation provides predator protection, basking, foraging and overwintering habitat (Bower *et al.* 2013; Garnham *et al.* 2015; Hamer *et al.* 2002b; Hamer & Mahony 2007; Pyke & White 1996; Valdez *et al.* 2015). Data on sex ratios show that females are not found regularly in, or close to, wetlands (in comparison to males), and it has been suggested they use terrestrial habitat or alternative habitats to males (Goldingay & Newell 2005; Goldingay 2008; Pickett 2009; Pickett *et al.* 2012; Valdez *et al.* 2016). Juvenile *L. aurea* disperse from natal wetlands after metamorphosis at a high rate, possibly avoiding cannibalistic adults (Bower *et al.* 2013) or areas of high conspecific density due to resource limitation (Berven 2009). For *L. aurea* tadpoles in ephemeral wetlands, an adequately long hydroperiod is essential to avoid clutch death (*Melanie James personal observation Chapter 3)*, as there is no evidence of developmental phenotypic plasticity leading to early metamorphosis in response to drying waterbodies (Hamer *et al.* 2002a).

Past findings from amphibian restoration programmes have established that incorporation of key ecological needs and preferences of a target species can improve conservation outcomes for initial colonisation and long-term persistence (Brown *et al.* 2012). Thus, integration of past learnings and research knowledge on *L. aurea* allows us to derive a list of key elements to improve habitat design. These include:

(1) *Mosaic of ephemeral and permanent water bodies.* It has been proposed that the key habitat criterion in habitat design should be the implementation of a mosaic of ephemeral and permanent water that accommodates dispersal and opportunistic breeding after rain events, but also provides drought refuges (Hamer *et al.* 2008; Hamer & Mahony 2010; Valdez *et al.* 2017b; Valdez *et al.* 2015). Ephemeral wetlands should have adequate hydroperiods to allow tadpoles to reach metamorphosis, while obtaining the advantage of frequent drying to remove predatory fish (Hamer *et al.* 2002a; Klop-Toker *et al.* 2017; Pollard *et al.* In Prep).

(2) *Limitation of dispersal of invasive fish (G. holbrooki).* Dirt bunds (walls of soil that encircle constructed waterbodies) may be constructed around wetlands to prevent the flooding that allows *G. holbrooki* dispersal across landscapes, although this carries the limitation of reducing the rainfall catchment size area to fill waterbodies.

(3) *Appropriate development of the terrestrial vegetation surrounding constructed wetlands* (should be viewed as a part of the constructed habitat). Terrestrial vegetation provides foraging habitat (especially for females, given their tendency to

disperse from waterbodies except in breeding events), facilitates differential sex and age based habitat selection including the use of terrestrial vegetation as overwintering habitat (Bower *et al.* 2012).

(4) *Connectivity*. Good connectivity allowing dispersal between existing populations and waterbodies through wetland systems improve chances of successful colonisation during dispersal of juveniles and adults (Hamer & Mahony 2010; Muir 2008). Constructed waterbodies close to other constructed and existing waterbodies are the optimal landscape configuration.

(5) *Construction of habitat with elevated salinity*. As elevated levels of salinity (in approximately the 2-6 ppt range) inhibits chytrid development and reduces disease mortality, habitat design can also incorporate novel methods such as salt addition or groundwater fed salt (Stockwell *et al.* 2012; Stockwell *et al.* 2015c). Although it may require additional effort at the design phase (including hydrological surveys of ground water), wetlands connected to ground water may provide a mosaic of salt concentrations to passively treat frogs (Hossack 2011; Hossack *et al.* 2013; Stockwell 2011) while salinity remains within tadpole tolerance thresholds (Christy & Dickman 2002; Stockwell *et al.* 2015c; Werkman 1999).

In this chapter, we report on a recent example of created wetland habitat for *L. aurea*, assess the newly constructed wetlands to determine if the site was successfully occupied by a breeding population of *L. aurea*, and assess success of habitat design using a number of ecological, population and behavioural metrics. The criteria for successful colonisation in the reported habitat construction programme included: (1) adult male and female *L. aurea* must colonise the site, use habitat for foraging, sheltering and breeding (2) the wetlands must support the successful growth, development and metamorphosis of tadpoles into juveniles (3) there should be similar to no difference in demographics and behaviour between the constructed habitat and similar existing habitats occupied by *L. aurea* in comparable wetland systems (4) *G. holbrooki* must be absent/excluded from the constructed wetland before and after construction, requiring adequate bunding that maintains its integrity and excludes landscape water drainage flows into the constructed waterbodies (5) water quality occurs within the ranges that are considered optimal for *L. aurea*.

5.3 Method

5.3.1 Study area including constructed habitat, and Litoria aurea distribution

This study was undertaken on Kooragang Island/Ash Island, within the Hunter Estuary of NSW, Australia (-32.862863 S, 151.728355 E) (Figure 15:A). The Kooragang Island/Ash Island complex retains one of the largest persisting populations of *L. aurea*. The islands are situated in the centre of the estuary of the Hunter River. The two islands are joined, with the southern Kooragang Island operating as an industrial harbour. The northern Ash Island forms the Ramsar-protected Hunter Wetlands National Park (HWNP) that was previously used as grazing pasture.

Litoria aurea (Figure 15:B) occurs on a number of sections of the island system, either as remnant population or as the result of translocations and reintroductions. The main remnant population of *L. aurea* occurs within water bodies in the industrialised zone of the island (Figure 15:D yellow area); *L. aurea* has occupied and bred extensively in vegetated wetlands formed from residual waterbodies left over from previous industrial activity. The population extends north to the Ash Island HWNP where *L. aurea* inhabit the mid-island section (indicated by green boundary (Figure 15:D); this area contains former grazing pastures prone to flooding and the formation of ephemeral waterbodies, the area being constrained by tidal saltmarsh and mangroves. These areas have a number of waterbodies, approximately 5 permanent, 12 semi-permanent and 11 ephemeral (Hamer *et al.* 2002a; Hamer & Mahony 2010). This mosaic of wetlands within flood prone, ephemeral fields is often used by *L. aurea* for chorusing and breeding (Hamer *et al.* 2002b; Valdez *et al.* 2015) and was the most northern extent of the remnant population in recent years. A trial habitat construction project for *L. aurea* involving the construction of four semi-permanent and six ephemeral wetlands, located at the north-west edge of the mid-section (Figure 15C black area) was undertaken by BHP Billiton, and in 2011, captive bred *L. aurea* were released inside and outside an enclosure to trial a soft release translocation in that constructed habitat. However, no breeding occurred in the fenced or unfenced sections of that translocation site, and in 2015 the population was considered zero within the fenced section of the constructed habitat, due to chytrid mortality and an absence of breeding by adults (Klop-Toker *et al.* 2016). North of this site, there was very little available permanent water. At the upper-north extent of the island (Figure 15:C blue area), a second habitat construction project built wetlands and

reintroduced *L. aurea,* establishing a new population with a continuing supplementation programme at the time of the current study (*Michael Mahony personal communication*).
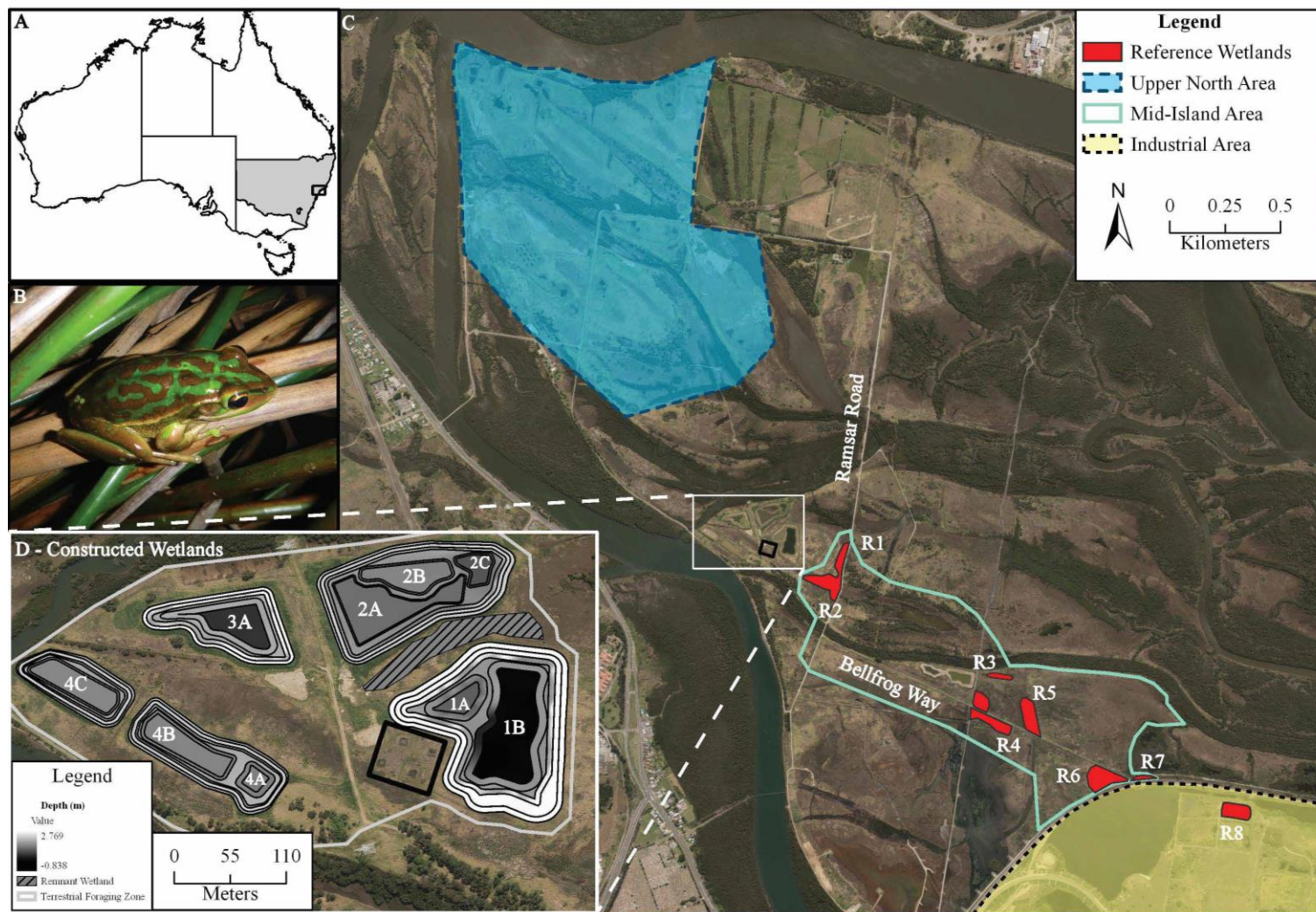
Figure 15: A) Australia with Ash and Kooragang Islands (square) located east coast of NSW (grey). B) Adult *Litoria aurea* C) Partial map of Ash and Kooragang Islands showing the industrialised areas (yellow) and mid-island (green boundary), both occupied by *L. aurea*. The upper north-west zone includes re-introduced *L. aurea* (blue). Surveyed waterbodies 'reference wetlands' (red) are indicated as R1-8. D) Constructed wetlands map - grey-scale represents waterbody depth in Australian Height Datum, ephemeral wetlands 1A, 2A,B,C and 4A,B,C, permanent wetlands 1B and 3A and remnant wetland.

7 *5.3.2 Collection of baseline data*

8 We collected baseline data on the *L. aurea* population on Ash Island by surveying the mid-
9 island belt (Figure 15:C green) in summer when *L. aurea* are most active and breeding
10 (August to March 2013-2014, 2014-2015), targeting ephemeral and permanent/semi-
11 permanent wetlands (Figure 15:C red) which are from here on referred to as reference
12 wetlands.

13 Nocturnal surveys of each wetland were conducted between 7:30 pm and 3:30 am involving
14 two or more observers. A standardised method was employed: each waterbody was
15 approached and an auditory survey conducted, involving 3 minutes of listening for frog calls.
16 The number of calling frogs was estimated and recorded for each species. The call of male *L.*
17 *aurea* were then imitated for one minute and the number of *L. aurea* were again counted over
18 another three-minute period. Immediately following auditory surveys, timed visual encounter
19 surveys were undertaken for each waterbody by searching within the water and scanning the
20 emergent vegetation for *L. aurea* using a head torch. Terrestrial areas were surveyed by
21 walking a strip transect around the outside of each wetland 5-7 m from the water's edge.
22 When an *L. aurea* was observed, the GPS location and presence/absence of calling was
23 noted. The observer also recorded the substrate on which the frog was found using Sainty and
24 Jacobs (2003), and height and distance from ground/water level. Observers then attempted to
25 catch each frog with a hand covered with a plastic bag. When the frog was captured, the bag
26 was inverted, filled with air and tied to a string necklace. Flagging tape was placed on
27 vegetation at the capture location and a unique identifying number placed on the tape and
28 bag. The observer then continued surveying.

29 After the survey was complete at each waterbody, captured frogs were weighed inside the 1.5
30 g capture bag using Pesola spring scales, the bag flattened to measure the (snout to vent
31 length (SVL) and right tibia length (RTL) using dial callipers. Any physical injury was
32 recorded. A Passive Integrated Transponder (PIT) tag, detectable with a TROVAN scanner
33 (Christy 1996), was inserted under the dorsal skin, providing a unique individual identifier
34 for future Capture-Mark-Recapture (CMR) studies. The sex of each individual was
35 determined by noting the secondary sexual characteristics; raised nuptial pads on the
36 forefinger of males, which were visible on males > 45 mm SVL. Absence of nuptial pads on
37 frogs > 45 mm were taken to indicate females. Frogs were then released at the capture
38 location and the bag disposed of (NPWS 2001a).

39   To determine whether the calling characteristics of *L. aurea* was similar between constructed

40   and reference wetlands, we undertook surveys in other locations throughout the state of

41   NSW, Australia, as a measure of habitat performance. Performance is therefore measured as

42   the constructed wetlands ability to attract breeding age males which form a chorus. Counts of

43   calling males were recorded from two other locations: (i) Avoca Lagoon (2014-2015) (-

44   33.462181 S, 151.434254 E) and (ii) Sydney Olympic Park (2012-2013) (-33.842402 S,

45   151.072504 N and -33.841155 S, 151.061090 N) (S1:Northern Water Feature and Brickpit

46   wetlands S2:12, S3:11s, S4:13, S5:C1, S6:S1) (James *et al.* (2015) and *unpublished data*).

47   *5.3.3 Habitat design and construction*

48   A wetland habitat construction program was undertaken in 2015 in the mid-island section of

49   the Kooragang Island/Ash Island system as a compensatory habitat programme associated

50   with a development approval on Kooragang Island. The compensatory wetland construction

51   programme was undertaken in a section of the mid-island that contained no permanent water

52   bodies, within ephemeral wet pasture dominated by introduced *Kikuyu* grass and native

53   saltmarsh grass (*Sporobolus virginicus*). This location was chosen to act as a habitat stepping

54   stone by increasing permanent water availability between the southern industrial/mid-island

55   population (Figure 15:D green), and the newly constructed northern habitats (Figure 15:D

56   blue).

57   The constructed wetland design consisted of nine waterbodies; two permanent wetlands (3A

58   and 1B) and seven ephemeral wetlands (1A, 2A, 2B, 2C, 4A, 4B, 4C) (Figure 15:D). These

59   were constructed around a remnant semi-permanent wetland (Figure 15:D). During the

60   design phase, hydrology assessments were undertaken by consultants to assess the optimal

61   wetland depth, so that the constructed waterbody would interact with the water table and

62   introduce groundwater salinity. Constructed ephemeral wetlands were designed to be shallow

63   enough to dry completely during dry periods of the climatic cycles and lowering ground-

64   water tables, and refill with the onset of heavy rains and rising ground-water tables. Soils

65   were assessed to detect and manage potential acid sulphate issues.

66   Prior to work start, frog proof fences were constructed and consultants undertook pre-

67   clearance surveys using visual encounter and capture to remove *L. aurea* from the site.

68   Construction of the wetlands occurred over a six-month period and were completed in

69   November 2015. As soil was dug from the site, it was used to form bunds surrounding the

70   waterbodies (a continuous, compacted soil mound surrounding each water body) (Figure

71   15:D); bunds ranged from 1 – 2 m in height. Bunds were constructed to prevent water

72   flooding in from the surrounding ephemeral wet pastures during periods of rainfall, and the

73   consequent movement of *G. holbrooki* into the water bodies from the surrounding wetland

74   system. Planting of native vegetation tube stock was completed by the end of November 2015

75   with the exception of wetlands 1A and 1B, which were planted-out during January 2016.

76   Plants were watered and maintained by contractors.

77   *5.3.4 Surveys after completion of the constructed habitats*

78   After wetlands were constructed, we undertook fish, tadpole and invertebrate trapping, visual

79   encounter and auditory surveys and habitat and water quality surveys. These were undertaken

80   in a range of areas dependent on survey requirements, including: the constructed wetlands,

81   remnant wetland, terrestrial foraging zone and reference wetlands on Ash Island.

82   *5.3.4.1 Gambusia holbrooki, tadpole and invertebrate surveys*

83   Constructed and reference wetlands were surveyed for the presence of *G. holbrooki* and

84   tadpoles (Figure 15 : R5 low *G. holbrooki* density and R7 high *G. holbrooki* density). We

85   used small mesh fish funnel traps (20×20×40 cm) to survey over a four-day period for *G.*

86   *holbrooki* (December 2015, January, February and March 2016) and for tadpoles over a two-

87   day period (January, February and March). In the evening (5pm), traps were tied to emergent

88   vegetation or wooden stakes at the perimeter of each wetland and in open water (Sanders *et*

89   *al.* 2015) at 50 m intervals in large wetlands (1B and 3A) and 20 m intervals in small

90   wetlands (1A, 2A,B,C 4A,B,C). Ten trout pellets and a source of light (glow sticks) were

91   placed in each trap as attractants. Traps were checked in the morning from 6am-10am by

92   opening traps and tipping the contents into a white tray. *G. holbrooki* presence/absence was

93   noted and each tadpole identified to species (Anstis 2013). All animals captured except *L.*

94   *aurea* were then released at the site of collection.

95   For *L. aurea* tadpoles, the Gosner (1960) table was used to assess tadpole developmental

96   stage. Tadpoles were then measured to determine SVL and stomach width. Stomach width

97   was taken at the widest part of the body as a proxy for body condition, as tadpoles lose

98   stomach width at this section of the body when not feeding (*James M. S. personal*

99   *observation*). Tadpoles were placed in ~1 cm of water in a shallow dish, dorsal up, and care

100 taken to straighten the tail and ensure the stomach shape was not distorted by pressing on the

101 dish. Stomach was measured at the widest point, before tapering back in to the tail.

102 As an indicator of constructed wetland suitability, we compared tadpole body condition

103 between constructed and reference wetlands. To provide comparable tadpole measurements

104 we undertook standardised dip-netting surveys to capture tadpoles in the mid-island

105 ephemeral belt (Figure 15:D) (Shaffer *et al.* 1994). Using a hand-held net (on a round steel

106 frame with a handle), individual observers swept the net through open water column and

107 through emergent vegetation for ~ 2 m. The net was emptied into a clean tub and tadpoles

108 identified and counted.

109 Wetland conditions were assessed using biological indicators. To achieve this, we

110 incorporated surveys of invertebrates, as they are sensitive to water quality and fluctuations in

111 water parameters (Awal & Svozil 2010), providing more information about the site

112 conditions. In February, five dip net sweeps were taken per waterbody, sampling the surface,

113 water column, base, and around vegetation to sample different invertebrate habitats.

114 Invertebrates were identified to order or family level (Gooderham & Tsyrlin 2002). Where

115 identification in the field was in doubt, samples of invertebrates were preserved in ethanol to

116 be identified in the laboratory. The white tray was then emptied.

117   *5.3.4.2 Auditory and visual encounter surveys*

118 Auditory and visual encounter surveys were undertaken in the constructed wetlands, remnant

119 wetland and in the terrestrial foraging zone between January and March 2016. In addition to

120 surveying constructed waterbodies, the remnant wetland and terrestrial foraging zone (Figure

121 15:D) were also surveyed.

122   *5.3.4.3 Habitat surveys*

123 Notes on wetland vegetation were recorded to provide information on site conditions over the

124 survey months. Additionally, the availability of various habitat types was recorded twice for

125 all surveyed wetlands using percentage coverage of habitat structure categories: bare ground,

126 grass, reeds, floating vegetation, water and tree. This was done for three sections per wetland:

127 within the water, 2 m surrounding the wetland and 20 m of terrestrial area from the

128 waterbody edge. A weighted mean of each substrate type in each zone was calculated based

129 on zone area using google maps (GoogleEarth 2012).

5.3.4.4 Water quality and depth

131   After wetland construction, the depth in constructed waterbodies was recorded at a

132   standardised point, using a marked stake to measure depth. As permanent wetlands were too

133   deep to measure depth at the deepest point, depth was measured at a specific reference point

134   within the water and the deepest point depth calculated from that measurement, using data

135   from construction schematics. Water quality readings were recorded monthly using a *YSI*

136   *Professional* water quality meter placed 15 cm from the surface. The salinity, temperature

137   and dissolved oxygen were recorded as key parameters and indicators of water quality for *L.*

138   *aurea* survival (Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman

139   1998; Pyke *et al.* 2002; Stockwell 2011; Werkman 1999). Readings were taken from 2 to 4

140   times for each constructed ephemeral waterbody at standardised points at the edge and centre,

141   however, permanent waterbodies were too hazardous to enter due to soft sediments and water

142   quality was recorded from within 2m of the edge.

143   **5.3.5 Descriptive and statistical methods**

144   *5.3.5.1 Tadpole diversity and macroinvertebrate abundance, diversity and sensitivity*

145   In order to assess tadpole and macroinvertebrate diversity in constructed wetlands , we used

146   the Shannon-Weiner diversity index, the index being a function of species richness and

147   abundance $H^1 = -\sum(P \times \ln(p))$ (calculated as a positive value) (Awal & Svozil 2010;

148   Nzengy'a & Wishitemi 2000). To assess which wetlands had higher macroinvertebrate

149   sensitivity we used the scoring system: Stream Invertebrate Grade Number – Average Level

150   (SIGNAL) (Chessman 2003). This provides pre-assessed scores for macroinvertebrate

151   sensitivity, in which the sum of the macroinvertebrate score for each order is the measure of

152   total sensitivity. To assess sensitivity and abundance, the sensitivity was multiplied by a pre-

153   set abundance weighted measure (Chessman 2003), to form the SIGNAL score. Each

154   measurement was done for each net sweep in each waterbody. A one-way ANOVA was

155   performed in the program JMP comparing water bodies for A) tadpole species diversity B)

156   macroinvertebrate diversity index, C) total sensitivity and D) weighted sensitivity.

       5.3.5.2 Comparison of constructed wetlands to reference wetlands: chorus size as a

158   *measure of habitat utilisation*

159 As a measure of habitat performance, we compared graphically the maximum chorus size

160 detected from the constructed wetlands to reference wetlands (2013-2014 and 2014-2015)

161 and auditory data from Avoca and Sydney Olympic Park, NSW, Australia.

162   *5.3.5.3 Comparison of constructed wetlands to reference wetlands: sex ratios, age*

163   *class structure and body condition*

164 We reported female-male sex ratios of constructed wetlands compared to reference wetlands.

165 In order to account for potential variability in habitat use, we assessed whether female-male

166 ratio differed between wetland type, separating count data and sex ratios for ephemeral,

167 permanent and remnant wetlands/terrestrial foraging areas. In order to estimate age-classes

168 (Skalski *et al.* 2005), we assessed females and males data for constructed wetlands and

169 reference wetlands separately, comparing the snout vent length of males and females to a

170 published growth chart (Hamer & Mahony 2007) using Rohatgi (2017) software to

171 determine age-size brackets from growth curves.

172 We used body condition as a proxy for health to compare health of adults, juveniles and

173 tadpoles between the constructed wetlands and reference wetlands. Indices of body condition

174 were estimated by a linear regression of two comparative variables using residuals (for a

175 critique and method see Green 2001; Jakob *et al.* 1996; Schulte-Hostedde *et al.* 2005). For

176 adults, the RTL was used for body condition instead of SVL as precision is often better

177 (*Melanie James unpublished data, Chapter 5*). We used the RTL and weight for adults and

178 juveniles (<30 mm SVL). For tadpoles, the SVL and stomach width at the widest part of the

179 body was measured.

180 Body condition was compared between the reference wetlands and constructed wetlands. As

181 the reference wetland dataset spanned from (August to March 2013-2014, 2014-2015), and

182 the constructed wetlands January – March 2016 we ran a regression on body condition by

183 date to ensure there were no confounding effects of season in the data, as has been found in

184 past research (Humphries 1979). Additionally, ontogenetic and age variation in development

185 stage and morphology can affect body condition comparisons. To avoid ontogenetic and age

186 variation from distorting data comparisons between the constructed wetlands and reference

187 wetlands (Grosjean 2005), tadpoles within the same Gosner stages were compared and a

188 visual assessment of adult age class structure identified a similar age class structure which

189 validates comparison between constructed and reference wetlands. Estimates of body

190 condition were then compared between the constructed wetlands and reference wetlands for

191 females and males using a one-way ANOVA in the program JMP (SAS 2016). We used a

192 significance level of $p<0.05$ for all analyses. All ANOVAs in the current study were tested

193 for normality, and also equal variance (Levene's test). Where necessary, a nonparametric

194 comparison Wilcoxon / Kruskal-Wallis Test (Rank Sums) and Wilcoxon comparison (*p*-value

195 pair comparisons) were undertaken. Thus median and upper and lower quartiles were

196 reported as measures of variability, as means and standard errors do not adequately reflect the

197 data where nonparametric statistics are required (Olsen 2003).

198 *5.3.5.4 Comparison of constructed wetlands to reference wetlands: habitat*

199 *availability and use*

200 To assess habitat segregation and utilisation in constructed versus reference wetlands, we

201 assessed the height at which males and females sat on a substrate for each location using one-

202 way ANOVA. For all habitat assessment, the constructed wetlands, remnant wetland and

203 terrestrial foraging zone was combined to assess the available habitat in the constructed area.

204 To analyse the effect of both structure and sex within a given wetland, a Generalised Linear

205 Model was used with Poisson distribution and log link function in R (RStudioTeam 2015).

206 The model main effects were substrate and sex and their interaction. Expected values for the

207 count of substrate use based on habitat areas were incorporated into the model with an offset

208 of log of the expected count (total number of frogs counted for a given sex*proportion of

209 substrate availability). To assess which substrates were used more or less than expected, we

210 used the lsmeans package in R Studio (RStudioTeam 2015).

211 To provide qualitative output for interpretation of preference, the model coefficients were

212 used to determine the ratio of observed/expected use for each of the substrate/sex

213 combinations and their 95 % CIs. Estimates were graphed for each sex, for 6 substrate

214 categories in the reference wetlands and 5 substrate categories for the constructed wetlands.

215 Results were graphed on a logarithmic scale for the observed/expected to manage large

216 values. As such, high values were moderated while maintaining the integrity of low values

217 (Labonne *et al.* 2003). A) To determine if males and females used particular substrates within

218 each location, significant interactions were examined by comparing the graph columns in the

219 model outputs. Where values were below 1, results were considered avoidance of that

220   substrate, above 1 as selection for that substrate and close to 1 as no selection, as use was

221   proportionate to availability. These estimates were considered as different if confidence

222   intervals did not overlap with the 1 line. B) Building from this, we then assessed the

223   difference between male and female preferences within each location to detect differential

224   habitat use between sexes (assuming different habitat use indicates sexual segregation or

225   differential habitat requirements). Where male and female graph columns sat on either side of

226   the reference line (1), this was considered different if confidence intervals did not overlap. C)

227   To determine if *L. aurea* use habitat differently in the constructed wetlands compared to

228   reference wetlands, comparisons between wetlands were undertaken in a similar manner,

229   contrasting male and female use by comparing the reference lines (1).

230   One substrate category was particularly low in availability, however, this showed

231   considerably higher than expected habitat use for one sex and zero use for the other sex. The

232   zero-value resulted in a very large SE (40,000+) causing a non-significant difference despite

233   the significant interaction term in the model (which is a shortcoming of GLM theory). As a

234   means of working around this problem the 0 count was converted to 1 and the analysis re-

235   performed which then resulted in a significant difference was now significant (p = 0.02),

236   confirming the original analysis showing no-significance (40,000+SE) had to be significant

237   as well as the difference was even larger. In the current study, equal probability was assumed

238   for substrate types.

239   *5.3.5.5 Site conditions and suitability: rain data and water quality for constructed*
240   *wetlands*

241   Rainfall in four-day increments (mm) was collected from BOM (2016) and graphed alongside

242   water quality and depth. To assess wetland suitability for *L. aurea*, we overlayed the optimal

243   water quality thresholds for *L. aurea* tadpoles on the water quality graphs generated for the

244   study period (Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman

245   1998; Pyke *et al.* 2002; Stockwell 2011; Werkman 1999).

246   **5.4 Results**

247   *5.4.1 Post-construction landscape description*

248   After wetland construction, the ground around all wetlands consisted of bare compacted soil.

249   Within a few weeks, introduced grasses and weeds colonised the site in ephemeral wetlands

250   2A,B,C and 4A,B,C providing fringing and emergent vegetation (habitat availability will be

251   discussed later in 'Habitat availability, use and island comparisons'). From the 3rd to the 7th

252   of January 2016, heavy rainfall occurred in the lower Hunter River region which produced

253   >200 mm rainfall within 24 hours. All ephemeral wetlands within the constructed wetlands

254   filled with water. Within the bunding, ephemeral waterbodies 2A, 2B and 2C within the same

255   bunded area flooded and joined into one wetland, with a shallow 2 – 4 cm deep area

256   connecting the three waterbodies.

257   ***5.4.2 Frog and macro-invertebrate data***

258   *5.4.2.1 Litoria aurea response*

259   After the rainfall and flooding event early January, a total of 34 male and 17 female *L. aurea*

260   were captured and marked within the newly constructed wetlands, the remnant wetland and

261   terrestrial foraging zone (January to March 2016). Male *L. aurea* mainly colonised the

262   constructed ephemeral wetlands 2A,B,C, 4B and 4C, and very few males were found in the

263   remnant wetland and terrestrial foraging zone (Figure 16:A). Female *L. aurea* were found in

264   the remnant wetland and in the terrestrial foraging zone with very few found in the ephemeral

265   wetlands (Figure 16:A). SVL for males ranged from 48 mm – 66 mm and for females from

266   45 mm – 67 mm (6 females over the size of 65 mm SVL). No captured frogs had been PIT-

267   tagged from previous studies on Kooragang/Ash Island, three males had abrasions on their

268   snouts with pink skin colouration.
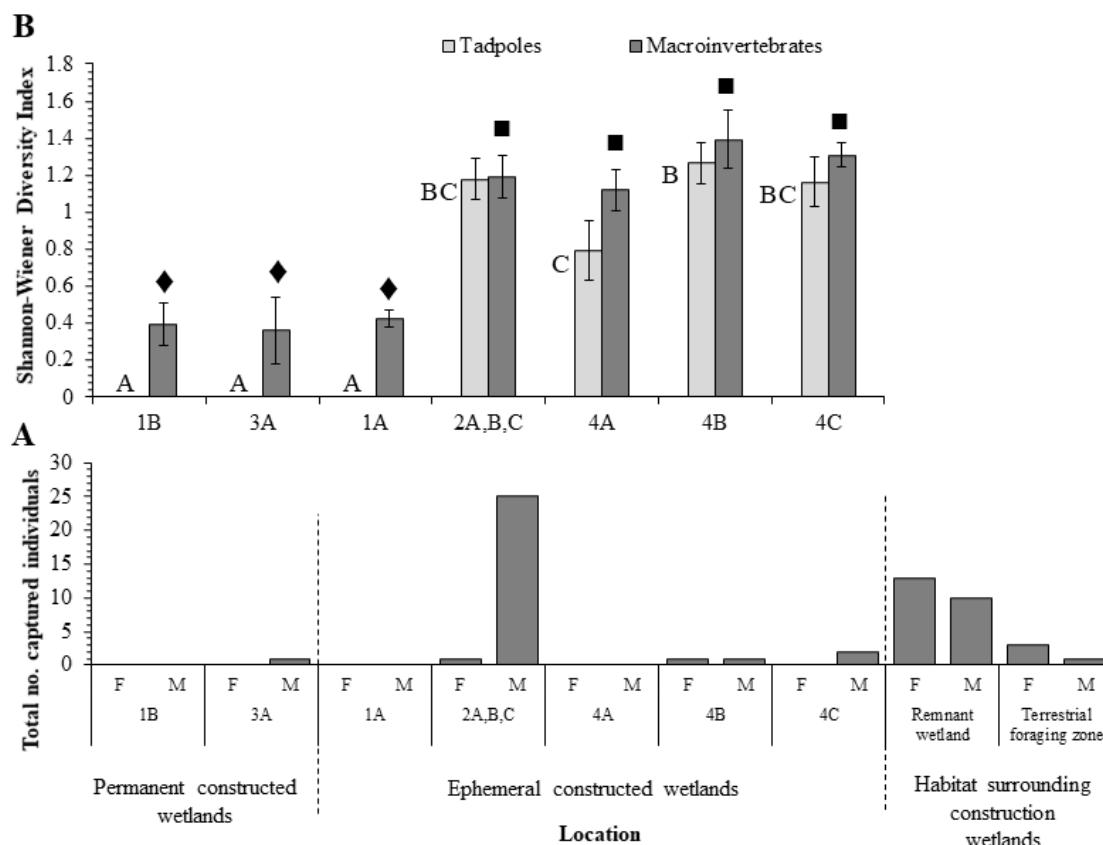
Figure 16: A) Total number of *L. aurea* captured grouped by sex and wetland for permanent, and ephemeral constructed wetlands, remnant wetland and the terrestrial foraging zone on Ash Island. B) Mean Shannon Weiner Diversity Index for tadpoles (all frog species) and macroinvertebrates in constructed wetlands with ANOVA post-hoc connecting letters report or non-parametric each pair Wilcoxon comparison (SAS 2016); tadpoles (A,B,C) and macroinvertebrates (♦,■) (means ±SE).

5.4.2.2 Tadpole species diversity and abundance

In February of 2016, *L. aurea* tadpoles were trapped in constructed ephemeral wetland 4C (Figure 16) with a total of 34, 16 and 23 individuals caught, with Gosner stages 34 to 39. A week later, 4 metamorphs were trapped (Gosner stages 43 to 45) and at the end of February, five juvenile *L. aurea* were captured ranging from 27 mm – 30 mm SVL. No juveniles or tadpoles were detected in visual encounter and trapping surveys in March 2016. Breeding on Ash Island outside the constructed habitat occurred at the same time as in the constructed wetlands provided tadpoles with similar Gosner (1960) stages were thus used for comparisons of tadpole body condition between constructed and reference habitats.

Five other frog species bred in the constructed wetlands, including *Litoria fallax, Litoria dentata, Litoria peronii, Limnodynastes peronii* and *Limnodynastes tasmaniensis* during January to March 2016. Wetlands 1B and 3A had no breeding for any species, and 1A had

288   only small numbers of *L. fallax*. Analysis indicated significant differences between wetlands

289   for frog species diversity (χ^2 = 42.81, df = 6, p = <.0001*), and post-hoc comparisons

290   indicated that ephemeral wetlands 2A,B,C and 4A, 4B and 4C had higher species diversity

291   compared to ephemeral wetland 1A and permanent wetlands 1B and 3A (Figure 16:B).

292   Invertebrate diversity varied significantly between wetlands (F=14.88, df = 6, p= <0.0001),

293   with the highest diversity in ephemeral wetlands 2A,B,C, 4A, 4B and 4C, and wetlands with

294   the lowest diversity were 1AB and 3A (Figure 16:B).

295   *5.4.2.3 Macroinvertebrate diversity and abundance*

296   Comparisons of SIGNAL scores between waterbodies (based on measures of

297   macroinvertebrate sensitivity) found that total sensitivity was not significantly different

298   between wetlands (F = 2.29, df = 6, p= 0.0632). Weighted sensitivity between waterbodies

299   (based on measures of macroinvertebrate sensitivity and abundance) was significant (χ^2 = 20,

300   df = 6, p = 0.003*), showing that ephemeral wetlands 2A,B,C, 4B and 4C had higher

301   weighted diversity than ephemeral wetland 1A (z = 2.72, p= 0.007*: z = 2.72, p = 0.007*: z =

302   1.83, p = 0.03* respectively). Ephemeral wetlands 2A,B,C, 4B, 4C had higher diversity

303   compared to permanent wetland 1B (z = 2.77, p = 0.006*: z = 2.40, p = 0.02*: z = 2.27, p =

304   0.02* respectively) and permanent wetland 3A had lower diversity compared to ephemeral

305   wetland 2A,B,C (z = -2.27, p = 0.02*).

306   Many invertebrates in the constructed wetlands were considered tolerant to variable

307   environmental quality i.e. low sensitivity: backswimmer (*Hemiptera*) water boatman

308   (*Hemiptera*), water scorpion/needle bug (*Hemiptera*), dragonfly nymph (*Odonata*),

309   bloodworm (*Diptera*), damselfly nymph (*Odonata*), midge larva and pupa (*Diptera*), and

310   semi tolerant diving beetle (*Coleoptera*) whirligig beetle and larvae (*Coleoptera; Dytiscidae*),

311   beetle larvae (*Coleoptera*). However, wetlands 4A,B,C and 2A,B,C had invertebrates within

312   the category sensitive (caddisfly larvae - *Trichoptera* and water mite - *Acarina*), and wetland

313   2A,B,C had very sensitive invertebrates (mayfly larvae - *Ephemeroptera*).

314   ***5.4.3 Comparison of habitat quality and use by L. aurea between constructed and reference***

315   ***wetlands on Ash Island and other sites***

316   A total of 98 males, 60 females and 21 juveniles were captured in the reference wetlands.

317   Male SVL ranged from 47.3 - 69.7 mm, female from 48.6 - 75.2 mm and juvenile from 26 −

318   45 mm.

5.4.3.1 Chorus sizes in Ash Island, Avoca and Sydney Olympic Park populations

320   On the 8th January 2016, chorusing *L. aurea* were detected in wetland 4C in the constructed

321   habitat. The chorus size in constructed wetlands 2A,B,C was similar to the maximum chorus

322   size of a reference wetland on Ash Island (R6; Figure 17: C) which had a maximum chorus

323   size of 23 (Figure 17). The chorus in 2A,B,C was considerably larger than other chorus sizes

324   found in wetlands across the mid-island area, as well as the populations surveyed in Avoca

325   and Sydney Olympic Park (Figure 17). Aggressive interactions occurred in sections of the

326   wetland where *L. aurea* were in high density. The size of the chorus in wetland 4C during

327   January was similar to choruses found on Ash Island, Avoca and Sydney Olympic Park. *L.*

328   *aurea* bred at the constructed ephemeral wetland 4C, Ash Island permanent wetland R6 and

329   Ash Island ephemeral wetland R1. In wetlands 4B and 4C, no aggressive interactions were

330   seen throughout surveys and males remained over 5 m from one another.

331
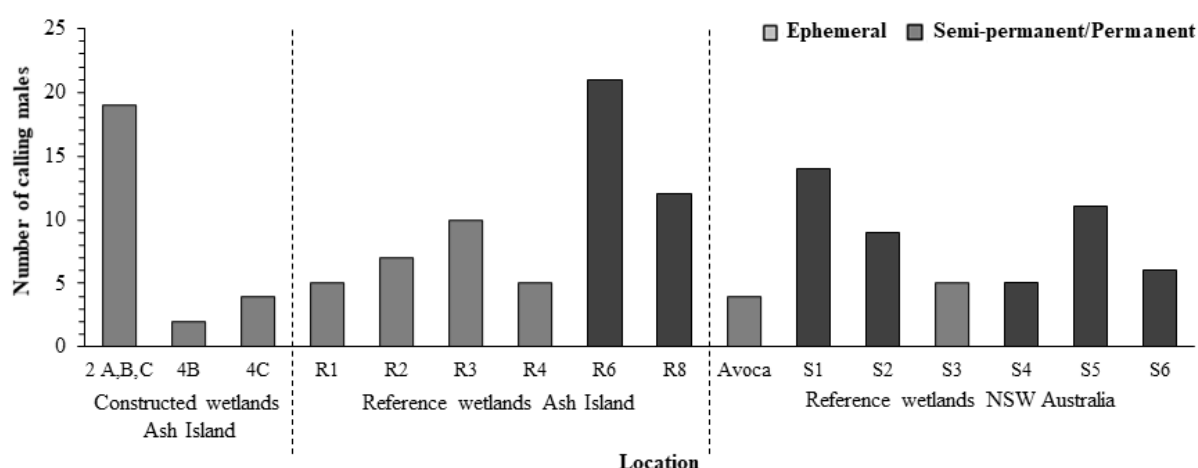
332   Figure 17: The maximum number of calling male *Litoria aurea* in a chorus per wetland based
333   on triangulation and visual encounter of chorusing males in constructed wetlands (January –
334   March 2016) and reference wetlands from August to March, on Ash Island (2013-2014,
335   2014-2015) and reference wetlands in NSW Australia; Avoca Lagoon (2014-2015) and
336   Sydney Olympic Park (2012-2013) (S1:Northern Water Feature and Brickpit wetlands S2:12,
337   S3:11s, S4:13, S5:C1, S6:S1) (James *et al.* (2015) and *unpublished data*).

338   *5.4.3.2 Sex ratio*

339   Overall, the number of males in the constructed ephemeral wetlands on Ash Island was

340   greater than females (Figure 18), and within the remnant wetland and foraging zone, females

341   slightly outnumbered males. In reference wetlands, males also outnumbered females in

342   permanent and ephemeral waterbodies and females were rarely found in chorusing ephemeral

343   wetlands, but were present in permanent waterbodies. The sex ratio of females to males in <u>*all*</u>

344 constructed wetlands and the remnant wetland was 1:2, compared to the broader Ash Island

345 which had more males at a 1:3 female to male ratio.

346 Comparing ephemeral wetlands alone, shows that the constructed ephemeral wetlands had a

347 female to male ratio of 1:20, and the ephemeral Ash Island wetlands had a 1:54 ratio.

348 Comparing permanent wetlands alone, on Ash Island female to male ratios were 1:2, in

349 constructed permanent wetlands only one male was found. In the remnant wetland and

350 terrestrial foraging area sex ratio of females to males was 3:2.

351
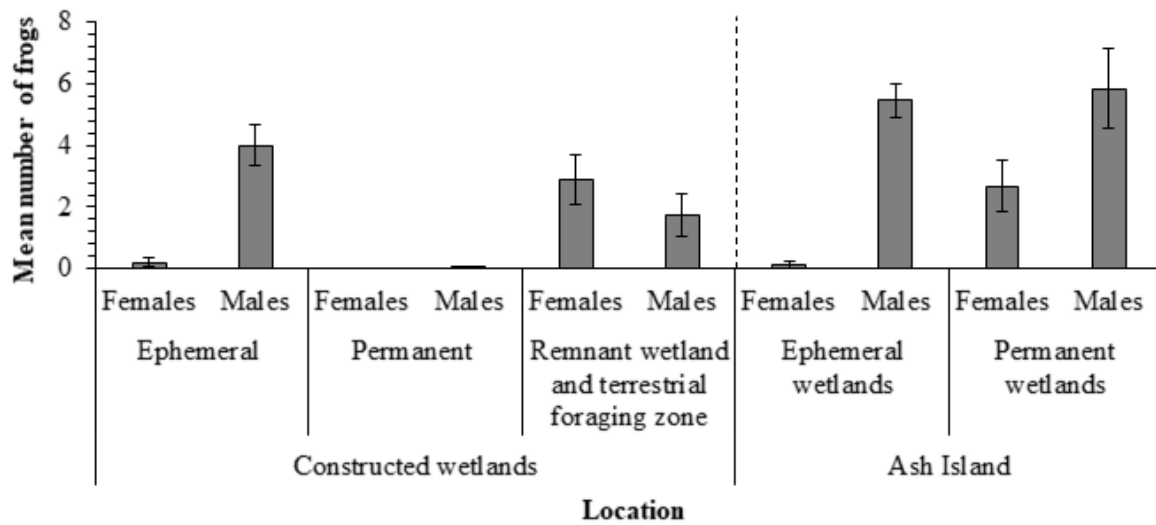
352 Figure 18: Sex ratios of captured adult *L. aurea*. Mean number of frogs per night by sex for
353 constructed wetlands, remnant wetlands, terrestrial foraging zone and Ash Island ephemeral
354 and permanent wetlands (means ±SE).

355 *5.4.3.3 Estimated age class structure of L. aurea in constructed habitat, Ash Island*

356 The age-classes of the males caught in the constructed wetland habitat were predominantly

357 under twelve months of age, with a small number around six months old. Females showed a

358 greater spread of ages, with females predominantly ranging from 6 − 18 months of age.

359 Generally, male and female age classes were similar in the constructed wetlands to the

360 wetlands across Ash Island, however, reference sites had some individuals from 12 − 24

361 months (Figure 19) (2013-2014 and 2014-2015 breeding seasons). No captured females

362 appeared to be gravid, indicated via a presence of abdominal egg masses detected by

363 candling, however three females appeared to be partially gravid.

364
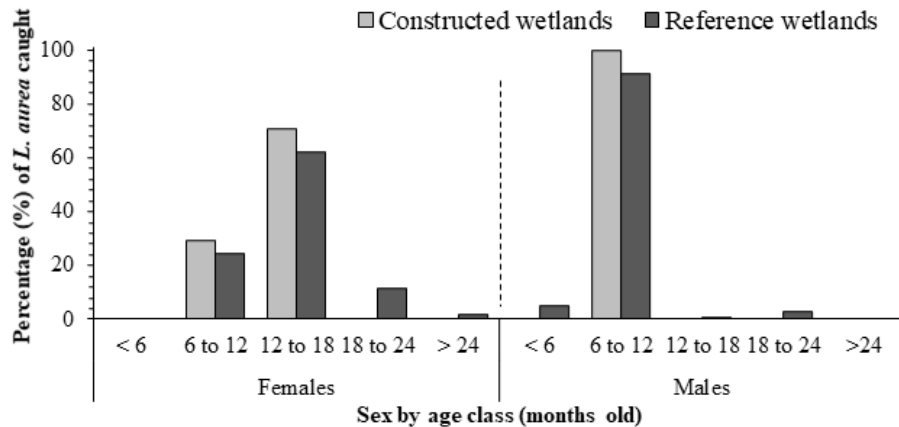
Figure 19: Age classes of female and male *Litoria aurea* in constructed wetlands in 2016
compared to reference wetlands from 2013-2014 and 2014-2015 breeding seasons on Ash
Island. Utilising size-age classes based on snout vent length from (Hamer & Mahony 2007),
where age is expressed as months post-metamorphosis. Constructed wetlands 2016 data
(N=17) and reference wetlands 2013 – 2015 data (N=60), and for males in the constructed
wetlands 2016 (N=34) and reference wetlands 2013 – 2015 (N=102).

5.4.3.4 Body condition of L. aurea in constructed and reference wetlands

Assessment of tadpole and juvenile body condition showed that both tadpoles and juveniles
in constructed habitat waterbody 4C (February to March 2016) had similar body condition to
the broader Ash Island population at the same development stage (Tadpoles: $t = 1.04$, df =
31.2, $p = 0.31$) (Juveniles: $t = -1.49$, df = 4.9, $p = 0.19$). Before comparisons of adult body
condition, assessment of body condition over the season (September – March) for reference
wetlands frogs showed no significant change in body condition by month (F Ratio = 0.1826,
df = 1, $F = 0.6697$) and therefore was appropriate to use for comparisons to constructed
wetland data from January to March 2016. Body condition differed for comparisons of the
constructed wetlands and reference wetlands for males and females ($F = 14.48$, df = 3, $p =$
<.0001*), and indicated that females in the constructed wetlands had higher body condition
(Figure 20) than constructed wetland males ($p=0.0005*$), reference wetland females
($p=<.0001*$), Ash Island males ($p=<.0001*$). Male comparisons between the constructed
wetlands and reference wetlands did not show a significant difference, however was close to
the significance level ($p = 0.062$).

386
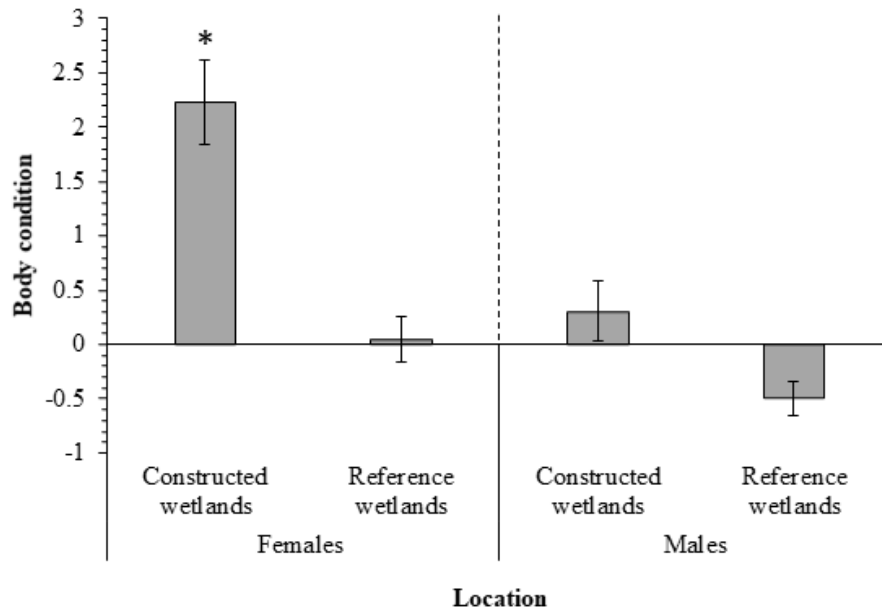
387 Figure 20: Mean body condition of *Litoria aurea* determined using regression residuals in
388 constructed wetlands 2016 compared to reference wetlands from 2013-2014 and 2014-2015
389 breeding seasons on Ash Island. Body condition determined for females in constructed
390 wetlands (N=17) and reference wetlands (N=60), and for males in constructed wetlands
391 (N=34) and reference wetlands (N=102) (means ±SE, *indicates significance, $p < 0.05$).

392   *5.4.3.5 Habitat structure comparison between constructed and reference wetlands*

393 Weeds and introduced grasses rapidly colonised the constructed habitat post construction,

394 providing fringing and emergent vegetation in ephemeral wetlands 2A,B,C and 4A,B,C.

395 Within 2A,B,C and in 1A, native vegetation *Phragmites* and *Bolboschoenus* established in

396 small patches. Natives planted after construction did not establish well. By March 2016,

397 terrestrial zone was dominated by dense introduced grasses (Figure 21:A). Wetlands 1B and

398 3A remained mostly bare of vegetation. The remnant wetland in the constructed habitat zone

399 contained more reeds, less bare ground (Figure 21:A) and little open water compared to

400 constructed wetlands and reference wetlands (Figure 21A). Within the constructed wetlands,

401 all aquatic vegetation was new growth and no decayed or floating vegetation was present.

402   *5.4.3.6 Habitat use in constructed and reference wetlands*

403 Habitat use (observations of vegetation type and height of vegetation in which animals were

404 found) indicated differences between males and females in the type and height at which they

405 were located. In particular, females were frequently found at higher positions in vegetation

406 than males, but there were some differences between constructed and reference wetlands.

407 Comparisons between male and female habitat use indicated that in constructed wetlands

103

408   females sat higher in vegetation compared to males (χ^2 = 3.74, df = 3, p = < 0.0158*) (z = -

409   5.23, p = <.0001), however, across the broader system, Ash Island females used similar

410   heights to males (z = -0.80, p= 0.4226) (Figure 21:B). Comparisons within sexes between

411   locations indicated that females sat higher in the constructed wetlands than on Ash Island (z =

412   2.88, p = 0.0039), but males sat lower in the constructed wetlands compared to Ash Island (z

413   = -2.19, p = 0.0282) (Figure 21:B). In the constructed wetlands, females in the remnant

414   wetland, were mostly observed sitting high in reeds, and in the terrestrial foraging zones were

415   found in trees and on the ground. Males mostly inhabited ephemeral constructed wetlands,

416   calling vigorously and moving actively on the surface of the water between flooded

417   vegetation such as native *Phragmites australis*, introduced grasses and a common weed

418   *Verbena bonariensis*. Males found in the remnant wetland were found on reeds and did not

419   call.

420   *5.4.3.7 Comparison of habitat use between sexes in constructed and reference*

421   *wetlands*

422   Habitat use analysis between males and females showed significant differences between the

423   sexes in use within the constructed wetlands (χ^2 = 72.8, df = 4, p = < 0.0001) and on Ash

424   Island (χ^2 = 54.6, df = 5, p = < 0.0001). Based on graphical interpretation (Figure 21: C and

425   D) and statistical analysis (significance levels in

426   Table 5: A), females in the constructed wetlands were positively associated with reeds and

427   trees, but on the broader Ash Island system were positively associated with water and trees

428   and negatively associated with grass. Males in the constructed wetlands were positively

429   associated with water and negatively associated with ground, and on reference wetlands were

430   associated with water but not grass when allowing for its abundance.

431   The interaction between substrate and sex was significant for both Ash Island (χ^2 = 16.5, df =

432   5, p = 0.006) and the Constructed wetlands (χ^2= 52.0, df = 4, p < 0.001). Based on graphical

433   interpretation (Figure 21: C and D) and statistical analysis (significance levels in

434   Table 5: B), females in the constructed wetland and reference wetlands used trees more than

435   predicted, compared to males. In the constructed wetlands, females were associated more

436   frequently with reeds, and males more associated with water.

5.4.3.8 Comparison of habitat use between constructed and reference wetlands

438 Both female (χ^2 =88.2, df = 10, p <0.001) and male (χ^2 = 173.1, df = 10, p <0.001*) habitat

439 use between reference and constructed wetlands was different. Based on graphical

440 interpretation (Figure 21: C and D) and statistical analysis (significance levels in Table 5:C),

441 females used ground less often and reed more often in the constructed wetland area. Males

442 used grass more often, ground and reeds less often in constructed wetland area.

443
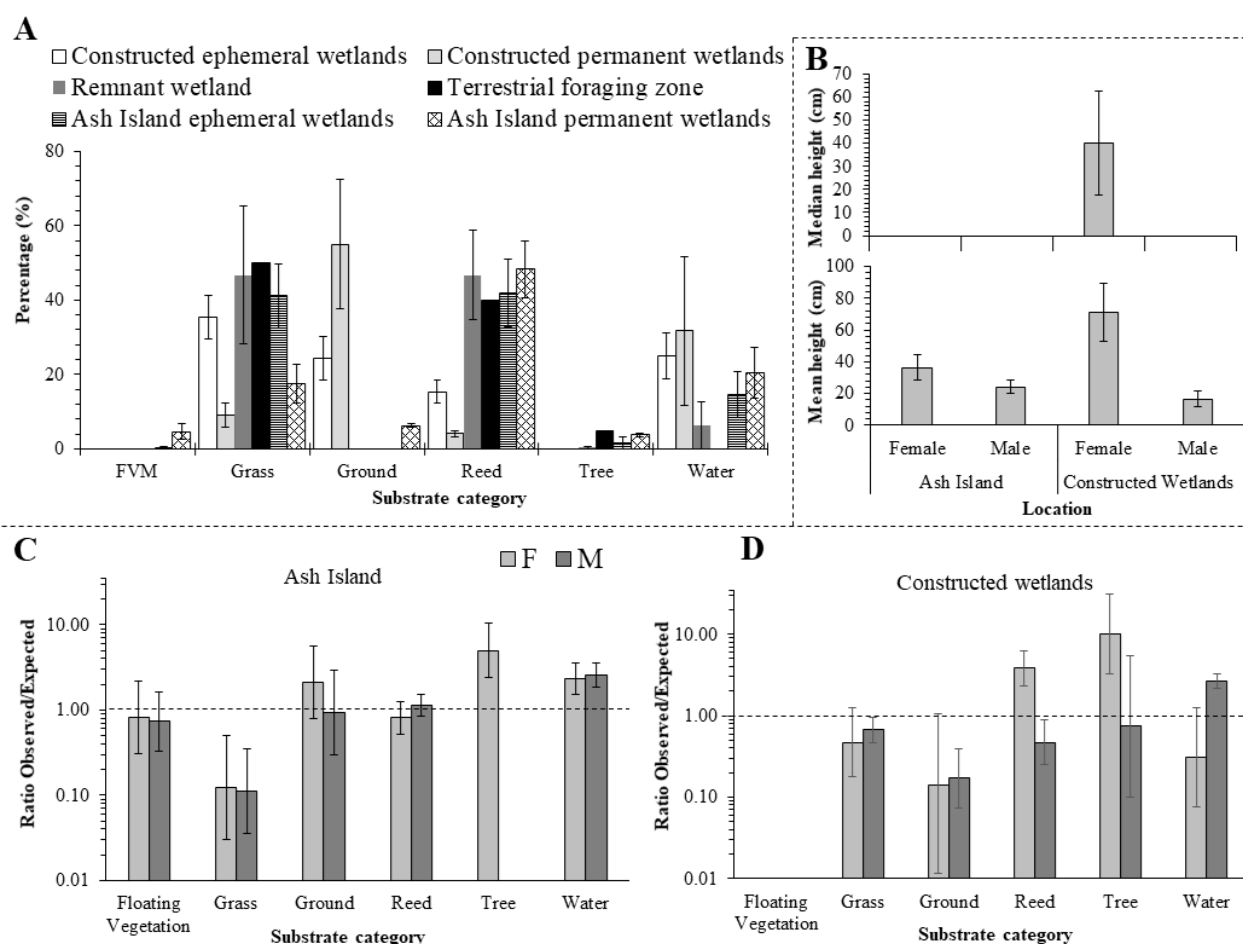
444 Figure 21: A) Mean percentage of available substrate type in constructed and remnant
445 wetlands, terrestrial foraging habitat and reference wetlands on Ash Island (mean ± SE). B)
446 Height at which male and female *Litoria aurea* positioned themselves on chosen substrate
447 (median ± quartiles; mean ± SE). C) and D) The observed count of *L. aurea* compared to
448 expected count per substrate type (relative to availability) for females/males in constructed
449 wetlands and in reference wetlands (95 % ± CI).
450 Table 5: Habitat selection analysis for *Litoria aurea* in constructed and reference habitats on
451 Ash Island. Output for habitat selection analysis showing the ratio of observed/expected
452 habitat use, based on Generalised Linear Mixed Model post hoc comparisons; calculated with
453 lsmeans package in the statistical package R (RStudioTeam 2015). **A)** Habitat selection
454 within each sex and habitat, and **B)** habitat use comparison between sexes and **C)** comparison
455 between male and female use between constructed wetlands and Ash Island.

		Substrate	Estimate	SE	Z Ratio	p-value
A **Habitat** **selection**	**Ash Island Female**	Floating Vegetation	-0.203	0.500	-0.406	0.685
		Grass	-2.092	0.707	-2.960	**0.003***
		Ground	7.444	0.500	1.489	0.136
		Reed	-0.207	0.223	-0.926	0.354
		Tree	1.609	0.378	4.258	**<0.0001***
		Water	0.859	0.218	3.934	**<0.0001***
	Ash Island Male	Floating Vegetation	-0.312	0.408	-0.765	0.444
		Grass	-2.190	0.577	-3.793	**<0.0001***
		Ground	-0.065	0.577	-0.112	0.911
		Reed	0.122	0.147	0.830	0.406
		Tree	-0.833	1.000	-0.833	0.405
		Water	0.943	0.162	5.813	**<0.0001***
	Constructed wetlands Female	Grass	-0.754	0.500	-1.508	0.132
		Ground	-1.902	1.000	-1.902	0.057
		Reed	1.338	0.250	5.350	**<0.0001***
		Tree	2.303	0.577	3.988	**<0.0001***
		Water	-1.163	0.707	-1.645	0.100
	Constructed wetlands Male	Grass	-0.394	0.186	-2.121	0.034
		Ground	-1.735	0.408	-4.249	**<0.0001***
		Reed	-0.747	0.316	-2.361	0.018
		Tree	-0.262	1.000	-0.262	0.793
		Water	0.973	0.108	9.024	**<0.0001***
B **Sex based habitat use comparison**	**M to F Ash Island**	Floating Vegetation	0.110	0.650	0.170	0.865
		Grass	0.100	0.910	0.107	0.915
		Ground	0.810	0.760	1.059	0.290
		Reed	-0.330	0.270	-1.230	0.219
		Tree	2.440	1.070	2.285	**0.0223***
		Water	-0.080	0.270	-0.311	0.756
	M to F Constructed wetlands	Grass	-0.360	0.530	-0.675	0.500
		Ground	-0.170	1.080	-0.155	0.877
		Reed	2.080	0.400	5.170	**<0.0001***
		Tree	2.560	1.150	2.221	**0.0263***
		Water	-2.140	0.720	-2.987	**0.0028***
C **Location habitat use comparison**	**Females Constructed wetlands compared to Ash Island**	Grass	-1.34	0.87	-1.54	0.1223
		Ground	2.647	1.118	2.367	**0.0179***
		Reed	-1.545	0.335	-4.605	**< 0.0001***
		Tree	-0.693	0.690	-1.004	0.315
		Water	2.022	0.740	2.732	**0.006***
	Males Constructed wetlands compared to Ash Island	Grass	-1.796	6.065	-2.961	**0.003***
		Ground	1.670	7.071	2.362	**0.018***
		Reed	0.869	3.489	2.491	**0.013***
		Tree	-22.873	4.225	-0.001	0.999
		Water	-0.030	1.948	-0.155	0.877

456

457   ***5.4.4 Water quality and Gambusia holbrooki surveys***

458   *5.4.4.1 Rainfall post-construction and occurrence of Gambusia holbrooki*

459   After construction, ephemeral wetlands dried out completely in October – December 2015

460   (Figure 22:B), and consequently would not contain *G. holbrooki*. Permanent wetlands 1A and

461   3B retained water from the pre-construction phase and were found to have no *G. holbrooki*

462   presence. Rainfall showed small peaks of rain <40 mm in early November and late December

463   (Figure 22:A). From the 3rd to the 7th of January 2016, heavy rainfall occurred in the lower

464   Hunter River region which produced >200 mm within 24 hours (Figure 22:A). After the

465   heavy rain from the 3rd to the 7th of January 2016, ephemeral wetlands filled to 0.33 m –

466   1.20 m depth (Figure 22:B) and permanent water bodies showed a small increase in depth

467   (1B ~0.1 m and 3A ~ 0.2 m). Constructed wetlands 2A, 2B, 2C flooded and joined, and

468   further rainfall on the 15th, 16th, 22nd and 23rd January 2016 increased waterbody depths of

469   all wetlands. Ephemeral wetlands remained charged until they dried in April-May 2016.

470   Bunding surrounding the wetlands successfully prevented overland flow of water and

471   trapping and dip-netting showed no indication of *G. holbrooki* presence in constructed

472   wetlands. *G. holbrooki* were successfully trapped in the reference wetlands in the mid-island

473   area (means R5 32 ± 7.8 SE, R7 25 ± 10.1 SE, R1 5 ± 5.5 SE).

474   *5.4.4.2 Abiotic water quality parameters*

475   Abiotic parameters indicated higher than anticipated levels of salinity (ground water derived)

476   in waterbodies post-construction, but the adjustment (decline) after major rainfall events

477   (Figure 22:A and C) to levels that were within tolerance limits for tadpoles and frogs,

478   indicating the hydrological dynamics of the system on Ash Island. The salinity of permanent

479   water bodies 1B and 3A was high and above the indicated threshold immediately post-

480   construction for salinity at 11.5 ppt and 15.5 ppt, respectively. However, after rain in January,

481   permanent wetland 3A reduced in salinity below the threshold to 3.57 ppt, although 1B

482   remained above threshold, at 7.38 ppt (Figure 22:C). In permanent wetlands, temperature and

483   dissolved oxygen remained within graphed tadpole tolerance thresholds, with temperature

484   ranging from 23.7 - 27.9 ^{o}C, and dissolved oxygen ranging from 6 - 15.7 mg/L. The pH level

485   of all wetlands ranged from 7.7 - 8.5 throughout the season. Ephemeral wetlands had salinity

486   within the acceptable thresholds from 0.06 - 1.5 ppt. The temperature for ephemeral and

487   permanent wetlands remained similar, ranging from 24 - 28.7 ^{o}C, and dissolved oxygen

488   ranged from 6 - 11.4 mg/L, all which remained within he suitable range for higher *L. aurea*

489   survival (Figure 22:D and E). Non-salinity parameters (pH, dissolved oxygen, water

490   temperature) were within acceptable limits at all times in the constructed habitat in the study

491   period (Figure 22).

492
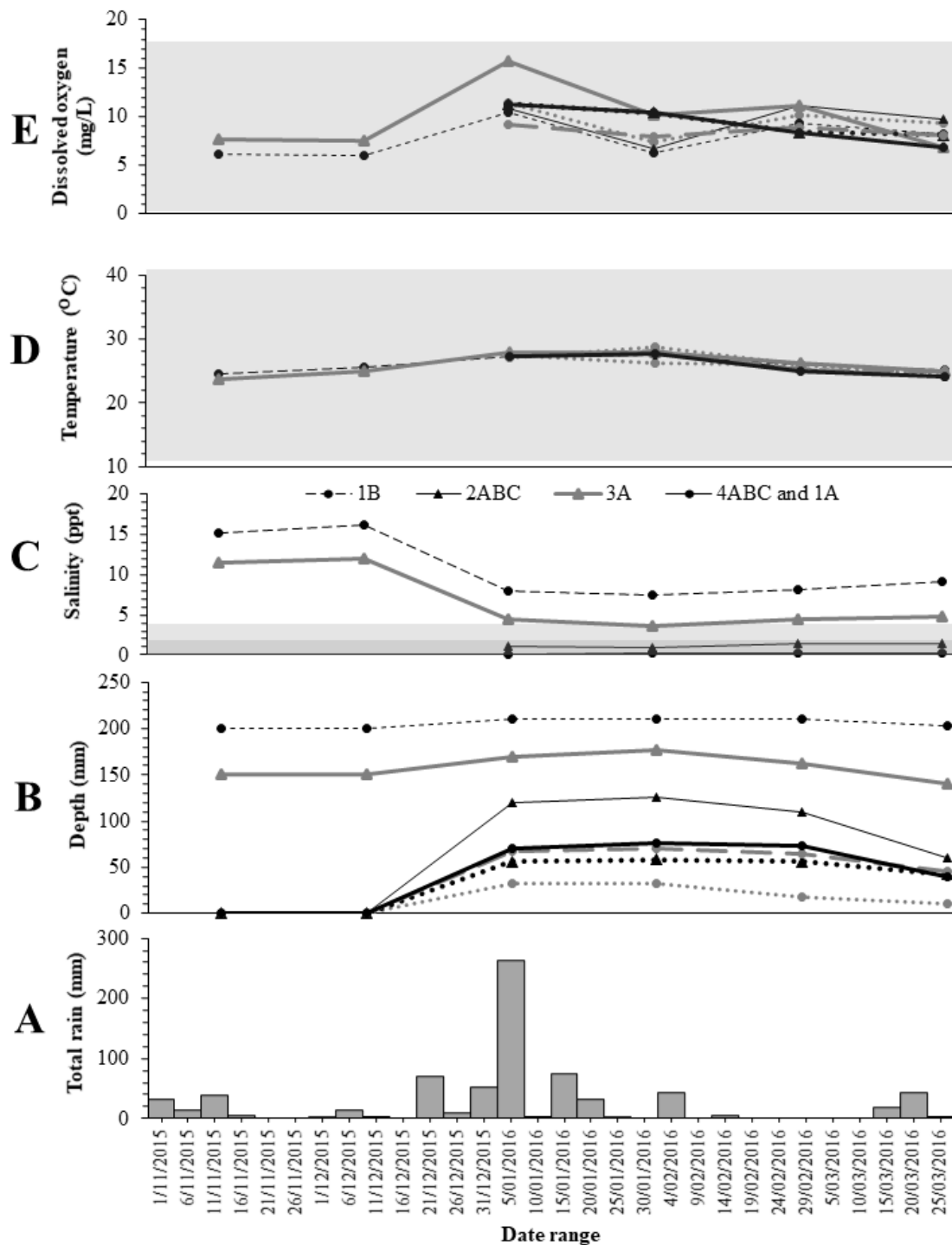
Figure 22: A) The total amount of rain (mm) in four-day periods recorded from the
Newcastle University Weather Station (No. 61390). B, C, D, E) Mean depth, salinity, water
temperature and dissolved oxygen, during 6 days of sampling from November 2015 to March
2016 in the compensatory wetlands on Ash Island. Grey shading indicates thresholds for
maximum tadpole survival and light grey in salinity indicates suitable salinity for adults
(Browne & Edwards 2003; Christy & Dickman 2002; Hamer 1998; Penman 1998; Pyke *et al.*
2002; Stockwell 2011; Werkman 1999). *In the references provided, tadpole survivorship
was not affected up to 4 ppt. However, in one study, tadpole survivorship was reduced at 2
ppt (Christy & Dickman 2002).

502   *5.5 Achievement of Key Performance Indicators for constructed habitat during the study*

503   *period (7/1/2016 – 30/3/2016)*

504   The key performance indicators were established for the wetland habitat construction

505   programme to benchmark habitat quality, viability and occupation by *L. aurea.* Most

506   benchmarks were achieved within the short time frame of the current study, largely

507   attributable to the occurrence of the major rainfall event in January, 2016. The major

508   benchmark not achieved during the period of the study were due to the absence of breeding in

509   the permanent waterbodies within the constructed habitat. This was followed by variability

510   between constructed wetlands reference wetlands on Ash Islandincluding variation in age

511   class (8) a comparative use of ephemeral and permanent wetlands (10) use of substrate (11)

512   and appropriate water quality for adults breeding in permanent constructed wetlands (19 and

513   20) (Table 6).

514   Table 6: Summary of results relating to Project Aims and Key Performance Indicators (KPI)
515   for constructed wetlands designed for *Litoria aurea* conservation on Ash Island, NSW
516   Australia.

Project Aims	No.	KPI	Achieved	Yet to achieve
Colonisation	1	Males onsite	X	
	2	Females onsite	X	
	3	Use of available habitat features	X	
	4	Breeding in ephemeral wetlands	X	
	5	Breeding in permanent wetlands		X
	6	Juvenile emergence	X	
Comparison to Ash Island	7	Chorus size similar or greater to other sites in NSW	X	
	8	Age classes similar between reference wetlands and constructed wetlands		X
	9	Body condition in constructed wetlands equal to or greater than reference wetlands	X	
	10	Use of ephemeral and permanent wetlands similar to reference wetlands		X
	11	Use of substrate similar to reference wetlands		X
Wetland design	12	Wetland bunding prevents flooding/*Gambusia holbrooki* movement	X	
	13	Ephemeral wetlands filled with water	X	
	14	Permanent wetlands filled with water	X	
	15	Ephemeral wetlands dried after breeding	X	
	16	Ephemeral wetland water quality appropriate for adults	X	
	17	Ephemeral wetland water quality breeding appropriate	X	
	18	Salinity in a range to treat chytrid	X	
	19	Permanent wetland water quality appropriate for adults		X
	20	Permanent wetland water quality breeding appropriate		X

517

5.5 Discussion

519   Many conservation programmes constructing habitat for threatened fauna focus on

520   reproducing the physical habitat used by the target species. Relatively fewer programmes

521   manage to incorporate novel designs to improve outcomes despite numerous examples where

522   behaviour has been demonstrated to play a vital role in successful persistence of a population

523   (Mahony *et al.* 2013; Sutherland 1998). In this chapter, I reported on a recent example of

524   created wetland habitat for *L. aurea* which showed initial, early signs of success.

5.5.1 Assessment of outcomes of constructed L. aurea compensatory habitat programme

526   Taken together, the outcomes of the current study indicate that KPI for the constructed

527   wetlands were achieved in a remarkably short timeframe. My results show rapid colonisation

528   of constructed wetlands by *L. aurea* and successful achievement of 14 of the 20 research KPI.

529   Male *L. aurea* rapidly colonised newly constructed ephemeral wetlands with one large and

530   two small choruses and a successful breeding event. Although few females were found in

531   constructed habitat, they used a nearby remnant waterbody and the terrestrial areas

532   surrounding constructed wetlands. Further evidence of habitat construction success included

533   successful development of tadpoles from metamorphosis to juvenile stage. Monitoring of the

534   colonising individuals to assess health indicated *L. aurea* in the constructed wetlands had

535   similar sex ratios and to the broader Ash Island, and that adults, tadpoles and juveniles were

536   in good condition, and that females in fact had an even better body condition than males (an

537   important factor, given the likely dependence of Kooragang Island populations on the few

538   females which survive to breeding age) (Hamer & Mahony 2007). The data taken together

539   indicates that the constructed wetlands are viable breeding habitat for *L. aurea*.

540   In addition to successful colonisation by *L. aurea*, the design and construction of the wetlands

541   can be considered mostly successful. Bunding successfully prevented flooding induced

542   invasion of *G. holbrooki*, and wetlands successfully dried, indicating waterbody drying may

543   protect against *G. holbrooki* infestation (through unanticipated events) in the future,

544   regardless of other potential sources of infestation (Figuerola & Green 2002). The water

545   parameters pH, DO and temperature (Pyke *et al.* 2002; Wassens *et al.* 2010), indicated that

546   all wetlands were suitable for adult occupation. The salinity in all ephemeral wetlands was

547   suitable for breeding. Although *L. aurea* only bred in one wetland, the breeding of other frog

548   species, colonisation of macroinvertebrates and presence of sensitive macroinvertebrates

549   further supports the suitability in all 7 ephemeral constructed wetlands (if flooded joined

550   wetland 2A,B,C is considered as 3 wetlands). Taken together, the constructed wetlands

551   habitats provided high quality environmental conditions for aquatic and terrestrial fauna.

552   In assessing outcomes of the habitat construction programme, five KPI were not met within

553   the first season (Table 6). Firstly, habitat use differed between reference and constructed

554   wetlands. Overall, there was a relative absence of reeds and floating vegetation masses in

555   constructed wetlands, however, dense reeds were available within the nearby remnant

556   wetland. As vegetation provides overwintering and sheltering habitat (Garnham *et al.* 2015),

557   it is recommended that restoration continue to establish fringing and emergent native reeds.

558   Secondly, use of ephemeral and permanent wetlands was different in the constructed habitat,

559   as evident in the low level of permanent waterbody occupation by *L. aurea*. This was likely

560   due to a lack of vegetation establishment in permanent waterbodies. Thirdly, constructed

561 habitat had minor differences in ages. Reference wetlands on Ash Island had very few

562 females over 18 months and few males over 12 months, whereas constructed wetlands had no

563 animals in these age classes. Despite this minor difference, overall age class structure of the

564 constructed wetlands was similar to reference wetlands. Lastly, although high salinity levels

565 suggest that wetland design successfully connected permanent wetlands with saline ground

566 water, salinity was generally above adult and breeding thresholds. Future work should

567 continue to monitor salinity to assess the interaction between rain, groundwater and

568 waterbody salinity, including the issue of stratification that can occur as a result of the

569 overlay of rainwater on waterbody strata (Turner & Erskine 2005). If stratification occurs, it

570 may be possible for tadpoles to use the lower salinity water on the surface of permanent

571 wetlands (Dobbs *et al.* 2012). Arguably, the timeframe of the study was too short to capture

572 breeding in permanent ponds as a metric, given the timing of the rainfall event so soon after

573 construction. Overall, despite the low levels of native vegetation and higher salinity in some

574 wetlands and slightly different age classes in occupied waterbodies, in light of these results as

575 a whole, the constructed wetlands, remnant wetland and terrestrial foraging areas have

576 formed effective habitat for *L. aurea*. 5.5.2 Response of other amphibians

577 In the current study, although the waterbodies were designed for *L. aurea*, a range of other

578 common species from the Hunter region were found using the constructed habitat. These

579 species ranged from ground frogs (*Lim. peronii* and *L. tasmaniensis*) to tree frogs (*L.fallax,*

580 *L.dentata, Lit. peronii*). All these species successfully reproduced within the constructed

581 waterbodies. For amphibians, habitat restoration or construction programmes have had

582 varying success, showing some species readily colonise and breed, where some species do

583 not (Lehtinen & Galatowitsch 2001; Pechmann *et al.* 2001). The current study indicates that a

584 number of species in the Hunter region are responsive to habitat construction, and future

585 programmes for conservation of these species will likely find similar designs successful at

586 attracting adults and facilitating breeding.

587 **5.5.3 Implications of research findings on L. aurea biology and ecology**

588 *5.5.3.1 Body condition of L. aurea in constructed wetlands*

589 The current study found that the body condition of females was higher in the compensatory

590 wetlands compared to reference wetlands. As frogs had only recently colonised the

591 waterbodies when the surveys in this study were undertaken, it is unlikely that the occupation

592   of constructed wetlands had resulted in an increased body condition in such a short period of

593   time. As the broader Ash Island data was collected in years prior to the current study, a major

594   limitation in comparing datasets is the variability in the body condition data that may occur

595   from year to year. Variability between years may increase or decrease body condition, such

596   as dry seasons and their effects on water and availability and prey availability (Brown *et al.*

597   2011; Greenville & Dickman 2005; Sperry & Weatherhead 2008). Nevertheless, as body

598   condition in the constructed wetlands was equivalent for males and better for females, the

599   comparable values for reference wetland body condition offer support for the view that

600   constructed wetlands support at least an equivalent comparable body condition to that in

601   reference wetlands. A further potential criticism of the use of body condition includes the

602   adequacy of regression residuals to measure this parameter (Peig & Green 2010). Body

603   condition can be affected by; sex due to differences in development rate or by differential

604   seasonal energetic investment, or between age classes due to ontogenetic variation in body

605   mass. To account for this in the current study, body conditions were not compared between

606   sexes. Additionally, distribution of age classes was reviewed to ensure an equivalent

607   distribution of frogs in each age class. This analysis was seen as adequate as it controls for

608   growth effects and the premise of size-independence (Green 2001; Peig & Green 2010).

609   *5.5.3.2 Age and breeding of L. aurea in constructed and reference habitats*

610   The size of *L. aurea* captured within this study in both reference and the constructed wetlands

611   consisted of young age-classes and a lack of older adults, which has implications for breeding

612   potential at the population level. In the wild, *L. aurea* typically reaches 6 years of age,

613   specifically the Broughton Island population which is not exposed to chytrid (*Stockwell M. S.*

614   *personal communication*). However, chytrid fungus prevents many individuals surviving to

615   older age classes, and in recent studies populations affected by chytrid fungus are typified by

616   few females reaching 18 months and few males reaching 12 months (Pickett *et al.* 2013;

617   Pickett *et al.* 2014; Pickett *et al.* 2016; Stockwell *et al.* 2015a; Stockwell *et al.* 2006a, b). As

618   males can breed at a younger age than females i.e. under 12 months of age (Mahony *et al.*

619   2013) males at the constructed wetlands were reproductively mature. Although eggs have

620   been recorded in young females as small as 53.4 mm SVL, egg masses were not well

621   developed (Christy 2000) and it has been suggested that breeding occurs later around 24

622   months of age, at 65 mm SVL or larger (Pyke & White 2001a). Although breeding occurred

623   at the constructed wetlands, the reproductive condition and fitness of the *detected* females is

624 uncertain, as the variability and thresholds for breeding age are not clearly understood. It is
625 also not currently well understood how age, body condition and environmental/disease
626 factors affect reproductive readiness. Nevertheless, given the comparative size distributions
627 between reference and constructed habitats in this study, it can be suggested that the
628 constructed habitat represents at least as viable a breeding habitat as the non-constructed
629 reference wetlands on Ash Island.

630 *5.5.3.3 Colonisation and dispersal of L. aurea at a landscape level – support from*
631 *this study for developing paradigms*

632 In addition to providing habitat for *L. aurea*, the current project also provides further support
633 for previously investigated biological and behavioural theories. Past research has suggested
634 *L. aurea* are an *r*-selected species (Hamer & Mahony 2007), based on high reproductive
635 output, rapid growth and development of offspring, colonising behaviour and the occupation
636 of habitats without established competitors or predators (Hamer 2008; Hamer & Mahony
637 2007; Mahony *et al.* 2013). From the current study, it is evident from preclearance surveys
638 and disturbance from construction activities that the construction site was not initially
639 occupied. Additionally, directly after construction ephemeral wetlands were dry, and
640 therefore not considered suitable for habitation. Consequently, the current study observed a
641 colonisation of new habitat after rain, adding to past examples of colonisation of waterbodies
642 in landscapes by *L. aurea* (Darcovich & O'Meara 2008; Stockwell et al. 2006a). The current
643 study also showed that colonisation is not necessarily dependent on the presence of
644 established native vegetation, as chorusing males used newly available weeds and grasses and
645 were observed sitting in low vegetation and on the water, indicating their opportunistic use of
646 habitat structures, including early succession vegetation structures. Consequently, the current
647 study shows that *L. aurea* will disperse to new or disturbed habitat, occupy new ephemeral
648 habitat, and respond rapidly to changing conditions.

649 Research to date has not clearly demonstrated whether *L. aurea* disperses or migrates,
650 however current research suggests this species disperses opportunistically in responses to
651 short term changes in environmental conditions. Some species of amphibians disperse to
652 breed, seeking newly available habitat after rain. Alternatively, some amphibian species
653 migrate to and from known habitat between seasons (Semlitsch 2008a; Sinsch 1990). In the
654 current study, the constructed wetlands were built in close proximity to an existing
655 population, but with no evidence of prior occupation on the constructed wetlands site,

656   suggesting that this location was not utilised (Hamer *et al.* 2008; Valdez *et al.* 2015) (*James*

657   *M. S. personal observation*). Additionally, several colonising males detected in the

658   constructed habitats showed abrasions on their snouts, similar to rubbing seen from escape

659   behaviour in captivity (*James M. S. personal observation*). This may indicate males moved

660   along boundary silt fences, scraping their snouts when moving into the constructed wetlands

661   from surrounding areas. As the constructed wetlands were newly available habitat in an

662   unoccupied space, dispersal and colonisation cannot represent utilisation of a known habitat.

663   This suggests that following large rain events *L. aurea* disperse widely to find suitable

664   breeding habitat, and movement is not migration. We have direct empirical evidence that

665   once male calling commences, this attracts other males to join the chorus (James *et al.*

666   2015)and other research on other species shows that frogs may also eavesdrop on

667   heterospecifics (Bee 2007; Phelps *et al.* 2007). Consequently, wide dispersal of males over

668   the landscape may be assisted by attraction behaviours (within and between species)

669   produced when an individual finds suitable habitat. Consequently, the evidence supports the

670   hypothesis that *L. aurea* opportunistically disperses as opposed to migrating to previously

671   used habitat.

672   The current study also showed differential habitat use across the landscape. At the

673   constructed wetlands and reference wetlands, females preferentially used permanent

674   waterbodies, the remnant wetland and terrestrial areas, and did not appear to regularly use

675   ephemeral waterbodies. However, males often used ephemeral waterbodies. There are a

676   number of potential reasons for this difference in habitat use. Research on other species have

677   shown that females may only attend a chorus waterbody when ready to breed (Lodé 2005).

678   This may be to avoid sexual harassment and associated energy loss (Thornhill & Alcock

679   1983), injury (Stockley 1998) or reduced density which may improve fecundity (Berven

680   1990). Alternatively, or additionally, females may mature later or breed less frequently and

681   thus have no need to spend time at breeding habitat (Berven 1990). Ephemeral habitat may

682   also provide less shelter and have high competition for prey (density dependent effects) due

683   to high male density. Consequently, chorusing habitats may be less attractive to occupy.

684   In addition to spatial segregation, some research has also found that segregation can be

685   facilitated by using different substrate heights (Keren-Rotem *et al.* 2006). The current study

686   showed that females in the remnant wetland sat higher than males. This may suggest height-

687   based segregation, however, as males and females in the reference wetlands sat at similar

688   heights, the height that females perched at in the remnant wetland is not likely segregation.

689   Two extra arguments also support this. Wetland 2A,B,C (chorusing waterbody) and the

690   remnant wetland were separate waterbodies approximately 20 m apart. Consequently, height

691   would not likely improve segregation as the distance between waterbodies acted as a form of

692   segregation. Also, differences between reed availability may have caused differences in

693   female use in the remnant wetlands. Tall reeds were readily available in the remnant wetland,

694   but were not readily available in waterbody 2A,B,C. Consequently, disparity in availability

695   likely caused differential heights between waterbodies.

696   In the current study, juvenile *L. aurea* dispersed from the natal wetland, as has been

697   previously reported for *L. aurea* in other populations (Bower *et al.* 2012). Juveniles quickly

698   dispersed and were not detected in terrestrial fringes around ephemeral wetlands. They were

699   also absent from the permanent wetlands in the constructed habitat, possibly due to low

700   vegetation density and connectivity. Currently, it is not known if juvenile *L. aurea* disperse

701   directionally (sensing water and moving towards it) (Semlitsch 2008a) or if dispersal is

702   random. As amphibian dispersal is risky and survival decreases with increasing dispersal

703   distance (Rittenhouse *et al.* 2009), dispersal behaviour should provide an advantage to

704   outweigh the risks of dispersal. For example, it is known that cannibalism occurs for *L. aurea*

705   (Crump 1991). If cannibalism provides enough mortality pressure, dispersal may have

706   developed as a behaviour to reduce juvenile mortality (Rudolf *et al.* 2010). Another possible

707   driver of dispersal is density dependence. In other amphibian species, high juvenile density

708   results in lower juvenile survival, delayed maturation, producing smaller adult body size and

709   reduced fecundity (Berven 2009). This is likely caused through competition for finite

710   resources such as microhabitats or food (Berven 2009; Pope & Matthews 2002; Reading &

711   Clarke 1995; Scott 1994; Scott & Michele 1995; Sztatecsny & Schabetsberger 2005).

712   Therefore, juvenile dispersal may occur to reduce local density and associated density

713   dependent effects. Juvenile dispersal may also prove to colonise wetlands alongside adult

714   dispersal, facilitating metapopulations (Hamer *et al.* 2008). As the causes for dispersal and

715   the methods in which juveniles find habitat are not known, this represents a major gap in

716   knowledge which cannot be fully addressed in habitat design at this stage.

717   Despite a lack of direct assessment on reasons behind female segregation and juvenile

718   dispersal, the movements and habitat use of females and juveniles raises further questions on

719   wetland design. To optimise habitat design in constructed landscapes, one strategy to cope

720   with juvenile dispersal and female segregation across the landscape may be to provide

721   wetlands close to the natal wetlands. Provision of more smaller waterbodies throughout the

722   local landscape as suggested in Klop-Toker *et al.* (2016) (but with protection from *G.*

723   *holbrooki*), would increase the chance of juveniles and females locating water at a short

724   distance away, but would maintain segregation from chorusing males. Additionally, research

725   into density dependent dispersal and novel ways to enhance habitat may further improve on

726   the currently successful design. For example, the presence of invertebrate food sources may

727   be enhanced by improving vegetation quality and habitat complexity (Almany 2004;

728   Hornung & Foote 2006; Kremen *et al.* 1993), factors which may be key to increase

729   survivorship from chytrid (Bower *et al.* 2014; Hornung & Foote 2006). Increasing vegetation

730   structure complexity may also reduce cannibalism by reducing encounter rates or provide

731   additional foraging resources to reduce the influence of resource availability on density

732   dependent dispersal.

733   Another interesting observation in the current study was that breeding occurred at the

734   waterbody with the smallest chorus (2 males), not the largest chorus (20 males). For many

735   frog species, females must detect and locate dispersed males, however, noise detection may

736   be difficult and limited to short distances from 70 – 100 m (Swanson *et al.* 2007). In the

737   current study, some females were found within 50 m of the breeding wetland, and as short

738   distance conspecific attraction has been confirmed for *L. aurea* (James *et al.* 2015) these

739   females may have been the source of breeding in the constructed wetlands. However, male

740   dispersal is known to occur from 300 – 500 m (Hamer *et al.* 2008), and it would seem logical

741   that females could detect signals at such a distance, however, this needs to be assessed. An

742   alternative option is that females may avoid larger choruses due to acoustic interference. If

743   *L. aurea* females use calling traits as proxies for male genetic quality (Klump & Gerhardt

744   1987; Welch *et al.* 1998) loud chorusing may limit their ability to identify attractive calls

745   amid chorus noise that interferes with mating decisions, known as the 'cocktail party

746   problem'. However, research has shown that some amphibians deploy methods to overcome

747   confusion in choruses (Lee *et al.* 2017; Tárano 2015). On Ash Island we detected *L. aurea*

748   breeding in wetlands with both large and small choruses. It is unlikely that females move

749   long distances between waterbodies to choose a mate due to energetic costs and exposure risk

750   in dispersal (Semlitsch 2008a). Consequently, it is more likely that females moved from an

751   area close to the chorus to participate in breeding. However, these assumptions could not be

752 evaluated in the current study, and female chorus preference, call preference and distance of
753 auditory detection still need to be resolved.

754 *5.5.4 Broad implications for habitat creation programmes*

755 Overall, the aim of habitat construction programmes should be to provide conditions that not
756 only provide habitat that increases survival and reproductive success. From this, a successful
757 programme would ideally have flow on effects to the surrounding population forming a
758 population source within the larger connected and populated landscape (for a discussion on
759 metapopulations in *Litoria raniformis* a similar species, see Heard *et al.* 2012). Although the
760 current study provides important information on initial colonisation, it cannot assess the
761 overall success of the programme. Long-term monitoring of the constructed wetlands and
762 reference wetlands on with new KPI could determine if *L. aurea* use more saline wetlands,
763 and assess if the increased salinity has assessable benefits for *L. aurea*. For example, salinity
764 and absence of *G. holbrooki* may decrease disease or predation on the site and increase
765 survival and breeding success. Further monitoring of the constructed habitat will be needed to
766 determine if there is increased survival of adults through a sustained shift in age class
767 structure and increased reproductive success.

768 **5.6 Conclusions**

769 This study showed that constructed wetlands can be rapidly occupied by *L. aurea* from
770 adjacent waterbodies, resulting in successful colonisation and breeding. However, to truly be
771 considered a success from a conservation perspective, future research needs to assess if the
772 wetlands improve long term population viability and persistence as a result of reduced
773 predation and availability of saline conditions to reduce disease. The current study was short-
774 term, but future longer-term monitoring of vegetation and frog community succession on the
775 Island should reveal whether features of the constructed habitat support the persistence of a
776 resilient population.

777 **5.7 Acknowledgements**

790 **Chapter 6.   Larger males get the girls: mating patterns help direct**

791 **captive breeding strategies for the endangered green and golden bell frog**

792 **(*Litoria aurea*)**

793 Melanie S. James, Michelle P. Stockwell, Alexander M. Wray-Barnes, Ligia Pizzatto, John

794 Clulow and Michael J. Mahony

795 **6.1 Abstract**

796 Captive breeding programmes are commonly used to facilitate wildlife translocations to

797 prevent species extinction, however, gaps in information on breeding behaviour can limit

798 programme quality. Mate choice and sexual selection can improve the genetics of

799 translocated individuals, however, failure to incorporate these breeding behaviours may

800 reduce animal fitness and translocation success. To include breeding behaviour in captive

801 breeding programmes one needs to understand how sexual selection and male-male

802 competition operates in a species. This task can be difficult, especially for species rarely

803 observed during breeding, thus, initial research findings can provide key information for

804 breeding programmes. In the wild, we studied mate selection in a species which is the subject

805 of numerous translocation programmes, the green and golden bell frog (*Litoria aurea*).

806 To assess male-male competition and sexual selection, we compared size and body condition

807 of naturally paired males and females (amplexed or grasped to breed), in comparison to males

808 and females that were not amplexed, but were available in the breeding waterbodies. We used

809 body size (snout vent length, right tibia length, body weight) and body condition indexes as

810 proxies for fitness. Body sizes and condition indices did not differ between amplexed females

811 and non-amplexed females. Sizes of amplexing male and female pairs were not correlated.

812 However, we found that amplexed males had larger size and better body condition compared

813 to non-amplexed but available males in breeding waterbodies. This suggests that either

814 females select for larger males or a trait that correlates with male size. Alternatively, a better

815 male body condition may provide an advantage in male-male competition. Regardless of the

816 mechanisms that generate this pattern, this information should be used to direct captive

817 breeding programmes. In breeding tanks, provide a range of male sizes to allow sexual

818 selection or competition between males, and possibly increase the fitness of released

819 individuals.

6.2 Introduction

821 A species' breeding behaviour has a strong link to the maintenance of a populations fitness

822 and the persistence of a species (Lorch *et al.* 2003; Quader 2005). However, information on

823 breeding behaviour for endangered species is often limited as many threatened species are

824 under-studied and breeding events are rarely observed (NPWS 2001b). This lack of

825 information poses major problems for endangered species recovery programmes, as we often

826 rely on captive breeding to produce animals for translocation with very little information on

827 breeding behaviour (where translocation is: the supplementation of small populations with

828 new individuals or reintroduction of the species into its former range (IUCN/SSC 2013;

829 NPWS 2001b). Breeding behaviour such as intrasexual selection (male-male competition)

830 and intersexual selection (mate choice) results in differential reproductive success, favouring

831 individuals with high quality traits (Andersson 1994; Quader 2005). However, within captive

832 breeding programmes, sexual selection is often excluded and animals are placed with

833 breeding partners to maximise genetic variability and prevent inbreeding depression (Quader

834 2005). Consequently, male-male competition and/or mate choice is limited, excluded or

835 unstudied, which may result in the loss of favourable traits that are required for individual

836 survival and reproductive success after translocation (reviewed in Chargé *et al.* 2014b and;

837 Snyder *et al.* 1996).

838 In the wild, *natural* and *sexual* selection purge deleterious genes from populations, however

839 within captive breeding programmes this is excluded and can reduce fitness. In the wild,

840 individuals which are unable to find resources, avoid predation, resist disease, compete for

841 breeding and attract a mate fail to survive and do not pass their genes to the next generation.

842 However, captivity often removes these natural and sexual selection processes (captivity

843 adaptation). Firstly, in captivity deleterious genes may be retained as survival is artificially

844 increased (reviewed in Williams & Hoffman 2009). Such genes, may confer neutral or

845 favourable traits in captivity but that are not favourable in the wild (Frankham & Loebel

846 1992; Williams & Hoffman 2009). Secondly, sexual selection pressures are limited or

847 excluded in captive breeding. In the wild, male-male competition can result in the

848 reproductive success of males with larger physical weapons, greater body size and good body

849 condition as they can monopolise available females, as has been shown in mammals,

850 invertebrates and amphibians (Halliday & Tejedo 1995; Preston *et al.* 2003; Savalli & Fox

851 1998; Yu & Lu 2012). Alternatively, female mate choice can remove deleterious mutations

852   by selecting traits which indicate male performance. For example, in amphibians females

853   may select a male on their choice of oviposition site (Howard 1978a, b; Kelly 2008) or

854   parental quality (Summers 1989). Females may also choose males for indirect benefits, such

855   as traits that indicate genetic qualities for offspring (good genes selection). For example,

856   some female birds select for brighter male plumage as an indicator of parasite resistance

857   (Folstad & Karter 1992; Moller 1990) or some amphibians select for call duration as a proxy

858   for increased offspring performance (Klump & Gerhardt 1987; Welch *et al.* 1998).

859   Alternatively, males may choose females for traits which will produce better quality or

860   quantity of offspring, such as maturity or size (Eddy *et al.* 2016; Swierk *et al.* 2013; Verrell

861   1989). Consequently, without natural selection and sexual selection acting on a captive

862   population, benefits from these processes are lost, posing issues for captive animal fitness.

863   There is direct evidence to suggest that removing selective processes have hindered

864   translocations. Salmon population supplementations which release captive born juveniles

865   have shown that on release, juveniles experienced low survivorship (Christie *et al.* 2012),

866   detrimental changes to physical traits and behaviour (Ford 2002) as well as decreased

867   fecundity in adulthood (Araki *et al.* 2008; Ford 2002) and that a lack of sexual selection may

868   decrease immunity to disease and parasites (reviewed in Bernatchez & Landry 2003). It has

869   been suggested that adaptation to captivity and a lack of sexual selection has hindered the

870   effectiveness of supplementation programmes (Anderson *et al.* 2013; Chargé *et al.* 2014a;

871   Chargé *et al.* 2014b; Quader 2005; Wedekind 2002). More alarmingly genetic changes as a

872   result of captivity can occur rapidly, in one generation (Christie *et al.* 2012; Heath *et al.*

873   2003), not only during long term captivity (Ford 2002; Kraaijeveld-Smit *et al.* 2006; Milot *et*

874   *al.* 2013; Woodworth *et al.* 2002).

875   Poor genes can, however, be purged through sexual selection and competition (Lorch *et al.*

876   2003; Whitlock & Agrawal 2009). For example, radiation induced mutations in beetles and

877   mites were removed when females were given a choice of mate and where competition

878   between males was available, but mutations were not removed in the instance of forced

879   monogamy, where sexual selection did not occur (Almbro & Simmons 2014; Radwan 2004).

880   Furthermore, small populations of bulb mites were more likely to survive when sexual

881   selection was active (Jarzebowska & Radwan 2010; Plesnar-Bielak *et al.* 2012). However, for

882   some species where intense sexual conflict causes injury, reproduction may improve in the

883   absence of intense conflict (Hollis & Houle 2011). Although we may not be able to use

884   sexual selection in captive breeding for highly aggressive species for ethical reasons or when

885   few individuals in the species remain, research on mites and beetles indicates that fitness can

886   be promoted by facilitating sexual selection in breeding programmes.

887   Reduced fitness of captive bred animals is a concern for taxa considered vulnerable to

888   declines such as amphibians (IUCN/SSC 2013), especially considering that translocation

889   attempts have had limited success (Dodd 2005; Dodd & Seigel 1991; Germano & Bishop

890   2009). Although much is known about amphibian mate selection, there are a wide range of

891   mating systems that vary within and between species, which includes male-male competition

892   and mate choice (Gerhardt & Huber 2002). As a result of reduced genetic quality in breeding

893   programmes and the positive role sexual selection can play in restoring genetic quality,

894   amphibian captive breeding programmes should seek to understand the mating system of the

895   target species. These mating systems should then be allowed to operate in captive breeding to

896   improve fitness post-translocation.

897   The first step in promoting sexual selection in captive colonies of endangered species is to

898   understand their mating system. The green and golden bell frog (*Litoria aurea*) has been the

899   focus of conservation efforts in New South Wales Australia and is considered globally

900   Vulnerable (IUCN 2015), nationally vulnerable (*Environment Protection and Biodiversity*

901   *Conservation Act 1999*), and endangered in NSW (*Threatened Species Conservation Act*

902   *1995*). Species recovery programmes have focused on habitat creation, captive breeding and

903   translocations with limited success (Mahony *et al.* 2013). Many translocation failures can be

904   attributed to an inability to remove original key threatening process such as predators and

905   disease (Daly *et al.* 2008; Pyke *et al.* 2008; Stockwell *et al.* 2008). However, these breeding

906   programmes do not specifically incorporate or monitor male-male competition or sexual

907   selection as potential increaser of fitness for released animals.

908   The aim of this study was to determine whether *L. aurea* display a random or non-random

909   mating pattern for male and female size as a preliminary indicator of female selection or male

910   competition, respectively. We expect that amplexing pairs will be larger than males and

911   females that do not achieve amplexus, and that male-female pair sizes will be correlated,

912   indicating size assortative mating. As morphometric measurements can be highly variable

913   between sample measurements due to body flexibility, we also compared repeatability of two

914   morphometric measures of snout vent length and right tibia. This will determine which

915   variable is the most precise to assess body condition.

6.3 Method

917 *6.3.1 Study species*

918 *Litoria aurea* has a prolonged breeding season and are reported to breed between August and

919 March in the spring and summer. They breed in permanent waterbodies as well as

920 opportunistically in ephemeral (flooding) waterbodies after heavy rain (Courtice & Grigg

921 1975; Hamer *et al.* 2008; Hamer & Mahony 2010; Mahoney 1999). This indicates that

922 throughout a breeding season some males and females in a population are capable of breeding

923 if climatic conditions are suitable, which is characteristic of a prolonged breeder such as a

924 permanent wetland or stream breeding frog (Wells 1977). This species is iteroparous,

925 breeding multiple times throughout their life, however, it is not known if this species can

926 breed twice within a single breeding season *(Mahony M. J. personal communication).*

927 Females produce minor vocalisations consisting of non-mating based grunts and squeals,

928 however, males produce grunts, squeals, and a droning call followed by a number of grunts

929 (Ford 1989) and choruses to attract a mate (James *et al.* 2015). This species has been noted as

930 an aggregating species, where aggregation is facilitated by acoustic communication (James *et*

931 *al.* 2015). Males and females of this frog are dimorphic; females being larger than males, and

932 males have no evolved weaponry for combat. Females are larger than males of the same age

933 and produce up to 3000 eggs (Pyke & White 2001a), making up one quarter of their body

934 weight, a large reproductive and energetic investment. When breeding, *L. aurea* deposit eggs

935 in a waterbody, and females often lay the eggs around aquatic vegetation where the eggs then

936 sink (Pyke & White 2001a). Most observations suggest that mating is monogamous (*Mahony*

937 *M. J. personal communication, James M. S. personal observation)***,** but it has not been

938 assessed if polyandry occurs, though this is considered rare in anurans (Lodé 2005).

939 Assessment of microhabitat selection has indicated that males use patches of vegetation to

940 call, sitting in the water and holding vegetation. Females use a range of microhabitats, but use

941 terrestrial vegetation higher than predicted based on availability (Chapter 2 and 3, Valdez *et*

942 *al.* 2016).

943 *6.3.2 Field method*

944 *Litoria aurea* were introduced in New Zealand in the 1870s and are considered invasive

945 (Ford 1986). Surveys were done at four independent green and golden bell frog breeding

946 waterbodies, herein referred as waterbodies one to four, located at Ocean Beach Road,

947 Whangarei, New Zealand. These waterbodies were man-made for different purposes:

948 waterbodies one and two were constructed as wildlife habitat by the landowner (both ~12 m^2

949 and 2 m at their deepest point), and waterbodies three and four were designed as the first and

950 second stage effluent filter from dairy sheds (both 1.5 m deep, 480 m^2 and 540 m^2,

951 respectively). Waterbodies contained fringing vegetation such as tall grasses and rushes, but

952 did not contain emergent reeds, and only waterbodies one and two contained a minor amount

953 of emergent grass. Weather readings were obtained from meteorological stations and the

954 virtual climate network (NIWA 2014). Climate data had a 5 km grid resolution, and data for

955 the survey location was interpolated from climate gradients taking into consideration terrain,

956 ground cover and slope angle.

957 Waterbodies were systematically surveyed from 8 pm to 4 am in timed visual encounter

958 surveys (Scott & Woodward 1994) over a four night period (15th – 18th December 2014).

959 Surveys involved searching the waterbody, aquatic, fringing and terrestrial vegetation for *L.*

960 *aurea* using head torches. After detection, each frog (including amplexing pairs) were caught

961 with a plastic bag over the hand and the bag inverted and tied to contain the animals. Time

962 and the presence or absence of calling was recorded. Flagging tape was tied to vegetation to

963 indicate capture location.

964 Sex of each individual was identified by the presence of nuptial pads on males. A passive

965 integrated transponder microchip (PIT tag) with a unique identification code was placed

966 under the skin of each frog between the vent and abdomen and codes scanned using a Trovan

967 LID-560ISO pocket reader. The snout to vent length (SVL) and right tibia length (RTL) were

968 measured with vernier callipers, making sure the frog's body and limbs were straightened as

969 much as possible to ensure accurate measurement. Each frog was then weighed using spring

970 balances (Pesola®). On three occasions amplexing pairs could not be separated, as males

971 continued to hold on to the female. For these pairs SVL and RTL were measured

972 independently, but weight was taken as a total weight of female and male. As these two males

973 were captured in the days before amplexus, and one also after amplexus, their weights were

974 assumed to be the same as during amplexus. We could then calculate the weight of the female

975 mates by discounting the males' weight from the total weight of the amplexing pairs. This

976 was only done for recaptures of males, as significant weight loss is expected for females after

977 dropping eggs. After data collection, animals were released at the location of capture.

6.3.3 Weight and size analysis

Animals that had been caught twice had their size averaged and only one record kept for the analysis. Indices of body condition were estimated using a linear regression of (1) weight against SVL and (2) weight against RTL, using the residual distance from the linear line (for a critique and method see Green 2001; Jakob *et al.* 1996; Schulte-Hostedde *et al.* 2005). The SVL, RTL, weight, and body condition indices were compared between amplexing and non-amplexing adults using *t*-test or Wilcoxon signed rank test, depending on data normality and heteroscedasticity of variances (SAS 2012) and means graphed (± standard error). All statistical analyses were done in JMP v. 11 (SAS 2016) with a significance level at $P < 0.05$. The SVL and RTL were both used to assess the size of adults. SVL is a good indicator of size, and also a good tool to assess age when the current study is compared with demographic studies. RTL is also a good measure of size and was assessed to determine if leg length provided some form of advantage in gaining mates, although the literature has not currently supported such assertions.

Gaining morphometric measurements of frogs can be difficult, as their bodies can flex, reducing accuracy of the measurement and precision of measurements between measuring events. It has been suggested that RTL is a more accurate and precise measurement as the rigidity of the tibia bone prevents flexibility for RTL measurements which is otherwise allowed by the spine in SVL measurements. We assessed the precision of SVL and RTL to determine which variable was more precise, for use in the body condition assessment. For animals which were caught multiple times, the variability in measurements (mm) between recaptures were recorded and graphed to indicate which measurement had the lowest precision (highest variability) and the highest precision (lowest variability). The results for SVL and RTL were then compared using a *t*-test. The measurement with the highest precision was used for the body condition indices.

6.3.4 Assortative mating

To assess size assortative mating, we correlated the body measurements of males with those from their amplexed females. Due to low sample size, we undertook a principal components analysis of the size variables of SVL, RTL and weight to develop a single size variable (principal component 1) calculated from all three measurements to be used in the correlation.

1008 *6.3.5 Behavioural observations*

1009 Opportunistically, over a two-hour period on the 17/12/2014, breeding behaviour was

1010 observed and notes taken on the movements of both males and females to detect if direct

1011 selection occurred or if satellite male behaviour occurred. Breeding occurred during the

1012 daytime. An observer sat quietly at the edge of the waterbodies and noted the number of

1013 times males 1) approached another male 2) the outcome of the approach (mistaken amplexus,

1014 direct movement of two males at each other or one male leaving the space he had occupied

1015 before being approached or the approaching male moving away from the approached male).

1016 The observer also noted the frogs entering the waterbodies, and whether they moved in a

1017 particular direction (no veering away from a direction). If amplexus occurred, the amplexus

1018 was categorised as 1) direct approach and no deviations to path, 2) varied movement and

1019 no/interrupted path and 3) amplexed by male which was not on the female's directional path.

1020 These were reported descriptively and may help to further characterise the bell frog mating

1021 system. Calls were classified as either a drone or grunt based on descriptions by (Ford 1989).

1022 **6.4 Results**

1023 *6.4.1 Climate*

1024 A large storm event preceded the surveys, with rain starting on the 11th and continued to the

1025 last day of surveys (Table 7). Temperatures ranged from 16.8^{o}C – 20.2 ^{o}C, relative humidity

1026 was high and wind increased over the survey nights.

1027   Table 7: Climate data for the survey nights from the NIWA Taihoro Nukurangi climate centre

1028   from interpolated data (closest weather station 25578) (NIWA 2014).

Date	MSL Pressure (hPa)	Rain (mm)	Relative humidity (%)	Temperature maximum (°C)	Temperature minimum (^{0}C)	Wind Speed (mps)
11/12/2014	1011.2	4.5	92	24	17.7	3.8
12/12/2014	1011.4	19.8	93	18.8	16.4	2.2
13/12/2014	1013.5	70.2	79	18.9	14.6	2.4
14/12/2014	1005	11.4	95	20.9	15	9.3
15/12/2014	1007.9	6.9	90	16.8	15.2	11.1
16/12/2014	1013	37.7	95	19.2	14.8	10.8
17/12/2014	1007.8	10.6	98	24.1	15.8	7.2
18/12/2014	1014.8	0	86	24.3	17	2.8

1029

1030   6.4.2 General descriptions

1031   Over the four-day survey period, 164 individuals of *L. aurea* were caught which included 12

1032   amplexing pairs. One male was caught on two different nights in amplexus with two different

1033   females. This resulted in 11 amplexing pairs for *t*-tests and a 12 amplexing pairs for the

1034   correlation of assortative mating.

1035   6.4.3 Variability in morphometric measurement precision

1036   Variability in precision between the morphometric measurements of SVL and RTL indicates

1037   that there was lower variability in measurements between recaptures for the RTL compared

1038   to the SVL (Figure 23) ($\chi^2 = 47.2$, df = 1, p = <0.0001*).
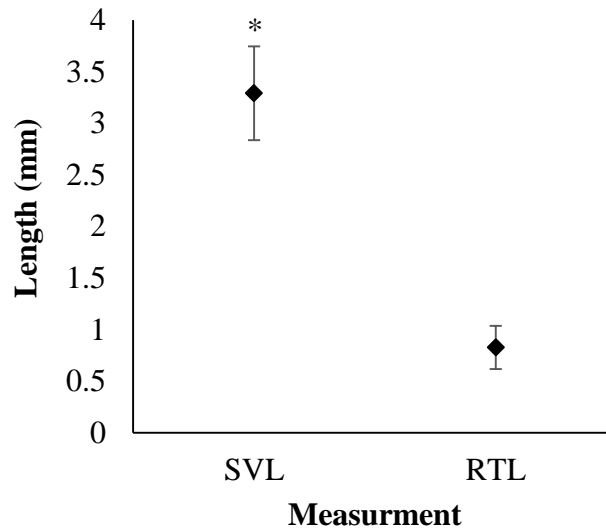
Figure 23: Mean snout-vent length (SVL) and right tibia length (RTL) variability for
recaptured *Litoria aurea* (± SE with statistical significance indicated by asterisks*).

6.4.4 Weight and size analysis

The SVL ($\chi^2 = 11.44$, df = 1, p < 0.001) and weight ($\chi^2 = 9.80$, df = 1, p = 0.002) were significantly greater in males that were caught in amplexus compared to those that were not in amplexus in the waterbody (Figure 24: A and C), however, the RTL did not differ between amplexing and non-amplexing males ($\chi^2 = 0.27$, df = 1, p = 0.61, Figure 24 B). Males in amplexus had a higher body condition than non-amplexing males ($\chi^2 = 11.41$, df = 1, p < 0.001= 0.0007). The SVL, RTL and weight measurements of females in amplexus were highly variable. There was no difference in weight ($\chi^2 = 0.7992$, df=1, p = 0.37), SVL ($\chi 2 = 0.9739$, df = 1, p = 0.32) and RTL ($\chi^2 = 0.5119$, df = 1, p = 0.47) and body condition ($\chi^2 = 0.4149$, df = 1, p = 0.52) between amplexing and non-amplexing females (Figure 25: A, B and C).

6.4.5 Assortative mating

Overall, the correlation coefficient indicated no relationship between male size and female size in the 12 amplexing pairs ($r = 0.197$, n =11, p = 0.54).
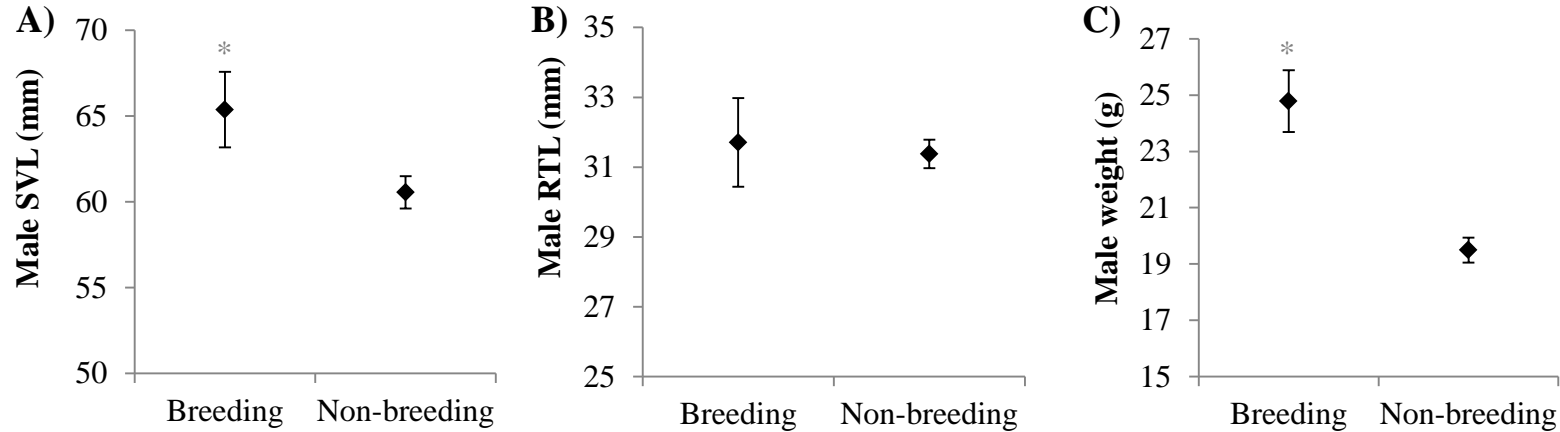
1056
1057 Figure 24: Size and weight measurements of male *Litoria aurea*. The A) snout vent length (SVL), B) right tibia (RTL) and C) weight of males in
1058 amplexus (SVL and RTL n=10, weight n=7) compared to males not in amplexus (n=91). Diamonds indicate averages ± SE with statistical
1059 significance indicated by asterisks (*).
1060
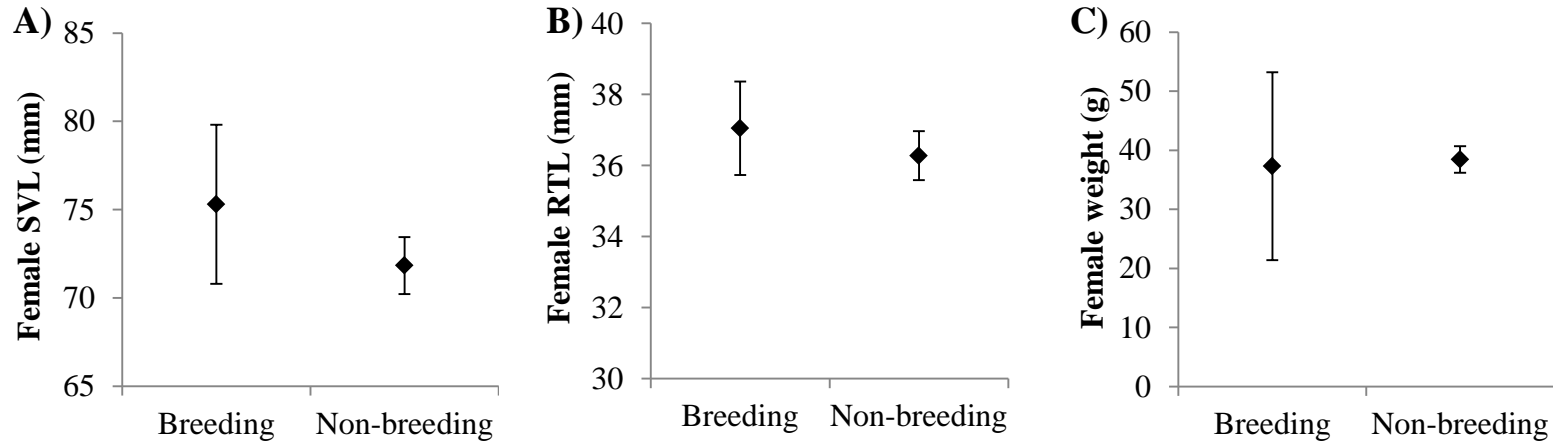
1061
1062 Figure 25: Size and weight measurements of female *Litoria aurea*. The A) snout vent length (SVL), B) right tibia (RTL) and C) weight of
1063 female in amplexus (SVL and RTL n = 11, weight n = 5) compared to females not in amplexus (SVL and RTL n = 52, weight n = 50).
1064 Diamonds indicate averages ± SE with statistical significance indicated by asterisks (*).

131

6.4.6 Behavioural observations

Males were observed floating on the water holding the algae and calling. On a number of occasions repeated low grunt like calls were emitted, two calling males would change calls from a drone-grunt to grunts only and one male (approaching male) would approach the other male by swimming and hopping in the water. The male being approached (resident male) would maintain position and jump at the approached male or move away prior to direct contact to a 20 – 30 cm distance. If the resident male maintained position, several movements and jumps were made towards the approaching male until the approaching male moved away. On several occasions the approaching male or resident male would jump on the other males back either without attempting amplexus (Figure 26: A) or sometimes grasping the other male. When males grasped other males, they continued calling, and males underneath made higher grunts in quicker succession than those displayed while calling. If males stayed in close proximity, grunting would continue and repeated moves would be made toward each other until one male moved away. Calling would then recommence.

During diurnal observations between the hours of 1 pm and 3 pm On the 17/12/14, three females were seen approaching the chorus over a one hour period (Waterbody 1). In two instances, females made directional movements into the waterbody toward calling males and a chorusing male grasped the female in amplexus on each occasion. One amplexing pair was approached by another chorusing male. The male used his head to nudge and pushing the amplexed male with their body. The amplexing male kicked back legs in the direction of the nudging male on several occasions. The amplexing male was dislodged, however, the female moved away without being amplexed again by the two males. On two further occasions, a male was seen to displace another amplexing male. On one occasion, a female making a directional movement towards callers was approached by a male from the left, where the male successfully grasped the female in amplexus with no indication of female selection (or rejection – no breeding was observed).

Over 28 minutes of this observation, of the 12 m^2 waterbody (90 % algae cover, 5 % open water and 5 % emergent individual grass shoots) at the site of amplexus two pairs laid eggs together in a 1 m^2 area in the 5 % emergent vegetation through algae (Figure 26: C and D). The third pair did not lay eggs during the observation period. During the PIT tagging process, breeding pairs stayed in amplexus despite being caught, measured and chipped with a PIT-tag

1096   (Figure 26: B). During measuring and weighing, males repeatedly kicked and made low short

1097   grunts.

1098

1099

1100
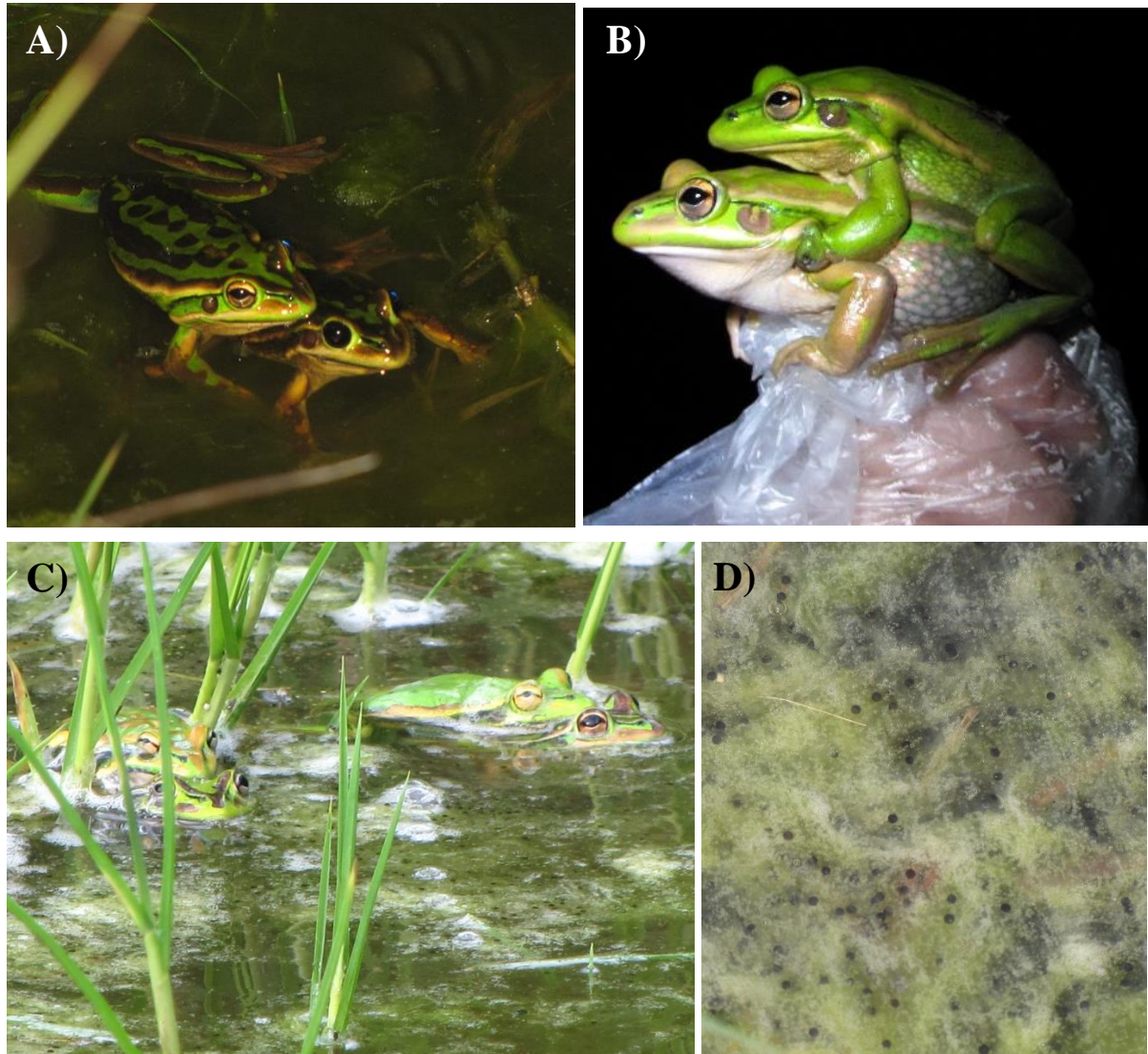
1101   Figure 26: Within the *Litoria aurea* breeding waterbody A) Two males during an approach
1102   after mirrored grunt calling without amplexus grasp, B) male maintaining amplexus during
1103   microchipping C) two amplexing pairs laying eggs in the same location within a chorus D)
1104   eggs layed in floating algae, around emergent grass. Photos by Melanie James.
1105

6.5 Discussion

1106

1107 Many studies on the conservation biology of threatened fauna focus on the physical and

1108 biotic habitat components related to occupancy to advise restoration projects. Relatively

1109 fewer studies consider the behaviour of the threatened fauna, despite numerous examples

1110 where behaviour has been demonstrated to play a vital role in successful persistence of a

1111 population (Mahony *et al.* 2013; Sutherland 1998). Studies on the endangered green and

1112 golden bell frog (*L. aurea)* is one such example with extensive studies on habitat tolerance

1113 and preference (Browne & Edwards 2003; Browne *et al.* 2003; Christy & Dickman 2002;

1114 Garnham 2009; Garnham *et al.* 2015; Hamer 1998; Klop-Toker *et al.* 2017; Mahony *et al.*

1115 2013; Pollard 2009; Pollard *et al.* 2016; Pollard *et al.* In Prep; Pyke & White 1996; Pyke *et*

1116 *al.* 2002; Stockwell 2011; Valdez *et al.* 2016; Valdez *et al.* 2015; Werkman 1999; White &

1117 Pyke 2015) and several large restoration and reintroduction programs structured around

1118 habitat requirements (Callen Unpublished; Darcovich & O'Meara 2008; Goldingay & Lewis

1119 1999; Klop-Toker *et al.* 2016; Pyke *et al.* 2008; Stockwell *et al.* 2008; Valdez *et al.* 2017b;

1120 White & Pyke 2008b). This is despite evidence that occupancy of wetlands by calling males

1121 may be driven by clustering and not habitat (James *et al.* 2015). This is most evident in the

1122 formation of calling choruses in constructed habitat with identical waterbodies (Callen

1123 Unpublished). These observations indicate that reproductive behaviour is an important

1124 component of occupancy and reproductive success, and that reintroduction and habitat

1125 restoration programmes require a detailed focus on the behavioural aspects of life history.

1126 As information on natural mating in *L. aurea* in NSW Australia is rare and interruption of

1127 breeding is unethical for endangered and declining populations, this study on mating pattern

1128 in New Zealand provides previously unreported insights on male-male competition and

1129 sexual selection. By assessing the pairings of male and female *L. aurea* in the wild, we

1130 demonstrate that *L. aurea* conforms to a non-random mating pattern in regards to male body

1131 size. Overall, amplexing males had a larger body length and better body condition (using

1132 right tibia) than males that were not amplexing, which is consistent with a number of

1133 amphibian species (Lee & Park 2009; Yu & Lu 2012). However, for females there was a

1134 random mating pattern where amplexing females had a range of sizes, weights and body

1135 conditions similar to non-amplexing females. Variations in female size may be due to age

1136 (Hamer & Mahony 2007) or history of movements and resource availability (Bower *et al.*

1137 2014). Overall, size assortative mating was not observed (no correlation between male and

1138   female pair size), nor was any evidence of polyandry and multiple paternal input. We found a

1139   form of direct competition between males, where a) males approach nearby callers and

1140   disrupt their calling location and b) that non-amplexing males dislodge an amplexing male

1141   (on two separate occasions).

6.5.1 Large male mating advantage?

1143   From this study, male-male competition and larger male amplexing success suggests that a

1144   larger body size and condition provides an advantage in competition. In some cases, size

1145   provides an advantage when displacing another male from a females' back (Berven 1981;

1146   Höglund 1989; Reading & Clarke 1983; Tsuji & Matsui 2002; Yu & Lu 2012). In the current

1147   study, we witnessed competition between males and displacement of a male from a females

1148   back on two occasions. Displacement behaviour was also been reported in *L. aurea* by Ford

1149   (1986). This suggests larger amplexing *L. aurea* males are a result of male-male competition

1150   and take overs. For example, larger males may be able to attend a chorus for longer periods

1151   of time due to higher energy reserves, thus increasing the chance of mating (Tejedo 1992),

1152   and they may also occupy the most desired micro-habitats with a wetland (Mathis 1990,

1153   1991b), e.g. reed patches for egg deposition (Chapter 4).

1154   Alongside male-male competition, females may also select larger males or a trait that

1155   correlates with larger size (e.g. call frequency or duration) (Klump & Gerhardt 1987; Welch

1156   *et al.* 1998). Although findings from this study suggest a male-takeover strategy occurs for *L.*

1157   *aurea*, future research should confirm if displacement and competition strategies are common

1158   for *L. aurea*. Additionally, as the current study was undertaken after a large rain storm event

1159   with high density of *L. aurea*, the reproductive strategy in this instance may represent an

1160   'explosive' breeding event, and it is currently not known if *L. aurea* employs different

1161   breeding strategies in different environmental contexts as has been found in some species of

1162   fish, birds and amphibians (Head *et al.* 2017; Robinson *et al.* 2012; Tejedo 1992). It is also

1163   unknown if strategy is affected by density (Höbel 2016), which are limitations in the current

1164   study.

1165   The next question is: are females choosy? Previous literature has suggested that the sex which

1166   provides more investment in their offspring (e.g. parental care or energy investment through

1167   egg production) will be choosier about those they mate with (Summers 1989; Trivers 1972).

1168   Females may also have a choice of a mate even when male-male size competition exists, by

1169 preferentially associating with larger males and avoiding smaller males (Houck *et al.* 1985;

1170 Mathis 1991a). There is no evidence in the literature to suggest that *L. aurea* invests parental

1171 care after oviposition, and we made no observation of egg guarding. Therefore, the only

1172 detected form of direct offspring investment is female energy through egg production. As egg

1173 masses make up a large proportion of female body size (1/4 of a females weight) (Pyke &

1174 White 2001a) and females reproduce once, maximum twice per season (*Mahony M. J.*

1175 *personal communication*) it would appear that females have the largest energetic investment

1176 lower potential reproductive rate and thus females should be choosy about their mate.

1177 Consequently, female choice could operate concurrently with the male-male competition seen

1178 in this study, as choice in amphibians may be mutual, one sided, or flexible in response to

1179 surrounding conditions and stimulus (Berglund *et al.* 2005).

1180 A factor in question from this study, is whether the male-male approach behaviour was

1181 mistaken sexual identity or aggressive male-male interaction. For some frog species, males

1182 amplex males and females without discrimination, and if a male is mistakenly amplexed, a

1183 release call is made (Wells 1977). In the current study, a call heard during the male-male

1184 amplexus may have been a release call, and the male-male interaction may have been

1185 mistaken identity or display of dominance (this has been recorded twice during previous

1186 fieldwork: *James M. S. personal observation*). However, the remainder of the male-male

1187 approach behaviour was unlikely mistaken identity as the other male was forced away from

1188 the interaction and males that approached other males did not attempt amplexus with the

1189 other male. This may suggest some competition to monopolise chorusing space or defence of

1190 a particular oviposition resource/site/territory (de Oliveira & Arturo Navas 2004; Wells

1191 1978).

1192 Some uncertainties also lie in the assumption of that large males of *L. aurea* result in greater

1193 mating success. Although this study shows that larger males successfully amplex available

1194 females, grasping does not necessarily indicate reproductive success, since it cannot be

1195 determined if females laid eggs. This is a distinct possibility, as Ford (1986) found that many

1196 amplexing adults parted without dropping eggs. This may be due to a lack of female interest

1197 in the male or current lack of reproductive readiness (Paterson 1979, Ford 1986).

1198 Consequently, size may determine amplexus success, but not necessarily reproduction. This

1199 may indicate that male-male competition and larger male mating advantage from regular

1200 takeovers does not necessarily exclude female selection.

1201   *6.5.2 Varied size of amplexing females*

1202   The information on amplexing female size provides insight to the broader reproductive

1203   behaviour of *L. aurea*. In the current study, the size of amplexing females varied

1204   considerably, which suggests that males are not choosy about female size, as some

1205   amphibians are (Eddy *et al.* 2016). Additionally, for *L. aurea*, it is likely that females are the

1206   limiting resource, as sex ratios in waterbodies are largely skewed to males (Pickett *et al.*

1207   2012). In a situation where the females are the limiting resource, males may be less choosy,

1208   competing for reproduction irrespective of female traits (Mathis 1991a) such as age and size

1209   (and therefore potentially a fitter breeder) (Eddy *et al.* 2016; Swierk *et al.* 2013; Verrell

1210   1989). However, this does not necessarily indicate that females in amplexus are capable of

1211   reproduction once they have reached a development stage that is below total growth potential,

1212   as actual breeding may not have occurred.

1213   *6.5.3 Captive breeding programmes*

1214   Currently, no published literature on *L. aurea* captive breeding has provided adequate

1215   information to ascertain if methods allowed for sexual selection (Daly *et al.* 2008; Pyke *et al.*

1216   2008; Stockwell *et al.* 2008; White & Pyke 2008b). From the current study, it is evident that

1217   *L. aurea* exhibits sexual selection resulting in larger males achieving amplexus, and this

1218   information may help guide captive breeding programmes. Considering the evidence that

1219   sexual selection is a key component for maintenance of population fitness and removal of

1220   deleterious traits (Almbro & Simmons 2014; Jarzebowska & Radwan 2010; Lorch *et al.*

1221   2003; Plesnar-Bielak *et al.* 2012; Radwan 2004; Whitlock & Agrawal 2009), it is pertinent to

1222   incorporate sexual selection behaviour within breeding programmes. In the case of *L. aurea*,

1223   sexual selection may be incorporated into breeding programmes by placing multiple males in

1224   breeding tanks. This would allow male-male competition and/or female selection to operate

1225   naturally. Detailed information on captive breeding designs and animal monitoring

1226   information should also be included in publications. This setup also offers an opportunity to

1227   study the reproductive behaviour of *L. aurea* in more detail. Due to the rarity of breeding

1228   events in the wild, breeding programmes have the opportunity to assess if frequent male

1229   takeovers occur, if females select a mate, and what she selects for in a male. Further research

1230   could also show if the performance of offspring in forced parings or sexually selective

1231   designs varied. Ultimately, this could help guide best practice for captive breeding.

6.6 Conclusion

As sexual selection can maximise genetic fitness of offspring, it is becoming more apparent that reintroduction programmes should consider sexual selection when breeding animals for release. The current conservation efforts in NSW Australia have an opportunity to research sexual selection in a captive population to determine if sexual selection is being excluded and to understand reproductive behaviour and potential changes in fitness, which is key to species persistence.

6.7 Acknowledgements

Chapter 7.   Synthesis

7.1 Summary

A fundamental aspect of conservation programmes is establishing an understanding of species biology and ecology that drives dispersal, settlement, site occupancy and breeding (Noss *et al.* 1997). Based on this understanding, conservation initiatives involving habitat protection, creation, breeding programmes and translocations have the potential to be more effective through informed decision making. Key aspects of biology and ecology that are not immediately apparent and can confound conservation efforts include: the influences of conspecific attraction, multilevel habitat selection and the sexually selective mechanisms driving mate choice (Campomizzi *et al.* 2008; Chargé *et al.* 2014b; McGarigal *et al.* 2016). On the other hand, where there is available information on distribution and breeding behaviour, conservation efforts can be refined and improved (Campomizzi *et al.* 2008). The endangered green and golden bell frog (*Litoria aurea*) has been the focus of numerous habitat construction and reintroduction programmes (Daly *et al.* 2008; Darcovich & O'Meara 2008; Klop-Toker *et al.* 2016; Pyke *et al.* 2008; Stockwell *et al.* 2008; Valdez *et al.* 2017b; White & Pyke 2008b). However, the breeding ecology and behaviour of *L. aurea* has not been explored in great detail, leaving a number of uncertainties for conservation initiatives, potentially resulting in lost or sub-optimal management opportunities.

Based on a number of uncertainties surrounding *L. aurea*'s reproductive ecology and the impact this gap may have on conservation programmes, the current studies tested four key elements: (1) the concept of landscape scale conspecific attraction (2) breeding site selection (3) sexual selection and (4) *L. aurea*'s response to newly available habitat. Specifically, I explored whether calls from speakers can manipulate site occupancy of calling males, whether calling male distribution is linked to particular habitat, if breeding pairs show mate selection and to report on the success of a habitat construction programme.

7.1.1 Conspecific attraction

The current thesis found that manipulation of *L. aurea* distribution using conspecific call broadcast on a landscape scale (Chapter 3) was not as successful as short distance manipulations (James *et al.* 2015). We found that abundance and calling did not significantly

1278   change. This was slightly different to findings in other research, which found that for two

1279   other amphibian species, one species colonised waterbodies with speakers and a another

1280   species did not (Buxton *et al.* 2015). Additionally, the calling seen across Ash Island was

1281   correlated with rainfall, supporting past research that has found similar associations (Hamer

1282   *et al.* 2008; Mortel & Goldingay 1998). Another key finding of this study is that a number of

1283   breeding events failed due to short waterbody hydroperiod. As we detected a low response to

1284   conspecific call broadcast, new uncertainties have arisen on the strength of conspecific

1285   attraction at a landscape level; these include whether *L. aurea* exhibit preferences for

1286   ephemeral or permanent waterbodies, and how competing conspecific attraction influences

1287   decision making. Despite these uncertainties, this study provides further information on the

1288   dynamics of landscape occupancy and further understanding of the strength of conspecific

1289   attraction over landscapes.

1290   *7.1.2 Microhabitat selection*

1291   Investigation into the microhabitat use of *L. aurea* found that males most regularly floated on

1292   the water amongst emergent vegetation or perched in vegetation, suggesting a preference for

1293   high vertical structural complexity. Calling males were occasionally found sitting on floating

1294   masses (vegetation or algae), but rarely floated in open water or perched on rocks or open

1295   ground. Consequently, *L. aurea* makes considerably more selective use of emergent

1296   vegetation than would be predicted from the availability of this habitat component around

1297   waterbodies. Comparisons between calling and non-calling locations indicated that overall,

1298   calling males preferentially used shallower areas within waterbodies with higher emergent

1299   vegetation and ground and less open water compared to available habitat. Additionally, males

1300   used locations with higher dissolved oxygen and lower salinity. This study also showed that

1301   males are willing to call independently within waterbodies. Overall, this study provides

1302   information on fine scale habitat selection of calling males.

1303   *7.1.3 Constructed wetlands*

1304   In the newly constructed wetlands, male *L. aurea* rapidly colonised and aggregated in one

1305   large and two small choruses in ephemeral waterbodies, where females used a nearby

1306   remnant wetland. During the period of this study, there was a successful breeding event in

1307   one ephemeral waterbody and tadpoles successfully developed into metamorph and juvenile

1308   stages. Overall, the demographics and behaviour at the constructed wetlands were equivalent

1309   to that across the broader landscape of Ash Island, with similar chorus sizes, sex ratios,

1310   estimated age, body conditions (and similar to other coastal populations removed from the

1311   Hunter Estuary). Integration of past learnings on habitat construction on Ash Island

1312   successfully created bunding to exclude overland flooding and subsequent transport of *G.*

1313   *holbrooki*, and ephemeral waterbodies successfully dried after *L. aurea* metamorphs left the

1314   waterbody. The water quality in all ephemeral wetlands was suitable for breeding, however,

1315   continued monitoring will determine if the high salinity in permanent wetlands will decrease

1316   with further rain. Taken together, this study describes the early success of a constructed

1317   wetlands programme.

1318   *7.1.4 Mating patterns*

1319   Investigations on the mating behaviour of *L. aurea* indicate that at high density chorusing

1320   after large rain events this species exhibits a larger male mating advantage, having both larger

1321   snout-vent length, weight and body condition. Large male mating advantage may be the

1322   result of male-male physical competition or larger males may be able to attend a chorus for

1323   longer (Bertram *et al.* 1996; Friedl & Klump 2005; Mathis 1990, 1991a). Alternatively,

1324   females may select larger males, either directly for size, or for size indirectly for a trait that

1325   correlates with size i.e. colour or call duration spectral, temporal or amplitude characteristics

1326   (Folstad & Karter 1992; Moller 1990; Welch *et al.* 1998). Considering the aggressive

1327   behaviour and displacement during amplexus observed within the waterbody, it is likely that

1328   larger males outcompete smaller males. In addition to these findings, the current study found

1329   that male size did not correlate with female size, indicating no size assortative mating pattern.

1330   Overall, this thesis provides insight on direct mating patterns, which have not previously been

1331   described in detail.

1332   **7.2 Synthesis of chapters and conservation implications**

1333   ***7.2.1 Microhabitat selection alongside aggressive territorial behaviour suggests guarding***

1334   ***of habitat or chorus space***

1335   This thesis demonstrated that *L. aurea* aggregate in particular habitat, possibly competing for

1336   specific resources. We detected aggressive interactions between males (Chapter 6) and

1337   preferential use of fringing vegetation with higher dissolved oxygen and lower salinity

1338   (Chapter 4). These two factors considered together suggest that firstly, within a waterbody,

1339   chorus locations aggregate around specific habitat for oviposition or adult protection. This

1340 thesis has shown that *L. aurea* prefer approximately 42 % emergent vegetation (Chapter 4),

1341 but a lack of dense vegetation does not restrict oviposition (Chapter 6). Consequently,

1342 vegetation use likely acts as protection from predators, and within a chorus, males compete

1343 for a resource such as space and breeding opportunities (de Oliveira & Arturo Navas 2004;

1344 Wells 1978).

7.2.2 The implications of conspecific attraction, chorus density, large male mating

1346 advionce advantage and the Allee effect

1347 These studies in this thesis detected a number of behavioural traits that highlight important

1348 issues facing *L. aurea* regarding low density, population dynamics and persistence. At high

1349 density, *L. aurea* displays aggressive male-male interactions that likely result in larger male

1350 mating success (Chapter 6). Generally, sexual selection and male-male competition provides

1351 a fitness benefit, either directly to breeding adults and/or indirectly to their offspring. For

1352 example, females may receive direct benefits from a male's oviposition site selection, or

1353 receive indirect benefits from choosing call duration as a proxy for improved offspring

1354 viability (Klump & Gerhardt 1987; Welch *et al.* 1998). Nevertheless, with respect to *L.*

1355 *aurea*, it seems unlikely females receive direct benefits from a male's oviposition site

1356 selection as although males chose particular areas of waterbodies, there was high availability

1357 of suitable habitat elsewhere, close by, in most of the waterbodies (Chapter 4). As an

1358 alternative explanation of aggregation in chorusing groups, it is was found that *L. aurea*

1359 display short distance conspecific attraction (James *et al.* 2015) but not strong long distance

1360 attraction (Chapter 3); consequently, short distance conspecific attraction may help drive

1361 high chorus density to facilitate male-male competition and larger male mating advantage.

1362 In low density populations, where only small choruses form, competition may not operate

1363 strongly and large male mating advantage may be lost. If large male mating advantage

1364 provides fitness benefits, loss of this form of sexual selection as a result of low density may

1365 decrease population fitness. A reduction in fitness with decreasing population density may

1366 result in an Allee effect if there is a decrease in population *per capita* growth rate

1367 (Courchamp *et al.* 2008; Stephens & Sutherland 1999). In the constructed wetlands, in the

1368 smaller chorus, our research did not detect direct physical competition between low density

1369 and distanced males (Chapter 5). However, breeding occurred in the constructed wetlands at

1370 the site of the smaller chorus. Consequently, it is unlikely that the four males involved in that

1371 breeding event had close competitive interactions that led to, or were indicative of, a large-

1372   male mating advantage. Thus, research on variable breeding strategies at different densities

1373   may determine if large male-mating success is a common occurrence or occurs alongside

1374   other forms of breeding behaviour. From this uncertainty, it is evident that more research is

1375   needed on the effects of large male mating advantage in aggregations, alternative strategies

1376   and potential Allee effects.

1377   **7.3 Recommendations for future research**

1378   The associations between animals and the habitat they occupy is one of the most studied

1379   areas of ecology particularly in light of habitat loss and population declines (Campomizzi *et*

1380   *al.* 2008). However, as discussed extensively in this thesis, habitat use is substantially

1381   influenced by a species' behavioural characteristics such as conspecific attraction, mate

1382   selection and a range of other behaviours that can confound conservation initiatives. As a

1383   result, habitat-based conservation planning can struggle to explain, predict and protect the

1384   extent of species movements, occupancy and ultimately survival.

1385   *7.3.1 Behaviour and Allee Effects*

1386   Based on the findings of this thesis, there a number of key gaps in knowledge for *L. aurea*'s

1387   behaviour that need to be addressed to direct conservation efforts. The major factor most

1388   pertinent to the persistence of *L. aurea* populations is the influence of Allee effects on

1389   individual fitness and population *per capita* growth rate. If conspecific attraction operates to

1390   increase chorus size and facilitate mate selection (i.e. male-male competition or female

1391   choice), then reduced population sizes and thus chorus sizes, may remove the benefits *L.*

1392   *aurea* receives from higher density. Currently, the presence of conspecific attraction in adult

1393   *L. aurea* has only been tested twice (James *et al.* 2015, Chapter 3), and no studies have

1394   attempted to determine why *L. aurea* aggregate. First, investigations should attempt to

1395   quantify the benefits received from aggregations, for example large chorus sizes improving

1396   male-male competition, mate choice, predator swamping reducing predation, or a

1397   combination of other factors discussed throughout this thesis. Second, research should assess

1398   the effects of reducing density on individual fitness and population *per capita* growth rate. In

1399   the absence of knowing why aggregatory behaviour occurs, step two can still be undertaken.

1400   Third, assess how low density is affecting wild populations. A key component of this

1401   research should include testing seasonal effects on large male mating advantage (Tejedo

1402   1992). Comparisons of breeding strategy should test prolonged and explosive breeding to

1403   determine what advantages and disadvantages are received at high and low chorus densities.

1404   As the Allee effect drives population decline and extinction vortexes (Courchamp *et al.*

1405   2008), assessing potential components of the Allee effect in declining populations should be a

1406   key focus of future conservation efforts for *L. aurea*.

1407   ***7.3.2 Resource Selection***

1408   Evidence of resource selection and aggressive breeding behaviour has identified key areas of

1409   focus for future research and conservation programmes. As the habitat within waterbodies is

1410   variable, and *L. aurea* preferentially uses vegetated, high dissolved oxygen and low salinity

1411   areas, future research on landscape occupancy should ensure water, vegetation and climate

1412   measurements are collected and recorded from a wide range of locations including

1413   microhabitats used by calling males. This could, in the future, further refine habitat

1414   assessment and predictive modelling (Blomquist & Hunter Jr. 2009; McGarigal *et al.* 2016).

1415   As vegetation likely provides protection from predation and is also important in low density

1416   for oviposition, a strong focus of habitat construction programmes should include provision

1417   of emergent and fringing vegetation, while still providing areas of open water. A better

1418   understanding of these factors will help improve conservation programmes and methods used

1419   to predict distribution and habitat use.

1420   ***7.3.3 Behavioural traits affecting dispersal and utilisation of natal and other breeding***

1421   ***habitats***

1422   The dispersal of *L. aurea* across a landscape may be influenced by a range of factors other

1423   than conspecific attraction, such as natal learning and behavioural variation in dispersal

1424   capacity, and their influence on habitat preference. The presence of natal learning and its

1425   influence on adult habitat preference has been detected in a number of animals (Benard &

1426   McCauley 2008; Ousterhout *et al.* 2014; Stamps *et al.* 2009). Although natal experiences may

1427   influence landscape movement and settlement decisions, currently this has not been assessed

1428   for *L. aurea*. Additionally, fields that have had no attention for *L. aurea* is the influence of

1429   within-species variation in behaviour, e.g. animal personality, and how hereditary variations

1430   in disperser behaviour can result in differential behaviour over landscapes (Brodin *et al.*

1431   2013), potentially influencing settlement patterns and habitat preference. Considering that *L.*

1432   *aurea* show preference for permanent waterbodies and dispersal resulting in ephemeral

1433   breeding (Hamer *et al.* 2002a), assessment of variability in individual dispersal capacity may

1434 provide more insight into the proximate drivers of landscape-scale movements. It has been

1435 suggested that *L. aurea* may possess a form of bet-hedging strategy that drives a bimodal

1436 breeding strategy (permanent and ephemeral preference) (Hamer *et al.* 2002a), however this

1437 has not been studied in light of personality or behavioural traits that may vary in expression

1438 across a landscape or operate as a stable bet-hedging strategy in the context of population

1439 dynamics.

1440 The presence of short distance conspecific attraction but a reduced effect of long distance

1441 conspecific attraction leaves a number of uncertainties for future research and conservation

1442 programmes. Firstly, a continuation of conspecific attraction research is necessary to

1443 determine the strength of attraction and aggregation across a landscape. A measure or

1444 parameter of the strength of conspecific attraction incorporated into modelling may improve

1445 predictive ability and provide routes to better understand dispersal and landscape movements

1446 (Campomizzi *et al.* 2008). Secondly, further research on the distance over which conspecific

1447 attraction operates and how competing conspecific attraction, natal learning and personality

1448 traits may interfere with this will help determine if conspecific call broadcast is viable

1449 management tool.

1450 In additional to landscape settlement, a key component to understanding the reproductive

1451 behaviour of *L. aurea* is assessing whether egg clutches gain advantages from selection of

1452 specific ovipositing sites (Mills & Barnhart 1999; Mills *et al.* 2001; Orizaola & Florentino

1453 2003; Seale 1982) and if this affects habitat selection in juvenile and adult stages (Davis &

1454 Stamps 2004; Stamps *et al.* 2009). As calling males were associated with emergent

1455 vegetation, shallower water, high dissolved oxygen and lower salinity, the next step is to

1456 further investigate these and other parameters that may be important for oviposition,

1457 development and survival. This can be done by directly assessing males and females through

1458 oviposition choice experiments (Iwai *et al.* 2007) and then assessing the development and

1459 survival of offspring in various conditions. Concurrently, varying male density in these

1460 experiments could assess the influence of male density on reproductive strategy and further

1461 explore female sexual selection and male-male competition as undertaken and reported in

1462 Chapter 6. The offspring from such experiments could then be raised and used in settlement

1463 experiments to test natal learning based on known oviposition and tank conditions at tadpole

1464 stages, and their choice of habitat as adults (Benard & McCauley 2008). In light of failed

1465 translocation programmes, this extension of research would provide insights into the way

1466 captive breeding influences the habitat selection of released individuals which are naïve to

1467 natural conditions (Chargé *et al.* 2014a; Chargé *et al.* 2014b; Scillitani *et al.* 2013).

1468 Ultimately, further understanding of microhabitat selection, natal learning and sexual

1469 selection are vital to maintaining the integrity of behavioural and survival traits for

1470 translocation programmes.

1471 **7.4 Conclusion**

1472 In conclusion, there has been extensive research on the biology and ecology of *L. aurea* that

1473 has led to an improved understanding of direct habitat requirements, key threatening

1474 processes and their management (Mahony *et al.* 2013). Nevertheless, despite extensive efforts

1475 to create habitat and reintroduce populations, there are still considerable barriers to the

1476 success of conservation programmes presented by key threatening processes and a lack of a

1477 more complete understanding surrounding behavioural ecology. These gaps in knowledge

1478 leave a number of uncertainties for species managers, breeding and habitat construction

1479 programmes, and continuing research on these key threatening processes and behaviours can

1480 help guide future conservation.

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