Improving ecological surveys of Australian frogs.

Mickayla Heinemann

[redacted]

Supervisors – Associate Professor Peter Murray

Dr Nick Hudson (University of Queensland)

Dr Megan Brady (Hidden Vale Wildlife Centre)

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Declaration of Originality

I declare the research carried out in the course of this investigation and the results presentedin this report are, except where acknowledged, the original work of the author, and all research was conducted during the Honours program.

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Abstract

Many of Australia's native frog species are threatened with extinction due to increasing pressure from disease, predation by invasive species and habitat destruction. As such, it is critical that ecological surveys provide accurate frog population diversity and abundance estimates to allow frog populations trends to be monitored over time, and conservation protocols implemented where possible. However, current survey methods to determine frog diversity are limited in their reliability and accuracy. Thus, it is critical that the limitations of current frog survey methods be recognized and reduced where possible. This study aimed to determine if survey methods from frog surveys could be improved by: (1) implementing artificial light as an attractant for frog prey species in conjunction with pitfall traps, (2) triallinga new method of surveying arboreal frog species, (3) testing the accuracy of a photographic identification software for individual toad identification; and by (4) providing observational data regarding potential occurrence of predation of frogs and other animals from pitfall traps.

A combination of pitfall traps in conjunction with artificial light (pit-light traps), and PVC pipe and bamboo refugia were used to survey frog populations across two trapping sites on a property in southeast Queensland. The resulting capture rate of native frogs in pit-light traps was used to determine the efficacy of artificial light to increase the capture rate of frog speciesin pitfall traps. PVC pipe and bamboo artificial refugia, as a method to survey arboreal frog species was trialled, demonstrating proof of concept, and opening pathways for future research. The use of photographic identification software (I^3S) was used to determine its viability as a non-invasive method of individual identification of cane toads. With further software development, and sufficient sample sizes, such methods may be utilised in mark-recapture studies to individually identify species that have unique patterns. Furthermore, the study

provided observational data that suggests vertebrate predation from pitfall traps may be a potential factor influencing frog capture rates in pitfall traps.

Methods to improve frog surveys were trialled, with limitations of each method highlighted and discussed. Further avenues of research were highlighted to further improve the reliability and accuracy of frog surveys. These methods provide a means to improve current knowledge of frog population trends and inform where conservation and habitat management projects are required.

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Keywords and abbreviations

Frog survey, pitfall traps, pit-light traps, camera traps, PVC pipe and bamboo culm, artificial refugia, limitations, photographic identification, species accumulation, environmental influence, mark-recapture

IUCN – International Union for Conservation of Nature

SEQ – southeast Queensland

NSW – New South Wales

PVC – Polyvinyl chloride

SVL – snout-vent length

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Chapter 1: Introduction

1.1. General Introduction

Native frogs in Australia are under increasing threat from disease, predation by invasive species, and habitat destruction (Department of Agriculture Water and the Environment 2021). However, current approaches to accurately determine frog diversity and abundance, are limitedin terms of the reliability of the survey methods used to determine their presence and thus calculate population estimates (Gillespie et al. 2020). Obtaining accurate and reliable population estimates, is vital in providing and understanding of population trends over time (Christie et al. 2012).

1.2. Frog diversity in Australia

There are approximately 7,404 recognised species of frogs and toads (hereby 'frogs') worldwide (Frost 2017). Comprising approximately 3.3 % of global frog species, Australia's 244 recognised species are representatives of five family groups: the Limnodynastidae (41), Microhylidae (22), Myobatrachidae (88), Pelodryadidae (or Hylidae) (88), Ranidae (1) and Bufonidae (1), (93 % of which are endemics) (Frost 2017; Cogger 2018; AmphibiaWeb 2021). Not only do frogs have intrinsic value as part of global biodiversity, but they also provide important ecosystem services (Sahasrabudhe & Motter 2011). These services include roles in food webs, as both predator and prey, and natural biological control of disease vectors (e.g. mosquitos), which pose significant risks to human health (Rubbo et al. 2011). Furthermore, frogs play a key role in the advancement of scientific knowledge as they have been a primary scientific model, used for developmental and physiological research from as early as the 18th century (Wells 1857; O'Rourke 2007). Additionally, due to their permeable skin and sensitivity to environmental pollutants, frogs are valuable indicators of environmental health (Hofrichter 2000; Llewelyn et al. 2019). The presence or absence of frogs in any ecosystem where they should be present, is indicative ecosystem health (Hofrichter 2000; Llewelyn et al. 2019).

Australian frogs inhabit regions ranging from the dense rainforests of north-east Queensland to the arid regions of central and western Australia (Anstis 2013). The greatest diversity of Australian frogs is found along the eastern coast, particularly north-east and south-east Queensland, and eastern New South Wales (Figure 1) (Slatyer et al. 2007). While some species are widespread, (e.g. *Litoria caerulea*, found across the entire northern region of mainland Australia) (Cogger 2018), some species are highly localised (e.g. *Pseudophryne corroboree*, found only in 400 m^2 habitat patches in sub-alpine regions of Kosciuszko National Park, south-east New South Wales) (Hunter 2012). Natural habitat types predominantly inhabited by frogs are wetlands, forests, and grasslands (Figure 2) (The IUCN Red List of Threatened Species 2021). Less common habitat types, such as salt marshes and estuaries, are primarily inhabitedby *Rhinella marina* and *Litoria aurea*, respectively (The IUCN Red List of Threatened Species 2021). Despite the broad range of habitats utilised by Australian frogs, many frog populations have experienced significant population declines in the last few decades (Allentoft & O'Brien 2010; Scheele et al. 2019).

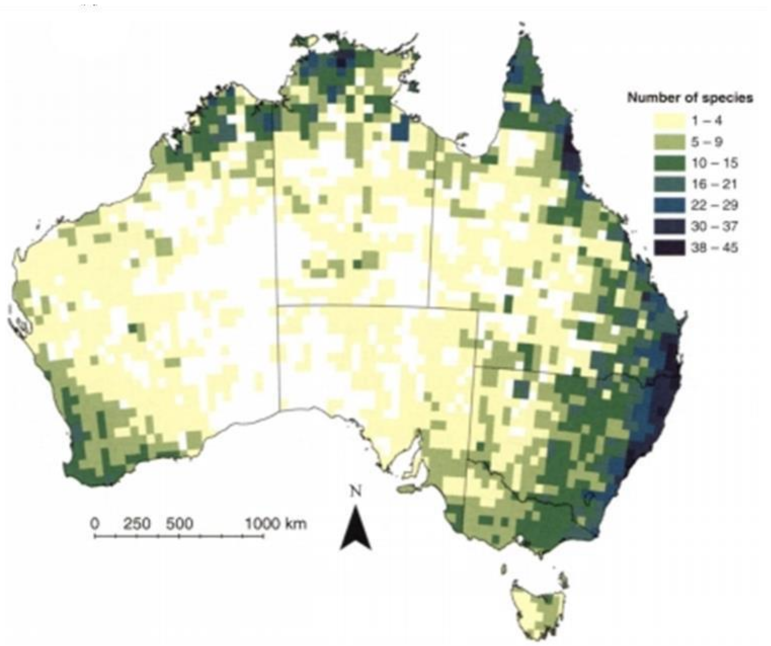

Figure 1. Species richness of Australian frogs (Slatyer et al. 2007).

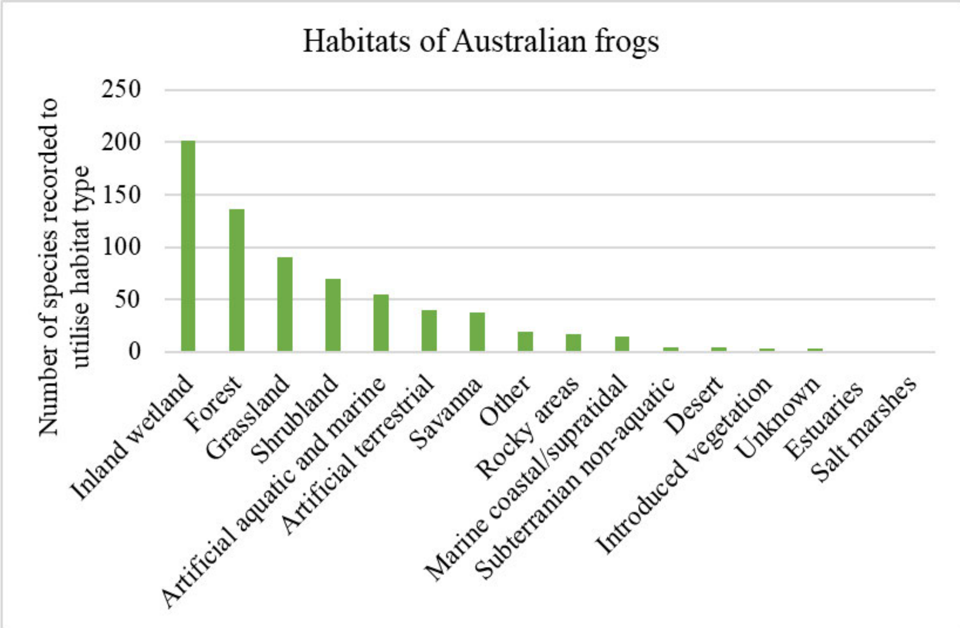

Figure 2. Habitat types utilised by Australian frogs (The IUCN Red List of Threatened Species 2021). 'Unknown' habitat type recorded for species whom full extent of habitat preference has not been fully researched. 'Other' habitat type refers to species which resideunderground (often in clay or sandy soil) except for breeding events during, or after heavy rainfall.

1.3. Frog population declines

Recent surveys have reported that over a third of all frog species world-wide are threatened with extinction (IUCN Red List of Threatened Species 2020). Of Australia's 244 species, 15 % are listed as endangered or critically endangered on the IUCN Red List, with 10 % listed at vulnerable or near threatened (Figure 3) (The IUCN Red List of Threatened Species 2021). Lack of reliable, accurate and recent population studies has meant that 5 % of Australian frog species are listed as data deficient (The IUCN Red List of Threatened Species 2021), with little known of the status of their populations (Figure 3). Furthermore, four species now listed as extinct are: *Rheobatrachus silus, R. vitellinus, T. acutirostris* and *T. diurnus* (Department of Agriculture Water and the Environment 2021; The IUCN Red List of Threatened Species 2021).

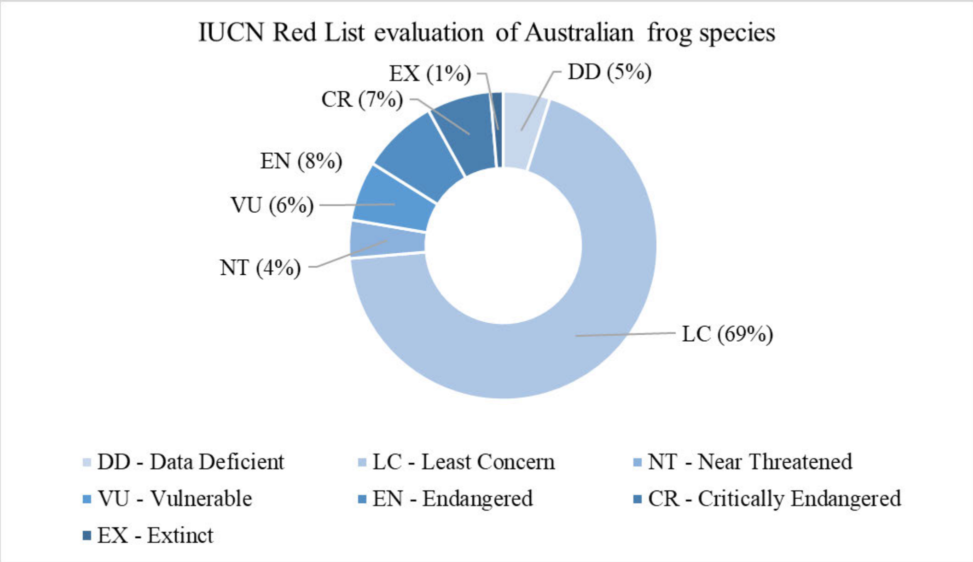

Figure 3. IUCN listing of Australian frogs (The IUCN Red List of Threatened Species 2021).

Factors attributed to these declines, include the spread of disease, predation by introduced species, habitat destruction, primarily due to agricultural expansion (Figure 4), and climate change (Blaustein et al. 2004; Wake & Vredenburg 2008; The IUCN Red List of Threatened Species 2021). The Australian landscape has been impacted by humans since First Nations people arrived in Australia over 65,000 years ago (Bowman 1998).

From the time of arrival, First Nations people used prescribed fires to systematically alter habitats, to selectively favour some wildlife species (Bowman 1998). Furthermore, European settlement led to the arrival of introduced species (e.g. cane toads (*Rhinella marina),* and cats (*Felis catus*)), as well as diseases such as chytrid fungal disease, which have all negatively impacted Australian frog populations (Woinarski et al. 2015).

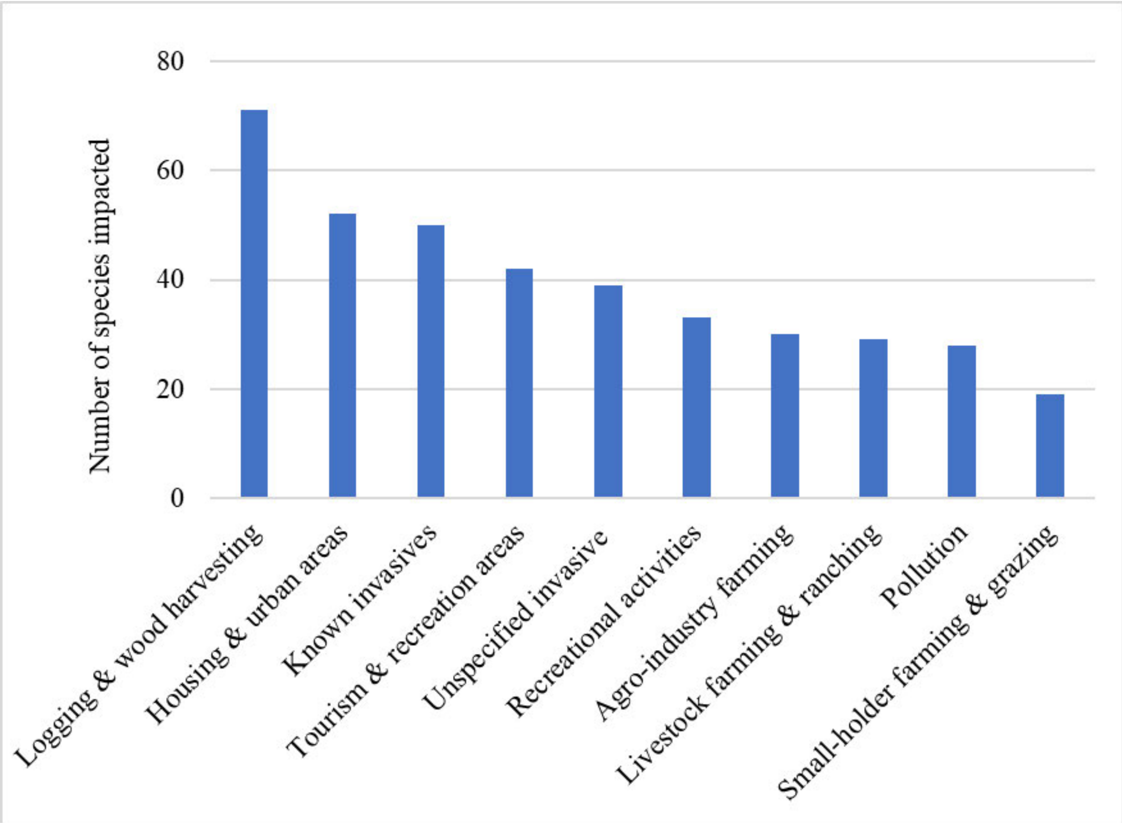

Figure 4. Major threats to natural habitats of Australian frogs (The IUCN Red List of Threatened Species 2021).

1.3.1. Disease

Amphibian chytrid fungal disease, or chytridiomycosis, (*Batrachichytrium dendrobatidis*(*Bd*)), is a fungal disease thought to have been brought into Australia through the internationaltrade of *Xenopus laevis* specimens for medicinal and scientific purposes (Weldon et al. 2004; Department of Sustainability Environment Water Population and Communities n.d.). The disease spreads through frog populations via contact with contaminated water or soil, causing hyperkeratosis and cytoplasmic degeneration of the frogs' epithelial cells (Campbell et al2012). Therefore, there is a reduction of osmotic transport through the skin and a reduction of electrolytes in the frogs' body, causing decreased cardiac function (Campbell et al. 2012). Furthermore, infected frogs develop pronounced lethargy, poor appetites, loss of their righting reflex, and excessive skin sloughing (Campbell et al. 2012), further resulting in high mortality rates in many infected populations, across a range of frog species (Department of Sustainability Environment Water Population and Communities n.d.).

1.3.2. Introduced species

The introduction of the cane toad (*Rhinella marina*), into Australia in 1935, has had negative effects of native frog populations, through the predation on, and competition with many frog species in locations where cane toads are now present (Shine 2010). The presence of cane toad tadpoles in breeding ponds, has also been shown to have strong negative effects on the development of at least three native frog species (*Limnodynastidae terraereginae, L. tasmaniensis* and *Notaden bennetti*) in south-east Queensland (Crossland & Shine 2010; Shine 2010). Of these three species, there was a clear reduced growth rate when in the presence of cane toad tadpoles, likely due to increased resource competition or potential toxicity of the cane toad tadpoles (Crossland & Shine 2010; Shine 2010). Furthermore, each adult cane toad can consume over 200 prey items per night, thus invertebrate populations diminish at an increasing rate as the population of cane toads increasing (Silvester et al. 2017). This potentially poses negative repercussions for native frog species because of increased resource competition (Shine 2010). Frog population declines caused by resource competition and developmental delays are further amplified by the increased predation by other introduced species (such as cats and feral pigs) (Gillespie et al. 2020; Woinarski et al. 2020).

1.3.3. Habitat destruction

With increased pressure due to disease and introduced species, habitat destruction (primarily for agricultural expansion, including logging and wood harvesting) is exacerbating the decline of frog populations across Australia (Blaustein et al. 2004; Cushman 2006; The IUCN Red List of Threatened Species 2021). The increase of land use for agriculture and urban development, has resulted in the complete or partial destruction of natural ecosystems, leading to habitat fragmentation and genetic isolation of frog populations (Figure 4) (Cushman 2006; The IUCN Red List of Threatened Species 2021). As examples, the southern and northern corroboree frogs (*Pseudophryne corroboree* and *P. pengilleyi*) are listed as critically endangered, having due to the impacts of severe habitat fragmentation, and reduced genetic diversity (Hunter 2012; Vanderduys 2012). Furthermore, the increased use of agricultural fertilisers and pesticides have resulted in decreased populations of many frog species (Hamer et al. 2004; Pulsford et al. 2019). Contamination of water bodies by agricultural run-off, causes an imbalance in nutrients and lower dissolved oxygen levels (Kremser & Schnug 2002). This then increases the prevalence of infertility, and can cause developmental abnormalities in frog populations, oftenaccompanied by high mortality rates (Hofrichter 2000; Tyrone et al. 2002; Egea-Serrano et al.2012).

1.3.4. Climate change

Global climatic changes are further contributing to the declines in frog populations through altering rainfall patterns, increasing ultra-violet (UV) radiation and global temperatures (McDonald & Alford 1999; Blaustein et al. 2004). Changes in rainfall patterns (e.g. lower average rainfall, increased short-duration heavy rainfall events), linked to climate change, reduces breeding periods for species reliant on consistent, heavy rainfall to breed, to which many species (e.g. *Helioporus australiacus, Neobatrachus sudellae* and *Notaden bennettii*) cannot adapt to (Broomhall 2004; Murray & Hose 2005; Vanderduys 2012; Bureau of Meteorology 2021b). While Australian frogs have evolved in a landscape with variable rainfall patterns, the increasing prevalence of extreme climatic events occurring within short time periods, result in compounded severity (Bureau of Meteorology 2021b). An example of the impact of changed climatic activity was observed across Australia with the 2019-2020 bushfires, where a combination of extreme heatwaves, after a period of long-term drought, saw the effects of extreme environmental stress, resulting in a severe, environmental catastrophe (Deb et al. 2020), that would have had a negative impact on frogs.

Additionally, the continuing depletion of the ozone layer has been attributed to increased levelsof UV radiation (Bernhard et al. 2020), with increased exposure found to result in increased tadpole mortalities in *Crinia signifera* and *Litoria verreauxii alpina* (Broomhall et al. 2000; Blaustein et al. 2003; Collins & Storfer 2003). Furthermore, increases in global temperature results in environmental stress which further contributes to increased susceptibility to disease, and increased rates of chytridiomycosis outbreaks in frogs (Blaustein et al. 2004).

1.4. Conservation action

Presently, majority of conservation efforts for native frogs involves recovery actions and strategies based on a species-by-species basis determined by threatened species lists (Meyer 2006; Clemann & Gillespie 2010, 2012; Hunter 2012). Limitations to the resources available for conservation often results in resource allocations made in favour of species that are high risk (critically endangered) or highly favoured by the public (Possingham et al. 2002).However, it is often inappropriate for greater resource allocation towards species with the highest probability of extinction due to the large recovery efforts needed, with small chance ofsuccess (Joseph et al. 2009). On the contrary, the recovery of other, less threatened species has relatively high feasibility. While threatened species lists provide information regarding the urgency of conservation action, they should not solely provide the basis for resource allocation(Joseph et al. 2009). Highly feasibly conservation strategies, such as habitat protection and management, focused on reducing development and ecological risk, whilst promoting biodiversity and ecological system conservation should be prioritised (Joseph et al. 2009).

Filling knowledge gaps about factors that influence frog population trends, within a given ecosystem can be vital to inform conservation strategies (Gillespie et al. 2020).

1.5. Importance of measuring frog populations

In the face of extinction, ecological surveys of frog populations are vital in securing knowledgeof the abundance and diversity of species within an ecosystem (Bower et al. 2014). From this data, populations can be monitored for fluctuations, and targeted conservation strategies implemented for frog species, particularly for species at risk of decline or extinction (Bower etal. 2014).

The acquisition of temporal and spatial data allows for population trends and distributions to be determined (Bower et al. 2014). With increasing pressure on ecosystems worldwide, frog populations are experiencing rapid declines in populations (Collins & Storfer 2003; Blausteinet al. 2004; Wake & Vredenburg 2008). Hence, there is increasing need to obtain reliable information to fill critical knowledge gaps on species abundance and diversity to inform appropriate management, including conservation projects (Bower et al. 2014).

However, current methods for surveying frogs are severely limited in their ability to reliably determine frog species presence and abundance. Such limitations are due to low detection probabilities of many species as a result of species behaviour (e.g. cryptic, arboreal or sedentary species), or the intrinsic bias of sampling technique in regard to taxon (e.g. arboreal species are not often recorded in terrestrial traps) (Hutchens & DePerno 2009). Thereby, there is a substantial need for the improvement of frog survey methodology, particularly for species that are often under surveyed or in low densities (e.g. arboreal, or rare species).

1.6. Factors that influence survey success

Species information is often limited by the type of survey (e.g. population level or individual level survey) conducted, which thus limits the data obtained (Hutchens & DePerno 2009; Clutton-Brock & Sheldon 2010). Population level studies are vital in the acquisition of data on population size, distribution and structure, and are vital in providing evidence of the effects of changes in climate on species populations (Clutton-Brock & Sheldon 2010). These types of studies are limited in that the proximal causes of population change is not always discernible, due to a lack of information regarding breeding success and survival rates of the frogs studied (Clutton-Brock & Sheldon 2010). Contrarily, long-term individual-based studies provide insights to the proximal causes of variation in growth, breeding success and survival (Clutton-Brock & Sheldon 2010). Optimal survey design maximises the data obtained whilst remaining representative of the true population (Scott et al. 1994a).

In addition to the overall study design, it is essential that principles of sampling theory are metto obtain accurate, and representative data to which a statistical estimate of the true populationcan be made (Scott et al. 1994a; Hankin et al. 2019). As a specialised component of statistical theory, sampling theory concerns itself with the development of procedures for random sampling of units, representative of a larger, finite population (Hankin et al. 2019) Primary factors of sampling theory include standardisation, randomisation, spatial scale and acknowledging and minimising sources of bias (Scott et al. 1994a; Hankin et al. 2019).

One of the primary factors of these principles is the necessity of standardisation of techniques between studies (Scott et al. 1994a; Hankin et al. 2019). The use of standardized techniques for population monitoring ensures that data collected across different studies is comparable, and accurately reflects actual species composition (Scott et al. 1994a; Hankin et al. 2019). As discussed by Scott et al. (1994b), standardisation between studies allows for a greater pool of comparable data, which therefore allows true changes in species composition to be recognized.

Considerations that must also be made are the spatial scale of the survey itself, to ensure the survey meets the requirements set by the research question, and to maximise the utility of the information gathered (Scott et al. 1994a; Hankin et al. 2019). Surveys conducted with a sub-optimal scale cannot accurately determine densities of widely dispersed species (Scott et al. 1994b). However, if a species is highly abundant in a small area, minimal variance in density estimates will occur between transects (Scott et al. 1994a). Furthermore, interpretations of data also hinge on the fundamental elements of randomization and bias (Scott et al. 1994a; Hankinet al. 2019). As no environment is ever truly homogenous, differing sampling procedures can give significantly different results (Scott et al. 1994a; Ryan et al. 2002). Random selection of survey points is necessary to ensure biodiversity estimations are not over or under-estimated due to environmental heterogeneity (Scott et al. 1994a). Additionally, the removal of sampling bias through randomisation within a habitat is optimal in removing the effects of environmental heterogeneity (Scott et al. 1994a; Ryan et al. 2002).

Another key component of survey design comes through replication, providing confidence in the data obtained and by minimising the effect of localised factors that can obscure site-wide variables (Scott et al. 1994a). Understanding the underlying assumptions of any project is vital for meeting the assumptions made in any statistical procedures (Hankin et al. 2019). Basic assumptions for most parametric statistical procedures are (1) the sampling is randomised and (2) observations are independent (Scott et al. 1994a). With these sampling considerations standardized across surveys, variability in survey success then falls to the survey method utilised (Scott et al. 1994b).

Lack of independence between samples results in occurrences of pseudo-replication, where the wrong statistical hypotheses, and therefore inferences are made (Hurlbert 1984; Hankin et al. 2019). Pseudo-replication occurs when statistical analyses tests for treatment effects on data that: (1) was not replicated, or (2) replicates are not statistically independent (Hankin et al. 2019). Sample units not replicated (or not statistically independent occur due to lack of acknowledgment that multiple observations have been taken on a single replicate of a treatment (Hankin et al. 2019). A true replicate, as defined by Heffner et al. (1996), is “the smallest experimental unit to which a treatment is independently applied”. The occurrence of pseudo-replication can be reduced by consideration of the structure and inherent randomness within the data when lack of independence occurs during the survey design (Millar & Anderson 2004). Appropriate survey design and the limitation of pseudo-replication in ecological studies is vitalin providing comparable data that meaningful statistical inferences can be made (Hankin et al. 2019).

With many frog species experiencing increasing environmental pressures and greater risks of extinction, there is an increasing need to obtain further data on frog populations. However, traditional frog survey methods are often limited by survey technology, and the intrinsic biases these methods have regarding the accuracy and representation of frog populations. Therefore, there is a significant requirement for additional survey methods to be identified to provide data that will in the gaps left by traditional frog surveys.

1.7. Thesis outline

Chapter 2 reviews literature on the traditional methods used to survey frog species, highlighting how these methods are limited in obtaining accurate diversity and abundance estimates. An alternative method, untested in Australian ecological surveys, is discussed for surveying arboreal frog species. Also discussed is an alternative method for individual identification, in mark-recapture studies, replacing traditional invasive, and often detrimental methods. Chapter 3 provides detailed description of the experimental design and methodology used for this research project, with Chapter 4 presenting the results. Chapter 5 discusses results in relation to the given hypotheses and research objectives. This chapter also discusses correlations between the number of frogs captured and environmental influences such as rainfall and daily temperature range. Chapter 6 provides conclusions from the research project and recommendations for further research.

1.8. Hypotheses and objectives

This study examined how frog survey methods and individual identification of native frogs canbe improved. The experimental hypotheses tested were:

1. Artificial light in conjunction with pitfall traps will increase trapped frog diversity and abundance estimates compared to traditional pitfall trapping.
2. Arboreal frog surveys using PVC pipe and bamboo culm as artificial refugia will increase frog diversity and abundance estimates compared to traditional pitfall trapping.
3. Photographic identification software is a reliable method for the individual identification of trapped, free-living frog species.
4. Predation of frogs and also their escape from pitfall traps results in inaccurate estimates of frog diversity and abundance.

The objectives of the study were to:

1. Improve frog surveys using pitfall traps by utilising lights as an attractant for prey species to increase capture of frogs in pitfall traps.
2. Determine if artificial refugia i.e., PVC pipe and bamboo culm, can be utilised as a sampling method for arboreal frog species.
3. Determine if frogs exhibit a preference for bamboo or PVC pipes as artificial refugia.
4. Use microchipped cane toads to determine the reliability of photographic identification software for frogs.
5. Use camera traps to record animal interactions around pitfall traps *in situ* to determine the prevalence of predation and escape of animals from pitfall traps.

Chapter 2: Literature review

2.1. Frog survey methods

The aim of this literature review is to discuss the importance of reliable ecological surveys to monitor frog populations for research and conservation planning. This literature review also discusses factors that influence the capture rates of frogs, including survey method, sampling intensity, frequency, sampling effort, biases, and standardisation factors. Furthermore, the review also discusses new methods that can be utilised to improve the reliability and scope of frog population surveying. The review focuses on monitoring techniques for adult frogs, excluding methodologies (e.g. dip-netting) that focus on tadpole surveying. The review also excludes novel practices such as detection dogs as a survey method, due to current limitations of available literature.

2.2. Traditional methods of anuran population surveys

Traditional methods of conducting frog population surveys include visual surveys, pitfall trapping, bioacoustics surveys, and camera trapping, each with their unique advantages and disadvantages (Table 1) (Scott et al. 1994b). Prior to the development of bioacoustics and camera trap technology, visual surveys and pitfall traps were the primary method for surveying frogs (Scott et al. 1994b).

Table 1 Summary of traditional frog survey techniques. Adapted from Scott et al. (1994b) and Ali et al. (2018).

Sampling method	Advantages	Limitations	Population data
Visual Encounter	Cost effective. Less destructive. Study area and survey intensity can be easilyaltered to fit constraints of study.	Animals can conceal themselves. Open to bias from researcher assumptions and experience. Requires extensive time in field. Influenced by habitat complexity.	Species richness, composition, relative abundance, and density
Pitfall Trap	Size can be altered to suit target species. Non-biased in what terrestrial species are captured. Effective for long term surveys. Data is comparable when averaged over a setnumber of trap events/days.	Does not account for the individuals that escape from or predated from the trap. Labour intensive to install and check frequently. Often excludes arboreal and fossorial frog species.	Distribution, relative abundance, and population estimates.
Bioacoustics	Detects species that are difficult to find otherwise. Reduced field work time. Non-invasive.	Only records vocalising individuals. Costly equipment. Does not account for female frogs or non-vocalising males.	Abundance, diversity, and richness
Camera trap	Can be used for long term surveys.Non-invasive.	Limitations in species captured, oftenexcludes small, fast moving, and poikilothermic animals. Time consuming to process.Costly equipment.	Distribution, abundance, density

2.2.1. Visual encounter surveys

Historically, the most common method for surveying frog species, has been through an active search of the habitat where the species was expected to be found (Campbell & Christman 1982; Ali et al. 2018; Fragoso et al. 2019). Visual encounter survey (VES) methods, consist of researchers walking throughout the selected survey site in transects, for a set time and recording the individuals observed (Scott et al. 1994a). This form of survey technique is utilized to estimate species richness, species composition, and estimate relative abundance of species within the ecosystem (Scott et al. 1994a). VES surveys are most extensively used for large forest areas for frogs and other small reptiles and are particularly ideal for species that are 'highly clumped', such as frogs and other aquatic species at ephemeral water sources (Scottet al. 1994b; Akre et al. 2019).

When used in conjunction with a mark-recapture method, repeated VES can be used to reasonably estimate species density (Table 1) (Ali et al. 2018). Furthermore, VES are largely cost-effective (measured against time spent during search) and relatively non-destructive in comparison to other methods (William & Harris 2005; Ali et al. 2018). VES are primarily useful for surveying species that are rare or unlikely to be caught by other trapping methods (Scott et al. 1994b).

The efficacy of VES versus quadrant surveys, for number of individuals and species per sampling effort, was examined in a study on rainforest herpetofauna in south-east Peru (Doan 2003). Sampling across five sites, the study determined that VES surveys recorded higher frogabundance than quadrant surveying and greater diversity of species recorded (Doan 2003). Over a longer-term survey (23 months), there was no significant difference reported in frog species richness across the two survey methods (Doan 2003). The study provided quantitative data on the efficacy of each method, concluding that neither method was better than the other for surveying all rainforest herpetofauna, as each method had their own advantages and limitations (Doan 2003). This study highlights a potential trade-off required between sample area and survey intensity with each method, and considerations that must be made to the research goals when selecting survey method (Doan 2003). The study further emphasises the importance of standardised sampling methods used within ecological surveys (Doan 2003).

Assumptions that must be made when conducting a VES include: (1) every individual has the same chance of being observed; (2) each species is equally likely to be observed; (3) an individual is only recorded once during the survey (no multiple encounters of the same individual); and, (4) there are no observer-related effects (e.g. assumptions at where individual are most likely to be, and experience in species identification) (Scott et al. 1994b; Ali et al. 2018). However, in most VES studies, the primary limitation is that these assumptions are not always upheld (Scott et al. 1994b). This is often due to unreliability in survey success across dissimilar habitat types, and the influence of environmental conditions (e.g. vegetation density, visibility, weather conditions) on capture success rates (Scott et al. 1994b).

VES methodology is further limited the lifestyle of a given frog species, as VES are unsuitable for many fossorial species (Doan 2003). Furthermore, the cryptic nature of many frog species, and limited active periods may provide unreliable data due to daily variation in when that species may be detected (Doan 2003). Because of this, VES is an unreliable method that does not always produce accurate, and reliable data that is appropriately representative of the true diversity of frogs in a given habitat (Scott et al. 1994a; Ali et al. 2018).

VES offer an effective frog surveying method when the study design has accounted for and minimised the limitations of this method (Scott et al. 1994b; Doan 2003). It is highly recommended that VES are used in conjunction with other standardised surveying methods, to provide the most accurate and comparable data (Scott et al. 1994a).

2.2.2. Pitfall traps

Developed in the early 20th century, pitfall traps are one of the most versatile and frequently used form of trapping technique for frogs (Luff 1975; Woodcock 2005). Since their initial development, pitfall traps have been used as a passive trapping method, valuable as a cost- effective method in surveying a wide range of vertebrates (including frogs), and invertebrate species (Ali et al. 2018; Ramírez-Hernández et al. 2018).

The basic design of a pitfall trap is an open plastic bucket, container, can, or pipe, buried to thelip of the container flush with the ground into which animals fall (Ali et al. 2018; Waudby et al. 2019). The design of pitfall trap used (in regard to depth and width) is highly dependent onthe purpose of the study and target species, ensuring it is large enough to sample targeted species, whilst minimising the possibility of escape (Woodcock 2005; Waudby et al. 2019). For example, a shallow-wide mouthed pitfall trap is often used for sampling a broader range of reptile species, in comparison to deep-narrow pitfall traps used for species with jumping or climbing abilities (Waudby et al. 2019). Consequently, significant considerations must go intothe design of the pitfall trap, as the design holds significant influence on the type of frogs captured, thus influencing diversity estimates and capture rates that can be derived from the data (Woodcock 2005; Waudby et al. 2019).

Pitfall traps are a common frog survey method, as they are relatively cost-effective, and are efficient from the time of installation to specimen collection (Ali et al. 2018; Waudby et al. 2019). Furthermore, they are beneficial in providing continuous sampling from the time of installation until they are emptied by the researcher (Woodcock 2005). For vertebrate trapping, pitfall trap capture of one individual does not necessarily prevent further captures of other individuals, with the exemption of predation events occurring within the trap (Waudby et al. 2019). This a critical comparison to standard vertebrate traps (e.g. Elliot traps) in which multiple captures are limited by trap design (Waudby et al. 2019).

Like other methods (Table 1), pitfall trapping is not without its limitations, as species specific and community specific sampling biases are a common limitation with pitfall trapping (Melbourne 1999). Biased population estimates are produced when the study does not account for the variation in the probability of capture between species (e.g. terrestrial versus arboreal frog species) and population densities within the habitat (Melbourne 1999; Engel et al. 2017). Like VES, success of pitfall trapping for frogs is further limited by the active period of the frog species being trapped (Doan 2003; Engel et al. 2017).

Sampling biases commonly found when using pitfall traps were quantified and multiple simulation experiments analysed with an allometric individual-based model, simulating 10 terrestrial arthropod species (Engel et al. 2017). Sampling biases decreased with increases in frog body mass, body temperature and number of pitfall traps used (Engel et al. 2017). The spatial arrangement of pitfall traps was also found to have a limited effect on sampling bias when estimating relative abundance (Engel et al. 2017).

A further limitation to pitfall trapping is the potential for frog, and other animal mortalities to occur once they are trapped. The rate of animal mortality varies depending on the species caught, the environment being sampled and the frequency in which the trap is checked (Enge 2001). Frogs in particular are more susceptible to mortalities in dry environments (e.g. inside the pitfall trap) due to their biological dependence on moist environments, or if the trap is not checked frequently (Enge 2001). High mortality rates (17-33 %) of the most common frog species caught in pitfall traps have been reported in one study of forest regions in New Hampshire, despite traps being checked every 1-2 days (Degraaf & Rudis 1990). Contrarily, mortality rates of <8 % have been reported during a survey along streams within hardwood forests in Florida, despite traps being checked every 5.5 days (Enge 1998).

Furthermore, daily trap checks in herpetofauna studies conducted in Florida and Connecticut resulted in mortalities of 4.7 % (Dodd Jr & Franz 1995) and 1.3 % (Gibbs 1998), respectively. While heat stress and dehydration can be mitigated by the provision of moisture in the pitfall traps (e.g. using moist leaf litter or sponges) (Petit & Waudby 2013), the most effective mortality reduction method is frequent trap checking. This however increases the cost-efficiency of pitfall traps through increased labour required (Waudby et al. 2019). There also remains potential for mortalities to occur due to predation from pitfall traps. While most evidence of predation from pitfall traps is anecdotal, there have been some studies that found predator species repeatedly attending the site with active pitfall traps. Due to the limited data on predation from pitfall traps available, there is no conclusive evidence that it is not a contributing factor to low pitfall trap capture rates, and the subsequent biases in frog population estimations (Ferguson et al. 2008).

Overall, pitfall traps are effective in obtaining information on spatial distribution, relative abundances, and population estimates (Table 1) (Woodcock 2005). However, pitfall traps are widely recommended for use in conjunction with an effective mark-recapture method, as individual identification of specimens is vital in obtaining accurate population data (Scott et al. 1994b; Woodcock 2005).

2.2.3. Mark-recapture studies

The mark-recapture method is a commonly used method to estimate the size of animal populations and composition within the survey area (Donnelly et al. 1994; Lettink & Armstrong 2003; Bardier et al. 2020). Widely used for vertebrate surveys, mark-recapture dataprovides more reliable data for species abundance estimates than alternate methods, as it accounts for individual detection probabilities (Karanth 1995; Fewster & Pople 2008; Bardieret al. 2020).

The series of assumptions that mark-recapture studies are based upon are: “(1) the animal population is closed, (2) animals do not lose their marks during the sampling periods, (3) all marks are correctly recorded, and (4) each animal has a constant and equal probability of capture on each trapping occasion” (Otis et al. 1978, p. 9). While the first assumption can be relaxed in many short term studies, changes in capture probabilities between sampling periods can bias population estimates (Otis et al. 1978; Lettink & Armstrong 2003). Seasonal effects and age demographics alter the probability of capture (capture heterogeneity), which results inbiased population estimates. Other sources of bias such as inappropriate trapping method or trap placement influences the data recorded, often resulting in an over or under estimation of animal abundance (Table 2). When accounting for all underlying assumptions, the mark-recapture model can effectively be used for vertebrate surveys, as long as the animals can be marked individuals identified (Donnelly et al. 1994; Lettink & Armstrong 2003).

Table 2. Consequences of unequal capture probabilities when estimating animal abundance(*N*). Taken from Lettink and Armstrong (2003).

Source of bias	Example	Consequence	*N*
Capture heterogeneity	Some animals are less likely to be caught (e.g.age-biased dispersal).	Marked animals have higher capture probabilities.	Under-estimated
Capture heterogeneity	Inappropriate trapping method	Precludes some individuals from capture if trap is already occupied.	Under-estimated
Capture heterogeneity	Inappropriate trap placement (e.g. traps inedge of home range instead of middle).	Animals less likely to be captured, hence fewer animals marked.	Under-estimated
Trap response	Trap-happiness (e.g. useof baited traps)	Animals caught once are more likely to be caught again.	Over-estimation
	Trap-shyness (e.g. animals learn to avoid traps in fixed places).	Animals caught once are less likely to be caught again.	Underestimation

In frog surveys, the primary method of identifying individual frogs for mark-recapture studies, is through toe clipping (Donnelly et al. 1994; Parris & McCarthy 2001; McCarthy & Parris 2004). This method relies on the strategic amputation of digits, up to a particular toe joint, in a corresponding number schematic, unique to that frog (Donnelly et al. 1994; Phillott et al. 2007). Toe clipping is a highly contentious method in herpetological studies (Parris & McCarthy 2001; Waddle et al. 2008; Perry, Gad et al. 2011), with many concerns expressed regarding the permanent disfigurement of the animal (particularly when more than one digit is clipped from a single limb) and the effect this has on the ongoing survival of the animal (McCarthy & Parris 2004). For example, a 1979 study on the dynamics of breeding population investigated the return rate compared to number of toes clipped across age demographic for 11 species (*Crinia signifera, C. parinsignifera, Limnodynastes tasmaniensis, L. dumerilii, Litoria aurea, L. peronii, L. raniformis, L. verreauxii, Neobatrachus sudelli, Pseudophryne bibroni* and *Uperoleia rugosa*), at a pond near Canberra, Australia (Humphries 1979).

Results from this study indicated that toe clipping reduces the probability of frogs being recaptured by 6-18 % for each toe clipped across all species recorded (Parris & McCarthy 2001). Reduced recapture rates of frog's post-toe clipping may be caused by increased risk of infection and thereby, mortality, or changes in their behaviour and movement around the study area (Parris & McCarthy 2001; McCarthy & Parris 2004). In the face of frog population declines worldwide, highly invasive methods of frog identification, such as toe clipping, remains ethically contentious in herpetological research, with the ongoing development of other individual identification methods encouraged (Donnelly et al. 1994; Brown 1997; Parris & McCarthy 2001; McCarthy & Parris 2004; Phillott et al. 2007; Perry, G et al. 2011).

Most alternative identification techniques for frogs also require invasive procedures, such as branding (e.g. tattooing, heat branding), or the insertion of passive identification transponders (PIT) tags and visible implant elastomer (VIE) tags (Donnelly et al. 1994; Brannelly et al. 2013; Sapsford et al. 2015). PIT tagging involves the subcutaneous implantation of electronic microchips that store a unique identification number that can be read by handheld PIT tag scanners (Brannelly et al. 2013). Standard PIT tags are approximately 10 mm in length and are unsuitable for small frog species (body length <30 mm) (Brannelly et al. 2013). In comparison, VIE tags are subcutaneous injections of coloured silicone which are illuminated through the skin via handheld UV light, to show a code unique to the individual (Brannelly et al. 2013). While this method is useful for identification of larger tadpoles, tag migration from the original implantation site can lead to misidentification or tag duplication (Brannelly et al. 2013).

While unique and effective identification methods, PIT and VIE tag use on frogs is often discouraged, as they are invasive, require specific equipment and relevant training to inject the tags, and are unsuitable for many frog species that do not meet the minimum size requirement for tagging (Brannelly et al. 2013; Bardier et al. 2020). Due to increased focus on animal welfare in the scientific community, there is a necessity for a reliable, non-invasive identification techniques for frogs, such as photographic identification (Lettink & Armstrong 2003; Bardier et al. 2020).

2.2.3.1. Photographic identification as alternative to marking techniques.

The use of photographs or sketching a frog’s skin colour or pattern to record its identification has been used for decades, with techniques being refined (McHarry et al. 2018; Bardier et al. 2020). The introduction and application of photo-identification and software recognition methods offers an alternative method for mark-recapture studies, with photographs considered as a ‘visual capture’ of an individual frogs identification (Lettink & Armstrong 2003).

Pattern recognition software is continually being improved and popularity in the use of identifying phenotypically unique individuals, having been utilised with a range of cetaceans and reptiles (Gomez-Salazar et al. 2011; Rocha et al. 2013; Amelon et al. 2017). The colouration and markings of each individual animal are mapped and added to a photograph database. These databases can then be used to reliably identify recaptured animals according to their individual markings (Bardier et al. 2020).

The development of freely available programs such as I^{3}S, Wild-ID, and HotSpotter allow for software recognition to be widely used in wildlife research on species such as tigers, dolphins, geckos, and frogs (Karanth 1995; Gomez-Salazar et al. 2011; McClintock et al. 2013; Rocha et al. 2013; Bardier et al. 2020). These programs are utilised to map identifying features for individual animals and will automatically recognise individuals based on information specific to that individual, stored in the database (Gomez-Salazar et al. 2011; Bardier et al. 2020). Moreover, photographic identification of frogs can identify recaptures with greater accuracy than traditional marking techniques (Bardier et al. 2020). The utilisation of this software allows for reliable, low-cost research on animal's populations (Bardier et al. 2020).

The performance of software-assisted photo-identification (Wild-ID and APHIS) was tested on the pacific horned frog (*Ceratophyrys stolzmanni*) against human visual recognition (Bardier et al. 2020). Whilst constrained to a singular species, the study analysed the rate at which photos were matched and the accuracy of human versus software identification (Bardier et al. 2020). The study found that software-assisted identification was a more time efficient, non-invasive method for photographic identification for both juvenile and adults of the sampled species, in contrast to human identification (Bardier et al. 2020).

The efficacy of photographic identification was further evaluated in a mark-recapture study using I^3S software and VIE tags (McHarry et al. 2018). The study found that without software assistance, 76 % of recaptured tadpoles were identified in contrast to 98 % recapture identification using the I^3S software (McHarry et al. 2018). Whilst accurate and time efficient, limitations in software identification arise when sampling species with minimal distinctive markings (such as *Litoria caerulea*) (McHarry et al. 2018). Such limitations can result in species exclusion from the mark-recapture study and unreliable data on frog species abundance and diversity (McHarry et al. 2018).

With further technological development, photographic, software assisted identification of frog species offers a potential method of individual identification of frogs which will improve the efficacy of mark-recapture studies, further enhancing frog survey methods (Pellitteri-Rosa et al. 2010; Andreotti et al. 2018).

2.2.4. Bioacoustics surveys

Bioacoustics is an interdisciplinary field, combining biological and acoustic science, in which devices are used *in situ* to record, store, and analyse recordings of animal communications (Mcloughlin et al. 2019; Penar et al. 2020). From a vast majority of known frog species, most adult male frogs (and in some species, adult female frogs) during breeding, are well documented to produce species-specific vocalizations to advertise their position to potential mates (Emerson & Boyd 1999; Wells 2007). These vocalisations provide a source of information about the ecological system in terms of species richness and diversity and aspects of animal ecology and behaviour (Obrist et al. 2010; Philippe et al. 2017; Penar et al. 2020). Primarily, audio surveys provide data on the relative abundance of calling frogs, species composition, as well as breeding phenology and microhabitat use (Scott et al. 1994b).

Frog monitoring programs often rely on surveys of male vocalizations to detect the presence of adult male frogs over time, often based on calling intensity rather than numbers of vocalizing males, due to difficult in determining the latter (Čeirāns et al. 2020). The metrics used for calling intensity are the frog calling index (CI), call latency (between start of the survey and the first detected call), call counts from recordings and the presence-absence of a particular species (Čeirāns et al. 2020). These metrics provide data that is weakly related to true population sizes, and that more accurate population data can be obtained by male count data rather than call intensity data (Čeirāns et al. 2020). However, the use of bioacoustics as a method for estimating minimum adult frog density (MAFD) is useful in estimating population densities and are increasingly accurate at low to medium population densities (Čeirāns et al. 2020).

The underlying assumptions for audio surveys are (1) researchers have knowledge to identify species-specific vocalisations and (2) vocalisations equate to breeding season and that only males are vocalising (Scott et al. 1994b). It is also accepted that audio surveys cannot be utilised to determine distributions or abundances of females, juveniles, or silent males (Scott et al. 1994b).

Despite being an emerging technology in ecological science, bioacoustics technology has quickly established itself as a useful tool in population monitoring for an array of species (Zwart et al. 2014; Mcloughlin et al. 2019). Research on the efficacy of the method has demonstrated the technology sufficient in obtaining data of species richness and diversity (Zwart et al. 2014; Čeirāns et al. 2020). Technological advancements are continually improving bioacoustics, making it more cost effective and accessible as a survey method (Obrist et al. 2010). Furthermore, the implication of bioacoustics technology is particularly valuable in regions that are unsuited for pitfall traps (e.g. marshlands, swamplands) or inaccessible for visual surveys (e.g. tropical rainforests, where there is high species richness and frogs inhabit many microhabitats at many strata (Scott et al. 1994b). Furthermore, the use of bioacoustics as a survey method reduces that need for human presences *in* *situ*, with the exception of setting up and removing the equipment, and allows for data capture of rare, or otherwise hard to find species (Penar et al. 2020).

A citizen science project, 'FrogID' is a recent project launched in November 2017 by the Australian Museum to facilitate the collection of frog biodiversity data across Australia (Rowley et al. 2019). The project utilises smartphone technology to allow citizens to submit recording of frogs vocalising and geo-referenced information on the respective habitat, into a national database (Rowley et al. 2019). These audio submissions are then expert-validated, providing broad-scale frog biodiversity data, including rare and threatened species, allowing for inferences to be made on species richness in each area (Rowley et al. 2019). In the first year, FrogID obtained more than 66,000 recording of frog vocalisations, providing revised knowledge, particularly on the distribution of many frog species (Rowley et al. 2019). FrogID was developed primarily to collect presence-only data, many frog recordings include multiple species (averaging 2.2 species per recording) (Rowley et al. 2019). This allows for inferences can be made from a collection of submissions in relation to missing species, correlations with weather conditions (e.g. temperature and rainfall), and time of day (Rowley et al. 2019). Furthermore, the use of bioacoustics technology in this project has allowed for public engagement and education, with ongoing, positive influences towards frog conservation(Rowley et al. 2019).

In contrast, Waddle et al. (2009) tested the efficacy of automated vocalisation recognition software (Song Scope^{TM}) in a frog survey. Across 200 hours of recordings, misidentification occurred between 2.7 % and 15.8 % per species and failed to detect frog calls in 45 % to 51 %of recordings (Waddle et al. 2009). The limitations in software advancement provides a trade-off between error type in vocalisation recognition, which can be minimally offset by changes to quality settings within the software (Waddle et al. 2009). Quality settings for minimum quality minimised false positives (misidentification of true calls) but produced false negatives (detection failures). This trade-off emphasizes the importance of cautious study design for monitoring programs and the need for software training and improvement (Waddle et al. 2009). However, overcoming these limitations, bioacoustics surveys have the potential to revolutionise research in frog population monitoring, as camera traps have in broader ecological surveys.

2.2.5. Camera traps

Becoming popular in the late nineteenth century, wildlife photography greatly inspired the development of photographic equipment specifically for wildlife monitoring and wildlife camera traps have revolutionised the world of wildlife monitoring (O'Brien 2011; Swann et al. 2011). As a remotely activated camera with a motion or infrared sensor, camera traps are usedto obtain data on a wide array of bird, mammal, and reptile species across an ecosystem (O'Brien 2011; Swann et al. 2011). Whilst recording the presence of species within anecosystem, camera traps provide data on the distribution, abundance, and behaviour of species (O'Brien 2011; Swann et al. 2011). Furthermore, this non-invasive, camera surveying technique is highly regarded due to the elimination of animal handling and welfare concerns (Burton et al. 2015). Additionally, camera traps offer a labour-cost effective survey method that reduces field work requirements (Palmeirim et al. 2019). The adoption of camera traps inecological surveys has allowed for improvements in the reliability in wildlife population data (Burton et al. 2015). Furthermore, the development of smaller, “field-savvy” technology has allowed for the ability to capture behaviours of undisturbed animals *in situ*, which was not previously possible (O'Brien 2011).

Camera traps predominantly rely on mobile species, such as birds or mammals, crossing the detection zone, triggering the camera to record images (Palmeirim et al. 2019). Camera traps are typically most effective when an animal with a body temperature 2.7°C warmer than the surrounding environment passes through the detection zone, allowing the camera to detect the temperature differences (Hobbs & Brehme 2017). The body temperature of poikilothermic animals, such as frogs, is not different enough from the ambient temperature, to trigger the camera to record images (Hobbs & Brehme 2017). This leads to an unreliable detection rate of frogs, and therefore an underrepresentation of frogs in camera trap surveys (Burton et al. 2015; Palmeirim et al. 2019). The utilization of the Hobbs Active Light Trigger (HALT), a 3 mm near-infrared beam was developed to help detect small animals, such as frogs during these surveys, (Hobbs & Brehme 2017). Due to patents pending on components of the HALT system, it is not yet used in ecological surveys, however it is anticipated to be available to researchers in 2018, improving the detection rate of frogs in ecological surveys (Hobbs & Brehme 2017).

Increasing the efficiency of detection of wildlife species is an ongoing problem, however the use of multi-camera arrays offers a potential solution (O'Connor et al. 2017). Factors influencing wildlife detectability in camera trap surveys include the size of camera arrays (number of cameras) deployed and length of deployment (O'Connor et al. 2017). These factors were evaluated by sampling four wildlife species (white-tailed deer, *Odocoileus virginianus,* bobcat, *Lynx rufus,* racoon, *Procyon loto*, and the Virginia opossum, *Didelphis virginiana*) of varying body size (and therefore assumed different detectability) (O'Connor et al. 2017). An increase from one to a two-camera array increased detection probability 80 % across all species (O'Connor et al. 2017). While this is promising, equipment costs remain a major factor limiting the implementation of multi-array camera surveys (O'Connor et al. 2017).

Furthermore, limitations in developing remote-access camera equipment impacts the long-term viability of camera surveys, with labour needed to maintain cameras (e.g. battery life, memory cards) over long periods, and identify animals in the recorded images (O'Brien 2011; O'Connor et al. 2017). Despite these limitations, camera traps offer a promising method in wildlife monitoring and surveying, however, they do not currently provide adequate data to replace all other sampling methods (Table 1) (O'Connor et al. 2017).

2.3. Improving frog capture rates

Whilst the traditional survey methods all possess their own advantages and limitations (Table 1), there is a need for improvements to these methods to reduce their limitations. Improvements to traditional surveying methods could include: the use of artificial light as a natural attractant of frog prey species, and the development of a method to survey arboreal frog species.

2.3.1. Artificial light

A method to improve pitfall trapping for frog surveys, is through utilising an artificial light source to attract prey items (e.g. moths) to the trap. In turn, this light source (and therefore an attractant of food) may attract frogs to the trap location, as frogs are natural predators of arthropods (Tyler 1999), and thus improve trapping success and the accuracy and reliability of the subsequent estimations of frog populations.

The use of artificial light has been documented to increase the presence of photo-taxic insects (e.g. moths) to the light source (Kim et al. 2019). The use of light traps has been used as an effective pest control method for a variety of arthropod species (Kim et al. 2019). Furthermore, the utilisation of artificial light during invertebrate surveys has been effective in increasing capture rates when sampling arthropod species (Hébert et al. 2000; Liu et al. 2007). Across multiple habitat types, the installation of a light source over a pitfall trap resulted in a significant increase of arthropods of different species captured in contrast to unlit arthropod traps (Hébertet al. 2000; Liu et al. 2007).

Across these studies, multiple light sources have been used, dependent on the requirements of each study (Hernandez 1999; Hébert et al. 2000; Ramírez-Hernández et al. 2018). Common light sources used in the literature include: an automatic light trap with a 15 W Blacklight-blue light (Axmacher & Fiedler 2004), a Luminoc® insect light, with a 1.8 W miniature fluorescent tube (Hébert et al. 2000), and a 8 W ultra-violet fluorescent tube (Heap 1988), among others.

Furthermore, a South Carolina study, using aquatic funnel traps, was successful at increasing larval amphibian capture rates through the use of glowsticks (Bennett et al. 2012).

While artificial light has been used for sampling tadpoles (Bennett et al. 2012) and other aquatic species (e.g. fish); (Hernandez 1999), extensive use of a light trap targeting juvenile and adult frog species is not evident in the literature currently available. While frogs primarily exhibit nocturnal behaviours, often due to hot and dry environmental conditions, activity levels are not dictated by photoperiod (Weil & Crews 2009). Artificial light is a substantial attractant of insects to pitfall traps, therefore, it can be reasonably expected that by using this approach, the diversity and abundance of frogs captured in pitfall traps will increase due to increased prey availability (Kim et al. 2019). This increase in frogs captured will be beneficial in multiple ways. Firstly, the increase in trap success, via use of light, will provide more data than passive pitfall trapping surveys. Secondly, the additional frogs collected, could result in shorter surveys, which in turn reduces the impacts pitfall trapping has upon the habitat (e.g. human disturbance, animals stressed by being trapped and handled). Thirdly, the use of light could potentially attract frog species, (e.g. arboreal species) that would otherwise not be caught in a pitfall trap under other circumstances. Further studies on insect attractants (and therefore frog attractants) are recommended to increase our understanding of improved methods to increase frog capture rates for population surveys.

While the use of artificial light may improve the capture rate of standard pitfall traps, many frog species remain under-surveyed in pitfall trapping surveys. By inadvertently excluding arboreal frog species from the species surveyed, population data remains inaccurate and is not representative of the true frog population in the location being surveyed. The development of new sampling methods targeting arboreal species will greatly improve the reliability of data collection of this group of frog species.

2.4. New method of tree frog surveys

In many locations, a lack of suitable habitat, e.g. tree hollows as refugia for tree frogs may provide the opportunity to introduce artificial hollows to attract tree frogs, such that they can then be found and included in surveys. Despite being classified as tree frogs, some arboreal frog species (e.g. *L. rubella*) are often found recorded vocalising in, or within proximity to water bodies during the breeding season (Sanders 2021). During periods of low activity, (e.g. during the day) frogs' shelter under tree bark, or in low vegetation structures (Anstis 2013; Sanders 2021). The implementation of artificial refugia into habitats where arboreal frog species are found, can improve the chances of these species being captured, and therefore included in population estimates. The use of artificial refugia as a sampling technique was firstpublished by Goin and Goin (1957). The study consisted of utilising empty tin cans placed fourfeet above the ground, and the tree frogs *Hyla squirrella* and *H. cinera,* utilised these as refugia (Goin 1958). This approach provides a simple method of monitoring arboreal species, otherwise under-surveyed by traditional methods (Goin & Goin 1957). Further development ofthe use of artificial refugia was reported by Drewry (1970) who studied frogs in the Puerto Rican rainforest utilising closed bamboo nodes as refugia. The use of artificial refugia as a sampling method has been successful in frog surveys across a variety of locations (Appendix A) (Glorioso & Waddle 2014). However, there is little published evidence that this method has used for surveying native Australian frogs, despite tree frogs composing over 30 % of all Australian frog species (Anstis 2013). Furthermore, other studies have used PVC pipe as refugia material, with fewer studies available to compare the effect refugia material has on theoccurrence of refugia use by frogs (Appendix B) (Glorioso & Waddle 2014).

2.4.1. PVC pipe and bamboo refugia

With widespread availability and relatively low cost (albeit location dependent), both PVC pipeand bamboo culm provide the most used materials for artificial refugia for frog sampling (Appendix B) (Martins et al. 2009; Glorioso & Waddle 2014).

Furthermore, PVC pipe has been found to be an efficient and cost-effective sampling method, with reduced installation labour and eliminating trap mortality in comparison to pitfall traps (Boughton et al. 2000). Additionally, both bamboo culm and PVC pipe traps have been used in studies of frog site tenacity and territoriality (Boughton et al. 2000). The use of PVC pipe and bamboo culm as artificial refugia provide a useful method of sampling tree frog populations, however, the efficacy of this method depends on the fidelity of individuals to their usual shelter (Windes 2010). Some species of frogs in America are known to exhibit extreme site fidelity, preferring to return to the same location each time they leave to breed or hunt for food (Windes 2010). Therefore, the use of artificial refugia, placed in the habitat where these frogs are found, is highly likely, once established.

The use of PVC pipe versus bamboo culm, by frogs may vary dependent on species, location of trap (e.g. on ground versus elevated above ground level), and size of the refugia, suggesting that frogs discriminate between potential refugia sites (Boughton et al. 2000). Further selectivity in refugia material may be due to PVC pipe not imitating the natural environment (Boughton et al. 2000). In comparison, bamboo refugia offers a more inconspicuous choice of shelter; however, further studies are required to accurately determine factors influencing refugia choice (Boughton et al. 2000).

By utilising the natural behaviours of tree frogs seeking shelter in darkened cavities, the use of PVC pipe and bamboo as artificial refugia is highly effective in sampling tree frog populations in other parts of the world (Glorioso & Waddle 2014). However, the lack of published information on the use of artificial refugia as a frog survey method in Australia suggests, that there has been few, if any, studies incorporating it as a survey method, leaving a gap in the scientific knowledge of Australian frog populations. Furthermore, survey methodologies widely used across Australia, are not designed to account for the wide diversity of arboreal frogs, resulting in capture bias, and therefore limiting the accuracy of population estimates derived from these surveys (Hoffmann et al. 2009). With 93 % of all Australian frog species endemic to Australia, the implementation of a new survey method is vital in obtaining data on under surveyed species (Anstis 2013). The introduction of this sampling method may be vital in improving the monitoring and conservation of native frog populations.

2.5. Conclusions

With increased pressure on frog populations worldwide, it is more important than ever to develop and improve methods to survey populations (Allentoft & O'Brien 2010; Cogger 2018). Traditional frog survey methods, such as pitfall traps, visual searches, bioacoustics and camera traps, all provide various benefits to assessing frog diversity and abundance (Scott et al. 1994b). However, each of these methods have their unique limitations, leaving gaps in the data provided. Relatively new survey methods, i.e. camera traps and bioacoustics surveys are providing ways to fill in some of the gaps of earlier survey methodologies.

Pitfall traps are a traditional method used in many ecological studies to obtain data on species diversity and abundance (Work et al. 2002; Woodcock 2005; Schirmel et al. 2010; Ali et al. 2018; Palmeirim et al. 2019). Even so, the method significantly under-represents many frog species within the ecosystem studied. The implementation of artificial light in conjunction witha pitfall trap offers the potential of significantly increasing the capture success of frogs in pitfall surveys. Furthermore, the implementation of new methods to survey tree frog species using artificial refugia is highly recommended in Australia. Testing the efficacy of the method with Australian species, will not only increase the amount of data available for the survey methodology, but open new research opportunities, given the limitations to our currentunderstanding.

To effectively help conserve frog species around the world, improving and developing surveying methods suited to a range of habitats is increasingly important. Further development on traditional survey methods is just as vital as the development of newer, novel approaches for frog surveys being developed. By improving capture success, and therefore survey methodology, population data can be obtained for many species otherwise overlooked during traditional surveying methods, and therefore better inform conservation projects for native frogspecies.

Chapter 3: Materials and methods

3.1. Study site and study period

The study was conducted on the Old Hidden Vale Property, 617 Grandchester Mount Mort Road, Grandchester, QLD, 4340, (Figure 5). Located within Ipswich local government area (LGA), approximately 80 km from Brisbane CBD, the property covers 12,000 hectares of native bushland, under ownership of the Turner Family Foundation. The two sites studied on this property were the Billabong and the Ridge (Figure 6) each with a matrix of 100 pitfall traps, each trap approximately 15-20 m apart. The Billabong site was characterised by a series of three interconnecting dams, with 100[1] pitfall traps located across various habitats across the site, ranging from the edge of the water bodies to a forested incline (Figure 7). Similarly, the Ridge site had 40 pitfall traps located on a slope as part of a forested ridge, and the remaining 60 pitfalls were located on a grassy plateau below the Ridge (Figure 8), dominated by blady grass (*Imperata cylindrica*).

Pitfall traps were open at the Billabong for 10 consecutive days from 5th to 15th January 2021, and pitfall traps were open at the Ridge from 18th to 28th January 2021.

[1] Five pitfall traps at the Billabong site were previously destroyed during land works at the site prior to field work and excess flooding. Another pitfall trap was removed from the sample due to excessive flooding, resulting in a total of 94 traps sampled at the Billabong.

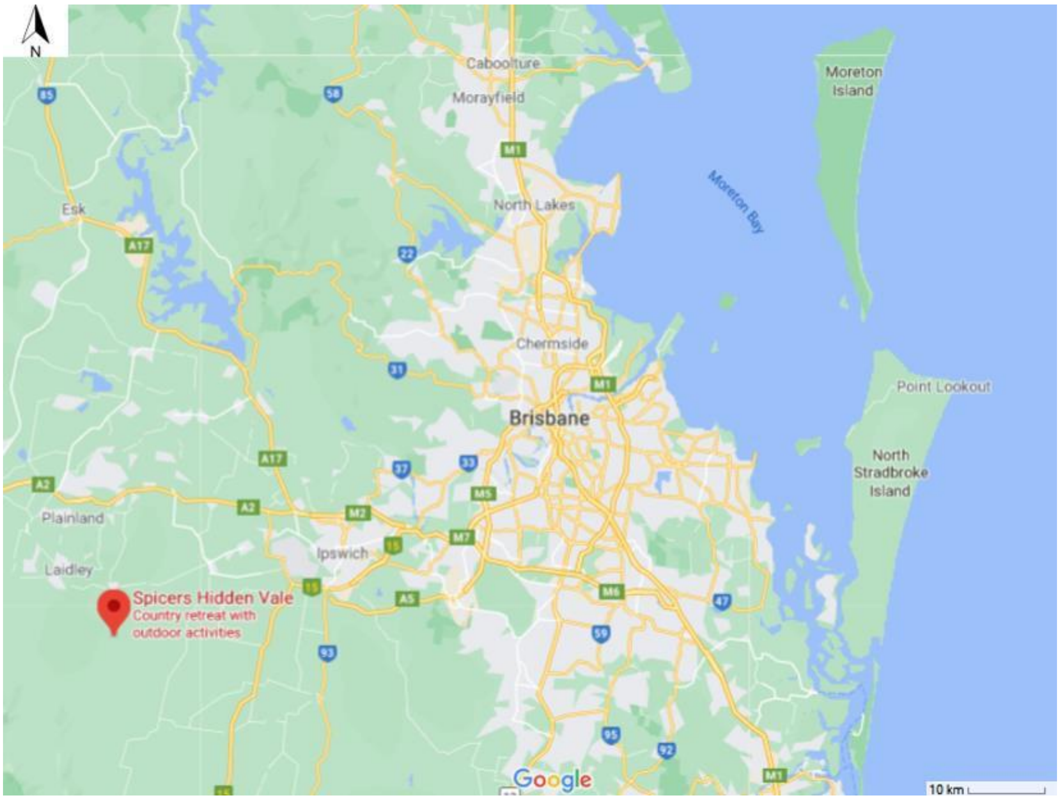

Figure 5. Location of Hidden Vale Property (Spicers Hidden Vale Retreat) (Google 2021).

Figure 6. Location of the two trapping sites and the Hidden Vale Wildlife Centre on the Old Hidden Vale property (Queensland Government Department of Resources 2021).

3.2. Summary of experimental design

The pitfall traps at each site were categorised into three habitat types, nested within each trapping site, and categorised by the dominant microhabitat type surrounding the pitfall traps. The nested habitat types at the Billabong were Dam (pitfall traps located <10 m from the edge of the water body), Grass (characterised by open wooded forest with prevalence of native grass species), and Hill (a relatively densely wooded area, on an inclined portion of the site). These habitat types comprised of 30, 43 and 21 pitfall traps respectively (Figure 7). The Ridge habitat types were Blady grass, Native pasture, and Slope with 60, 10 and 30 pitfall traps respectively (Figure 8). Unequal habitat classification of pitfall traps was utilised to represent the dominant habitat types of each trapping site.

30 pairs of PVC pipe and bamboo culm refugia, and 30 solar lights, were allocated to pitfall traps in each nested habitat type at each trapping site (Table 4). The pitfall traps allocated pipe refugia and solar lights were selected to ensure minimal light interference between allocated locations. A further 30 pairs of PVC pipe and bamboo pipe refugia were attached to trees around each trapping site where frogs were expected to be found, predicted by predominant microhabitat (Figure 9).

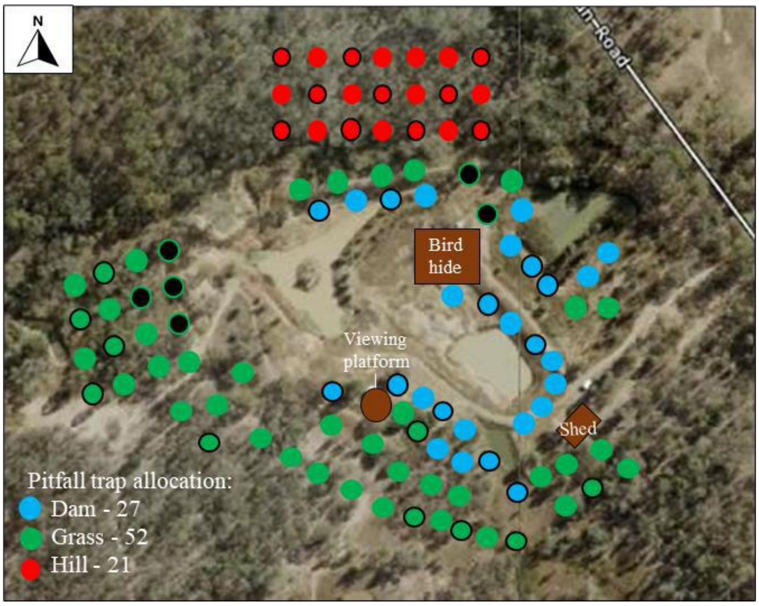

Figure 7. Number of pitfall traps allocated to each habitat type at the Billabong site. Locations outlined in black indicate pitfall traps allocated lights, PVC pipes and bamboo refugia and camera traps. Locations marked black indicate pitfall traps that were not used in the survey.

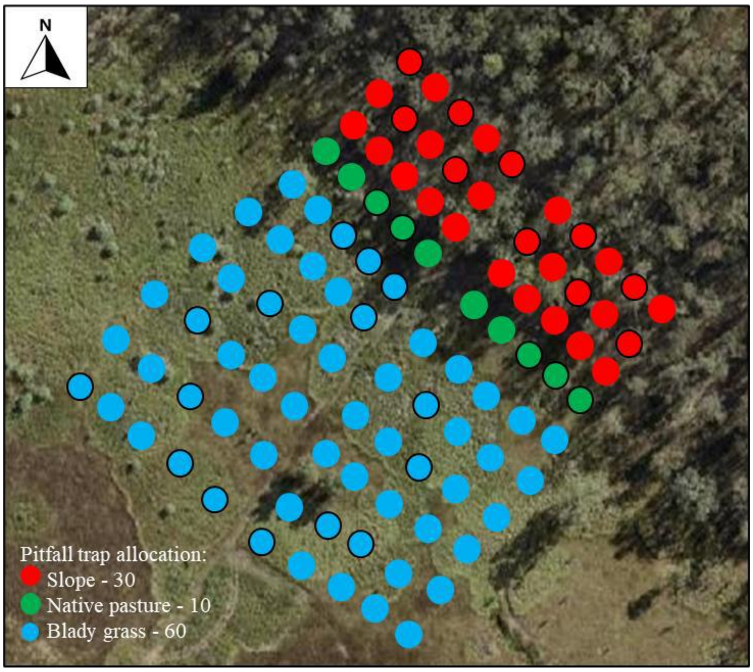

Figure 8. Number of pitfall traps allocated to each habitat type at the Ridge site. Locationsoutlined in black indicate pitfall traps allocated lights, PVC pipes and bamboo refugia and camera traps.

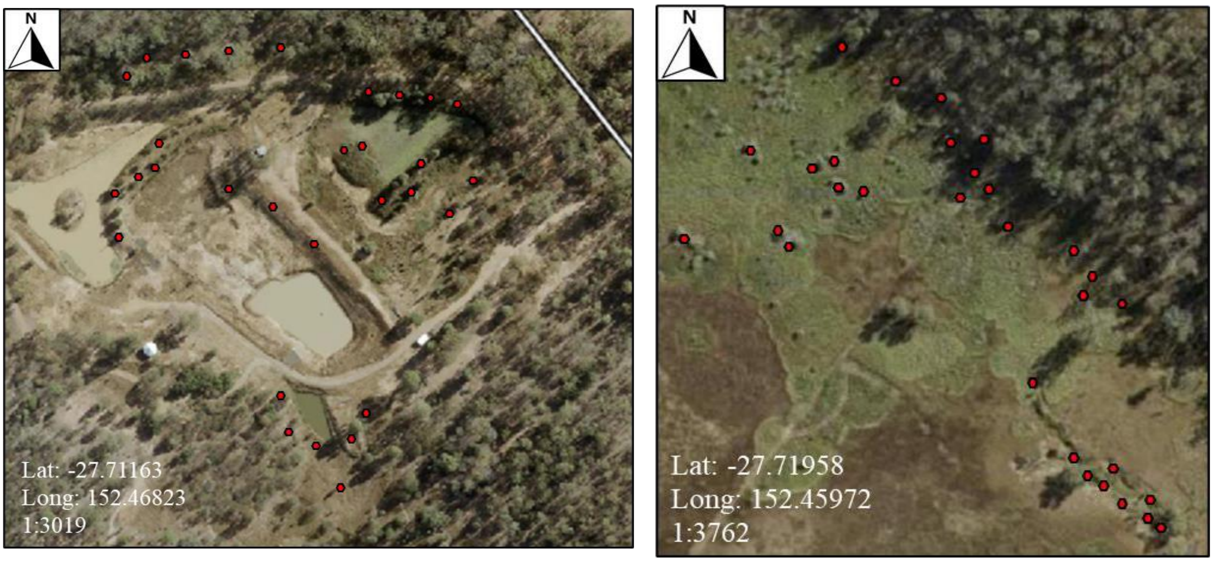

Figure 9. Locations of tree pipe (PVC and bamboo) refugia located at the Billabong (left) and the Ridge (right) trapping sites.

Table 3. Summary of trap locations across both trapping sites.

Site: Billabong	**Nested habitat type**			**Total number**
Trap type	**Dam**	**Native pasture**	**Hill**	**of traps**
Pitfall traps	20	33	11	64
Pit-light traps	10	10	10	30
Total pitfalls	**30**	**43**	**21**	**94**
Pipe refugia*	10	10	10	30
Tree refugia				30
Total artificial refugia				**60**
Camera traps				**30**
Site: Ridge	**Nested habitat type**			**Total number**
Trap type	**Blady grass**	**Native pasture**	**Slope**	**of traps**
Pitfall traps - no light	45	5	20	70
Pit-light traps	15	5	10	30
Total pitfalls	**60**	**10**	**30**	**100**
Pipe refugia*	15	5	10	30
Tree refugia				30
Total artificial refugia				**60**
Camera traps				**30**

*Pipe refugia as allocated to pitfall trap locations

Pitfall traps were active for 10 consecutive days, checked twice daily (morning and late afternoon) (n = 2,100). Lights were active every second night (n = 300). Pipe refugia (pitfall trap and tree allocated), were continuously active during this period, checked on days 4, 7 and10, (n = 4,800), to reduce the possibility of negatively influencing animal relationships with the refugia.

Table 4. Summary of trapping events.

Treatment	Number of traps	Number of sites	Time periods		Total number of trapping events
			Day	Night	
Light	30	2	-	5	300
No Light	70*	2	10	5	2,100
Camera	30	2	10	10	1,200[a]
PVC pipe	60	2	10	10	2,400
Bamboo culm	60	2	10	10	2,400
Total					8,400
Adjusted total*					8,310*

*Removal of 6 pitfall traps at the Billabong site resulting in an adjusted sample size (n = 64) for pitfall traps (no light); [a] The camera traps were active continuously across trapping period.

3.2.1. Pitfall traps

Pitfall traps (n = 100) made from 150 mm diameter by 500 mm lengths of PVC pipe were usedin each location. Each pitfall trap contained a plastic shelter (half of a 15 cm diameter polystyrene dish) and a piece of Styrofoam (approximately 80 cm^2) to act as a floatation devicein the event the pitfall trap was flooded. The pitfall traps were opened at the beginning of the 10-day study period and left open for the duration of that period. The pitfall traps were checked twice daily, early morning and late afternoon (to determine nocturnal and diurnal species), and any trapped vertebrates were identified and morphometric and other data about them recorded(as discussed in section 3.2.).

3.2.2. Lights

The lights were turned on, on alternate nights to assess the influence of light on individual trap success with the unlit traps as a control. At the Billabong, 10 pitfall traps in each of the three habitat types were selected and fitted with a 'Solar Magic' garden light (solar powered, 30 lumen, white LED light). The lights were tested prior to the commencement of the study and at night, when at full charge, produced light for 7-8 hours for approximately two weeks, givenadequate light to recharge. Lights were installed 1.2 m above the ground level, to illuminate the area in and around the pitfall trap (Figure 10). The lights were distributed across the habitatsto reflect the abundance of each habitat type (Table 3), with 15 lights in the blady grass habitat, 10 lights in the slope habitat, and 5 lights allocated to the native pasture habitat.

3.2.3. Pipe refugia

PVC pipes (n = 60) were built from 300 mm lengths of 40 mm diameter PVC pipe, with a capfitted at each end (not glued). Each pipe length had a 30 mm diameter hole cut in the middle section. Another 60 refugia were made from bamboo culm, each approximately 300 mm in length, approximately 4-6 cm in internal diameter, with a 30 mm diameter hole cut in the middle section (Figure 11). Pairs of one PVC and one bamboo pipe were attached to 30-star pickets (with the pipes located such that they were 85 cm from ground level to the middle of the entrance hole of the refugia) using garden twist ties at each pitfall trap location that was allocated a light and camera (Figure 10). Another 30 pairs of pipe refugia were attached to trees(such that they were 85 cm from ground level to the middle of the entrance hole), using gardentwist ties. These pipes were installed around each trapping site where frogs were expected to be found, such as along the edge of each dam at the Billabong, and along the creek line and amongst long grass at the Ridge site (Figure 9). Tree species allocated pipe traps included *Eucalyptus maculata* and *E. tessellaris, Alphitonia excelsa*, and *Acacia falcata.*

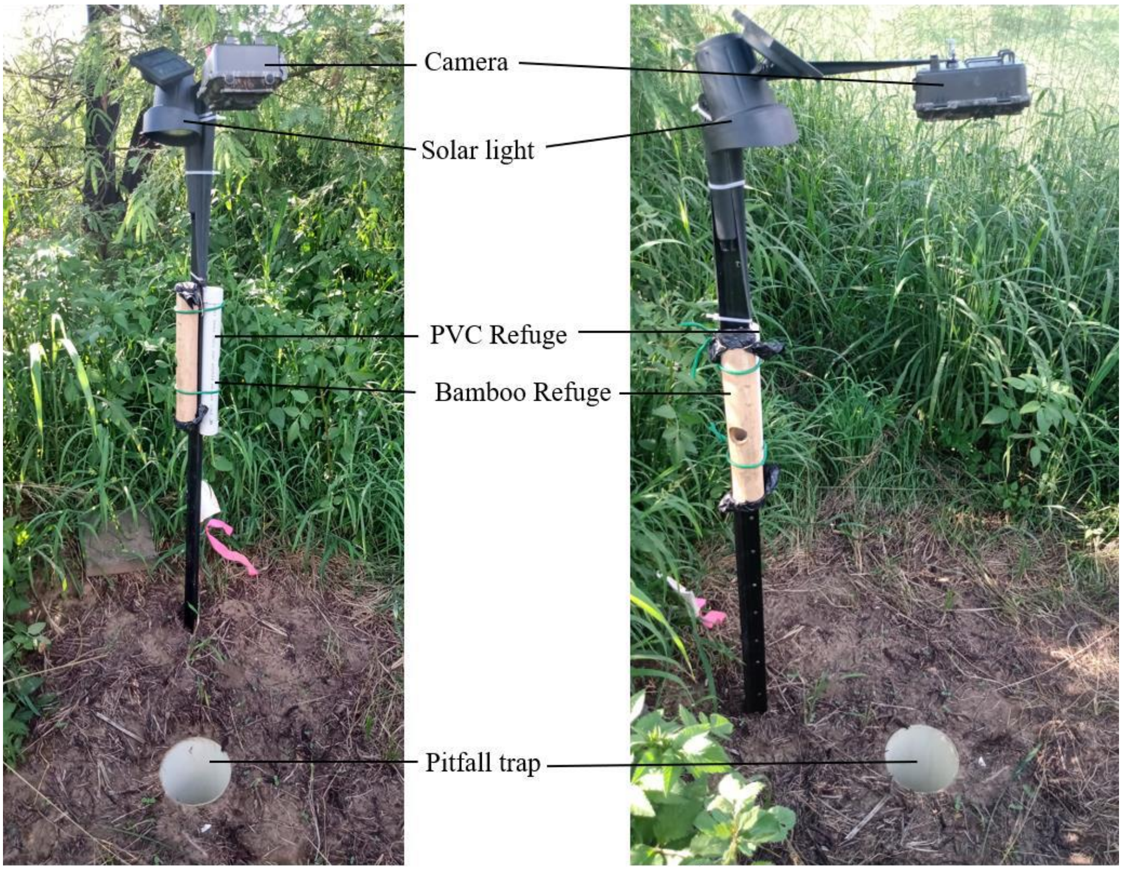

Figure 10. Set up of all equipment at each pitfall trap allocated a camera, light and refugia pipes. Cameras were installed vertically, approximately 1.2 m above pitfall traps, ensuring thepitfall trap was in the field of view, with minimal interference from surrounding vegetation. Solar powered LED lights were installed 1.2 m from ground level, downward facing, illuminating the pitfall trap. PVC and bamboo pipes were installed, entrances lateral facing, 85cm from ground level.

Figure 11. PVC and bamboo pipe refugia for arboreal frog species. Black plastic squares attached to bamboo culm using rubber bands to close the pipe with a removeable covering. Arboreal frogs could enter the pipes via the 3 cm diameter hole in the middle section.

3.2.4. Camera traps

Camera traps (20 Swift Enduro and 10 Reconyx Hyperfire 2 cameras) were installed above pitfall traps that were allocated a solar light (Figure 10). At the Billabong site, 10 camera traps were allocated to each microhabitat type. At the Ridge site, the camera traps were allocated to the trap selected for a light source (10 cameras in the hill habitat, 5 to the native pasture habitatand 15 in the blady grass habitat) to account for the larger number of pitfall traps in the blady grass habitat due to blady grass being the more dominant habitat type at this site. The cameras were vertically set, approximately 1.25 m from ground level, to capture rapid-fire images and short videos, and to observe animal interactions with pitfall traps.

3.3. Processing of trapped animals

Each pitfall trap was checked in the early morning and late afternoon every day. Each trap wasfirst checked by gently lifting the plastic shelter with a stick. Traps were cleaned of any debris or leaf litter and left open. For traps containing animals, all individuals were identified, measurements recorded, and photographed before release as per the following sections. Data on day, date, time (AM or PM), trap number, animal weight (if applicable), and body measurements, as per Duellman (1970), were recorded for each trap visit. Processing procedures for mammal and reptile species are described in Appendix C.

3.3.1. Processing of frogs in pitfall traps

Frogs were removed from the pitfall and handled using clean latex gloves for each animal. Animals, depending on their size, were gently placed in either a 90 or 140 mm diameter clean petri dish on a laminated sheet with a 1 mm grid and photographed as per the following section. Seventeen body measurements were recorded for all individuals with a snout-vent length of greater than 3 cm as per Duellman (1970) (Figure 12). Individuals shorter than 3 cm snout-vent length were recorded as too small to measure in the field and were later measured using the grid in the photographs. Once processed, frogs were wet using moisture from the surrounding vegetation (either dew or rain drops) and released in a sheltered area near the pitfall trap from where they were captured. After processing animals, a photograph was taken of the trap number to record where they had been trapped.

3.3.2. Processing frogs in pipe refugia

PVC and bamboo pipes were checked every three days in the morning trapping session. Pipe refugia allocated at a pitfall trap were checked alongside the pitfalls, and the tree allocated refugia were checked after all pitfalls were checked and all animals released. PVC and bamboo pipes were checked by removing one end of the pipe covering to see if there was anything inside. PVC and bamboo pipes that did not contain any animals had the end covering reinstalled and returned to their original position. Whilst wearing clean disposable gloves, traps that contained animals were removed from their position on the star picket or tree and the animal removed from the trap with by gently jolting the animal from its position within the refugia. All frogs removed from PVC and bamboo refugia were processed and photographed.

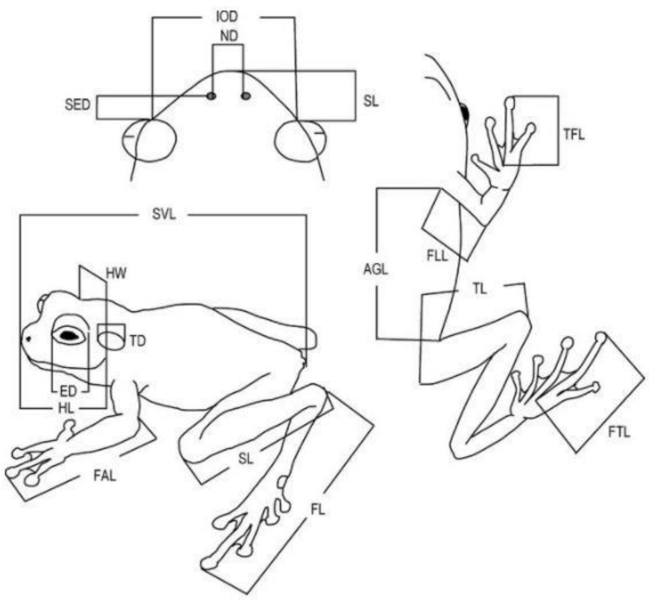

Figure 12. Morphometric measurements taken from frogs (mm): snout–vent length (SVL); head length (HL); head width (HW); snout length (SL); internasal distance (ND); interorbital distance (IOD); eye diameter (ED); snout–eye distance (SED); tympanum diameter (TD); forelimb length (FLL); forearm length (FAL); third finger length (TFL); axilla–groin length (AGL); thigh length (TL); shank length (SL); foot length (FL); fourth toe length (FTL) (Duellman 1970).

3.3.3. Microchipping cane toads

After being processed as per the above section, trapped cane toads that had a snout-vent length (SVL), greater than 80 mm were microchipped using 1.8 x 8 mm ISO FDX-B Mini Microchips. The toads were held firmly by the hind legs in the non-dominant hand, and the microchip was inserted in the left caudal lymph sac (Australian Veterinary Association Ltd. 2016). A SureSense Microchip scanner was used to confirm functionality of the microchip, and all details of the chip recorded on the respective data sheet. The toad was placed in a zip-lock bag for 1-2 minutes for observation, to ensure the microchip was not expelled and was then released near its site of capture.

3.3.4. Photographing animals

Once body measurements were recoded, the animal was photographed to record dorsal and ventral markings. The animals contained within a petri dish were placed over a clear laminated piece of 1 mm grid paper on a flat surface. The petri dish was closed to minimise movement ofthe animal and reduce the chance of it escaping. Animals were photographed using either a Panasonic Lumix DMC-ZS110, a Motorola One Macro phone or a Galaxy S10 mobile phone camera. Lateral profiles of animals were taken if the species was not able to be identified in the field, capturing identifying features for later identification.

3.3.5. Disinfecting equipment

When moving between habitat types and trapping sites, all petri dishes were sprayed and disinfected following the hygiene protocol for frog handling (Queensland Government n.d.). Petri dishes were thoroughly sprayed with a 1 % bleach disinfectant solution and left to soak for 2-5 minutes. Petri dishes were then rinsed thoroughly with warm water and dried with papertowel.

3.4. Software-assisted recognition

For each individual frog photographed, the best dorsal image obtained (regarding image sharpness, focus and position of the animal etc.) was cropped from snout to vent, to showcase the region of interest for photo identification purposes. Software assisted photo identification was performed on all image in the database (n = 22 images). The I^{3}S Pattern software was chosen as it is an easy to use, freely available and open-source program that has been applied to frog species previously (Reijns & den Hartog 2020). The software uses a key point extractionalgorithm which automatically extracts all key points within the region of interest (Reijns & den Hartog 2020). The region of interest was determined by setting the same three reference points in all photos (snout tip, left and right inguinal regions) and functions to correct for variation in viewing angle, rotation, and scaling

(Reijns & den Hartog 2020). Key points within the region of interest are automatically extracted (n = 35 points per image) and annotated where necessary to ensure all distinguishable patterns are selected and saved as the "fingerprint" of the individual. These fingerprints are compared to those of other images and the software produces a list of similar patterned individuals each scored by similarity (Reijns & den Hartog 2020).

3.5. Data analyses

Influence of habitat and time-period on trapping success was calculated with a two-way ANOVA (with a 0.95 confidence level) using the emmeans library package in R studio (version 4.0.5). This analysis compared the number of captures (scaled to 100 trap 'events' for comparability) in each habitat type at the Billabong site, with the time-period of capture (morning or afternoon). This process was then repeated for data collected at the Ridge site. Due to data from each site not being statistically comparable, analyses directly comparing trapping sites was not conducted.

The number of captures were scaled per 100 trap events using the following:

Trapping effort = number of traps x number of trapping events (days and nights).

Average per 100 trap events = number of frogs captured / trapping effort x 100.

A secondary analysis was conducted to evaluate the influence of artificial light on pitfall trap capture rate. Paired t-tests for sample means were used to identify the significance of artificial light on capture rates, using Microsoft Excel (version 2108).

Chapter 4: Results

4.1. Species Diversity and Abundance

Pitfall traps and pipe refugia were deployed for 10 consecutive days at the Billabong site fromthe 5th to 15th January and the Ridge site from the 18th to 28th January 2021 for a total of 7,200trapping sessions. During this period, there were 617 frogs captured, 98.8 % in pitfall traps, and 1.2% in the artificial refugia (Table 5). Bycatch of this project (61 mammals, 27 small reptiles and 1 bird) were not included in data analyses. Of the 617 frogs captured, four family groups were represented: Bufonidae (1 species, 186 individuals), Limnodynastidae (4 species, 401 individuals), Myobatrachidae (1 species, 1 individual) and Pelodryadidae (6 species, 29 individuals) (Figure 13). Data tables showing the number of each species caught per trap type and time-period at each site can be found in Appendix D and Appendix E.

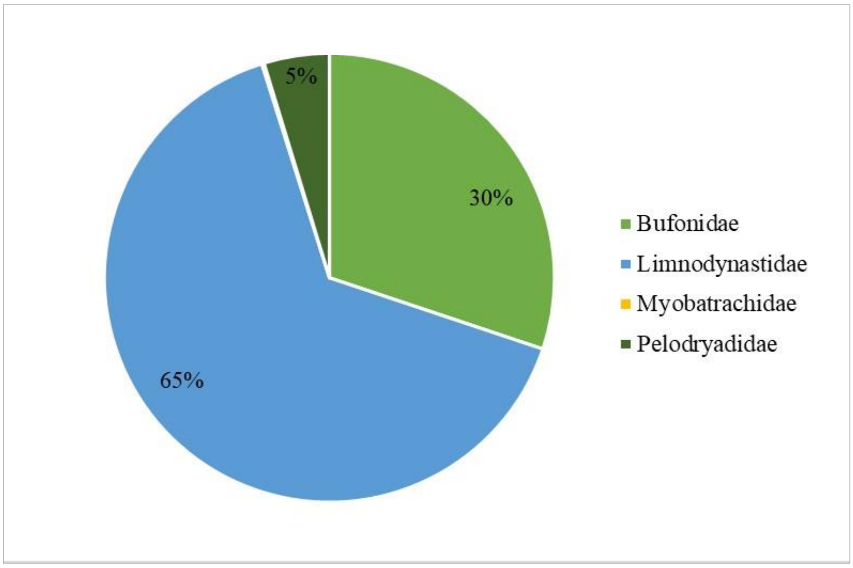

Figure 13. Percentage of each family group from total captured frogs. One individual fromthe Myobatrachidae family was captured, representing 0.0016 % of total captures.

Table 5. Frogs captured across both trapping sites and in different trap types.

Species	Pitfall traps			Pipe refugia		Total
	Light	No light	Total	PVC	Bamboo	
Pelodryadidae						**29**
Cyclorana brevipes	3	4	7	0	0	7
Litoria brevipalmata	0	3	3	0	0	3
L. caerulea	0	1	1	0	0	1
L. nasuta	0	1	1	0	0	1
L. peronii	2	4	6	0	0	6
L. rubella	1	3	4	6	1	11
Limnodynastidae						**401**
Limnodynastes salmini	0	1	1	0	0	1
L. tasmaniensis	38	87	125	0	0	125
L. terraereginae	11	24	35	0	0	35
Platyplectrum ornatum	80	160	240	0	0	240
Myobatrachidae						**1**
Pseudophryne major	1	0	1	0	0	1
Bufonidae						**186**
Rhinella marina	60	126	186	0	0	186
Total	**196**	**414**	**610**	**6**	**1**	**617**

Across both sites, 610 frogs were captured using pitfall traps, 504 frogs at the Billabong, and 106 at the Ridge (Figure 14). The number of frogs caught at each site were averaged per 100 trapping events, to account for the difference in number of pitfall traps between the two trapping sites. The Shannon diversity index recorded species richness scores for the Billabong (H = -1.2056) and the Ridge (H = -1.5404). Simpson's diversity index recorded species evenness scores for the Billabong (D = 0.327) and the Ridge (D = 0.0987).

The Billabong recorded the highest number of frogs caught on the third trapping day, with 4.26 captures per 100 trap events (Figure 14). In comparison, the highest number of frogs caught atthe Ridge occurred on day one, with 1.6 captures per 100 trap events (Figure 14). The lowest number of frogs captured at each site occurred on day two at the Billabong, with 1.54 captures per 100 trap events recorded, and on four, with 0.05 frogs captured per 100 trap events (Figure 14). Data on the number of each species caught at the Billabong and the Ridge can be found inAppendix F.

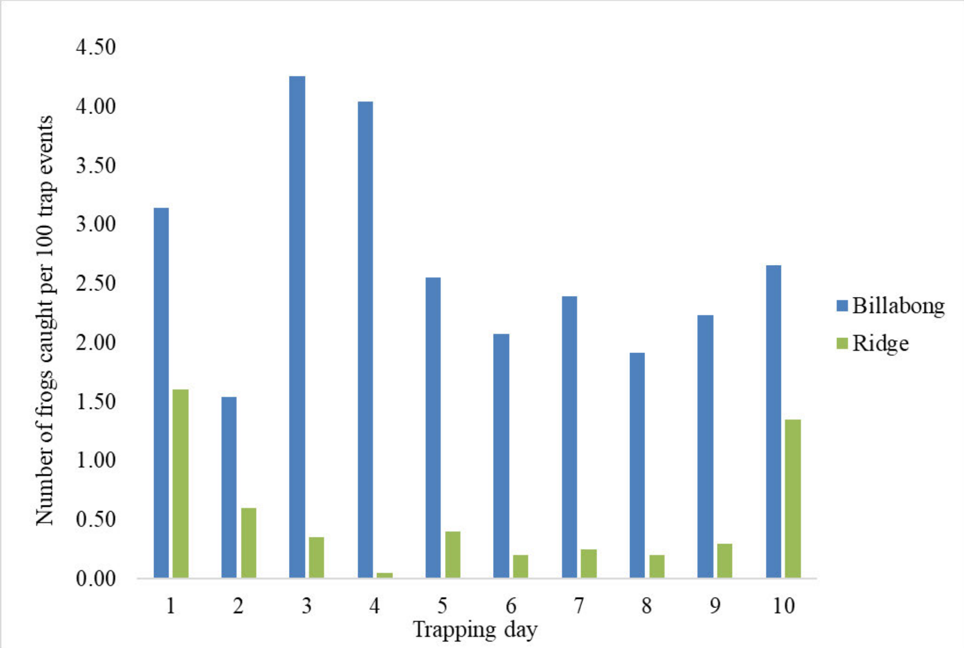

Figure 14. Number of frogs caught at each site, averaged over 100 trap events.

4.1.1. Species Accumulation curves

The number of additional species caught each day was used to calculate an accumulation curve for each survey site across the 10-day period (Figure 15). The Billabong had a total of 9 species captured over the 10-day period, plateauing between days 8 to 10 (r^2 = 0.9204) (Figure 15). The period with the highest accumulation of species occurred between trapping days 3 and 6, with a 44 % increase in total species captured (Figure 15). In comparison, the Ridge had a total of 9 species captured, plateauing between trapping days 3 and 8 with 4 species accumulated, before a 56% increase in species captured between days 8 and 10 (r^2 = 0.5981) (Figure 15).

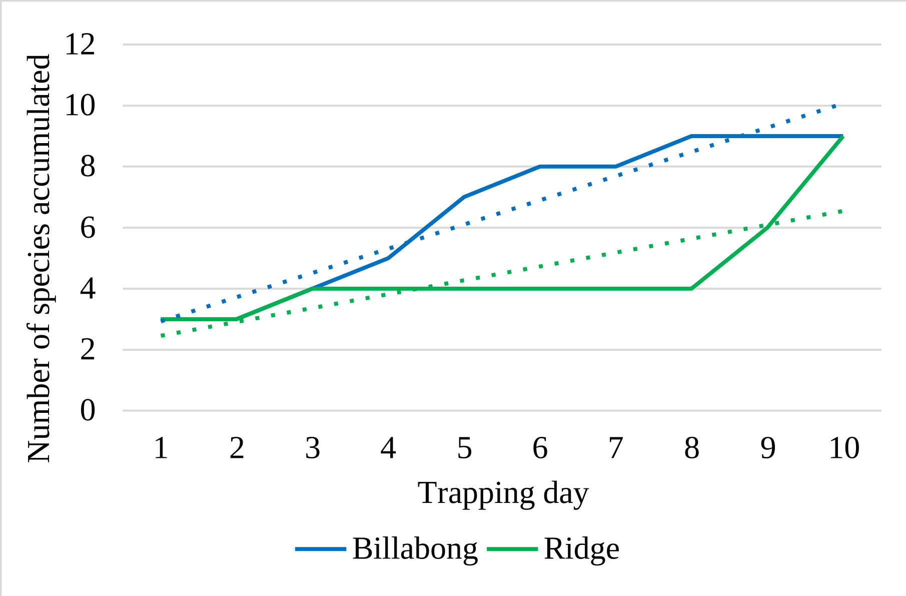

Figure 15. Frog species accumulation over 10-day period at the Billabong (blue), and theRidge (green).

4.2. Environmental influence

4.2.1. Rainfall

Rainfall data was obtained from the Bureau of Meteorology, University of Queensland, Gatton, station 040082 (Bureau of Meteorology 2021a). Approximately 8.5 and 47 mm of rainfall was recorded on the 6^{th} and 7^{th} of January respectively. The presence of precipitation in the region resulted in high numbers of frogs captured at the Billabong in subsequent days (Figure 16). In comparison, approximately 20.5 mm of rainfall was recorded on the 19^{th} of January, correlatingwith the highest number of frogs captured (per 100 trap events) for the study site on that day. In contrast to the Billabong site, frogs captured were not sustained in the days following precipitation, averaging 0.2 frogs captured per 100 trap events, from the 20^{th} to 27^{th} (Figure 17). The final survey period (28^{th} January) was an anomaly to this occurrence, with 1.35 frogs captured per 100 trap events, and 0.8 mm of rainfall (Figure 17).

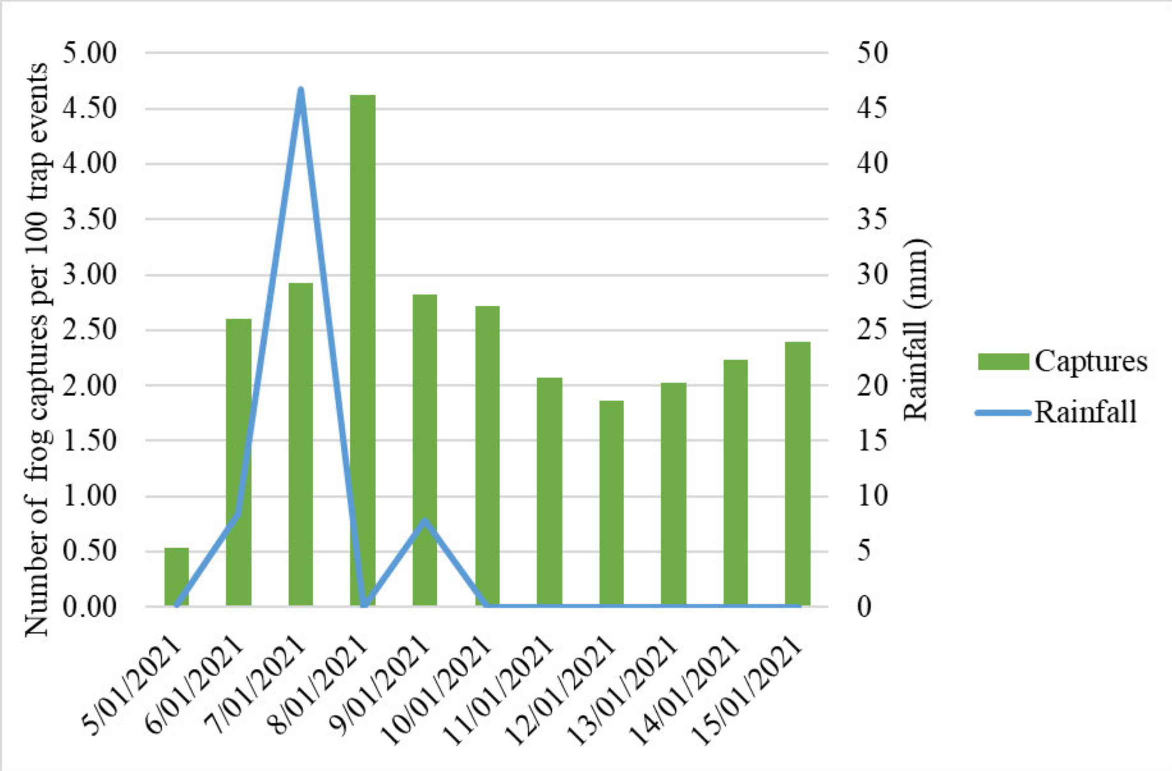

Figure 16. Correlation between rainfall (mm) and the number of frogs captured per 100 trapevents at the Billabong.

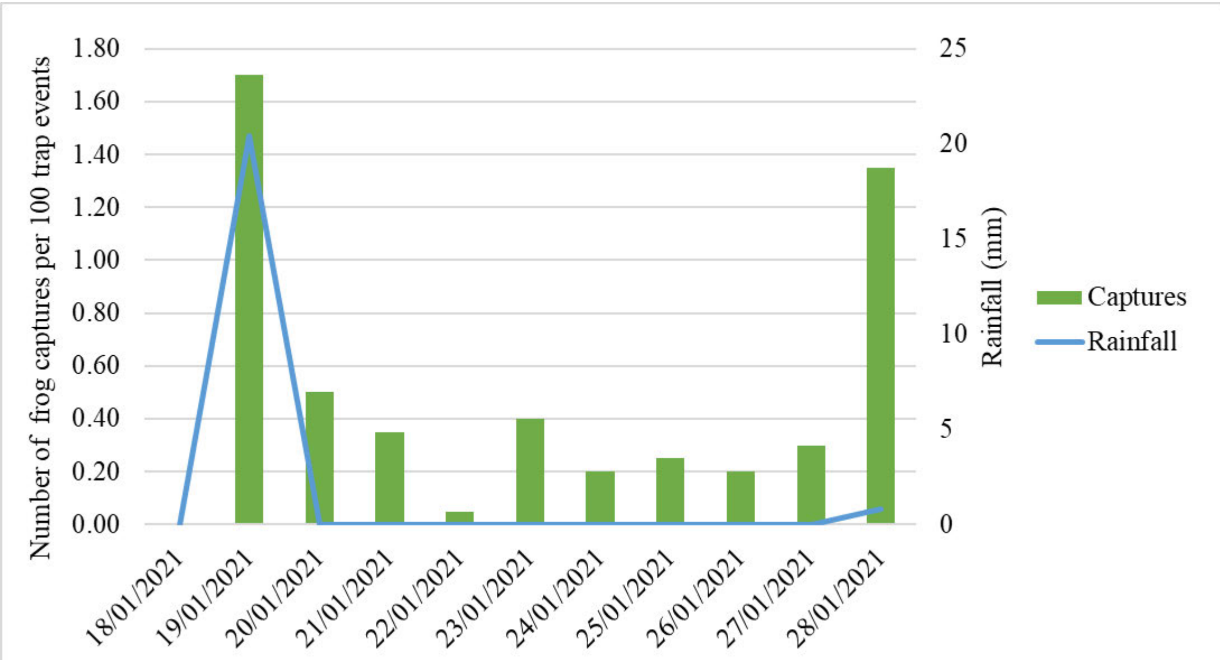

Figure 17. Correlation between rainfall (mm) and the number of frogs captured per 100 trapevents at the Ridge.

4.2.3. Ambient temperature

Across the trapping period, the ambient temperature range was also obtained through the Bureau of Meteorology, University of Queensland, Gatton, station 040082 (Bureau of Meteorology 2021a), to determine correlations between ambient temperature, and number of frogs captured at each site.

During the Billabong trapping period, minimum daily temperatures ranged between 15.2 °C and 21.6 °C, with and average minimum temperature of 18.3 °C across the 10-day period (Figure 18). Maximum daily temperatures ranged between 23°1 and 37.4 °C, with an averageof 30.9 °C (Figure 18). During this period, the highest number of frogs captured occurred on the day when maximum temperatures peaked at 23.1 °C, and the lowest number of captures onthe day when temperature was highest (Figure 18).

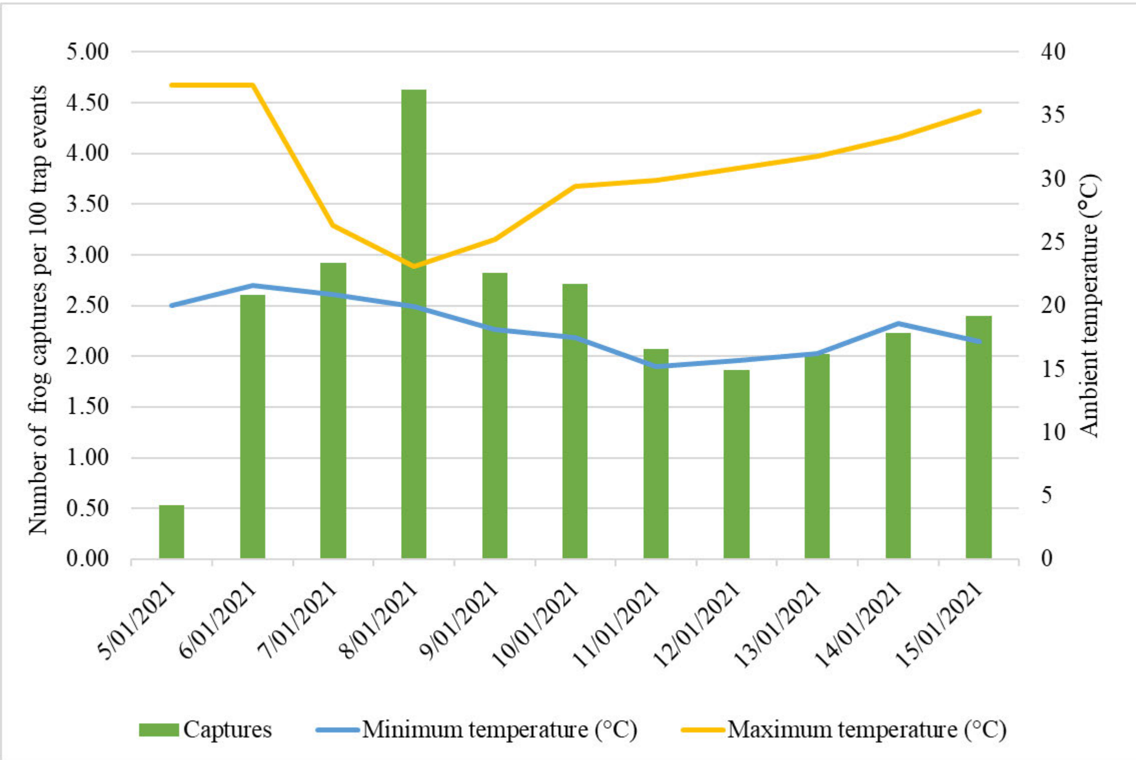

Figure 18. Relationship between ambient temperature and the number of frogs captured per100 trap days at the Billabong.

In comparison, during the second trapping period, the Ridge site recorded minimum daily temperatures between 14.3 °C and 22.2 °C, with an average minimum temperature of 18.4 °C across the 10-day period (Figure 19). Maximum daily temperatures ranged between 26.9 °C and 34.4 °C, with an average of 31.7 °C (Figure 19). In contrast to the Billabong site, the highest number of frogs captured at the Ridge (per 100 trap events), occurred on warmer days (19th and 28th) when temperatures were around 33 °C (Figure 19). Similarly, the lowest number of frogs captured occurred on days where the maximum temperatures were recorded above 30 °C (Figure 19).

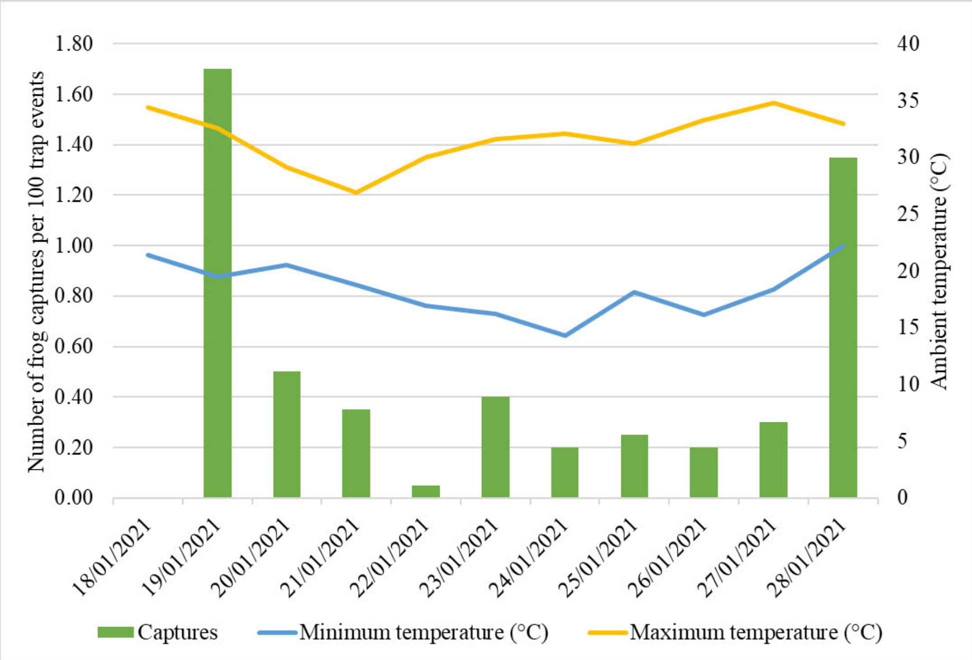

Figure 19. Relationship between ambient temperature and the number of frogs captured per100 trap days at the Ridge.

4.3. Correlation between nested habitat type and time-period

An ANOVA based on the estimated marginal means (EMM) was modelled to evaluate the influence of time and habitat type on the number of captures (per 100 trap events). There was no significant relationship between capture rate and time (Pr(>F) = 0.0564). There was also no statistically significant relationship between habitat type and capture rate (Pr(>F) = 0.3966).

However, the relationship between capture rate and time scored closer to statistically significance than habitat types.

At the Ridge trapping site there was no statistically significant relationship between capture rate and time (Pr(>F) = 0.0692). There was also no statistically significant relationship between habitat type and capture rate (Pr(>F) = 0.4202). However, like the Billabong site, the relationship between capture rate and time of capture, was closer to statistically significant than habitat types.

4.4. Efficacy of pit-light traps

Pit-light traps (n = 30 at each site) recorded a total of 167 frogs captured across both trapping sites, 75 captured on nights with the light on (n = 5 nights), and 92 captured on nights with the light turned off (n = 5 nights) (Figure 20). Only data observing the individuals captured overnight (‘AM’ recorded time-period) was used to avoid biasing the results in favour of non-light trap events.

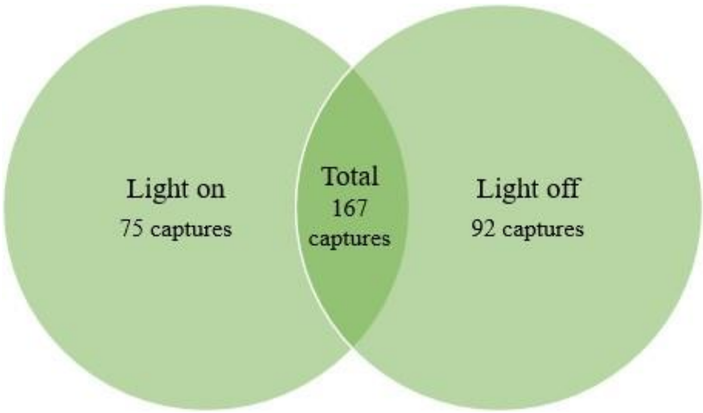

Figure 20. Total number of frogs captured in pit-light traps with lights on and off.

The Billabong site had a total of 143 frogs captured in pit-light traps (n = 30) across the 10 trapping days, 63 captures with the light on, and 80 with the light off (Figure 20). Within the nested habitat types, the dam had 41.48 frogs captured per 100 trap events (14.81 with light on, 26.67 without light), the grass habitat had 16.96 frogs captured per 100 trap events (7.36 withlight on, 9.57 without light) and the hill habitat had 45.72 frogs captured per 100 trap events (24.76 with light on, 20.95 without light) (Figure 21). The Ridge site had 24 frogs captured inpit-light traps (n = 30) during the 10-day trapping period (Figure 21). Of these, 12 frogs were captured with the light on, and 12 captured with the light off. The blady grass habitat had the most captures in pit-light traps, with 7 frogs captured per 100 trap events, (3.67 with the light on and 3.33 with the light off) (Figure 21). The native pasture habitat had 3 frogs captured per100 trap days, (1 with light on, 2 without light). The slope habitat had no frogs captured in pit-light traps across the Ridge trapping period (Figure 21).

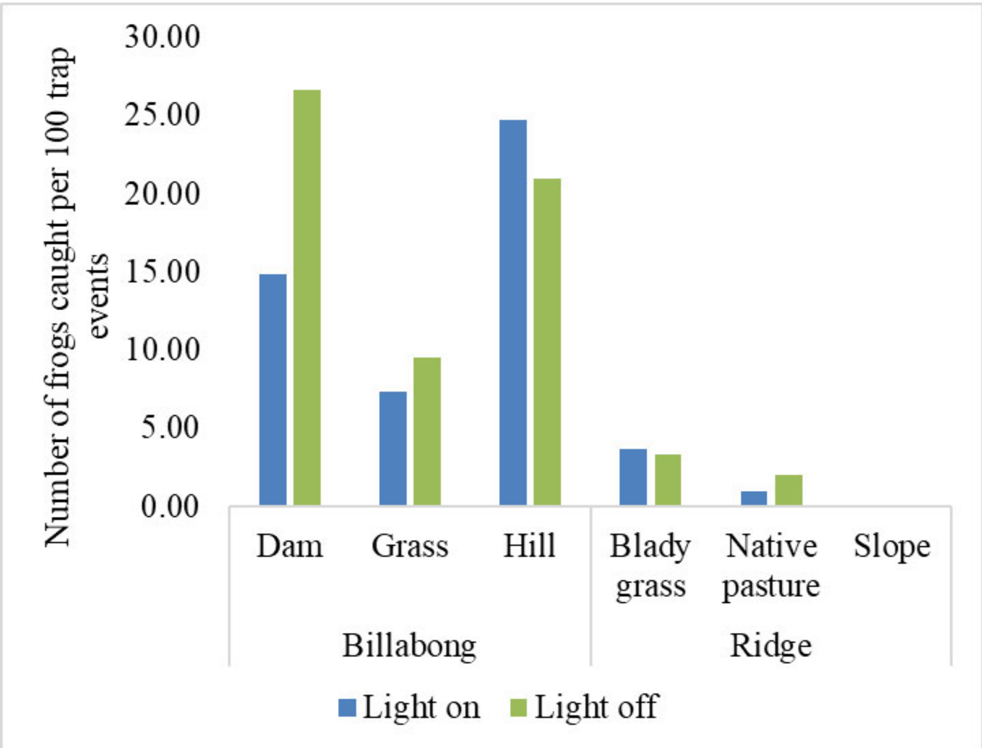

Figure 21. Frog capture rate of pit-light traps per 100 trap events.

A paired t-test for sample means was used to determine if artificial light influenced the capturerate of frogs in pitfall traps. In a small sample (n = 30), frog capture rates at the Billabong decreased when artificial light was used (M = 2.1, SD = 2.8), compared to the capture rate without light (M = 2.7, SD = 3.1). No statistically significant relationship between capture rateand the use of artificial light was found ($t(29) = 1.6$, $p = 0.15$).

At the Ridge, the capture rate of frogs in pitfall traps with artificial light (M = 0.4, SD = 1.3), was compared to the capture rate without light (M = 0.4, SD = 0.6). No statistically significant relationship between capture rate and the use of artificial light was found ($t(29) = 0$, $p = 0.5$).

4.5. Photographic identification software

Across the 10-day period, a total of 179 cane toads were captured at the Billabong site. While majority of these individuals were young juveniles (< 2 cm SVL), 15 % of captured toads were greater than 8 cm in body length and were microchipped and entered in the database to analyse photographic identification software. Due to there being no recaptures of cane toads at the Ridge site, toads captured and microchipped at the Ridge site were not included in the I^3S database.

Dorsal and ventral photographs of each microchipped cane toad (n = 22) were used to create a database in the I^3S software of annotated images saved as the 'fingerprint' of animal. Due to the abundance of cane toads at the Billabong site, only five (17.5 % of total) microchipped cane toads were ecaptured, while the Ridge site had no recaptures of cane toads.

I^3S software comparisons for recaptured toads (n = 4), resulted in an output of the 10 closest matched fingerprint images. Two of the four images (Toad 02 and Toad 03) returned with scores between 49 and 52, however the fingerprint image they were matched to were incorrect (Toad 01 and Toad 02 respectively). The higher scored matches were between Toad04 matched to Toad14, and Toad01 matched to Toad16. Database comparisons found Toad01 (microchip number 7139) fingerprint matched closest to (score: 110.96) Toad16, Toad02 (microchip number 7140) fingerprint matched closest to Toad01 (score: 51.9), Toad03 (microchip number 7144) matched closest to Toad02 (score: 49.04) and Toad04 (microchip number 7145) matched closest to Toad14 (score: 123.26) (Figure 22). Despite the fingerprint matches being incorrect, the low-level score for both Toad02 and Toad03 indicated that the software match was close to the correct match.

Figure 22. Image sets of; (a) original microchipped animal (1-4), (b) recaptured animal(microchip matched), and (c) closest scored individual from I^3S dataset.

4.6. PVC pipe and bamboo culm as artificial refugia survey method

The artificial refugia method captured a total of seven frogs captured of *L. rubella*, six at the Billabong site, and one at the Ridge site. Of the pipe refugia allocated to pitfall traps at the Billabong site, one *L. rubella* was found in a PVC pipe refugia. Of the refugia placed in trees around the Billabong site, four *L. rubella* were found in PVC pipes and one *L. rubella* in a bamboo pipe (Figure 23). At the Ridge site, one L. rubella was found in a PVC pipe allocated to a tree around the site, and none at allocated pitfall traps. The variation of microhabitat preference of *L. rubella* is reflected by the similarity in the number of captures in both pitfall traps (n = 6) and artificial refugia (n = 7) across both trapping periods. Furthermore, there was no clear indication that capture rates of artificial refugia allocated to a pit-light locations were influenced by the artificial light source. Due to the limited data record on frog use of pipe refugia in this study, no data analysis was conducted.

Figure 23. *Litoria rubella* in bamboo pipe refugia.

4.7. Camera traps

4.7.1. Billabong

At the Billabong site, 13 image captures were taken from 12 camera traps. The species were primarily mammals (10), with two bird species (Pacific black duck, *Anas supericiliosa* and an Australian magpie, *Gymnorhina tibicen*) and one reptile species (Eastern bearded dragon, *Pogona barbata)*. Mammal species captured by the camera traps included five, small, unidentified mammals, two long-nosed bandicoots (*Perameles nasuta*), a black rat (*Rattus* rattus), a common brushtail possum (*Trichosurus vulpeula*), and one European rabbit (*Oryctolagus cuniculus*) (Figure 24). Seven photo events were captured showing *R. rattus, P. nasuta, O. cuniculus* and *A superiliosa*. These interactions were recorded at pitfall traps across all three habitat types at the trapping site. *P. barbata* was not recorded interacting with the pitfall traps.

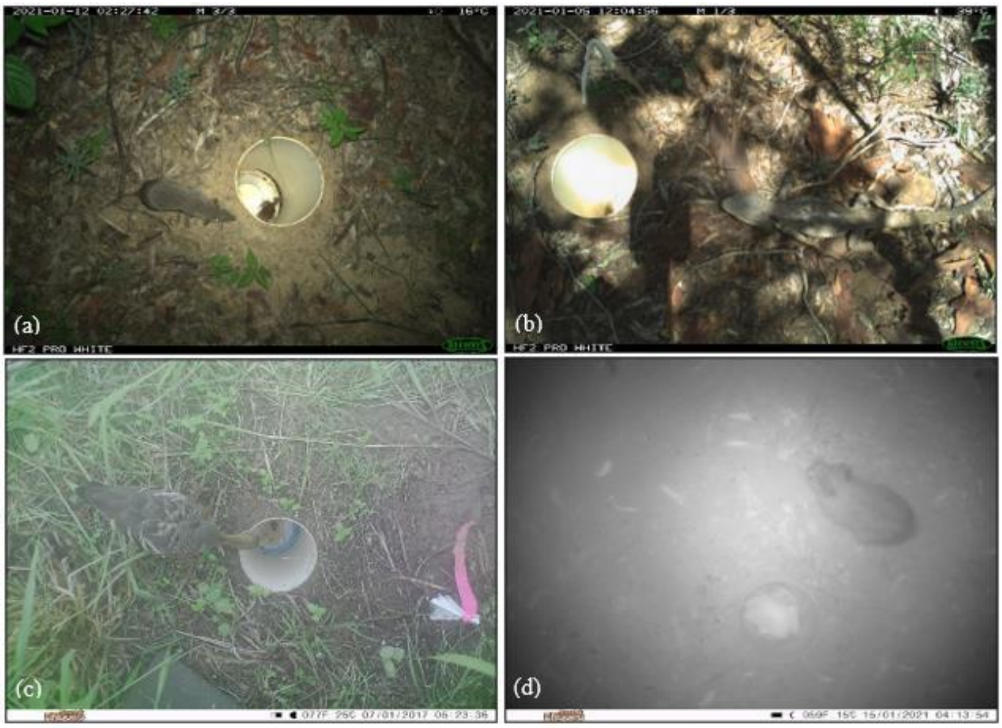

Figure 24. A selection of species observed interacting with pitfall traps at the Billabong trapping site; (a) Black rat (*Rattus rattus*), (b) Eastern bearded dragon (*Pogona barbata*), (c) Pacific black duck (*Anas superciliosa*), and (d) European rabbit (*Oryctolagus cuniculus*).

Further observation of the photo series shows a frog (<5 cm SVL) captured by the pitfall whichmay provide some context for the fixation on the pitfall. Further comparison with pitfall capture data shows that the offspring of this animal was recorded as bycatch in this pitfall trap in the subsequent trap-checking session, offering further context for the prolonged period this animal was recorded for.

An observed form of pitfall trap predation within this study was due to invertebrate species predating on small (>2 cm) frogs captured within the pitfall trap.

4.7.2. Ridge

At the Ridge site, 27 image captures were taken from 15 camera traps. The species were primarily mammals (17), with 2 bird species (Australian magpie, *Gymnorhina tibicen* (7), and a pheasant coucal, *Centropus phasianinus*) and one reptile species (Lace monitor, *Varanus varius*). Mammal species captured by the camera traps included eastern grey kangaroos (*Macropus giganteus*) (4), whiptail wallabies (*Notamacropus parryi*) (2), long nosed potoroos(*Potorous tridactylus*) (5), and common brushtail possums (*Trichosurus vulpeula*) (3), (Figure 25). Three small, and one medium sized mammal were also captured, but unable to be identified due to positioning, lack of distinguishable features, or movement of the animal when image was captured. Across all habitat types, 15 photo events were captured showing animals interacting with the pitfall traps. The mammal species investigating the pitfalls included *M giganteus, N parryi, T. vulpeula* and *P. tridactylus,* as well as an unidentified small mammal. The bird species investigating pitfall traps were *G. tibicen* and *C. phasianinus*. *P. barbata* and *V. varius* did not investigate the open pitfall.

Figure 25. A selection of species observed interacting with pitfall traps at the Ridge trapping site, (a) Eastern grey kangaroo (*Macropus giganteus*), (b) & (c) Whiptail wallaby(*Notamacropus parryi*), (d) & (e) Common brushtail possum (*Trichosurus vulpeula*), (f) Long-nosed potoroo* (*Potorous tridactylus*), (g) Australian magpie (*Gymnorhina tibicen*), (h) Pheasant coucal (*Centropus phasianinus*), (i) Lace monitor (*Varanus varius*), and (j) Unidentified small mammal species.

Chapter 5: Discussion

Traditional frog survey methods have many limitations to the data that is collected, and therefore result in biased population diversity and abundance estimates. To gain broader understanding of the status of Australian frogs and their population trends, the limitations of current survey methods need to be addressed. Chapter 2 highlighted the limitations of traditional survey methods, and proposed methods to reduce the impacts of these limitations.

This study hypothesised that the use of an artificial light source will act as a natural bait for arthropod species, the primary prey food of frogs, and therefore increase the capture rate of frogs in pit-light traps. At both trapping sites, there was no statistical significance in the capture rates of pit-light traps. However, as a pilot study for this method, there are many factors (discussed in section 5.2.), that may have influenced this result. With further research, the use of artificial light in conjunction with pitfall traps offers a potential solution to reduce the limitations of traditional pitfall trapping surveys to obtain more accurate, and representative data of frog populations.

Traditional methods of individual frog identification for mark-recapture studies have been becoming increasingly contentious in the last decade (Donnelly et al. 1994; Parris & McCarthy 2001; Perry, Gad et al. 2011). Due to the invasive methods commonly used (e.g. toe clipping, insertion of implants, and branding), concerns for animal welfare and survivability are common, as this may be negatively influencing the rate of return of marked individuals, thus biasing the survey results. It was hypothesised that microchipped cane toads will provide a verifiable dataset of animals (each with unique markings), allowing for photographic identification software (I^3S), to be tested for accuracy of identification. As discussed in section 5.4., the sample size of toads used in this study was too small to be able to confidently report the accuracy of the software program. While the software did not correctly identify recaptured toads (based on key points of their ventral pattern) with 100 % accuracy, the software scored 50 % of recaptured toads as the ‘next closest match’ with majority of key points matched. Withfurther research, and a larger sample size, the use of photographic identification software provides a potential method to replace traditional frog identification techniques. Replacing traditional identification techniques with photographic identification reduces the potential of inadvertently spreading disease between frogs, a vital concern that must be accounted for in the face of the chytrid fungus epidemic.

Many Australian frog surveys utilise terrestrial trapping methods (e.g. pitfall traps), which often do not consider the diversity of arboreal frog species that may be found in the given habitat. This often results in the under-representation of arboreal frogs in species diversity and abundance estimates that are then used to direct conservation projects. To improve the diversity of species captured in ecological surveys, to reduce the impacts of such limitations, a new method of surveying arboreal frog species was proposed. Based on research conducted on arboreal frog species in other regions of the world, the use of PVC pipe and bamboo culm as artificial refugia was trialled as a method to survey Australian arboreal frogs. It was hypothesised that the use of artificial refugia as a survey method will increase the number of arboreal species captured in any given habitat. As the first study to use this method inn Australian, this study provides proof-of-concept that arboreal frogs utilise PVC pipe and bamboo culm as artificial refugia. Further discussed in section 5.3., due to the small sample size recorded, there was no conclusive evidence of frog preference for either PVC pipe or bamboo culm as artificial refugia. The development of a new method to survey arboreal frog species, (used in conjunction with traditional survey methods) will allow for otherwise under-represented frog species to be surveyed and accounted for in species diversity estimates, thus lessening the impact of limitations of traditional survey methods.

Finally, the prevalence of vertebrate predation from pitfall traps is under-researched, with predator attendance at pitfall traps often reported as anecdotal (Ferguson et al. 2008), despite being a potential factor biasing survey results. Predation from pitfall traps potentially reduces the accuracy of survey data, and therefore may be negatively impacting population diversity and abundance estimates. This study hypothesised that the use of camera traps, in conjunction with pitfall traps offers a method to observe the prevalence of potential predation from pitfall traps. Observations of animal interactions around active pitfall traps, showed that vertebrate attendance at pitfall traps may be an indication of potential predation from pitfall traps. The observations from this study (section 5.5.), did not provide conclusive evidence on the occurrence of predation of frogs from pitfall traps. However, substantial interest in pitfall traps by some species (e.g. *Rattus rattus*) may indicate potential of predation events occurring. Future research should utilise camera traps to observe the occurrence of predation events from pitfall traps, to provide more accurate frog population and abundance estimates for ecological surveys.

5.1. Species diversity and abundance

The two study sites on the Hidden Vale property revealed a reasonable diversity of frog species, with frogs in 12 species captured during the trapping period. Southeast Queensland (SEQ) bioregion is home to approximately 64 frog species, 32 of which are commonly reported within the Ipswich LGA. The 12 species recorded during this study accounted for approximately 37.5 % of species known to occur in the LGA, and 18.74 % of SEQ frog species. Overall, the most diverse family group recorded during the study were frogs of the Pelodryadidae family, with 6 different species (Figure 13). The number of Pelodryadidae frog species recorded during this study reflects the diversity of the Pelodryadidae species found in Australia in comparison to other family groups. As one of Australia's most diverse frog families, the Pelodryadidae consists of 88 species, 36.7 % of all Australian frogs. Species of this family are found across most of the country (apart from some southern deserts), with diverse lifestyles (e.g. arboreal, fossorial, or terrestrial) varying by species. The Pelodryadidae species caught during this project, are predominantly terrestrial species (*Cyclorana brevipes, Litoria brevipalmata, L. nasuta, L. peronii*), with two arboreal species (*L.*

caerulea, *L. rubella*).

Despite being classified as 'tree frogs' *L. rubella* are often found calling from ground level alongside water sources, which provides an explanation to the 6 captures of *L. rubella* in pitfalltraps. An unusual capture recorded was a *L. caerulea* individual captured in a pitfall trap at theBillabong site. Like *L. rubella*, despite being arboreal frogs, *L. caerulea* often shelters in lower vegetation levels, such as in rock crevices and under fallen vegetation. Recording an individualof this species in a pitfall trap is unlikely, due to their ability to escape from a pitfall trap, due to their large toe pads, and climbing ability. Previous trapping surveys at the Billabong site recorded a single tree frog species over a five-year period (Associate Professor Peter Murray, personal communication, 2020), so the capture of a *L. caerulea* individual was particularly interesting.

Similarly, species of the Limnodynastidae family found across Australia are either terrestrial, or burrowing species. Frogs in the Limnodynastidae caught in this study (*L. salmini, L. tasmaniensis, L. terraereginae*) were all terrestrial species apart from *P. ornatum*, which is a burrowing species. This was reflected by the number of captures of Limnodynastidae. The lifestyle of different frog species is a primary factor contributing to the number of individualscaptured in pitfall traps, with arboreal frog species less frequently caught. The Limnodynastidae recorded the highest number of individuals caught with 401 frogs captured, In comparison, 186 toads in the Bufonidae, 29 frogs in the Pelodryadidae and one Myobatrachidae frog were recorded. Overall, while the Limnodynastidae family recorded highest abundance of species, the Pelodryadidae resulted in the highest diversity of species captured.

Terrestrial and fossorial frog species are more likely to be captured in pitfall traps, due to the strata level they reside in. In comparison, arboreal frog species that spend minimal time on lower strata levels are potentially less likely to be captured in these types of traps. The data collected in this study, and the significant absence of arboreal species captured in pitfall traps, provides an example of the intrinsic bias of pitfall traps regarding capture probabilities. These biases severely restrict the accuracy of estimations of species diversity and abundance, resulting in estimates that do not sufficiently represent the ecological community.

5.1.1. Species accumulation curves

Conservation recovery plans for frog species often require accurate estimates of species diversity within the relevant habitat. However, obtaining such data can often be difficult due to the limitation of many traditional survey techniques used in traditional ecological surveys. Species accumulation curves are a theoretical basis for understanding the relationship between trapping effort and the number of species collected, providing a widely accepted predictive tool for ecological studies (Thompson et al. 2003). Species accumulation curves plot the cumulative number of species recorded against a measure of trapping effort (e.g. number of trapping 'events') (Colwell & Coddington 1994). As the trapping effort increases, the likelihood of capturing additional species decreases. This plot can then be used to determine the appropriate amount of trapping effort required to obtain sufficient data on species richness.

Within the constraints of this study, 55 % of the total species trapped at the Billabong, were recorded by day four, and 88 % of species recorded by day six (Figure 15). The plateau in species accumulation in the last two days of the trapping period indicates that all prevalent species at this site were recorded.

In comparison, the Ridge site had 45 % of total species trapped by day three, followed by a five-day plateau in species accumulation. Initial assumptions were made that all species for this site had been recorded, however, a 55 % increase in frogs captured occurred in the last two trapping days. Such plateaus often conclusively show that all prevalent species have been accounted for.

However, the drastic increase of species captured in the final two trapping days at the Ridge site, clearly indicates that there are significantly more species abundant in the habitat than the data reported.

This shift in species accumulation could be attributed to many factors such as environmental influences on frog activity levels, or species ecology (e.g. arboreal vs terrestrial species or fossorial species) influencing capture probabilities. Furthermore, the trapping effort utilised for this study may not be conducive for accurate population assessments. Increases in trapping effort can significantly reduce the likelihood of underestimating populations (Guimarães et al. 2014). Thus, an increase in the number of trapping events, until a stable number of cumulative species are recorded, may be required to obtain more accurate data.

While species estimates reflect the density and diversity of frog species occurring in a given habitat, a reduction in the sample area, or a more concentrated cluster of traps within the sample area may also aid in improving the accuracy of population estimates for the given habitats. The species accumulation data collected from both trapping sites provides a tool vital in understanding the unique trapping requirements needed to collect adequate data. As a pilot study in population abundance and diversity at these sites, this study provides the framework of knowledge that can be further utilised to design more comprehensive population studies.

5.2. Environmental influence

5.2.1. Rainfall and temperature influence on capture rates

Environmental factors such as rainfall and temperature are well known to be a driving factor of frog activity (Williams & Hero 2001). Many frogs found in southeast Queensland are seasonal breeders, reliant on rainfall to stimulate behaviours in relation to the onset of breeding seasons (e.g.

male vocalisations, and movement towards breeding sites) (Anstis 2013). Fossorial species found in this region (e.g. *Platyplectrum ornatum*) are opportunistic breeders, reliant on the occurrence of heavy rainfall to stimulate the emergence from underground burrows for breeding (Penman et al. 2006; Anstis 2013). Periods during and just after heavy rainfall are the only time these species emerge from underground and are otherwise not easily found during ecological surveys (Penman et al. 2006).

Ambient temperature also significantly impacts frog activity levels, and therefore, the capture probabilities of frogs. Due to their permeable skin, frogs (excluding *R. marina*) are highly susceptible to desiccation in warmer, dry regions, such as SEQ during periods of little rainfall (Anstis 2013; Anstis et al. 2016). Across this study, *P. ornatum* was the most common species at both trapping sites in the Hidden Vale property. The abundance of this frog species is due to its burrowing behaviours, which allows the species to survive in a wide range of habitat types, and throughout dry periods (Anstis et al. 2016). Seasonal breeding species often exhibit reduced activity during the cooler months, entering a state of torpor (Staples 2011). Furthermore, the primarily nocturnal behaviour and reduced activity levels of terrestrial and arboreal frogs during warmer periods offers a level of resistance against dehydration.

These behaviours coincide with what was found during this study, with significantly more animals captured on days when it rained (Figure 16, Figure 17), due to the moist environment being conducive for increased frog activity. Frog capture rates at the Billabong followed what was expected based on knowledge of the relationship between ambient temperature and frog activity. On nights when the minimum temperatures were lowest, fewer frogs were captured in pitfall traps, indicating that the optimum temperature range for frog species of this region had been exceeded. This follows what could be expected by seasonal breeding frog species, as these species primarily enter torpor to reduce bodily metabolism during the cooler months.

It was expected that similar capture rate patterns would be found at the Ridge site in relation to temperature and rainfall recordings. Capture patterns followed what was expected in regard the influence of rainfall, with the highest capture rates occurring on days when rainfall was recorded. However, the data shows that the capture rates at the Ridge site did not coincide with these expectations regarding temperature recordings, with capture rates primarily following rainfall patterns, and less influenced by temperature. The highest number of frogs captured (34)occurring on trapping day two, when maximum temperatures reached 32°C, with minimum temperature reaching 19 °C. The second highest number of frogs captures for the trapping period, occurred on the last trap day, where temperature ranged from a 33 °C maximum and 22 °C minimum. In comparison, the lowest number of frogs captured occurred at a maximum temperature of 30 °C, and minimum of 16 °C. This suggests that the occurrence of a rainfall even on the second, and last day had a greater influence on capture rates than temperature. Furthermore, this data suggests that there is an optimal ambient temperature range in which frog activity is highest, offering increased detection probabilities in ecological surveys. Further studies on temperature preferences and species activity could provide further information on how to design frog surveys based on environmental conditions.

5.3. Nested habitat types

This study utilised three nested habitat types across each trapping site and different time periods(AM and PM collection) to evaluate the variation in capture rate, and species diversity across habitat type and time. The morning trapping periods at each habitat at the Billabong recorded 75 %, 76 % and 66 % of all frogs captures at the dam, grass, and hill habitats respectively. Similarly, the Ridge site recorded 100 %, 95 % and 100 % of morning trapping periods at the blady grass, native pasture, and slope habitats respectively. The dam habitat at the Billabong, recorded 41 % of all frogs captured across the site, with 35 % caught at the hill, and 23 % in the grass habitat. The

Ridge site recorded 50 % of frogs captured in the native pasture habitat, 35 % in the blady grass habitat and 15 % in the slope habitat.

The frog species recorded at the Billabong are primarily seasonal breeders (except for *P. ornatum*). With the sampling period occurring during January, these species are in the middle of their breeding season, during which some species (e.g. *Limnodynastes tasmaniensis* and *L. terraereginae*) migrate closer to a water body for breeding. Therefore, the probability of frogs being captured in pitfall traps closest to the water source was higher than pitfall traps further away. Based on this knowledge, it was expected that the dam habitat would have the highest number and diversity of captured frogs, due to the proximity of the pitfall traps to the water. Capture data confirmed that habitat type, and therefore, proximity to water, was a primary factor influencing the capture rate of pitfall traps across both trapping sites.

Furthermore, the most frogs were captured overnight, as expected based on knowledge of frog behaviour and activity. This importance of accounting for habitat variability and species behaviour when designing ecological surveys to determine frog activity. Without considerationof these factors, the survey will likely underestimate species diversity and abundance, and will not be a true representation of the ecosystem.

5.4. Pit-light traps

With a total of 600 trap events (n = 300 light on, n = 300 light off) across both trapping sites, the pit-light traps recorded approximately 27.8 % of total pitfall trap captures. Of these, 55 % of frogs captured were in unlit traps and 45 % of frogs captured when the light was on (Figure20).

The absence of statistical significance observed between frog capture rate and pit-light traps, could be explained by several factors. The increased number of frogs caught at the Billabong with pit-light traps off, could be due to the time of year the survey occurred. Many of Australia's frog species have breeding seasons spanning the entire summer period, with their breeding season well under way by January. While increased activity of frogs looking for breeding partners may increase the number of captures in passive pitfall traps, the influence of artificial light and the lure of prey items at lit pitfall traps may not be sufficient to divert frogs towards the trap. Further research is needed to understand the relationship between pit-light capture probabilities, species activity, and habitat type.

Similarities between the capture rate of frogs, in pit-light traps, between the slope and native pasture habitats at the Ridge were observed, regardless of whether the light was on or off. These similarities could be explained due to the vegetation prevalent to each habitat types. Both the slope and the native pasture habitat were less densely vegetated then the blady grass habitat, with the slope habitat being the least vegetated. The frog species found at this trapping site are primarily terrestrial species and rely upon vegetation structures for shelter during their inactive periods. Therefore, it would be expected that these habitats are less densely populated with frog species in comparison to the more densely vegetated blady grass habitat. The low level of species richness in these habitats is reflected by the low numbers of frogs captured in pit-light traps in both the slope and native pasture habitats.

Light traps used in invertebrate studies use a range of different light-trap models, and therefore light sources (Heap 1988; Axmacher & Fiedler 2004; Bennett et al. 2012). However, with no standard-use light in invertebrate surveys, and a lack of such surveys conducted in similar ecosystems to this study site, the optimal light for pit-light trapping of frogs is unknown; future studies on the efficacy of pit-light traps should aim to consider and assess the impact that light source has on insect (and therefore, frog), behaviour. Testing a variety of light types will determine the most effective light source to use in conjunction with pitfall traps.

5.5. Photographic identification as an alternative to marking techniques

Mark-recapture studies are vital in providing an understanding frog species abundance within a given habitat. However, these types of studies require capturing a frog, and marking the animal in some way, before releasing the animal to hopefully be recaptured again.

This study assessed the accuracy of I^{3}S software in individually identifying cane toads based on their unique ventral patterns, with microchips implanted in the toads to verify identificationof each toad. Due to the small sample size, there was not enough data to provide conclusive evidence on the efficacy of the I^{3}S software as an individual identification method for toads. Alarger sample size of recaptured toads would have provided an improved accuracy of the software. The number of recaptured, microchipped cane toads is likely due to the size of the population at the Billabong site, therefore resulting in a decreased probability of microchippedtoads being recaptured.

Regardless, this type of identification technique has potential use for mark recapture studies, and further studies evaluating the efficacy of the software is required, ideally with a small, closed population of toads of frogs. While the number of recaptured, microchipped toads in this study were insufficient to answer the hypothesis, other studies evaluating softwareprograms have found that photographic identification can be successfully used for ecological surveys (Andreotti et al. 2018; Bardier et al. 2020; Dunbar et al. 2021). The margin of error by the computer program, reflects the requirement for human identification to visually confirm matches produced by the software, however improvements to the software will likely result in the reduction of this error margin. Furthermore, the improved accuracy of photographic identification software will provide a means of conducting mark-recapture studies without theuse of invasive methods, often detrimental to the ongoing survival of the captured frog. Development and verified efficacy of non-invasive techniques will allow ecological surveys toobtain accurate data on species abundance and diversity, as well as obtaining data on individualanimals within a population.

5.6. Artificial refugia as a viable survey method

This research assessed the efficacy of artificial refugia as a survey method for Australian arboreal frog species. While only seven captures of *Litoria rubella* were recorded in PVC pipeand bamboo refugia, the repeated use of the refugia by this species demonstrates proof of concept for the viability of artificial refugia as a survey method for Australian arboreal frog species. By taking advantage of the natural behaviours of arboreal frogs, the use of artificial refugia, as a survey method provides a way of reducing trap capture bias and improving captureprobabilities of arboreal species (Hoffmann et al. 2009). By accounting for these factors in survey design, the accuracy and reliability of population estimates will be improved and allowfor conservation and management of native frogs to be better informed (Donnelly & Guyer 1994; Scott et al. 1994a).

5.7. Observations of animal interactions with pitfall traps

Camera traps allocated around pit-light traps provided a total of 14 photo events (a series of three rapid-fire photographs taken, with each event being a minimum of five minutes apart). These photo events included: 11 mammals, two birds and one reptile at the Billabong site, with 54.5 % of the mammals and 50 % of the bird species actively investigating the pitfall traps. Photo events from the Ridge site recorded 19 mammals, eight birds and one reptile, with 67.9 % of mammals and 28.6 % of bird species actively investigating the pitfall traps.

Prolonged attention by the rat to this pitfall trap indicates a heightened interest of theinvestigating animal. While there was no recorded predation from the pitfall traps, the interest of passing mammals in the presence and potential content of open pitfall traps suggests that predation from pitfall traps from vertebrate predators may be occurring.

The occurrence of pitfall trap predation can potentially negatively impact populations of frog species, particularly endangered species that have low numbers in wild populations. The predation of these individuals (particularly breeding individuals) due to confinement within a trap is detrimental to the overall population (Ferguson et al. 2008). Predation from pitfall traps also negatively impacts data (not) collected, and therefore population estimates. Further studies on the occurrence of vertebrate predation from pitfall traps, is needed to assess the impact this has on population estimates, and methods that can be developed to reduce these impacts.

Chapter 6: Conclusions and recommendations

With many of Australian frog species listed as endangered, or at risk of extinction, there is a need for more accurate estimates on frog populations and abundance to better inform conservation planning and habitat management for Australian frogs (Blaustein et al. 2004; Gillespie et al. 2020). This study has highlighted the limitations of traditional survey methods, such as the under-representation of arboreal frog species, habitat complexity and environmental factors influencing trap success, and traditional marking techniques that can have negative implications on the survival and rate of return for some frog species. Areas in which these limitations can be reduced were identified, and methods to improve survey techniques were proposed and trialled.

The impact of artificial light on the capture rate of pitfall traps was not conclusive in this pilot study, however, many factors have been highlighted that play a potential role in the success of pit-light traps for frog surveys. This study has provided many more areas of research that are required for this method to increase the capture rate of frogs in pitfall traps, and therefore improve species population estimates. Furthermore, this study is the first to provide evidence that PVC pipes and bamboo as artificial refugia, are a viable method of surveying arboreal treefrog species in Australia. Previous studies utilising artificial refugia have demonstrated that PVC pipe is an effective method to capture arboreal frog species in many regions around the world. However, as artificial refugia has not been utilised to survey Australian arboreal frog species, further studies are recommended to determine the viability of the method. Further studies could be conducted across different habitat types, such as dense rainforests, where natural crevices for frog refugia are highly abundant and the efficacy of artificial refugia may be limited.

Additionally, this study has valued the application of the photographic identification software I^3S, to individually identify cane toads by their unique ventral patterns. While a small sample size resulted in an inconclusive evaluation, limitations of the software were identified. This study proposes that with further development of such software, and larger sample sizes, photographic identification of frogs may be an adequate replacement for traditional, invasive marking procedures used for mark-recapture studies. Further research is necessary to identify methods of individually identifying frogs that do not have unique patterning between individuals. Future studies would also benefit by comparing different identification software, (e.g. HotSpotter, WildID), to highlight the benefits and limitations of each program regardingfrog identification.

Furthermore, this study has highlighted the potential occurrence of vertebrate predation from pitfall traps. Few studies have previously acknowledged to potential of vertebrate predation influencing the capture rate of pitfall traps (Ferguson et al. 2008). This study provides evidencethat omnivorous vertebrate species (such as *R. rattus*) attend and investigate active pitfall traps, suggesting that there may be potential predation occurring from pitfall traps. Additional studieson the prevalence and impact of predation from pitfall traps should be conducted.

This study has highlighted areas in which current frog surveys are limited, and providing inaccurate, under-representative data on frog populations. Used in this study have the potentialto improve current frog survey methods, allowing for more accurate data on population diversity and abundance to be obtained allowing conservation and habitat management programs to be better informed. This knowledge is a vital tool in the fight against the extinction of threatened and endangered frog species, not only in Australia, but world-wide.

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Appendices

Appendix A

Locations where artificial refugia has been used for frog surveys. Adapted from Glorioso and Waddle (2014).

Location	**No. of studies**	**Source**
Brazil	2	Silva and Rossa-Feres (2007); Ferreira et al. (2012)
Costa Rica	1	Laurencio and Malone (2009)
Equatorial Guinea	1	Layman (2011)
Japan	1	Hirai (2006)
Peru	2	Waldram (2008); von May et al. (2009)
Puerto Rico	8	Drewry (1970); Stewart and Pough (1983); Townsend (1989); Woolbright (1989); Stewart and Rand (1991); Fogarty and Vilella (2001, 2002, 2003)
United States	69	
North Carolina	10	Moulton (1996, 1997); Lamb et al. (1998); Matthews and Cook (2004); Tomasek et al. (2005); Hall (2006); Pittman and Dorcas (2006); Pittman et al. (2008); Tomasek and Matthews (2008); Hutchens and DePerno (2009)

South Carolina	6	Phelps (1993); Phelps and Lancia (1995); Perison et al. (1997); Schurbon (2000); Schurbon and Fauth (2003); LaBram et al. (2007)
Georgia	8	Moseley et al. (2003); Borg et al. (2004); Muenz et al. (2006); Smith, Barichivich, et al. (2006); Smith, Steen, et al. (2006); Liner et al. (2008); Farmer et al. (2009); Kirkman et al. (2012)
Florida	31	Goin (1955); Goin and Goin (1957); Goin (1958); O'Neill (1995); Meshaka (1996); Boughton (1997); Boughton et al. (2000); Donnelly et al. (2001); Meshaka (2001); Brandt et al. (2003); Zacharow et al. (2003) Bartareau (2004); Wyatt and Forys (2004); Rice et al. (2006); Waddle (2006); Hoffmann (2007); Dawson and Hostetler (2008); Piacenza (2008); Waddle et al. (2008); Harding et al. (2009); Hoffmann et al. (2009); McGarrity and Johnson(2009); Campbell et al. (2010); Glorioso et al. (2010); Haggerty (2010); Johnson et al. (2010); McGarrity and Johnson (2010); Windes (2010); Granatosky and Krysko (2011); Rice et al. (2011); Elston et al. (2013)
Mississippi	2	McComb and Noble (1981); Langford et al. (2007)
Louisiana	3	McComb and Noble (1981); Buchanan (1988); Pham et al. (2007)
Missouri	7	Johnson (2005b, 2005a); Johnson et al. (2007); Mahan and Johnson (2007); Gordon (2008); Johnson et al. (2008); Leach (2011)
California	1	Myers et al. (2007)
Hawaii	1	Beard et al. (2009)

Appendix B

Materials used in artificial refugia studies on frogs (ABS = acrylonitrile butadiene styrene). Adapted from Glorioso and Waddle (2014).

Material	No. of studies	Source
Tin can	3	Goin (1955); Goin and Goin (1957); Goin (1958)
Bamboo culm	12	Drewry (1970); Stewart and Pough (1983); Townsend (1989); Woolbright (1989); Stewart and Rand (1991); Fogarty and Vilella (2001, 2002, 2003); Waldram (2008); Beard et al. (2009); von May et al. (2009)
Wooden nest box	1	McComb and Noble (1981)
PVC pipe	60	Buchanan (1988); Phelps (1993); O'Neill (1995); Phelps and Lancia (1995); Meshaka (1996); Moulton (1996, 1997); Lamb et al. (1998); Fleet and Autrey (1999); Brandt et al. (2003); Moseley et al. (2003); Schurbon and Fauth (2003); Zacharow et al. (2003); Bartareau (2004); Borg et al. (2004); Matthews and Cook (2004); Wyatt and Forys (2004); Tomasek et al. (2005); Hall (2006); Hirai (2006); Muenz et al. (2006); Pittman and Dorcas (2006); Rice et al. (2006); Smith, Barichivich, et al. (2006); Smith, Steen, et al. (2006); Waddle (2006); Hoffmann (2007); LaBram et al. (2007); Langford et al. (2007); Myers et al. (2007); Pham et al. (2007); Silva and Rossa-Feres (2007); Dawson and Hostetler (2008); Gordon (2008); Liner et al. (2008); Piacenza (2008); Pittman et al. (2008); Tomasek and Matthews (2008); Waddle et al. (2008); Beard et al. (2009); Farmer et al. (2009); Harding et al. (2009); Hoffmann et al. (2009); Hutchens and DePerno (2009);

		Laurencio and Malone (2009); McGarrity and Johnson (2009); Miranda and Wilczynski (2009); von May etal. (2009); Campbell et al. (2010); Glorioso et al. (2010); Haggerty (2010); Johnson et al. (2010); Windes (2010); Granatosky and Krysko (2011); Layman (2011); Leach (2011); Rice et al. (2011); Ferreira et al. (2012); Kirkman et al. (2012); Elston et al. (2013).
ABS pipe	5	Johnson (2005b, 2005a); Johnson et al. (2007); Mahan and Johnson (2007); Johnson et al. (2008)

Appendix C

Processing of small mammals and reptiles

Prior to removal from pitfall trap, animals were photographed whilst in the trap for identification in event of escape. Small mammals were removed from the pitfall trap by using a calico bag to reach into the pitfall trap, securing the animal in hand firmly. Calico bags were tied, and the species of animal trapped recorded on the data sheet. Each animal was weighed whilst in the calico bag, using the appropriate scale size (20, 60, 100, or 600 g scales). Animal body weight was then calculated using bag weight with and without the animal. Each animal was gently manoeuvred to record body measurements (head and body, tail, hind foot, and ear length) using a plastic ruler, and all other relevant information recorded (i.e., the animals' sex, unique markings). Animals were gently placed in a clean 14 mm diameter petri dish and photographed on top of a 1 mm grid. Animals exhibiting signs of stress (e.g. increased respiratory rate) were not fully processed, and were released immediately near their site of capture, only recording the name of the species and where it was captured.

Small reptiles were removed from pitfall traps using a zip lock bag or 9 cm diameter petri dishto reduce handling of the animal. The bottom of the petri dish was lowered into the pitfall, the animal encouraged into the dish by gently nudging, and the lid placed on to capture the animal. Once secured in a zip lock plastic bag or petri dish, animals were photographed over a grid pattern to obtain measurements of the animal later. Measurements of reptiles were not taken in the field to reduce the risk of stressing the animal. The trap numbers were recorded and species name if known, before releasing the animal near where it was trapped. For unknown reptile species, other photographs e.g. magnified head were taken to assist with identification later. Once processed and all data collected, the animals were released in a shaded area, within 1-3 m of the trap they were caught in. Disposable glove and used zip lock bags were placed in a rubbish bag, and petri dishes cleaned with alcohol wipes.

Appendix D

Species captured with pitfall trapping and in artificial refugia at the Billabong trapping site

	C. brevipes	*L. brevipalmata*	*L. caerulea*	*L. peronii*	*L. rubella*	*L. tasmaniensis*	*L. terraereginae*	*P. ornatum*	*R. marina*
Pitfall Total	**7**	**3**	**1**	**2**	**2**	**85**	**3**	**222**	**179**
Dam	**4**	**2**	**0**	**2**	**2**	**56**	**0**	**80**	**59**
AM	4	2	0	2	2	55	0	58	42
PM	0	0	0	0	0	1	0	22	17
Grass	**0**	**1**	**1**	**0**	**0**	**26**	**0**	**70**	**76**
AM	0	1	1	0	0	22	0	52	60
PM	0	0	0	0	0	4	0	18	16
Hill	**3**	**0**	**0**	**0**	**0**	**3**	**3**	**72**	**44**
AM	3	0	0	0	0	3	3	35	41
PM	0	0	0	0	0	0	0	37	3
PVC Pipes	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**	**0**
Bamboo pipes	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**

Appendix E

Species captured with pitfall trapping and in artificial refugia at the Ridge trapping site.

		L. nasuta	*L. peronii*	*L. rubella*	*L. salmini*	*L. tasmaniensis*	*L. terraereginae*	*P. major*	*P. ornatum*	*R. marina*
Pitfall total		**1**	**4**	**2**	**1**	**40**	**32**	**1**	**18**	**7**
	Blady Grass	**1**	**4**	**1**	**1**	**25**	**18**	**1**	**17**	**5**
	AM	1	4	1	1	25	18	1	17	5
	PM	0	0	0	0	0	0	0	0	0
	Native	**0**	**0**	**1**	**0**	**5**	**8**	**0**	**1**	**2**
	AM	0	0	1	0	4	8	0	1	2
	PM	0	0	0	0	1	0	0	0	0
	Slope	**0**	**0**	**0**	**0**	**10**	**6**	**0**	**0**	**0**
	AM	0	0	0	0	9	6	0	0	0
	PM	0	0	0	0	1	0	0	0	0
PVC Pipes		**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Bamboo pipes		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Appendix F

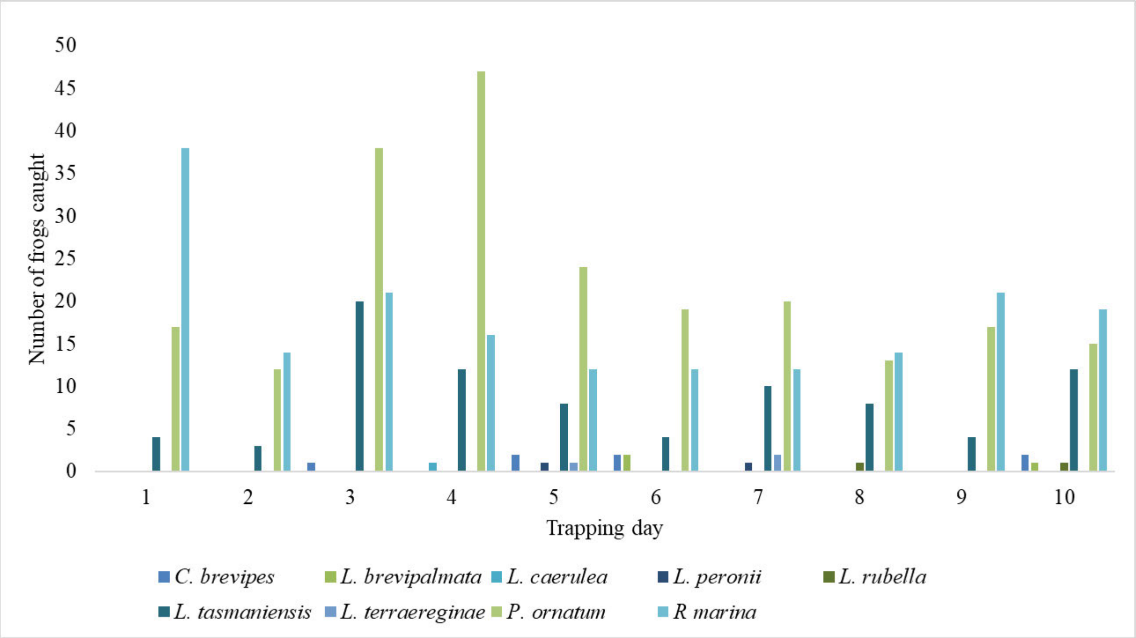

Figure 26. Abundance of frog species caught across trapping period at the Billabong.

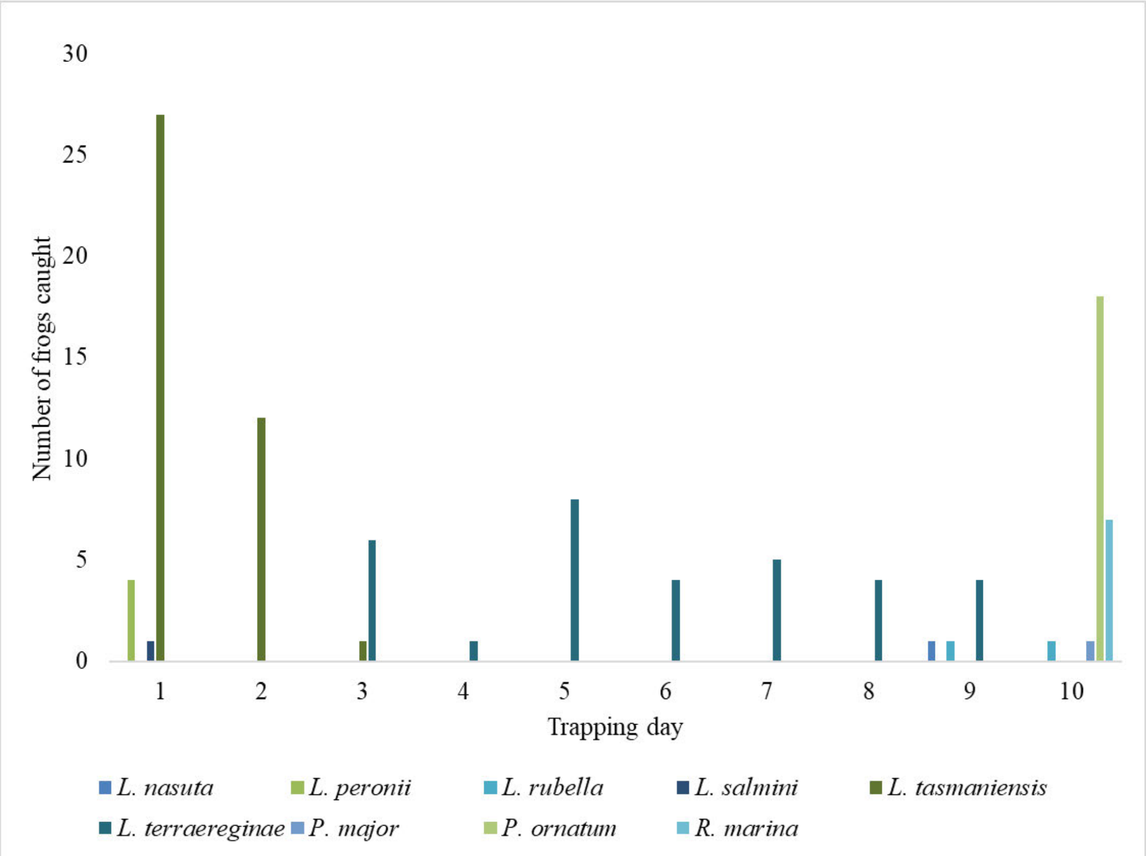

Figure 27. Abundance of frog species caught across the trapping period at the Ridge.