

Final Project Report

1. Contestant profile

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2. Project overview

Title:	Does the Kables Sands Plant promote local amphibian biodiversity?
Contest:	Quarry Life Awards 2016
Quarry name:	Kables Sands, Australia
Prize category: (select all appropriate)	<input checked="" type="checkbox"/> Education and Raising Awareness <input checked="" type="checkbox"/> Habitat and Species Research <input checked="" type="checkbox"/> Biodiversity Management <input type="checkbox"/> Student Project <input type="checkbox"/> Beyond Quarry Borders

Abstract

Anthropogenic disturbances to habitats influence the fitness of individual animals, the abundance of their populations, and the composition of their communities. Wetlands in particular are frequently degraded and destroyed, significantly impacting the animals that inhabit these important ecosystems. Consequently, the importance of artificial wetlands – those created deliberately or inadvertently by human activities – has become more and more critical to the management of wildlife. The creation of artificial wetlands during and following sand extraction processes is inevitable, and thus, sand quarries have the capacity to support considerable aquatic animal populations and enhance local and regional biodiversity. There is, however, a distinct paucity of empirical studies investigating the suitability of quarry wetlands for various taxonomic groups. If these wetlands are significantly impacting the fitness of individual animals, negative impacts could flow on to population-level effects and influence population persistence within quarries. Given amphibians are currently experiencing dramatic declines around the world, with approximately 40% of species facing the threat of extinction, focusing research efforts on this sensitive taxon is imperative.

Here, I conduct nocturnal call surveys at numerous wetlands within the Kables Sands quarry in New South Wales, Australia, and also within surrounding reference wetlands. In addition, I quantify levels of developmental instability (DI) in the frog populations inhabiting these two wetland types as a proxy for fitness. Quarry and reference sites were similar in morphology, and although water pH and salinity differed, this difference is not likely of biological significance for amphibians. Despite comparable vegetation structure, quarry wetlands harboured significantly more frog species (mean \pm se; 1.7 ± 0.48 , max = 3 species) than reference wetlands (0.4 ± 0.25 , max = 1 species). The only incidence of successful breeding was observed within quarry wetlands. Using unsigned asymmetry as a measure of DI, I show that frogs from the quarry wetlands exhibited significantly lower levels of DI compared to reference wetlands, indicating that quarry wetlands provide habitat conducive to comparably high levels of fitness. Levels of DI within quarry wetlands also compare favourably to data from healthy frog populations extracted from the literature. Finally, I produce a guidebook that was distributed to 35 local schools and other stakeholders. The book contains basic information about the quarry and amphibians in general, details how the quarry aims to promote and enhance local amphibian biodiversity, and includes biological information for the 25 amphibian species that inhabit the Blue Mountains Region, Australia.

Wetlands within the Kables Sands quarry are providing a valuable resource for local amphibian populations. Further enhancing their suitability would require little effort, with potentially significant increases in biodiversity. Developing the emergent vegetation surrounding several of the more sparsely vegetated wetlands, as well as ensuring all wetlands are connected to natural terrestrial habitat will provide greater access and breeding areas for many of the native frog species present at the quarry. Identifying amphibian species presence and quantifying their fitness through measuring limb lengths is an economically and logistically feasible method to assess the health of quarry wetlands. Levels of DI could provide early warning indicators of potential issues, allowing mitigation strategies to be implemented prior to population impacts. Overall, the methods outlined here provide a powerful, yet simple, tool to assess the overall health of quarry wetlands that could be easily adopted by non-scientists at quarry sites throughout the world.

Final report

Introduction

Humans are altering natural environments at unprecedented rates, with significant negative impacts for animals (MEA 2005, Pereira et al. 2010). Wetlands in particular harbour highly diverse biological communities and provide extensive ecosystem services, yet are frequently degraded and destroyed, with over 50% of global wetland surface lost during the last century (Zedler and Kercher 2005, Mitsch and Gosselink 2007). Consequently, declines of wetland-dependent species are some of the greatest recorded (MEA 2005). Concurrent with these losses, humans are rapidly constructing wetlands to harness the ecosystem services they provide, such as those constructed in urban areas to treat stormwater and settlement dams created in mining areas to store processed materials (Hammer 1989, Odum 2016). In addition, many artificial wetlands are inadvertently formed during mining extraction processes at quarries (Spencer and Griffith 2012).

Although typically not designed to support and conserve wildlife, these artificial wetlands (termed secondary wetlands; Dolny and Harabis 2012) often attract animals as they superficially resemble natural wetlands and contain the cues used by animals when selecting habitats (Bendell-Young et al. 2000, Thiere et al. 2009, Scheffers and Paszkowski 2013). Therefore, secondary wetlands may provide critical habitat, enhancing landscape-level connectivity and promoting biodiversity and population persistence (Thiere et al. 2009). In some circumstances, properly managed secondary wetlands have been shown to provide habitat superior to that of natural wetlands. For example, Dolny and Harabis (2012) observed more than twice as many dragonfly species in mine subsidence pools than in reference wetlands, positing that this was due to enhanced environmental heterogeneity resulting from abiotic succession processes occurring as a direct consequence of mining. Given this potential, there has been considerable interest in simultaneously satisfying human needs and promoting wildlife within secondary wetlands (i.e. multi-objective management; Benyamine et al. 2004).

However, since secondary wetlands are often designed to store contaminated water, or are in areas prone to contamination and human interference, animal inhabitants may experience compromised fitness (e.g. reduced survival and reproduction; Laposata and Dunson 2000, Dods et al. 2005). Therefore, despite being conceptually appealing, multi-objective management may prove problematic if individual fitness within secondary wetlands is impacted, and particularly if individual fitness impacts scale up to affect population persistence. By quantifying community composition, population sizes and individual fitness, management efforts to maintain and enhance the conservation potential of secondary wetlands will be greatly improved.

Determining the suitability of secondary wetlands for currently threatened taxa in particular should be at the forefront of conservation science. Amphibian populations are currently experiencing dramatic declines around the world, with approximately 40% of species facing the threat of extinction (Whitfield et al. 2007, Monastersky 2014). Amphibians are often considered particularly sensitive to environmental contamination due to their physiology and biphasic life cycle (Wake and Vredenburg 2008). Although numerous factors have been implicated in current population declines such as climate change, disease and pollution, habitat loss clearly stands out as one of the main threats facing amphibians (Cushman 2006). As such, secondary wetlands such as quarry wetlands may provide vital habitat for frogs and toads. Relative to other mining industries, such as fossil fuel mining (which can significantly impact wildlife; Rowe et al. 1996, Anderson and Arruda 2006), sand quarry wetlands may be more suitable for amphibians due to the comparably lower environmental impact of this industry. There is, however, a severe paucity of empirical studies investigating how quarry wetlands function as habitat for amphibians or indeed any other animals (although see Catchpole and Tydeman 1975, Eversham et al. 1996, Spencer and Griffith 2012).

During this study, I aim to (1.) determine how quarry wetlands are functioning as suitable habitat for amphibian communities, populations and individuals, (2.) investigate the biotic and abiotic factors that may be influencing these factors, and (3.) promote community awareness and education of the issues facing amphibians and how quarries are attempting to aid in enhancing biodiversity within their region. To do this, I measure various environmental variables, and quantify amphibian population density, species richness and levels developmental instability (DI) within sand quarry wetlands and reference wetlands. I also compile an educational guidebook on the local amphibian fauna of the Kables Sands Quarry region, and distribute this informative book to stakeholders and schools in the region.

Methods

Study site selection and description

The Kables Sands Quarry (33°27'27.51"S, 150°14'26.04"E) is located in Clarence, New South Wales,

Australia (Figure 1). The extraction of sandstone and production of sand began here 60 years ago, with current production approximately 350,000 tonnes per annum. Mining activities have inadvertently lead to the formation of wetlands filled *via* groundwater, and other wetlands have been created for sand washing and other production purposes. The four accessible wetlands within the quarry were surveyed (Figure 1): the settlement dam (SD), quarry swamp (Swamp), tailings storage cell (T6) and the reservoir (T3). Surrounding quarry wetlands are areas undergoing rehabilitation, and the age of the four wetlands range from 10 to 50 years old (see Table 1). The adjacent suburb, Lithgow (33°28'51.19"S, 150°09'26.67"E), was searched using high resolution aerial imagery to locate potential reference wetlands. Those accessible were selected for surveying, with highly isolated sites within densely forested or fenced areas excluded due to safety concerns with conducting nocturnal surveys alone. This limited reference sites to five wetlands within suburban areas (Figure 1): Geordie St Wetland (Geordie), Laidley St Wetland (Laidley), Vale of Clwydd Wetland (Clwydd), Lake Pillans Wetland 1 (LP WL1) and Lake Pillans Wetland 2 (LP WL2).

Local habitat variables

Wetland area and perimeter were calculated using the spatial analysis tools on www.nearmap.com. During surveys, I measured variables that may influence amphibian populations and communities including: date, time, cloud cover, wind speed, current rainfall, water temperature, salinity/conductivity, pH, and the presence of amphibian predators (e.g. mosquitofish *Gambusia holbrooki* and yabbies *Cherax destructor*). Local air temperature, humidity, and total rainfall during the survey period was extracted from the Bureau of Meteorology website (www.bom.gov.au). I also estimated the percentage cover of vegetation in the fringing, emergent, open, submerged and floating regions of each wetland.

Amphibian surveys

Each site was surveyed for amphibians 3–5 times over two distinct periods: March 30th–April 1st and July 19th–July 22nd, 2016. The sequence that wetlands were visited was randomised and stratified according to wetland proximity since wetlands occurred in clusters (i.e. quarry vs reference). Adopting standard nocturnal search methods (see Parris et al. 1999), I listened for the advertisement calls of male frogs at a site for a minimum of 15 min, and then searched the wetland and surrounding banks and vegetation with a headlamp for a minimum of 30 min. Frogs were captured, identified to species level, weighed and measured (snout-vent and limb lengths).

Developmental instability

Quantifying DI is a relatively novel method for determining the sub-lethal effects of habitat quality, and thus, provides a proxy for fitness. To calculate DI, I examined the total unsigned asymmetry of each frog (i.e. the difference between the left and right forelimbs, plus the corresponding value for the hindlimbs) using the blind protocol method developed by Alford and colleagues (1999). This procedure was conducted using digital calipers on 19 frogs collected by hand from quarry and reference wetlands.

Data analysis

Local habitat characteristics and environmental variables were separated into survey period, and compared between quarry and reference wetlands using t-tests. Species-specific abundance was estimated by categorising advertisement call intensity according to the index provided by Pope and colleagues (2000): (0) no individuals calling; (1) individual(s) can be counted with calls not overlapping; (2) calls of <15 individuals can be distinguished, but there is some overlapping; and (3) >15 individuals are calling. Indexed abundance data and species richness data were also compared between quarry and reference wetlands using t-tests.

To compare quarry and reference populations in terms of DI, I first partitioned the variation representing the different kinds of asymmetry (i.e. directional, fluctuating and antisymmetry) and measurement error. By calculating F-statistics from the appropriate Mean Square estimates (see Alford et al. 1999), I determined:

- (1.) Whether the degree of asymmetry can be used as an index of DI (Directional asymmetry: $F = MS_{\text{SIDE}} / MS_{\text{SIDE} \times \text{IND}}$). If $p < 0.05$, the degree of asymmetry cannot be used as an index of DI, as the ideal degree of symmetry cannot be known (i.e. structures appear to not be ideally bilateral).
- (2.) Whether measurement error is small relative to developmental instability (Fluctuating asymmetry + antisymmetry: $F = MS_{\text{SIDE} \times \text{IND}} / MS_{\text{SIDE} \times \text{IND} \times \text{REP}}$). If $p < 0.05$, measurement error is small relative to the levels of fluctuating asymmetry and antisymmetry in the data, and thus, DI can be compared between populations.
- (3.) How levels of DI differ between quarry and reference frog populations (Quarry vs Reference: $F = MS_{\text{SAMPLE} \times \text{IND} \times \text{SIDE}} / MS_{\text{SAMPLE} \times \text{IND} \times \text{SIDE} \times \text{REP}}$). If $p < 0.05$, levels of DI differ between populations.

SAMPLE: quarry or reference wetland; SIDE: left or right limbs; REP: replicate measurement number; IND: individual frog number. All analyses were performed on R, Version 3.3.1 (R Development Core Team 2016).

Educational guidebook and community engagement

I designed and created a detailed, 24-page guidebook titled 'Local amphibian fauna of the Kables Sands Quarry region' (Appendix 2). The guidebook includes introductory information about the quarry and amphibians, and details how the quarry aims to promote and enhance local amphibian biodiversity. Following this, basic biological information, a photograph of the species, and the IUCN conservation status is presented for the 25 amphibian species known to inhabit the Blue Mountains Region. Biological data were extracted from Antis (2013) and online sources (www.frogs.org). The guidebook finishes with proposed methods that individuals can easily adopt to promote amphibian biodiversity within their local community. The guidebook has been sent to 35 schools in the region surrounding the quarry (see Appendix 3 for a list of schools), as well as to quarry managers for further distribution to relevant stakeholders (e.g. staff, NGOs and local community members).

Limitations and biases

It is important to note that the reference wetlands selected for this study do not represent pristine natural sites, but rather were artificial wetlands in suburban areas. Therefore, there may be additional pressures on these wetlands, such as elevated pesticide or heavy metal levels, that quarry wetlands are not exposed to. Reference wetlands were chosen primarily for safety reasons, and despite not being pristine still serve as relevant and useful comparators to determine the suitability and ecological importance of quarry wetlands. In addition, I was limited in the number of potential species identified during surveys as the time-frame for the project did not include the spring/summer breeding season when most Australian species are active and calling (Anstis 2013).

Results

Site characteristics

Quarry and reference wetlands were similar (mean \pm standard error) in terms of their surface area (Q: 6650 \pm 2391m; R: 2200 \pm 566m; $T_7=1.57$, $p=0.15$) and perimeter (Q: 394 \pm 61m; R: 298 \pm 67m; $T_7=1.03$, $p=0.34$). Although the precise age of several reference wetlands was unknown, all quarry wetlands were younger than reference wetlands (Table 1). During the 1st and 2nd survey periods, water temperature was consistent between quarry and reference wetlands (1st: $T_6=0.92$, $p=0.39$; 2nd: $T_6=1.21$, $p=0.27$), whereas pH and salinity differed significantly. Quarry wetlands were consistently more acidic (1st: $T_6=3.03$, $p=0.02$; 2nd: $T_6=6.85$, $p<0.001$) and less saline (1st: $T_6=6.13$, $p<0.001$; 2nd: $T_6=9.65$, $p<0.001$) than reference wetlands during both survey periods (Table 1). The proportion of vegetation within each spatial region (i.e. open-water, submerged, emergent, fringing and floating) was comparable between quarry and reference wetlands (Table 1; $p>0.10$ for all comparisons). The detection of non-amphibian animals was low during all surveys: mosquitofish were observed within LP WL1 and LP WL2, trout were observed at Laidley, yabbies were observed at Geordie, and a tiger snake and water dragon were observed at the quarry wetland, T3 (Table 1).

Amphibian populations

Three amphibian species were heard calling and subsequently captured during the two survey periods: the Common Eastern Froglet *Crinia signifera*, the Southern Brown Treefrog *Litoria ewingii*, and the Striped Marsh Frog *Limnodynastes peronii* (Figure 2a). All quarry wetlands contained at least one species (mean richness = 1.8 ± 0.48), whereas only two of the five reference wetlands contained frogs (0.4 ± 0.25 ; $T_7=2.68$, $p=0.03$). Using the abundance index proposed by Pope and colleagues (2000), the mean abundance of *Crinia signifera* (the only species with sufficient presence to conduct formal analyses) was similar between quarry (1.5 ± 0.64) and reference wetlands (0.8 ± 0.49 ; $T_7=0.88$, $p=0.41$; Figure 2b). A breeding pair of *Crinia signifera* were observed in amplexus at the quarry wetland, T6. This was the only breeding observed during the survey periods.

Developmental instability

Only data for *Crinia signifera* were formally analysed. Preliminary analyses revealed no evidence for directional asymmetry in the frog population measured (forelimb: $F_{1, 15}=0.93$, $p=0.35$; hindlimb: $F_{1, 15}=0.33$, $p=0.58$), indicating that ideally, frog limbs should be symmetrical. Furthermore, measurement error was low relative to levels of fluctuating asymmetry and antisymmetry (forelimb: $F_{15, 30}=7.38$, $p<0.001$; hindlimb: $F_{15, 30}=7.14$, $p<0.001$), indicating that a comparison between populations is permissible. DI was lower within quarry wetlands (unsigned asymmetry (mm) = 0.14 ± 0.03) relative to reference wetlands (0.40 ± 0.04 ; Figure 2c), with both forelimb ($F_{5, 10}=11.78$, $p<0.001$) and hindlimb assymetry ($F_{5, 10}=4.11$, $p=0.027$; Table 2) significantly different between populations. Informal comparisons with values extracted from Alford and colleagues (2007) were made in order to consider the data from a more biologically relevant perspective (Figure 2c). Unsigned asymmetry in the quarry wetlands (0.14) was similar to the control group from Alford (0.14 and 0.21 for two frog species), whilst unsigned asymmetry in reference wetlands (0.40) was similar to the impact group from Alford which suffered population declines following a period of increasing DI (0.44 and 0.55).

Discussion

The number of high-quality natural wetlands available to aquatic animals has declined significantly in recent decades. Wetlands created for, utilised for, or inadvertently impacted by, human activities may be the most, or even the only, available inhabitable water body in many areas. Therefore, determining how these wetlands support wildlife is crucial for effective conservation planning to maintain populations and biodiversity. Here, I show that the quarry wetlands within the Kables Sands plant not only attract multiple frog species, but the individuals inhabiting these sites have high fitness and are breeding.

Of the three species present at the quarry, only *Crinia signifera* was present within reference wetlands. The mean abundance of *Crinia signifera* is greater at larger wetlands and at those with greater connectivity (Hamer et al. 2012). I found the site with the highest abundance of *Crinia signifera*, T3, to be the second smallest quarry wetland by area, but importantly, it had the greatest perimeter. Given that this species calls from the waters edge, a greater perimeter may permit more individuals to be present and calling at any one time (Antis 2013). Whilst salinity and pH was significantly higher within reference sites, levels found within all nine wetlands were well within the range deemed suitable for a significant proportion of amphibian species (Sadinski and Dunson 1992, Smith et al. 2007, Kearney et al. 2012). Although the vegetation structure surrounding and within quarry and reference wetlands was similar, sample sizes were fairly low with substantial variability among quarry wetlands. Therefore, comprehensive multivariate analyses to fully determine the environmental factors affecting amphibian presence and population sizes were not possible. Similar studies conducted within Australian wetlands, however, may shed light into the factors driving patterns and also provide information on how to best manage quarry wetlands. Both *Limnodynastes peronii* and *Litoria ewingii* occurrence probabilities correlate with the proportion cover of vegetation in the emergent and submerged zones, whereby at wetlands with >80% cover these species have a >0.8 probability of being found (Hamer et al. 2012). In addition, *L. peronii* occurrence is correlated to the depth of wetland shores, where gently sloping shores are preferred over steep drops (Hamer et al. 2012). The shoreline of one of the reference wetlands, LP WL1, was made up of a concrete wall, largely inappropriate for successful breeding in ground frog species (Parris 2006). Within the quarry, there was considerable inter- and intra-wetland variability in the steepness of the shoreline, and given other species within the region (e.g. *Limnodynastes dumerilii*) prefer steep shores, this variability likely enhances year-round biodiversity in quarries.

In addition to quantifying trends in species presence and population sizes within quarry wetlands, effective management also requires detailed knowledge of amphibian fitness. Since developmental stability occurs when genotypes repeatedly produce the same phenotype under the same environmental conditions during development (Zakharov 1992, Tracy et al. 1995), levels of deviation from stability (i.e. DI) provide information on environmental quality and individual health. Indeed, studies have shown that DI increases as health decreases, and amphibian population declines have preceded periods of increasing DI (Alford et al. 2007). I provide two lines of evidence suggesting quarry wetlands are providing a high-quality resource. Firstly, relative to reference wetlands, frogs within quarry wetlands had significantly lower levels of DI. Secondly, DI rates within quarry wetlands were comparable to those from stable frog populations observed elsewhere (see Alford et al. 2007). Importantly, quantifying DI provides only one estimate of fitness. Further research quantifying survival and reproduction, and determining how individual fitness translates to population level responses will more definitively evaluate the overall suitability of quarry wetlands.

Management implications and maximising the potential of quarry wetlands to enhance biodiversity

Empirical studies that monitor the relationship among population size, community composition, individual fitness and environmental factors is imperative to the long-term persistence of amphibians in quarry wetlands. I show that quarry wetlands provide good habitat for amphibians, and thus, they should be managed to enhance their suitability and attractiveness. Gallagher and colleagues (2014) suggest ensuring high-quality wetlands are connected to other wetlands, with their hydroperiod and vegetation managed to promote survival and reproductive success. Measures to remove exotic predators, such as mosquitofish and carp, will also greatly enhance amphibian populations and communities, with probable improvements to individual fitness (Maezono and Miyashita 2004, Tsunoda et al. 2010). To further promote and enhance amphibian biodiversity within quarries, it is important to provide substantial environmental heterogeneity; a mosaic of waterbody types and terrestrial refuges. Creating a diverse mosaic of wetlands that vary in size and depth will help ensure amphibian breeding at a proportion of quarry wetlands each season, thereby enhancing overall population persistence (Rannap et al. 2009). Creating wetlands with a minimum of 80% emergent vegetation would help ensure a high probability of many species occurring, including *Limnodynastes peronii* and *Litoria ewingii* (Hamer et al. 2012). In quarries with existing wetlands, such as Kables Sands, implementing topographical modifications and strategic

planting of native vegetation will ensure sufficient heterogeneous habitat to cater for as many species as possible. Although the quarry wetlands studied here were fairly well connected to one another and also to quality terrestrial areas, two of the larger wetlands – the settlement dam and T6 – had sparse vegetation in the emergent and submerged zones. Given many frogs oviposit their eggs within this vegetation, which in turn provides refuge and protection to tadpoles, planting native vegetation around and within these wetlands would greatly increase the probability of colonisation and, thus, enhance biodiversity within the site.

Project implementation

Identifying amphibian species presence and population size, and quantifying fitness through measuring limb lengths are economically and logistically feasible methods to assess the health of quarry wetlands. Each amphibian species has a distinct call, so quarry staff could be trained to identify and catalogue species presence. Alternatively, a dictaphone could be used to record calls and the audio sent to an expert for identification. Ideally, surveys would be conducted year-round in order to identify all the species present at each site. Repeat surveys would also allow the detection of endangered amphibian species that may inhabit quarries. Measuring DI is also a relatively simple process that could be taught and employed periodically throughout the year. Coupled with the water quality data often already collected on-site, levels of DI could provide an early warning indicator of potential issues, allowing mitigation strategies to be implemented prior to population declines. As researchers elsewhere adopt DI as a fitness proxy, their data can be used to compare levels observed in quarry wetlands, and ultimately, evaluate population health.

Providing the general public and schools with guidebooks such as the one created here will not only act to enhance the perception of the quarry, but will enhance overall interest in the conservation of local wildlife. These informative references require only basic biological information and are easily produced by non-scientists. Although specific costs of implementing these proposed methodologies is site dependent, I estimate that one or two survey nights per month conducted by one employee would be sufficient to document species presence and quantify levels of DI. Compiling a guidebook should similarly be a quick process given the wealth of freely available information online and in textbooks. Designing, assembling and distributing eBook copies could potentially be accomplished within one week at little cost. Overall, the methods outlined here provide a powerful, yet simple, tool to assess the overall health of quarry wetlands that could be easily adopted and implemented at quarries anywhere in the world.

Conclusion

The creation of wetlands during and following mining processes is inevitable. Given the rate at which natural wetlands are being lost and degraded, the ecological importance of these wetlands has never been more important. Here, I show that quarry wetlands within the Kables Sands plant are not only attracting amphibians, but also providing conditions conducive to high fitness and breeding activity. Relative to other wetlands in this region, quarry wetlands consistently harboured more species of frog, as well as healthier individuals. Several management practices could further enhance the role quarry wetlands play in promoting amphibian biodiversity, such as planting out emergent zones with native vegetation, and creating a mosaic of wetland types. Implementing basic surveys and promoting public awareness and education at other quarries around the globe is an economically and logistically feasible strategy to ensure quarry wetlands are, and remain, high-quality ecosystems capable of adding to local and regional biodiversity.

Acknowledgements and involvement

Although I conducted this research alone, there are a number of people who greatly assisted me throughout the whole process. I would firstly like to thank all employees from the Kables Sands Plant who gave me access to their site to conduct surveys, especially Paul and Wayne. Secondly, a big thanks to all associated with the Quarry Life Award, including HeidelbergCement, the national and international judges, and the Sustainability Manager, Paul Timmins. Best of luck to the other participants, Elizabeth, Luke and Michelle; I hope your projects were all hugely successful. Finally, thank you to my supervisors in Melbourne for allowing me the time off to conduct research for this valuable initiative.

Table 1. Site specific characteristics including: general wetland information, water quality data, weather data, the percentage cover of vegetation in various regions within wetlands, and the presence of non-amphibian species (see methods for descriptions of variables). Reference wetlands (ref) – Geordie: Geordie St Wetland; Laidley: Laidley St Wetland; Clwydd: Vale of Clwydd Wetland; LP WL1: Lake Pillans Wetland 1; LP WL2: Lake Pillans Wetland 2. Quarry wetlands (Quarry) – SD: Settlement Dam; Swamp: Quarry Swamp; T6: Tailings Storage Cell; T3: Reservoir.

	Geor- die	Laidley	Clwydd	LP WL1	LP WL2	SD	Swamp	T6	T3
Site type	Ref	Ref	Ref	Ref	Ref	Quarry	Quarry	Quarry	Quarry
Area (m ²)	1667	461	2678	3888	2305	12420	1831	8588	3760
Perimeter (m)	272	134	514	369	199	453	225	391	507
Approx. age (years)	100+*	100+*	100+*	105	105	10	10	30	50
<i>Survey period 1 (30/03/16 - 01/04/16)</i>									
Water temp (°C)	15.7	15.8	15.0	17.9	17.2	16.5	-	17.5	17.0
pH	7.58	7.76	7.22	7.63	7.40	7.35	-	6.10	6.22
Salinity (µS)	133.2	88.1	157.0	147.2	166.6	30.1	-	25.4	20.8
Mean min air temp (°C)					10.9				
Mean max air temp (°C)					19.5				
Mean humidity (%)					70.0				
Total rainfall (mm)					0.0				
<i>Survey period 2 (20/07/16 - 21/07/16)</i>									
Water temp (°C)	10.1	10.4	10.9	11.0	11.7	10.8	-	10.1	10.1
pH	7.02	7.49	7.14	7.60	7.51	6.00	-	5.80	5.10
Salinity (µS)	176.4	188.8	199.2	138.5	148.7	20.4	-	16.5	23.8
Mean min air temp (°C)					8.1				
Mean max air temp (°C)					15.9				
Mean humidity (%)					98.0				
Total rainfall (mm)					19.8				
<i>Percentage vegetation</i>									
Open-water	5%	20%	90%	10%	50%	0%	90%	0%	0%
Submerged	0%	20%	60%	10%	0%	0%	90%	0%	0%
Emergent	10%	20%	90%	20%	80%	10%	100%	3%	30%
Fringing	80%	100%	100%	100%	100%	5%	100%	30%	90%
Floating	0%	0%	20%	0%	0%	0%	0%	0%	0%
<i>Non-amphibian animal presence</i>									
Mosquitofish				Y	Y				
Tiger snake									Y
Trout		Y							
Water dragon									Y
Yabby	Y								

*Age of wetlands could not be precisely determined. However, all are in-line wetlands connected to long-established creeks or manmade channels formed or constructed, respectively, over 100 years ago.

Note: Water quality variables could not be measured within the swamp due to safety concerns with entering this site.

Table 2. ANOVA for determining the presence of directional asymmetry and fluctuating asymmetry (FA) plus antisymmetry for both forelimbs and hindlimbs. A significant p-value for directional asymmetry indicates that the degree of asymmetry cannot be used as an index of DI, as the ideal degree of symmetry cannot be known (i.e. structures appear to not be ideally bilateral). A significant p-value for FA + antisymmetry indicates that measurement error is small relative to the levels of fluctuating asymmetry and antisymmetry in the data, and thus, levels of developmental instability can be compared between populations (see Table 3). SIDE: left or right limbs; REP: replicate measurement number; IND: individual frog number.

Factor	df	Forelimb	Hindlimb
		Mean Square	
SIDE	1	0.023	0.013
REP	2	0.000	0.002
SIDE * REP	2	0.000	0.001
IND	15	1.233	3.199
SIDE * IND	15	0.025	0.041
REP * IND	30	0.003	0.006
SIDE * REP * IND	30	0.003	0.006
Directional asymmetry ($MS_{SIDE} / MS_{SIDE*IND}$)		$F_{1,15}=0.93, p=0.35$	$F_{1,15}=0.33, p=0.58$
FA + antisymmetry ($MS_{SIDE*IND} / MS_{SIDE*IND*REP}$)		$F_{15,30}=7.38, p<0.001$	$F_{15,30}=7.14, p<0.001$

Table 3. ANOVA for comparing levels of developmental instability in the Common Eastern Froglet (*Crinia signifera*) from quarry and reference wetlands (SAMPLE). To statistically compare samples, a full four-way ANOVA is conducted and the variance components are extracted. The F-statistic is calculated from the Mean Square of the three-way interaction of sample, side and individual divided by the Mean Square of the four-way interaction of sample, side, individual and replicate measurement. A significant p-value indicates a difference between quarry and reference sites. Abbreviations as in Table 2

Factor	df	Forelimb	Hindlimb
		Mean Square	
SAMPLE	1	0.475	1.931
SIDE	1	0.023	0.013
REP	2	0.000	0.002
IND	9	1.582	4.362
SAMPLE * SIDE	1	0.013	0.019
SAMPLE * REP	2	0.001	0.001
SIDE * REP	2	0.000	0.001
SAMPLE * IND	5	0.756	1.360
SIDE * IND	9	0.019	0.047
REP * IND	18	0.004	0.007
SIDE * REP * IND	2	0.001	0.002
SAMPLE * IND * SIDE	5	0.038	0.034
SAMPLE * IND * REP	10	0.003	0.007
SIDE * REP * IND	18	0.004	0.005
SAMPLE * IND * SIDE * REP	10	0.003	0.008
Quarry vs Reference ($MS_{SAMPLE*IND*SIDE} / MS_{SAMPLE*IND*SIDE*REP}$)		$F_{5,10}=11.78, p<0.001$	$F_{5,10}=4.11, p=0.027$

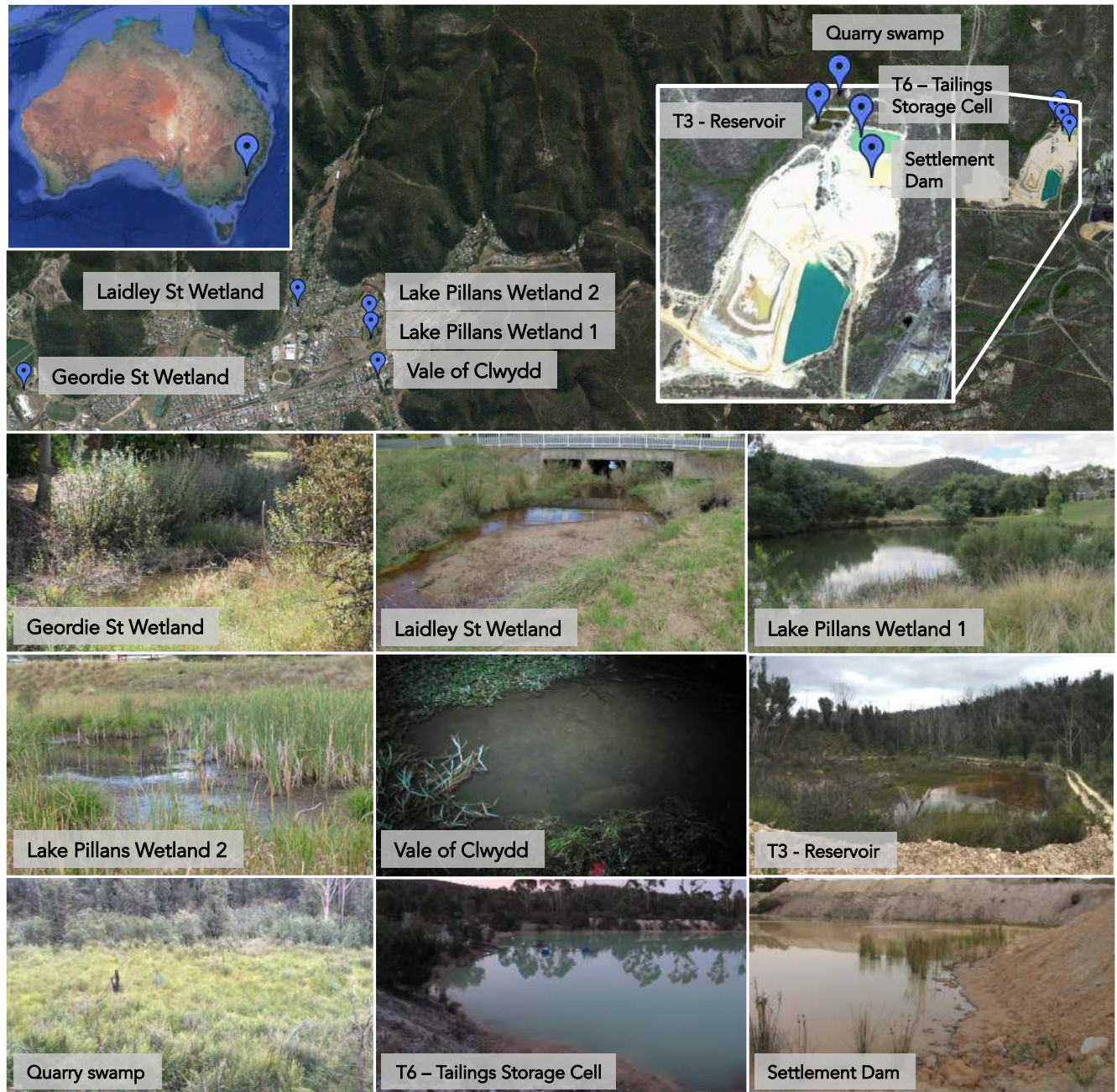


Figure 1. Map of the study area (the suburbs of Lithgow and Clarence, New South Wales, Australia), with the Kables Sands Quarry expanded inset, showing site location. Photographs of the nine wetlands surveyed for amphibian community richness, population density and developmental instability throughout this study. Reference wetlands: Lake Pillans Wetland 1, Lake Pillans Wetland 2, Laidley St Wetland, Georgie St Wetland and Vale of Clwydd Wetland. Quarry wetlands: T3 – Reservoir, Settlement Dam, T6 - Tailings Storage Cell and Quarry Swamp.

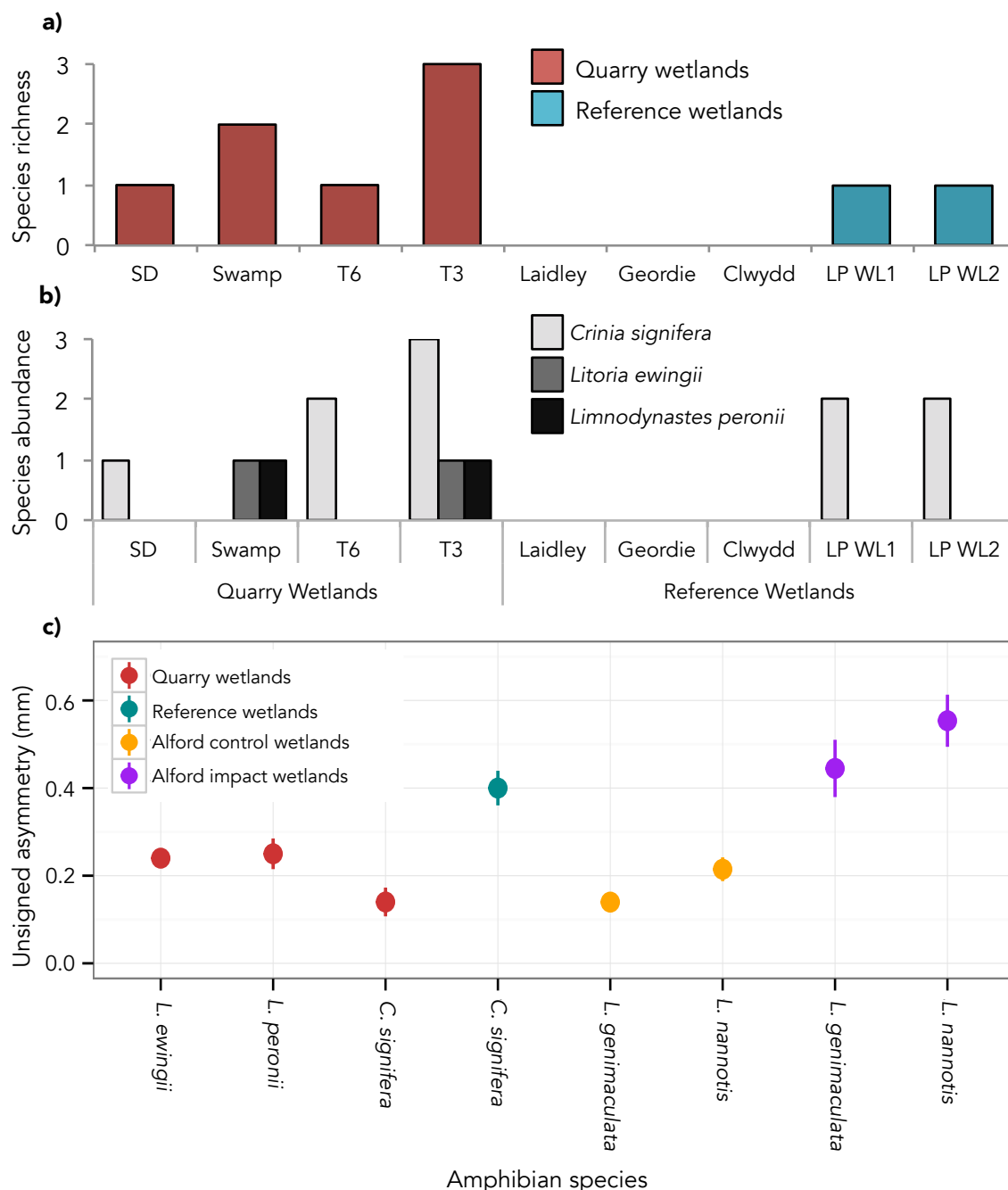


Figure 2. Mean (\pm standard error) amphibian (a) species richness, (b) species abundance and (c) developmental instability (DI; quantified as unsigned asymmetry) within quarry and reference wetlands. Abundance was estimated by categorising advertisement call intensity according to the index provided by Pope and colleagues (2000): (0) no individuals calling; (1) individual(s) can be counted with calls not overlapping; (2) calls of <15 individuals can be distinguished, but there is some overlapping; and (3) >15 individuals are calling. DI values are also shown from Alford et al 2007 for informal comparison. Frog populations from Alford control sites remained stable, whilst those from impact sites exhibited substantial declines following a period of increasing DI. Formal statistical analysis was conducted comparing *C. signifera* individuals from quarry and reference sites, with quarry frogs having significantly lower levels of DI ($p < 0.05$).

To be kept and filled in at the end of your report

Project tags (select all appropriate):

This will be used to classify your project in the project archive (that is also available online)

Project focus:

- ☒ **Biodiversity management**
- ☐ Cooperation programmes
- ☒ **Education and Raising awareness**
- ☐ Endangered and protected species
- ☐ Invasive species
- ☐ Landscape management - rehabilitation
- ☐ Rehabilitation
- ☒ **Scientific research**
- ☐ Soil management
- ☐ Urban ecology
- ☐ Water management

Flora:

- ☐ Conifers and cycads
- ☐ Ferns
- ☐ Flowering plants
- ☐ Fungi
- ☐ Mosses and liverworts

Fauna:

- ☒ **Amphibians**
- ☐ Birds
- ☐ Dragonflies & Butterflies
- ☐ Fish
- ☐ Mammals
- ☐ Reptiles
- ☐ Spiders
- ☐ Other insects
- ☐ Other species

Habitat:

- ☐ Cave
- ☐ Cliffs
- ☐ Fields - crops/culture
- ☐ Forest
- ☐ Grassland
- ☐ Human settlement
- ☐ Open areas of rocky grounds
- ☐ Recreational areas
- ☐ Screes
- ☐ Shrubs & groves
- ☐ Soil
- ☐ Wander biotopes
- ☒ **Water bodies (flowing, standing)**
- ☒ **Wetland**

Stakeholders:

- ☐ Authorities
- ☒ **Local community**
- ☒ **NGOs**
- ☒ **Schools**
- ☐ Universities

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Local amphibian fauna of the Kables Sands Quarry region

New South Wales
Australia

Created by Michael Sievers

Kables Sands Quarry

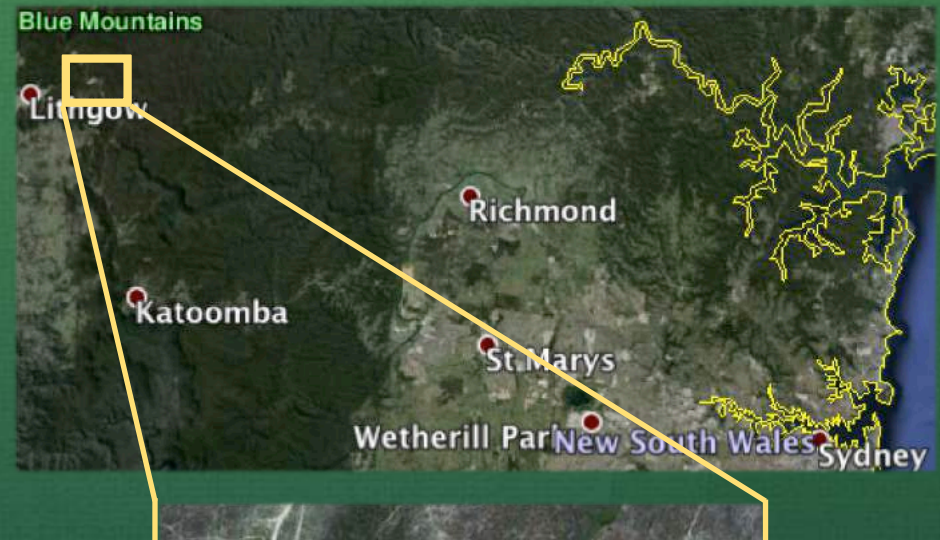
Clarence, NSW Australia

- Owned by HeidelbergCement:
More than 45,000 people employed in 40 countries
- Extraction of sandstone and the production of sand at the Clarence site began 60 years ago in 1956
- Approximately 350,000 tonnes extracted every year
- The site is continually undergoing extensive environmental / ecological restoration



Kables Sands Quarry

- During extraction and preparation processes, various wetlands are constructed or inadvertently form
- These wetlands can be a valuable resource to local animals, in particular those heavily reliant on water to complete their lifecycle such as amphibians



Amphibians

- Frogs, toads, newts & salamanders
- Over 7,000 species worldwide
- Aquatic larval stage & semi-aquatic adult stage
- Smallest: PNG frog – less than 1cm
- Biggest: Chinese Giant Salamander – 180cm



Amphibians – Frogs and toads

- They live in lots of places: wetlands, lakes, streams, rivers, ponds, marshes, swamps and maybe even your backyard!
- Important for a healthy environment

Egg → Hatchling → Tadpole → Frog



Life cycle of the Spotted Marsh Frog - *Limnodynastes tasmaniensis*

Amphibians in decline

- Globally, amphibian populations are in decline
- Numerous threats:
 - Habitat loss
 - Habitat fragmentation
 - Pollution
 - Chytrid fungus
 - Climate change
- We need to study amphibians in order to preserve and protect them



The highly vulnerable
Green and Golden Bell Frog

Quarries and amphibian conservation

HeidelbergCement's corporate mission is not only to excel in terms of economic performance, but also to act in an ecologically and socially responsible way.

- Given habitat loss is a primary threat facing amphibians, the wetlands created at quarries provide much needed habitat for frogs and toads
- Unlike other mining industries (e.g. for fossil fuels), water quality at sand quarries is relatively high quality and suitable for amphibian breeding



Amphibians of the Kables Sands Quarry and surrounding regions: Using this guidebook

Scientific name – Genus
and species

Common name – What
most people call it



Adult length: Full grown size

Call: The sound male amphibians make to attract a female to breed with

Breeding: The main seasons of the year the species is breeding

Conservation status based on the International Union for Conservation of Nature (IUCN) Red List



Tree frogs - Hylidae

Litoria aurea
Green and Golden Bell Frog



Adult length: Males to 69mm, females to 108mm

Call: "cr-a-a-aw-a-a-awk cra-a-a-awk crok crok"

Breeding: Spring to summer

Litoria booroolongensis
Booroolong Frog



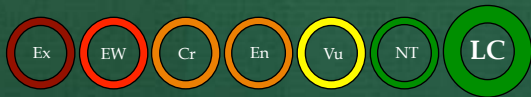
Adult length: Males to 42mm, females to 54mm

Call: "purrrrrrrrr"

Breeding: Spring to summer

Tree frogs - Hylidae

Litoria caerulea
Green Tree Frog



Adult length: Males to 77mm, females to 110mm

Call: "crawk, crawk, crawk..."

Breeding: Spring to autumn

Litoria citropa
Blue Mountains Tree Frog



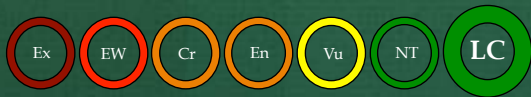
Adult length: Males to 57mm, females to 65mm

Call: "war-r-r-rk cruk-cruk cruk-cruk cruk-cruk cruk"

Breeding: Late winter to early summer

Tree frogs - Hylidae

Litoria dentata
Bleating Tree Frog



Adult length: Males to 40mm, females to 44mm

Call: "b-r-r-r-e-e-e-e-e-e"

Breeding: Spring to summer

Litoria ewingii
Southern Brown Tree Frog



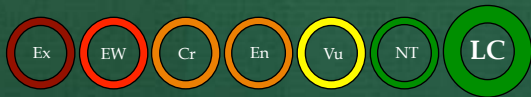
Adult length: Males to 40mm, females to 46mm

Call: "creeeeeeee creee creee creee creee"

Breeding: All year

Tree frogs - Hylidae

Litoria fallax
Eastern Dwarf Tree Frog



Adult length: Males to 26mm, females to 32mm

Call: "wre-e-e-e-k, pip-pip"

Breeding: All year

Litoria latopalmata
Broad-palmed Frog



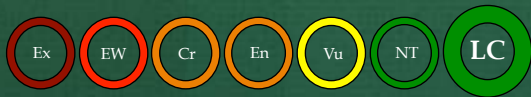
Adult length: Males to 39mm, females to 42mm

Call: "yap, yap, yap..."

Breeding: Spring to summer

Tree frogs - Hylidae

Litoria lesueuri
Lesueur's Tree Frog



Adult length: Males to 40mm, females to 61mm

Call: "creww crewwk crewwwwk crewwwwk"

Breeding: Spring to summer

Litoria littlejohni
Littlejohn's Tree Frog



Adult length: Males to 51mm, females to 68mm

Call: "creeeep creeeep creeeeeeep" or "reet reet reet"

Breeding: Late winter to spring

Tree frogs - Hylidae

Litoria peronii
Peron's Tree Frog



Adult length: Males to 53mm, females to 70mm

Call: "cra-ah-ah-ah-ah-ah-ah-ahhk"

Breeding: Early spring to early summer

Litoria phyllochroa
Green Stream Frog



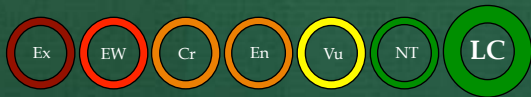
Adult length: Males to 32mm, females to 40mm

Call: "ik, ik, ik, iiiiii-k, iiiiii-k"

Breeding: Spring to summer

Tree frogs - Hylidae

Litoria verreauxii
Verreaux's Tree Frog



Adult length: Males to 36mm, females to 36mm

Call: "tweee tweee tweee twee...."

Breeding: Late winter to summer

Litoria wilcoxii
Eastern Stony Creek Frog



Adult length: Males to 48mm, females to 69mm

Call: Repeated 'soft trill', a soft "whirring" or a "soft purring".

Breeding: Spring to summer

Ground frogs - Myobatrachidae

Crinia parinsignifera
Eastern Sign-bearing Froglet



Adult length: Males to 22mm, females to 23mm

Call: "peep, ke-chik, ke-chik, ke-chik"

Breeding: Late winter to autumn

Crinia signifera
Eastern Common Froglet



Adult length: Males to 25mm, females to 29mm

Call: "crick crick crick crick crick"

Breeding: Autumn to spring

Ground frogs - Myobatrachidae

Limnodynastes dumerilii
Eastern Banjo Frog



Adult length: Males to 70mm, females to 73mm

Call: "bonk bonk bonk bonk"

Breeding: Spring to autumn

Limnodynastes peronii
Striped Marsh Frog



Adult length: Males to 69mm, females to 73mm

Call: "tock" or "poc"

Breeding: Spring to autumn

Ground frogs - Myobatrachidae

Limnodynastes tasmaniensis
Spotted Marsh Frog



Adult length: Males to 42mm, females to 47mm

Call: "click, click, click"

Breeding: Spring to autumn

Mixophyes balbus
Southern Barred Frog



Adult length: Males to 65mm, females to 100mm

Call: "kook kook kook kra-a-ak kruk kruk"

Breeding: Spring to autumn

Ground frogs - Myobatrachidae

Mixophyes fasciolatus
Great Barred Frog



Adult length: Males to 65mm, females to 101mm

Call: "wark, wark, wark..."

Breeding: Spring to autumn

Platyplectrum ornatum
Ornate Burrowing Frog



Adult length: Males to 41mm, females to 42mm

Call: "unk, unk..."

Breeding: Spring to autumn

Ground frogs - Myobatrachidae

Pseudophryne australis
Red-crowned Toadlet



Adult length: Males to 28mm, females to 30mm

Call: "nee-ak"

Breeding: All year

Pseudophryne bibronii
Rbibron's Toadlet



Adult length: Males to 30mm, females to 32mm

Call: "ark, ark..."

Breeding: All year

Ground frogs - Myobatrachidae

Uperoleia laevis
Smooth Toadlet



Adult length: Males to 28mm, females to 32mm

Call: "yerp, yerp, yerp..."

Breeding: All year

What can you do to help frogs and toads?

- Below are some handy tips for helping your local amphibians. Consult the internet for many more good ideas!

Protect existing habitat through education

- Educate others about the importance of protecting existing natural surroundings and how to keep your watershed healthy.

Landscape naturally

- Design your backyards in a way that helps local amphibians: plant natives and have wet areas.

Create your own pond

- Building or buying a pond is one of the best ways to increase the habitat available to local amphibians.

Reduce, reuse and recycle

- Reducing, reusing and recycling all forms of material goods will reduce our reliance on the environment and ultimately help save amphibian habitat.

Create a frog or toad house

- Amphibian houses can be created as simply as turning a ceramic pot upside down, cutting an entrance and placing it in an appropriate place.

Reduce the use of chemicals

- Pesticides and herbicides in particular impact wild amphibians. Avoid using them in your backyards.

Do not release pets

- Releasing pet fish and amphibians into the wild can introduce diseases into natural populations and severely impact the environment.

Help scientist

- Participate in scientific monitoring projects in your local area.

Acknowledgments

This guidebook would not have been possible without Wikimedia Commons and the following authors/photographers:

Abribus

Bidgee

Benjamin444

Donna Flynn

Froggydarb

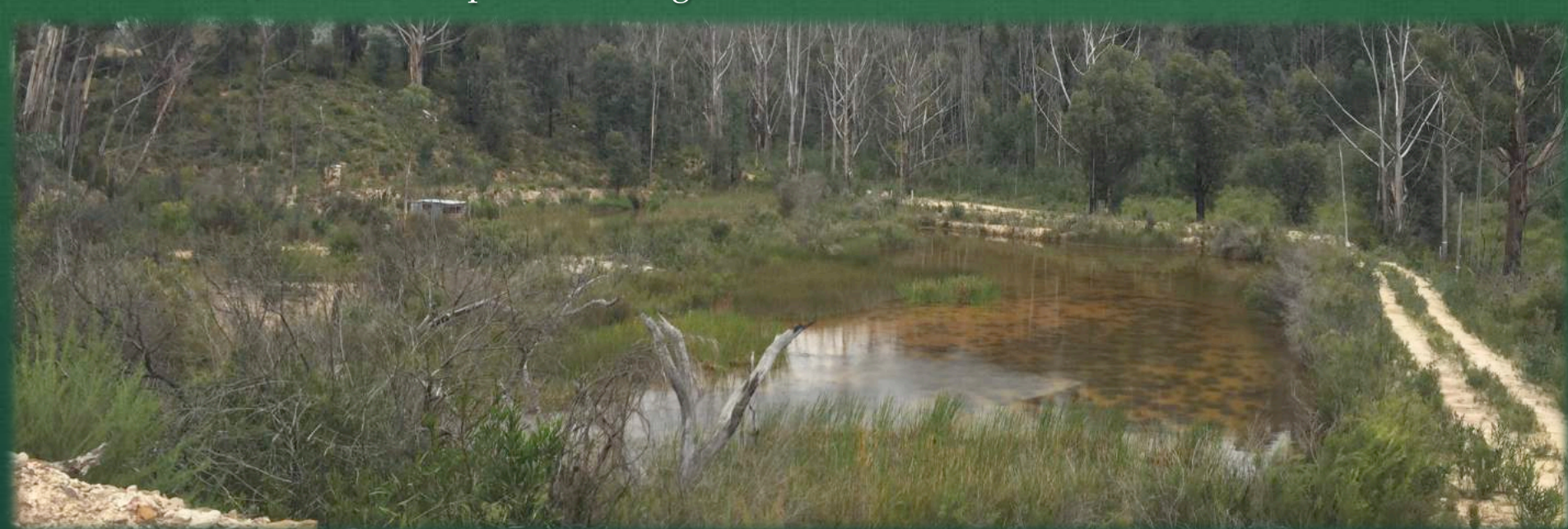
LadyofHats

LiquidGhoul

Retama

Tnarg 12345

Anstis, M. 2013. Tadpoles and frogs of Australia. New Holland Publishers. CSIRO.





Local flora and fauna



Appendix 3. List of the 35 schools that received the amphibian guidebook, *Local amphibian fauna of the Kables Sands Quarry region*.

School	Address	E-mail address
All Saints' College	70 Eglinton Rd, Bathurst NSW 2795	admin@saints.nsw.edu.au
Bathurst High Campus	Hope St, Bathurst NSW 2795	bathurst-h.school@det.nsw.edu.au
Bathurst South Public School	251 Havannah St, Bathurst NSW 2795	bathursts-p.school@det.nsw.edu.au
Bathurst West Public School	Suttor St, Bathurst NSW 2795	bathurstw-p.school@det.nsw.edu.au
Blue Mountains Grammar Preparatory School	Tusculum Rd, Valley Heights NSW 2777	registrar@bmgs.nsw.edu.au
Blue Mountains Grammar School	3 Matcham Ave, Wentworth Falls NSW 2782	registrar@bmgs.nsw.edu.au
Blue Mountains Steiner School	83 Clearview Parade, Hazelbrook NSW 2779	info@bluemountainssteiner.nsw.edu.au
Carenne School	158 Browning St, Bathurst NSW 2795	carenne-s.school@det.nsw.edu.au
Cooerwull Public School	319 Main Street, Lithgow NSW 2790	cooerwull-p.school@det.nsw.edu.au
Cullen Bullen Public School	15-23 Castlereagh Hwy, Cullen Bullen NSW 2790	cullenbull-p.school@det.nsw.edu.au
Holroyd School	Willara Ave, Merrylands NSW 2160	holroyd-s.school@det.nsw.edu.au
Holy Family Primary School	French Smith Place, Kelso NSW 2795	holyfamilykelso@bth.catholic.edu.au
Jack And Jill Preschool	Corner of Beaufort St & Bren St, Lithgow 2790	contact@jackandjillpreschool.com.au
Katoomba Public School	Merriwa St, Katoomba NSW 2780	katoomba-p.school@det.nsw.edu.au
Kelso Public School	19 Gilmour St, Kelso NSW 2795	kelso-p.school@det.nsw.edu.au
Korowal School	54 Hall Parade, Hazelbrook NSW 2779	info@korowal.nsw.edu.au
La Salle Academy	96 Rabaul St, Littleton NSW 2790	lasallelithgow@bth.catholic.edu.au
Lawson Public School	Adelaide St, Lawson NSW 2783	lawson-p.school@det.nsw.edu.au
Leichhardt Public School	Marion St, Leichhardt NSW 2040	leichhardt-p.School@det.nsw.edu.au
Lithgow High School	1A Pau Street, Lithgow NSW 2790	lithgow-h.school@det.nsw.edu.au
Lithgow Public School	Lithgow Primary School Mort St, Lithgow NSW 2790	lithgow-p.school@det.nsw.edu.au
MacKillop College	Gormans Hill Rd, Bathurst NSW 2795	mackillop@bth.catholic.edu.au
Meadow Flat Public School	2630 Great Western Hwy, Meadow Flat NSW 2795	meadowflat-p.school@det.nsw.edu.au
Mount Carmel Catholic College	210 Spitfire Dr, Varroville NSW 2566	info@mcccdow.catholic.edu.au
O'Connell Public School	15 Blacks Mill Ln, O'Connell NSW 2795	oconnell-p.school@det.nsw.edu.au
Portland Central School	Vale St, Portland NSW 2847	portland-c.school@det.nsw.edu.au
Raglan Public School	Nelson St, Raglan NSW 2795	raglan-p.school@det.nsw.edu.au

Springwood Public School	Burns Rd, Springwood NSW 2777	springwood-p.school@det.nsw.edu.au
St Joseph's School Portland	95 Williwa St, Portland NSW 2847	stjosephportland@bth.catholic.edu.au
St Stanislaus' College	220 Bentinck St, Bathurst NSW 2795	registrar@stannies.com
Stella Maris College	52 Eurobin Ave, Manly NSW 2095	administration@stellamaris.nsw.edu.au
The Scots School	1 Col Drewe Dr, South Bowenfels NSW 2790	scots@scots.nsw.edu.au
The Scots School Bathurst	4173 O'Connell Rd, White Rock NSW 2795	scots@scots.nsw.edu.au
Wallerawang Public School	Barton Ave, Wallerawang NSW 2845	wallerawan-p.school@det.nsw.edu.au
Zig Zag Public School	23 Victoria Ave, Lithgow NSW 2790	zigzag-p.school@det.nsw.edu.au
