Technical advice for the development of frog-monitoring guidelines informing community-led surveys in Melbourne Water's catchment management area

Prepared for Melbourne Water

Report 18033, Version 5.0
July 2026

Abzeco Pty Ltd. ABN 93113245435 – Suite 1, 4 Brisbane St. Eltham 3095. T:(03) 9431 5444 F:(03) 9431 5443 www.abzeco.com.au

Contents

Cover photo: Community members surveying for frogs using Melbourne Water's Frog Census app (photo courtesy of Sharon Blance). **Inset:** A Common Froglet *Crinia signifera* making advertisement calls (hence a male), showing its inflated vocal sac (photo courtesy of Craig Cleeland).

Version Control

Version	Responsibility	Name	Date	Signature
1.0	First draft	David De Angelis	27 October 2025	
2.0	Second draft	David De Angelis	15 January 2026	
3.0	Third draft	David De Angelis	29 April 2026	
3.0	Review	Kirsten Paris	14 May 2026	
4.0	Fourth draft	David De Angelis	9 June 2026	
5.0	Fifth (final) draft	David De Angelis	15 July 2026	

Acknowledgements

Thanks to Jane Petch and Will Steele (Melbourne Water) for providing background information on the current and previous projects to develop community monitoring guidelines for Melbourne Water's Frog Census program. Photos not taken by the author were provided by Sharon Blance, Craig Cleeland, Josh Griffiths (EnviroDNA) and Brendan Casey, acknowledged in the relevant sections of this report. Zoe Daniel and Lachlan King assisted with frog call data collation and analysis. Kirsten Parris (University of Melbourne) reviewed and provided feedback on an earlier draft of these guidelines.

1 Introduction

Across its catchment management region, Melbourne Water manages a large network of reserves, waterbodies and waterways that provide habitat for a range of native aquatic and semi-aquatic species, including frogs. Through Melbourne Water's Frog Census program, members of the public have the opportunity to become citizen scientists, contributing valuable data on the occurrence and distribution of frogs in the greater Melbourne area and surrounds, including for threatened species and some that have been introduced there. These data have the potential to inform the management of wetlands and waterways for these species, including through regulating water levels, guiding appropriate revegetation and weed control works, and the creation of new waterbodies and surrounding terrestrial habitat for frogs.

The development of guidelines for the community-led monitoring of frogs is mainly intended to inform the establishment of longer-term monitoring programs and more intensive survey efforts by community groups and individuals interested in obtaining data on the occurrence of frogs in areas they work in or regularly visit, across different times of the year. This builds on work by Hamer *et al.* (2010) intended to inform community frog-monitoring options for Melbourne Water. While several aspects of that work were not ultimately used to inform Melbourne Water's Frog Census program, other recommendations were, or have otherwise been added to this guidance with acknowledgment.

It is important to note that depending on the main question/s being asked by the community group or individual (e.g., assessing if a particular species is likely to be present at a wetland vs tracking the local distribution or calling intensity of a frog population over time), the monitoring methods used or other important survey considerations (such as seasonal timing and duration) are likely to vary. Melbourne Water's online frog monitoring tool, informed by this document, is intended to guide these choices. However, it is also recognised that survey regimes need to be reasonably user-friendly and achievable for members of the community working with limited time, site accessibility and expert knowledge.

In addition to methods readily useable by citizen scientists, a number of techniques are also mentioned that usually require a higher level of expertise and permissions to carry out (e.g., surveys to gauge breeding success, levels of disease, and obtain more quantitative abundance data). While these additional methods are touched on, rather than explaining them in great detail, relevant references are provided if readers are keen to find out more information. In any case, such methods can only be used by community groups and individuals who have adequate expertise and hold the necessary authorisations, or more likely, are able to partner with experts who do.

Melbourne Water's guidance also needs to be aimed at informing and reducing risks associated with monitoring locations where threatened species are known to occur or might otherwise be present, including the risks of spreading disease (mainly the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis*), disturbing habitats during surveys, and divulging those locations to members of the public who might result in those impacts.

Although the advice in this document is tailored to the Melbourne Water Catchment Management Authority (CMA) area, it is likely to be more broadly applicable (e.g., to nearby parts of the ranges of the various species mentioned, if not beyond). Some of it might also be useful for researchers working on frog monitoring projects alongside community groups or volunteers, or in some cases help guide professionals to additional information on aspects of frog monitoring being planned independently of community efforts.

2 Choosing a question or reason and considering requirements for monitoring

Although often associated with a specific question or scientific aim and corresponding experimental design, not all frog monitoring needs to be structured in such a way to be of value (e.g., for informing habitat protection, if a threatened species happened to be detected during a single opportunistic recording at a wetland using Melbourne Water's Frog Census app). In such cases, the objective might be more about contributing occasional or sporadic recordings to the broader Frog Census pool of data, and have an educational focus (i.e., providing opportunities to increase your familiarity with local frog species and safely recording their calls).

However, to maximise the use of monitoring data (e.g., for informing conservation and broader ecological questions), it needs to be collected through a well-designed and scientifically-informed framework, for example maintaining consistent sampling protocols, accounting for observer variation and ensuring accurate record keeping (Lindenmayer and Likens 2018).

If it is determined that a monitoring design is actually needed (e.g., the question being asked hasn't already largely been looked into by others, there is a real prospect of using the data to inform management, and a perceived or real need to gain new knowledge cannot be addressed through an alternative approach), additional things that need to be considered include the availability of funding or in-kind support, having access to expertise and ongoing commitment by key personnel, and having contingencies in place (e.g., to minimise interruptions to the data collection in the case of unexpected events) (Lindenmayer and Likens 2018).

Once it is determined that monitoring is needed, the most appropriate type of survey will need to be considered based on the objective or question being asked. This might either be an ongoing census or inventory (assessing species occupancy, as the Frog Census does), surveillance for disease (e.g., the Amphibian Chytrid Fungus) or responses of populations to environmental changes, and comparing the trajectory of populations against baseline or background abundance data (Wilkinson 2015). Once the type of survey and methods have been determined (recommended to be done in collaboration with an expert), you will then need to consider what equipment (including for hygiene) and other resources will be needed (including time and funding), if any and which permissions are likely to be required, how you will collect the data, and what the resulting outputs will be. These guidelines and Melbourne Water's online frog monitoring tool will help to inform this to an extent, although additional expertise should be sought wherever it is deemed it might be helpful.

3 Establishing and surveying monitoring sites for frogs

3.1 Choosing sites to monitor

Where the objective is to maximise the potential to record a number of different frog species or one or more specific targets, participants should aim for potential breeding sites (where most frogs are expected to be calling from or nearby). This means standing, slow-flowing or still water for most frog species in the Melbourne area (e.g. ponds, dams, lakes and pools in slow-flowing creeks) (Figure 1a). However, for the few "land-laying" local frogs that call (and breed) mainly in autumn (the Victorian Smooth Froglet *Geocrinia victoriana*, Brown or Bibron's Toadlet *Pseudophryne bibronii* and Southern Toadlet *P. semimarmorata*), breeding sites often consist of seasonally dry drainage lines, the shallow edges of dams, or shallow depressions that are usually dry in summer and autumn (Figure 1b). Conversely, Lesueur's Frog or the Stony Creek Frog *Rhyaconastes* (formerly *Litoria*) *lesueuri* is the only species recorded in Melbourne Water's catchment management area that regularly breeds near or within pools or runs alongside faster flowing sections of rivers and creeks, usually where exposed rocks or cobbles are present (Figure 1c).

Figure 1. Different types of breeding habitats used by frogs species found in the greater Melbourne area. (a) Still, isolated, near-permanently hydrated waterbody suitable for most local frog species (artificially constructed wetland in Bundoora). (b). Dry, seasonally-inundated area suitable for autumn-breeding species that lay their eggs out of water (upper Yarra River catchment). Photo courtesy of Craig Cleeland. (c) Rocky, in-stream habitat used by Lesueur's Frog in the north-west of greater Melbourne.

If aiming for statistical independence between sites (i.e., sites separated by distances over which individual frogs are unlikely to regularly journey), it is recommended to maintain a minimum of 500 m between still or isolated waterbodies, and 500 m between different tributaries (e.g., separate creeks) or 2000 m between sites within the same tributary (Hamer *et al.* 2010). However, sometimes monitoring clusters of wetlands where individual waterbodies are situated much closer to each other can be valuable, for example to try and determine which of them might be more favourable for frogs to call from and potentially breed in (e.g., in the situation where some but not other nearby waterbodies might have been affected by pollution, other changes in water chemistry or structural habitat being altered).

Importantly, participants should avoid entering breeding sites, and instead record calls from around the edges and where there is a low risk of damaging surrounding terrestrial habitat by trampling vegetation or spreading disease (mainly the Amphibian Chytrid Fungus) on footwear and equipment. Spotlighting with the aim of directly observing frogs can also be useful to detect some species, although this is only to be done in very small groups when accompanied by someone with demonstrated expertise, holding appropriate permits and ethics approvals where relevant, and with strict protocols in place to minimise the risk of spreading disease and disturbing habitat.

3.2 The best times and conditions to monitor

Although many local frog species are occasionally heard calling during the day, they are overwhelmingly nocturnal, so it is best to start surveys from dusk if not dark onwards. The air temperature is recommended to be a minimum of 10°C or warmer in autumn and 12°C or warmer in spring, based on key (threatened) species of interest that are often most easily detected during those seasons. Avoid nights with moderate to stronger winds or very heavy rain (Hamer *et al.* 2010), although surveying in light rain or a night or two after heavy rain can often be productive. While visually recording frogs is not a main priority for the Frog Census, frogs are also often more readily seen during rain, including away from water, even if they are not calling.

The main calling periods of frogs recorded in the greater Melbourne area based on records filtered for calling frogs from the Victorian Biodiversity Atlas (including many that have come through Melbourne Water's Frog Census), are shown in Table 1. Methods for determining core calling periods for each species largely followed Lemckert and Mahony (2008). In most cases, this was based on the period containing > 90% of the records (i.e., eliminating months containing up to 10% of the total records, starting with the month having the lowest number of records and then adding months with the next lowest numbers of records to the point before 10% was exceeded). If months close to core calling months contained a smaller number of records than those further away from core calling months, and eliminating these exceeded 10% when added to the other non-core months, they were also excluded from the core calling period. Months adjoining core calling periods that contained a substantially higher number of records than months further away were considered notable calling times outside of the core period. Records for which the month was uncertain (e.g., having a date range spanning several months rather than a more specific date being provided) were excluded from the analysis.

As insufficient call data were available from within Melbourne Water's catchment management area to define core calling periods for the Red-groined (Haswell's) Froglet *Paracrinia haswelli* and Lesueur's Frog *Rhyaconastes lesueuri*, and as these species are known to be seasonal in their calling activity, records were used from Victoria more widely (shown in brackets) to inform this. Additionally, records for Bibron's Toadlet *Pseudophryne bibroni* were examined for the whole state as well as Melbourne Water's catchment management area, given the very small number of (25) call records available for this species locally, and as there is increasing interest in surveying for this threatened species more widely in Victoria.

A core calling period was not assignable to the Common Spadefoot Toad *Neobatrachus sudellae* as this species is known to often breed opportunistically after rain and hence its call records tend to be more evenly spread across seasons.

Based on the data, it is recommended that for surveys aiming to broadly record the diversity of frog species that might be calling at a given site in greater Melbourne, at least two nighttime surveys are undertaken in spring, and another two in autumn. If only relying on hearing frogs calling, however (i.e., rather than visually searching for them), a greater number of surveys would be needed in the appropriate season to increase the likelihood of detecting some species such as the Growling Grass Frog *Ranoidea* (formerly *Litoria*) *raniformis*.

Table 1. Peak calling times for frog species recorded in the greater Melbourne area based on records obtained from the Victorian Biodiversity Atlas.
Data in brackets (for species with few call records in the greater Melbourne Area) are based on call records for the whole of Victoria. Black shading indicates core calling periods and grey shading indicates what may be notable calling times outside of those periods.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Ground frogs (Myobatrachidae)													
Plains (East. Sign-bearing) Froglet *Crinia parinsignifera*	5	3	6	5	0	2	2	25	49	65	106	19	287
Common Froglet *Crinia signifera*	290	215	704	906	1002	1378	1407	2154	2967	3484	2678	458	17643
Victorian Smooth Froglet *Geocrinia victoriana*	12	13	158	353	150	18	11	5	16	5	3	5	749
Red-groined (Haswell's) Froglet *Paracrinia haswelli*	0 (45)	0 (28)	0 (33)	0 (35)	4 (5)	3 (7)	0 (21)	3 (98)	9 (178)	7 (157)	2 (166)	0 (44)	28 (817)
Brown (Bibron's) Toadlet *Pseudophryne bibronii*	0 (3)	0 (1)	3 (16)	4 (70)	13 (151)	3 (15)	1 (2)	0 (5)	0 (6)	1 (8)	0 (6)	0 (3)	25 (286)
Southern Toadlet *Pseudophryne semimarmorata*	3	2	98	754	218	9	1	0	4	0	0	0	1089
Marsh frogs (Limnodynastidae)													
Eastern Banjo Frog (Pobblebonk) *Limnodynastes dumerilii*	693	197	202	35	8	3	1	95	769	1951	1821	518	6293
Striped Marsh Frog *Limnodynastes peronii*	488	201	287	83	26	4	15	448	930	1034	1173	335	5024
Spotted Marsh Frog *Limnodynastes tasmaniensis*	508	170	222	87	53	4	16	558	1140	1545	1510	296	6109
Common Spadefoot Toad *Neobatrachus sudellae*	1	4	5	4	30	7	2	4	11	8	5	0	81
Tree frogs (Pelodryadidae)													
Eastern Dwarf Tree Frog *Drymomantis fallax*	58	38	13	2	1	0	1	0	6	66	135	54	374
Peron's Tree Frog *Pengilleyia peronii*	145	38	2	1	1	1	0	1	13	196	582	243	1223
Growling Grass Frog *Ranoidea raniformis major*	37	10	8	1	0	1	1	2	7	56	148	113	384
Southern Brown Tree Frog *Rawlinsonia ewingii*	215	169	400	460	613	786	973	1355	1352	1383	1746	410	9862
Whistling (Verreaux's) Tree Frog *Rawlinsonia verreauxii verreauxii*	125	37	118	62	53	19	33	135	239	395	658	214	2088
Lesueur's Frog *Rhyaconastes lesueuri*	0 (3)	0 (1)	0 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (7)	0 (24)	0 (26)	0 (19)	0 (85)

Several of the core calling periods indicated in Table 1 can be further refined to reflect the best times to undertake targeted acoustic surveys or monitoring for particular species, based on additional months supporting high numbers of records (i.e., defining the period based on substantial drop offs in call data to either side of consecutive months with the most records), as well as the author's understanding of peak calling activity in southern Victoria and a study on calling phenology in north-west Melbourne by Herd *et al.* (2015). Noting the latter study examined the local calling periods of the Common Froglet, Eastern Banjo Frog, Spotted Marsh Frog, Southern Brown Tree Frog and Whistling Tree Frog.

An accordingly adjusted and simplified version of the core calling periods for these and the other species listed in Table 1 is provided in Figure 2.

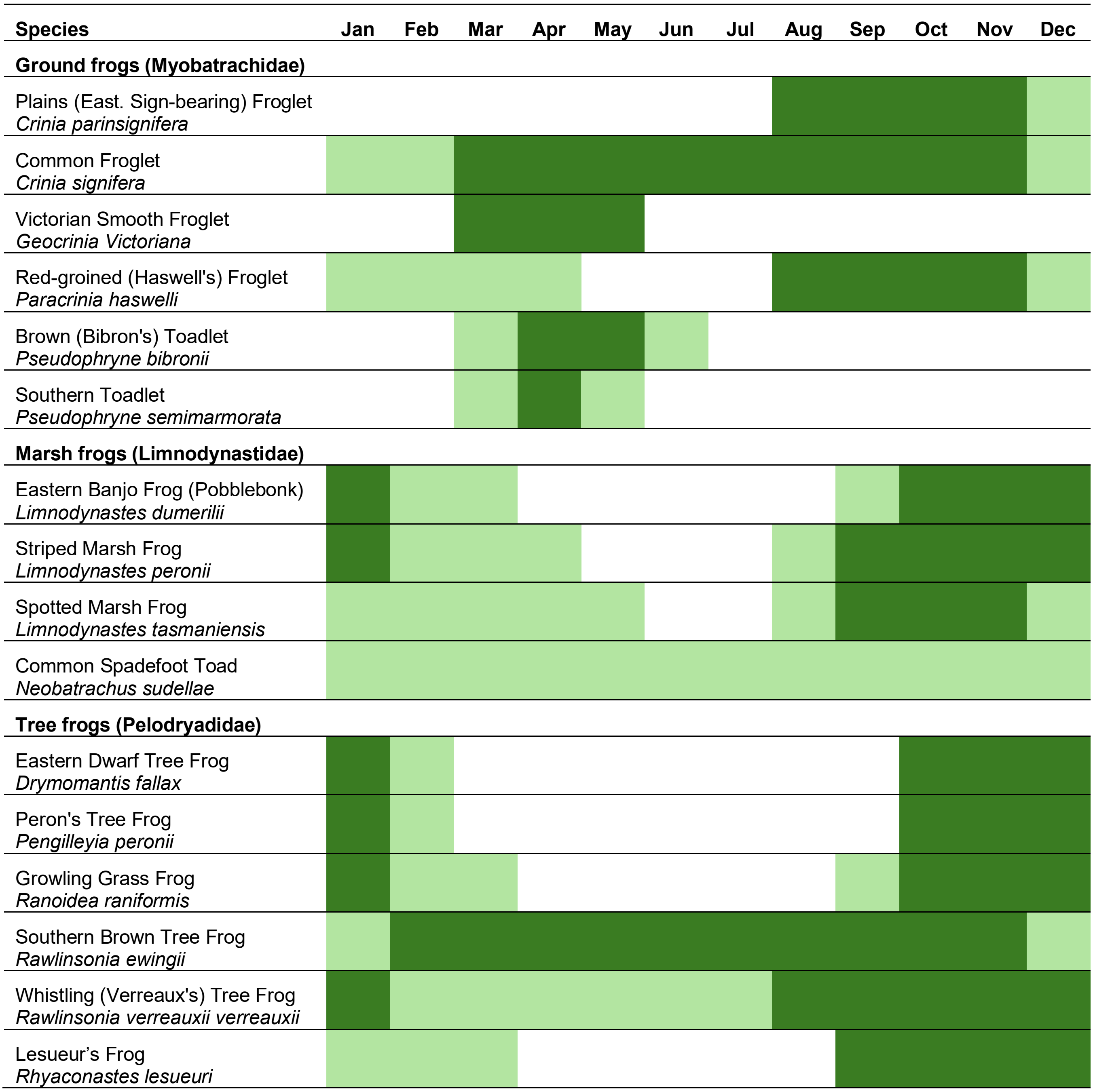

Figure 2. Adjusted main calling periods for frog species recorded in the greater Melbourne area largely based on the data in Table 1, Heard *et al.* 2015 and observations by the author.
Non-core periods which may still have a reasonable chance of recording the species are shown in lighter green.

It should also be noted that within the main calling periods indicated, there are likely to be peaks for each species (i.e., times during those periods when calling tends to be the most intense). For example, long-term monitoring data from a cluster of wetlands in Melbourne's northern suburbs has shown that calling there by the Victorian Smooth Froglet usually peaks in April, dropping off in May and not being heard as commonly during most of March (R. Bender unpubl. data).

3.3 Duration and method of call recording

Record calls using Melbourne Water's Frog Census app. In addition to this, consider obtaining a separate recording using the voice recorder app on your phone, or a hand-held audio recorder. This may have added benefits, including getting a clearer recording for other purposes (e.g., creating higher resolution waveforms and spectrograms for visual call analysis), and enabling you to retain a copy of the recording on file and forward it to others.

As recommended by Hamer *et al.* (2010), record for at least 5 minutes at each site on each survey, regardless of whether you can hear any frogs calling or not (as 'absence' data is still informative).

Using an external (e.g., unidirectional) microphone often gives the best results, and headphones or earplugs can be used to more accurately hear what you are recording in real time.

When you arrive at a site, wait a couple of minutes before you start recording, in case you have disturbed frogs and it takes a little while for them to start calling again. Also ensure you have as clear a line of sight between the recording device and calling frogs as possible (e.g., try to avoid recording in front of particularly thick, tall vegetation growing along the banks), minimise unwanted noise from roads and other human activity, and remain as still and quiet as possible for the duration of the recording.

3.4 Surveys with potential to detect the Growling Grass Frog

In accordance with recommended survey protocols for the threatened Growing Grass Frog by Heard *et al.* (2010), two surveys done on separate nights from October to the end of December may have a 95% chance of detecting the species if it is present. Three surveys on separate nights from January to March would achieve the same level of certainty at that later stage of the season. However, this is based on searching for frogs by spotlighting as well as listening for calls and doing call-playback to try and elicit a response. This is only to be done for existing or planned monitoring programs specifically targeting this species, in the company of an expert with demonstrated experience spotlighting for the Growling Grass Frog, holding appropriate permits and ethics approvals where relevant, and with strict protocols in place to minimise the risk of spreading disease (mainly the Amphibian Chytrid Fungus) and disturbing habitat.

On each survey, spotlighting would done for one "person hour" at each site (waterbody or slow-flowing section of creek), so if two people were involved for example, the actual spotlighting duration would be 30 minutes. Small ponds or dams could be surveyed for less time if they are able to be comfortably walked around in under an hour. Scan the water surface and shallow edges from the banks, and land within approximately 10 m. Look for eyeshine as well as movement and the silhouettes of frogs. Undertake surveys when it is dark, when the air temperature is 12°C or higher, and in little or no wind. This species' calls should also be played intermittently (e.g., from the Frog Census app) to encourage frogs to call back in response, while also listening for a total of 10 minutes at each site on each of the survey nights (Heard *et al.* 2010).

Otherwise, surveys for this species are to be limited to recording calls for the Frog Census, for the recommended 5 minutes at each site, over at least two but preferably many more nights from October to the end of December.

3.5 Surveys with potential to detect threatened toadlet (*Pseudophryne*) species

The Southern Toadlet *P. semimarmorata* and Bibron's (or Brown) Toadlet *P. bibronii* are usually most easily detected in autumn, with calling by the former species typically peaking in April and sometimes continuing in the week or two before and after. Bibron's Toadlet also appears to have its core calling period in April but commonly extending into May, with a closer examination of call records from Victoria suggesting that the peak in a given season, assuming suitable conditions, could continue into about the third week of May. Both species may also shift their calling periods by a few weeks earlier or later in a given season, depending on environmental conditions (e.g., Terry 2022). Surveys for these species are not usually done with the aim of sighting frogs given how cryptic they are, but instead listening for and recording their calls for 5 minutes on each survey. In accordance with recently updated interim survey guidelines for detecting these species (De Angelis and Cleland 2026), this should be repeated on two nights during the peak period when these toadlets are expected to be calling. A third survey is recommended if surveys are undertaken at the start or tail end of the peak calling period, or if conditions are particularly dry during the typical peak calling time.

There might be limited to no additional benefit to spotlighting at frog monitoring sites in autumn, although it is possible species such as the Common Spadefoot Toad *Neobatrachus sudellae* and Eastern Banjo Frog *Limnodynastes dumerilii* might be seen (but not necessarily heard) in damp conditions at that time of year.

3.6 Surveys to detect frog species introduced to the Melbourne area

Two frog species are so far known to have been introduced and established breeding populations in the Melbourne area, although potential impacts to locally indigenous frog species or other aspects of Melbourne's biodiversity have not yet been studied.

Peron's Tree Frog *Pengilleyia* (formerly *Litoria*) *peronii* was recorded for the first time in greater Melbourne in Yan Yean in 1985 (Littlejohn and Harrison 1987), while the Eastern Dwarf Tree Frog *Drymomantis* (formerly *Litoria*) *fallax* was recorded in the region for the first time in Moorabbin in 1999 (Gillespie and Clemann 2000). In Victoria before 1985, Peron's Tree Frog was mostly found in the wider Murray River catchment north of the Great Dividing Range, and in East Gippsland (ALA 2026). Before being introduced to Victoria, Albury and making its way further down the New South Wales coast, the Eastern Dwarf Tree Frog was distributed along the east coast of Australia from about Cairns south to Wollongong (ALA 2026).

Both of these species are mainly active in spring and summer, with their core call calling periods spanning from about October into January. Surveys targeting them are therefore recommended to be done on warmer nights in and between these months, and ideally following rain.

It is possible other non-indigenous frog species could also end up establishing populations in Melbourne in the future (e.g., from individuals intentionally or accidentally released in the wild after coming down in produce), in which case surveys for them should be timed to take place within their main activity and calling periods (e.g, refer to Anstis 2026).

3.7 Using remote acoustic recorders

Remote acoustic recorders such as AudioMoths or Song Meters (Figure 3) can be used to survey potential breeding sites, although cannot necessarily be relied on where large areas need to be covered and only a limited number of recorders are available (e.g., at multiple points along extensive gullies or waterways or particularly large waterbodies). Where multiple potential breeding sites need to be surveyed in these situations, visiting them repeatedly to listen for calling frogs (and spotlighting where appropriate) is often more efficient and cost-effective than setting up, collecting and processing data from a large number of remote recorders. Saying that, it is recommended to train sound editing software or use previously trained artificial intelligence programs to recognise calls where large amounts of data need to be sorted through. Additionally, larger numbers of remote acoustic recorders could be arranged in grids or arrays (at regular spacings) to try and detect calls where breeding sites might be less obvious (e.g., seasonally dry depressions in the landscape that may be used by *Pseudophryne* species).

Figure 3. Song Meter SM4 (Wildlife Acoustics) secured to a branch overhanging a creek pool.
Photo courtesy of Brendan Casey.

Surveying large wetlands (riverine weir pools) for the Growling Grass Frog may require three remote recorders placed out for 12 weeks to achieve 95% likelihood of detection (Mathwin 2024), whereas only a handful of nights spotlighting in addition to listening for calls in appropriate environmental conditions would likely achieve similar detection probability (Heard *et al.* 2010). However, it is possible that regional and habitat differences might influence the probability of detecting this species using these methods, with the former study based on the Murray River in South Australia (on the northern subspecies *Ranoidea raniformis raniformis*), and the latter in more urbanised catchments in the greater Melbourne area (on the southern subspecies *R. r. major*).

Conversely, a study using paired remote acoustic recorders to survey for frogs at four locations around Melbourne's urban fringe found that detections of additional species plateaued after six days, although recommended up to 10 days of recording (Gration 2019). Recorders were set to record for 30 min every hour over 24 hours each day. It was, however, recommended that around larger waterbodies, paired recorders be placed at a considerable distance from each other, and set to record for a minimum of two 5-minute periods per hour (so total of 10 minutes per hour) throughout the day and night.

Recorders should otherwise be set to record during peak calling times (i.e., typically in the evening) to reduce the amount of time taken to sort through the data and/or space taken up on the data card, and are recommended to record for longer periods (e.g., 30 minutes per hour) when focussing on detecting a specific (target) species. Remote acoustic surveys for a particular species should also target its preferred habitat the appropriate season for detecting its calls (Gration 2019). Depending on the habitat, background noise and frequency of frog calls being targeted, the effective recording distance of different types of recorders (e.g., AudioMoths vs Song Meter models) should also be considered when setting them up.

Remote recorders may be more effective than in-person listening surveys at sites that are otherwise difficult to access at night, and to better understand the abundance and calling behaviour of a breeding population over a season or multiple years (if that is desired beyond simply assessing whether the target species is present). If remote recorders are used, they should be set up as close as possible to the edge of the potential breeding site, ensuring the distance between the site and recorder is reasonably clear of thick vegetation while trying to keep the recorder hidden from public view.

3.8 Collecting environmental DNA (eDNA)

Reference sequences for specific genetic markers are available for many frog species, theoretically allowing them to be identified through the analysis of environmental DNA (eDNA) in water, and potentially (although much less commonly at this stage) for some species on land (e.g., Burns *et al.* 2020).

The collection of eDNA from water samples can be done in a variety of ways, although commonly involves collecting 2-3 or more samples at each waterbody or section of waterway, at the time of year when the target frog species are expected to be breeding (for most species that lay their eggs in standing water) or have tadpoles present. Sampling for eDNA should also focus on appropriate microhabitats for the frog species being targeted within the stream or waterbody, such as isolated or slow-flowing pools or amongst aquatic vegetation.

A sampling method often used locally is to collect up to 400 ml of water with a syringe (e.g., as many as possible or up to eight lots of 50 ml using a 60 ml syringe) (Figure 4), on each occasion passing it back out through a 0.22 µm filter unit attached the end of the syringe (e.g., Burns *et al.* 2020; Berran *et al.* 2025). This process should be repeated at least two or ideally three if not more times (i.e., so that this number of filtered samples ends up being collected) at each precise location surveyed. Alternatively, the use of larger filter sizes (e.g., up to 5 or 6 µm) and/or self-preserving filters has been found to increase the likelihood of detecting amphibian species in some cases (Goldberg *et al.* 2011; Berran *et al.* 2025).

New disposable gloves are to be worn between taking samples at different locations to reduce the risk of contaminating them. The filters are to be temporarily stored frozen or treated with a DNA preservation buffer before being transported to a lab for DNA extraction and sequencing (the lab engaged to analyse the samples will provide specific instructions that will need to be followed, as preferred methods can vary between operators).

Figure 4. Collecting a water sample to enable the filtration of DNA for later analysis in the lab. Photo courtesy of EnviroDNA.

It should be noted, however, that the detection of many amphibian species using eDNA can be highly seasonal, and even when certain species are spawning and tadpoles are in the water, it is not always reliable (including for the Growling Grass Frog and Southern Toadlet; W. Steele pers. comm.). This could possibly be due to one or a combination of issues around high levels of environmental inhibition or contamination (Stoeckle *et al.* 2017; Chen *et al.* 2023), high levels of DNA dilution or dispersal (Pilliod *et al.* 2014), relatively quick degradation (e.g. in warm, sunny conditions or extremes in water chemistry) (Pilliod *et al.* 2014; Strickler *et al.* 2015), and/or low levels of DNA being present at breeding sites (e.g. for species that lay their eggs on land or are otherwise mostly terrestrial as adults) (Wikston *et al.* 2023). It may be worth trialing the use of larger filter sizes or self-preserving filters as per Berran *et al.* (2025) to increase the likelihood of detecting target frog species in such cases.

3.9 Assessing abundance

Beyond monitoring for occupancy (i.e., whether certain frog species are present or in low abundance if not absent), a greater level of survey effort or data interrogation is usually needed to get reasonable estimates of abundance.

Melbourne Water's Frog Census adopted the Amphibian Calling Index (ACI) outlined by Weir and Mossman (2005) as a rapid way of gauging calling intensity as an indication of abundance (Table 2). This relies on assessing the intensity of advertisement calls made by male frogs. While the scoring categories are very broad, even estimates based on more precise counts of calling males are subject to imperfect detection of all the individuals in the population, limited for example by not being able to survey for females or juveniles by call, and male vocalisations varying between species and depending on the time of the survey (Čeirāns *et al.* 2020). Lek-mating species with discrete breeding territories (e.g, Southern Toadlet and Brown Toadlet) may also lend themselves to making more accurate estimates of abundance based on male calling than explosive breeders (e.g., Growling Grass Frog) (Čeirāns *et al.* 2020), and the tendency for breeding populations of species like the latter to fluctuate widely between seasons (e.g., depending on differences in the weather or hydrology between years) can make it difficult to determine 'real' declines and their likely cause beyond this. Longer-term data documenting background fluctuations in abundance (e.g., over a 6 year period) can therefore provide more robust pre-defined population levels to assess changes in abundance against (Wilkinson 2015).

Table 2. Amphibian Calling Index based on that outlined by Weir and Mossman (2005).

Index	Description
0	No frogs calling
1	Individual frogs can be counted, with space (i.e., no overlap) between their calls
2	Calls of individual frogs can be distinguished from each other, but with some overlap
3	Constant, overlapping calling, making it hard to distinguish individual frogs

Beyond auditory surveys, visual counts (typically coupled with auditory surveys) will provide greater reliability assessing numbers for species that are often in more open habitats when they are active (e.g., Growling Grass Frog) (see Section 3.4), as opposed to those that remain mostly concealed for much of their activity (e.g., Southern Toadlet and Bown Toadlet). However, to minimise the risk of spreading disease and unduly disturbing habitat, visual counts must only be done if frogs are observed incidentally (i.e., if frogs happen to be observed while recording their calls), or by an expert engaged to lead the surveys. Similarly, detailed counts of calling males for species with discrete breeding territories such as the Southern Toadlet and Brown Toadlet (e.g., by temporarily flagging their calling spots to avoid double-counting during a survey) must only be done by one or a very small number of experts engaged to do this, and avoid having larger numbers of volunteers enter the frogs' breeding areas.

While count-based methods have the potential to produce substantially biased estimates of abundance, they may still provide a baseline against which future comparisons can be made, as long as the methods and effort are kept consistent between surveys. However, more accurately quantifying population size requires mark-recapture techniques (in other words, having a system by which individuals of the same species can be distinguished from each other when they are recaptured on repeat surveys, based on each being given or otherwise identified by a unique feature) (Williams *et al.* 2002). Such data can also potentially be used to help inform questions relating to demography (e.g., longevity and age structure), dispersal rates, and spatial ecology (e.g., distances moved).

Mark-recapture often involves marking individual frogs, for example by microchipping larger individuals using passive integrated transponder (PIT) tags, or smaller ones using visible implant alphanumeric (VIA) or elastomer (VIE) tags (e.g., following Heard *et al.* 2008 and Sapsford *et al.* 2015). Alternatively, for some species with distinctive patterns that show limited change over time (e.g., belly pattern on the Southern Toadlet; C. Cleeland unpubl. data), taking photos of the pattern on each individual in a population and comparing it with photos taken of individuals captured on future surveys can be used in a similar way (analogous to comparing fingerprints among a human population). However, using each of these techniques requires specific expertise along with revenant animal ethics and research permit approvals.

For the most part, however (i.e., unless engaging an expert who holds relevant animal ethics and research approvals), community volunteers including Frog Census participants will be limited to using the Amphibian Calling Index or trying to make more precise estimates of the number of calling males by listening from a distance.

3.10 Surveys to confirm breeding

Surveys to confirm breeding often involve searching for tadpoles and/or recently emerged frogs at the final stages of metamorphosis (metamorphs). However, as these surveys typically involve more intensive searching in and around breeding habitats, they must only be done by hired expert contractors or people otherwise experienced in these techniques, and then only if the appropriate animal ethics approvals and research permits are held.

Tadpoles are often surveyed for by any or a combination of dip netting, fyke netting, and funnel or bait style (e.g., bottle, nylon mesh funnel other box-type) trapping (Figure 5). However, as tadpoles are protected wildlife in Victoria, these techniques require animal ethics approval as well as a research permit under the Wildlife Act, issued by the Victorian Department of Environment (currently DEECA). In the case of bait traps and fyke nets, while the entrances need to be submerged for the traps to work, it must also be ensured the top corners of the trap and end of the net respectively are held above the water surface so air-breathing bycatch such as adult frogs can survive until the traps are checked in a timely way (typically in the morning if traps are set on the prior evening).

Figure 5. Funnel (bait or box-style) trapping for tadpoles done by an experienced surveyor under animal ethics approval and wildlife research permit.

The tadpoles of many species are also sometimes identifiable by observing them in-situ from land, although in any case, can be hard to distinguish from each other and often need further effort or expert help to identify them. Clear photos can be helpful, although usually if taken of the sides of the tadpole while it is in water so that the tail remains unfurled, as well as the top and underneath, noting that ethics and research permit approvals are also needed to do this.

Metamorphs can usually be searched for visually by spotlighting around the edges of the water and several meters further out, although only by persons experienced in doing this, employing strict hygiene protocols for chytrid fungus and as much as possible, avoiding trampling the habitat. To further minimise this risk, no more than two observers are recommended to canvass a given area of habitat.

Anstis (2026) should be consulted to inform the best timing for tadpole or metamorph surveys based on when they are usually present for the species being targeted, although more specific guidance for the Growling Grass Frog (also in relation to trap effort) is provided by Heard *et al.* 2010.

Where tadpoles or metamorphs are otherwise observed incidentally at survey sites (e.g., while recording frog calls), their presence should be noted.

3.11 Surveys to monitor frogs for disease

Beyond watching out for and reporting seemingly ill or injured frogs (e.g., to Wildlife Health Surveillance Victoria at the University of Melbourne's Werribee Campus), diagnosis requires specialist analyses done in collaboration with a laboratory familiar with the relevant procedures. If it is desired to swab frogs for the amphibian chytrid fungus (most relevant where susceptible threatened species or managing their habitat is concerned), an expert experienced in swabbing frogs for the disease who holds relevant animal ethics approvals and research permits would need to be engaged to do it. Methods should largely follow Hyatt *et al.* (2007).

The Amphibian Chytrid Fungus can also often be detected by sampling the habitat for eDNA, including by citizen scientists (refer to Section 3.8). However, the presence chytrid in any given wetland in the greater Melbourne area could almost be assumed, and frogs may still need to be swabbed if it is desired to quantify infection load in addition to assessing the pathogen's presence.

3.12 Recording weather conditions

Recording weather and other environmental conditions can help give clues as to why certain frog species might or might not be calling at that particular time.

If participants have access to a hand-held weather meter, or a thermometer, hygrometer and wind impeller, it is worth recording air temperature, relative humidity and average wind speed at the start of each survey at each site (Table 3). Data from the Bureau of Meteorology could be used otherwise. It is also suggested to record cloud cover, moon phase, water temperature (if standing water is present and someone has a thermometer to measure it), and previous or current rain (e.g., mm within the past 24 hours). Make sure any equipment used in the water is disinfected between sites (e.g., using alcohol wipes).

Table 3. Weather and other environmental conditions recommended to be recorded at frog monitoring sites at the time of survey or otherwise where applicable, using data from the Bureau of Meteorology.

Environmental variable	Measure	Environmental variable	Measure
Air temperature	°C	Rain in past 24 hours (from BOM)	ml
Water temperature	°C	Average wind speed	km/h
Relative humidity	%	Wind direction (where coming from)	N, E, S, W
Moon phase	New, full, quarter, waxing or waning crescent or gibbous	Cloud cover over site	%
Rain at the time of survey	None, light, moderate, heavy	Average water depth (estimate)	cm

3.13 Assessing frog habitats

If participants are keen, daytime habitat assessments could be done just once for each site, and repeated in future if/when desired. This would arguably be more important for sites where species of particular conservation significance or other interest are found.

As well as recording the type of waterbody (e.g. creek or river, seasonally wet gully, pond, dam, lake), habitat assessments should include indications of the substrate type, water level, flow rate, covers or rocks, logs and bare ground, aquatic vegetation covers (emergent, floating and submerged), and covers of surrounding terrestrial vegetation (e.g., within 10 m of the water, separately for low-growing ground vegetation and overhanging woody plants). Habitat variables recommended to be recorded for both aquatic (breeding) and surrounding terrestrial habitat at survey sites are listed in Table 4.

Table 4. Aquatic and terrestrial habitat variables recommended to be recorded at frog monitoring sites.

<table>
<tr><th>Land habitat variable</th><th>Measure</th><th>Aquatic habitat variable</th><th>Measure</th></tr>
<tr><td>Bare ground</td><td rowspan="11">% cover</td><td rowspan="3">Habitat type</td><td rowspan="3">Dry gully, creek, river, drain, isolated pool, swamp, sedge wetland, culvert, dam, lake, reservoir, billabong, spring, anabranch</td></tr>
<tr><td>Rocks</td></tr>
<tr><td>Logs (≥10 cm wide)</td></tr>
<tr><td>Branches (<10 cm)</td><td rowspan="2">Substrate</td><td rowspan="2">Bedrock, boulder, cobble, gravel, pebble, coarse or fine sand, loam, clay, mud, silt, fine organic matter</td></tr>
<tr><td>Leaf litter</td></tr>
<tr><td>Fine organic debris</td><td>Water presence</td><td>Dry, isolated pools, inundated</td></tr>
<tr><td>Herbs</td><td rowspan="2">Flow type (for waterway)</td><td rowspan="2">Cascade, rapid, run, riffle, glide, pool, backwater, surface</td></tr>
<tr><td>Long grass (>10 cm)</td></tr>
<tr><td>Short grass (<10 cm)</td><td>Water level</td><td>None, low, medium, high, flood</td></tr>
<tr><td>Shrubs (1-5 m high)</td><td>Sediment</td><td rowspan="10">% cover</td></tr>
<tr><td>Canopy (>5 m high)</td><td>Rocks</td></tr>
<tr><td rowspan="8">Fire history</td><td rowspan="8">Year, burn type (e.g., prescribed), and intensity</td><td>Logs (≥10 cm wide)</td></tr>
<tr><td>Branches (< 10 cm wide)</td></tr>
<tr><td>Leaf litter</td></tr>
<tr><td>Fine organic debris</td></tr>
<tr><td>Submergent vegetation</td></tr>
<tr><td>Emergent vegetation</td></tr>
<tr><td>Floating vegetation</td></tr>
<tr><td>Overhanging bank</td></tr>
</table>

4 Hygiene, habitat-protection measures and permissions

Please ensure that frog habitats are not unduly trampled or otherwise disturbed, so participants need to avoid walking in the water and through vegetation along the edges of breeding (calling) sites. They should instead stand somewhere that is largely clear of vegetation or other habitat that frogs are most likely to use while recording calls.

It must also be ensured that specific locations supporting any threatened species are not mentioned publicly, including on social media, to avoid contributing to the risk that pathogens might be spread or habitat damaged by individuals who might go searching for threatened frogs (e.g., to get photos of or less commonly, poach them).

Hygiene protocols to reduce the risk of spreading Amphibian Chytrid Fungus during surveys are outlined in 'Hygiene protocols for the control of diseases in Australian frogs' (Murray *et al.* 2011) and 'Hygiene protocol for the control of disease in frogs' (Wellington and Haering 2008). However, aspects of these guidelines relevant to citizen science monitoring are summarised here.

It can be tricky deciding on the boundaries of sites between which you should disinfect footwear and other soiled or wet equipment. For example, the distance between sites which chytrid might be easily transmitted can be affected by the species of frogs and other potential vectors present, the time of year, and habitat factors influencing infection load and transmissibility including water chemistry and landscape barriers. However, as a precaution and general rule, different waterways and upstream locations (e.g., the latter separated by 500 m) should be considered separate sites, as should any waterbodies separated by dry land (Murray *et al.* 2011).

Any damp soil of mud should first be brushed off any footwear or other items carried between sites. Disinfectants then applied to the soles of footwear and other soiled gear (either by spraying or using a foot bath as appropriate) should consist of at least 70% ethanol (e.g., minimally diluted methylated spirits) or household bleach diluted to 1% sodium hypochlorite, and then be left for at least one minute.

Any clean, wet footwear, clothing or equipment can also be left to dry completely, preferably in the sun, before being used to survey between sites (if they are surveyed on different nights), as total drying will kill the Amphibian Chytrid Fungus.

Additionally, if community groups or individuals partner with experts who are using methods that could result in coming into contact with frogs, tadpoles or their habitat, they would need to hold the necessary authorisations for doing so, at a minimum usually including animal ethics approval, research permit under the *Wildlife Act 1975*, and an access agreement if surveying on land managed by Parks Victoria. In addition to government-managed land, the former two authorisations are also needed whenever working on any type of privately-owned land, along with permission from the landowner.

5 References

ALA (2026). Atlas of Living Australia. Available from: http://www.ala.org.au. Accessed on 9 June 2026.

Anstis, M. (2026). *Tadpoles and Frogs of Australia* (3rd ed.). New Holland, Sydney.

Berran, M. S., Weeks, A. R., Coleman, R. A., Connelly, F., Song, S. V. and Tingley, R. (2025). A comparison of environmental DNA sampling strategies for detecting anuran populations in wetlands. *Hydrobiologia* **852**: 3485–3501.

Burns, T. J., Clemann, N., van Rooyen, A. R., Scheele, B. C., Weeks, A. R. and Driscoll, D. A. (2020). Environmental DNA sampling in a terrestrial environment: Methods to detect a critically endangered frog and a global pathogen [preprint]. *bioRxiv.* doi: 10.1101/2020.03.01.968693.

Chen, Y., Tournayre, O., Tian, H. and Lougheed, S. C. Assessing the breeding phenology of a threatened frog species using eDNA and automatic acoustic monitoring. *PeerJ* **23**: 11:e14679. doi: 10.7717/peerj.14679.

Čeirāns, A., Pupina, A. and Pupins, M. A new method for the estimation of minimum adult frog density from a large-scale audial survey. *Scientific Reports* **10**: 8627. doi: 10.1038/s41598-020-65560-6.

De Angelis, D. and Cleeland, C. (2026). Interim survey guidelines for toadlet *Pseudophryne* species in Victoria, Version 2. Ecological Consultants Association of Victoria, Melbourne.

Gillespie, G. and Clemann, N. (2000). The Eastern Dwarf Tree Frog *Litoria fallax* (Peters) (Anura: Hylidae): a recent introduction to Victoria?. *The Victorian Naturalist* 117: 60-62.

Goldberg, C. S., Pilliod, D. S., Arkle, R. S. and Waits, L. P. (2011). Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS ONE* **6**: e22746. doi: 10.1371/journal.pone.0022746.

Gration, R. (2019). Frog species detection guidance note. Report prepared for Melbourne Water by Symbolix and EcoAerial Environmental Services, Melbourne.

Hamer, A., Ainley, J. and Lechner, A. (2010). Melbourne Water Frog Program: Frog Data Consolidation and Frog Monitoring Options. Report prepared for Melbourne Water by the Australian Research Centre for Urban Ecology, University of Melbourne.

Heard, G. Scroggie, M. P., and Malone, B. S. (2008). Visible Implant Alphanumeric tags as an alternative to toe-clipping for marking amphibians case study. *Wildlife Research* **35**: 747–759.

Heard, G., Scroggie, M. and Clemann, N. (2010). Guidelines for managing the endangered Growling Grass Frog in urbanising landscapes. Technical Report No. 208, Arthur Rylah Institute for Environmental Research, Department of Sustainability and Environment, Melbourne.

Heard, G., Canessa, S. and Paris, K. (2015). Interspecific variation in the phenology of advertisement calling in a temperate Australian frog community. *Ecology and Evolution* **5**: 3927–3938.

Hyatt, A., Boyle, D. G., Olsen, V., Boyle, D. B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, J-M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F. and Colling, A.

(2007). Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. *Diseases of Aquatic Organisms* **73**: 175–192.

Lemckert, F. and Mahony, M. (2008). Core calling periods of the frogs of temperate New South Wales, Australia. *Herpetological Conservation and Biology* **3**: 71–76.

Lindenmayer, D. B. and Likens, G. E. (2018). *Effective Ecological Monitoring* (2nd ed.). CSIRO Publishing, Melbourne.

Littlejohn, M. and Harrison, P. (1987). *Litoria peroni* (Anura: Hylidae): an addition to the amphibian fauna of Melbourne. *The Victorian Naturalist* **104**: 137–140.

Mathwin, R. (2024). The establishment of frog monitoring methods and sites to inform ecological targets of the Weir Pool Manipulation Strategy. Report prepared for the South Australian Department for Environment and Water by Rupert Mathwin Ecology, Belair, South Australia.

Murray, K., Skerratt, L., Marantelli, G., Berger, L., Hunter, D., Mahony, M. and Hines, H. (2011). Hygiene protocols for the control of diseases in Australian frogs. Report prepared for the Department of Sustainability, Environment, Water, Population and Communities by James Cook University, Townsville, Queensland.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S. and Waits, L. P. Factors influencing detection of eDNA from a stream-dwelling amphibian. *Molecular Ecology Resources* **14**: 109–116.

Sapsford, S. J., Alford, R. A. and Schwarzkopf, L. (2015). Visible Implant Elastomer as a viable marking technique for Common Mistfrogs (*Litoria rheocola*). *Herpetologica* **71**: 96–101.

Stoeckle, B. C,, Beggel, S., Cerwenka, A. F., Motivans, E., Kuehn, R. and Geist, J. (2017). A systematic approach to evaluate the influence of environmental conditions on eDNA detection success in aquatic ecosystems. *PLoS One* **12**: e0189119. doi: 10.1371/journal.pone.0189119.

Strickler, K. M., Fremier, A. K. and Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation* **183**: 85–92.

Terry, W. (2022). A note on the calling behaviour of brown toadlet, *Pseudophryne bibronii*, at a site in central Victoria. *Australian Zoologist* **42**: 733–737.

Weir, L. and Mossman, M. (2005). North American Amphibian Monitoring Program (NAAMP). In: *Status and Conservation of Midwestern Amphibians*, pp. 307-313. Ed. M. J. Lanoo. University of Iowa Press, Iowa City, Iowa.

Wellington, R. and Haering, R. (2008). Hygiene protocol for the control of disease in frogs. DECC Information Circular Number 6. Department of Environment and Climate Change, Sydney South, NSW.

Wikston, M,, Breton, B-A. A., Vilaça, S. T., Bennett, A. M., Kyle, C. J., Beresford, D. V., Lesbarrères, D., Wilson, C. C., Green, D. M., Fortin, M-J. and Murray, D. L. (2023). Comparative efficacy of eDNA and conventional methods for monitoring wetland anuran communities. *Frontiers in Ecology and Evolution* **11**: 1179158. doi: 10.3389/fevo.2023.1179158

Wilkinson, J. W. (2015). *Amphibian Survey and Monitoring Handbook*. Pelagic Publishing, Exeter, UK.