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Tasmanian Frog and Chytrid monitoring 2014:

Sound recording, capture-mark-recapture and Chytrid status

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I. Executive summary

- Chytrid fungus (*Batrachochytrium dendrobatidis* [Bd]) was first described in 1998 and is associated with frog population declines as well as frog population extinctions in Australia, Africa, Central, South, and North America. Anthropogenic processes have played a large role in the worldwide spread of Bd (Skerratt, Berger et al. 2007, Collins 2010), and the impact of chytridiomycosis on frogs is considered by some to be the most spectacular loss of vertebrate biodiversity due to disease in recorded history (Skerratt, Berger et al. 2007).

- In 2004 Bd was first detected in Tasmania (Obendorf and Dalton 2006), and is widespread across the state with the exception of the Tasmanian Wilderness World Heritage Area (TWWHA), where Bd has only recently begun to invade (Philips, Wilson et al. 2010, Cashins, Philips et al. 2013). Presently, the TWWHA makes up greater than 75% of the distribution of the endemic Tasmanian tree frog (*Litoria burrowsae*: Driessen and Mallick 2003). *L. burrowsae* has high susceptibility to Bd infection in laboratory trials (Voyles, Phillips et al. 2014), and climatic conditions are thought to be particularly favourable for Bd in the TWWHA (Murray, Rosauer et al. 2010).

- We report the results from three actions/components proposed in the Tasmanian Chytrid Management Plan (Philips et al. 2010): (i) a capture-mark-recapture study at two ponds, (ii) a remote sound recordings analysis across 36 ponds taken in 2013, and (iii) 2014 chytrid testing at Hartz Mountains and Birchs Inlet. We also review these actions and provide recommendations for ongoing implementation.

- A capture-mark-recapture study was used to determine survival and population growth in the Tasmanian tree frog (*Litoria burrowsae*) at two ponds, one at Lune River (chytrid positive from 2013) and the other at Melaleuca (chytrid negative). At both ponds, annual survival of males was very low, and was combined with a high degree of variation in frog abundance both within years (at Lune River) as well as across years (at both Lune River and Melaleuca). Survival analysis further indicated that frog movement (recruitment, immigration) is probably an important component of frog population dynamics at these sites; further study of frog movements across Tasmanian landscapes is needed to confirm these findings.

- Population growth from 2012 to 2013 was negative at Lune River, and then positive from 2013 to 2014 despite a change of status from chytrid negative to chytrid positive in 2013. At the chytrid-free site (Melaleuca), population was positive from 2012–13, then strongly negative from 2013–14. Frog population dynamics are often characterised as having a large degree of natural variation in abundance, and further longitudinal capture-mark-recapture study in additional future years would be needed to untangle any differences between chytrid impact on population growth and survival and naturally occurring population variation. Capture-mark-recapture studies could provide some of the strongest demonstrations of chytrid impacts on frogs in the TWWHA, but are also labour-intensive/costly.

- Remote sound recording units deployed in 2013 were used to assess frog population activity in 36 ponds across Tasmania's southwest. Brown tree frog *(Litoria ewingi)* were observed in every pond sampled, but call activity was much lower and variable for common froglet (*Crinia signifera*), Tasmanian froglet (*Crinia tasmaniensis*), and Tasmanian tree frog. Seventeen ponds had no or very little Tasmanian tree frog calling activity, 18 ponds had no

or very little common froglet calling activity, and 18 ponds had no or very little Tasmanian froglet call activity.

- Other sound data currently exist (2011 & 2012), and we present data on maximum call activities documented from previous years along with 2013 data in map form. For 2014, recordings exist but have yet to be coded. Preparing these other three years of acoustic data would allow for estimation of trends of call indices at ponds with and without chytrid.

- Further sound recordings should be taken in 2015, and sound analysis of previously collected data should be immediately performed. With the addition of the three available years of sound recordings, trends in call activity at ponds with chytrid versus those without may be discovered. Remote sound recordings appear to be the most cost-effective monitoring tool currently available.

- At Hartz Mountains chytrid was detected adjacent to the track past the bootwash station. Further incursion of chytrid at Birchs Inlet was observed as well. In both cases, understanding how to improve use of already existing bootwash stations, restricting public access to sensitive areas, and/or public education is recommended.

- Prevention, rather than managing the disease, is without a doubt the most powerful management tool currently available. Bootwash stations combined with an effective education program currently represent the best management tools available that can be used to prevent further chytrid movement into the TWWHA. Any actions that can be taken to deploy and manage more bootwash stations, along with actions designed to improve their use and associated education program, are likely the most powerful actions that could be taken to prevent the incursion of chytrid into the TWWHA.

II. Introduction

IIA. General background

Modern-day extinction rates in amphibians are staggering relative to other vertebrate taxa. Based on the geologic background rate of extinctions, less than 1 amphibian species extinction has been predicted to have occurred since 1980 (McCallum 2007). What has actually been observed, however, is that over 100 amphibian species have gone extinct since the 1970s, and around one-third of the world's approximately 6000 species of amphibians are now considered threatened (Stuart, Chanson et al. 2004). This has resulted in a modern day observed extinction rate 136–2707 times greater than that predicted by models (McCallum 2007). The potential ecological factors that are thought to be responsible for amphibian decline include habitat degradation and loss, introduced species, pollution, contaminants, pathogens, climate change, and of course, synergistic effects amongst several of these factors (Collins and Crump 2009).

One particular pathogen, chytrid fungus (*Batrachochyutrium dendrobatidis* [Bd]), has been strongly implicated in the worldwide decline of amphibians (Daszak, Cunningham et al. 2003). Bd was first described in 1998 and is associated with frog declines as well as extinctions in Australia, Africa, and in Central, South and North America. Anthropogenic processes have played a large role in the worldwide spread of Bd (Skerratt, Berger et al. 2007, Collins 2010), and the impact of chytridiomycosis on frogs is considered by some to be the most spectacular loss of vertebrate biodiversity due to disease in recorded history (Skerratt, Berger et al. 2007).

Chytridiomycosis results from Bd invasion into keratinised tissue of an amphibian and causes hyperkeratosis (Longcore, Pessier et al. 1999). Hyperkeratosis disrupts cutaneous function (Voyles, Young et al. 2009), compromising the host's immune system and normal cardiac functioning (Campbell, Voyles et al. 2012). While the majority of amphibian species appear to be highly susceptible to destructive Bd infection, some species appear to not be affected by chytrid (Collins 2010). In some cases, an amphibian species may host Bd, but are themselves not lethally impacted by the disease; these species can exist as 'reservoir species' whereby chytrid can persist, even when primary host density is low. Other populations may even recover from chytrid impacts, at least on short-term time scales (Newell, Goldingay et al. 2013). In general, it is difficult to predict how populations of amphibians may vary in their responses to Bd (Pilliod, Muths et al. 2010).

In 2004 Bd was first detected in Tasmania (Obendorf and Dalton 2006), and is widespread across the state with the exception of the Tasmanian Wilderness World Heritage Area (TWWHA), where Bd has only recently begun to invade (Philips, Wilson et al. 2010, Cashins, Philips et al. 2013). Presently, the Tasmanian Wilderness World Heritage Area (TWWHA) makes up greater than 75% of the distribution of the endemic Tasmanian tree frog (*Litoria burrowsae*: Driessen and Mallick 2003). *L. burrowsae* has high susceptibility to Bd infection in laboratory trials (Voyles, Phillips et al. 2014), and climatic conditions are thought to be particularly favourable for Bd in the TWWHA (Murray, Rosauer et al. 2010).

Three other species of frog are thought to be of importance in terms of potential Bd dynamics and likely impacts on amphibians in the TWWHA: the brown tree frog (*Litoria ewingi*), common froglet (*Crinia signifera*), and Tasmanian froglet (*Crinia tasmaniensis*). All three of these species are widespread in Tasmania. The brown tree frog and common froglet are thought to be potential

'reservoir' species for Bd (i.e. they will host Bd but appear to suffer no direct, observable adverse effects). Susceptibility to Bd is unknown for the endemic Tasmanian froglet (Philips, Voyles et al. 2010).

IIB. Overall aims

The aim of this report is to assess the results of three components of research and monitoring of the Tasmanian Chytrid Management Plan 2010 (Philips, Voyles et al. 2010). The first component is a capture-mark-recapture study conducted on Tasmanian tree frogs during their breeding season across three years. The second component is a report of frog calling activity across a wide geographic range in Tasmania currently under threat by Bd. The final component of research reported here is the current chytrid status at two locations at the edge of the TWWHA: Hartz Mountains National Park and Birchs Inlet. This report also aims to provide recommendations for ongoing implementation of these research and monitoring components.

IIC. Capture-mark-recapture studies

Capture-mark-recapture studies are a powerful tool for conservation managers, and can be used in any situation where individual animals can be marked and detected later by capture or sighting. Capture-mark-recapture methods can be used to evaluate impacts of threats (such as Bd), record population trends, collect information for population viability analyses, set performance targets against which responses to management can be measured, and highlight areas where further research is needed.

The design of a capture-mark-recapture study is very important, and will determine what the results can be used for. An important distinction can be made between open and closed population studies. A closed population remains constant in size and composition during the study, while an open population is subject to animals leaving and entering the population through births, deaths, emigration and immigration. Since all animal populations are more or less subject to open population demographic processes, it is usually only possible to have closure by conducting a study over a short time frame (e.g. a week).

Closed population models are normally used to estimate the number of animals in a population. In addition to the assumption of closure, an important component of these models is capture probability (Lettink and Armstrong 2003). If all animals do not have the same probability of being caught, capture heterogeneity needs to be modeled explicitly to obtain accurate and useful estimates of abundance. However, estimates of absolute abundance of animal populations via closed population models are considered imprecise, and in many cases population growth (positive or negative) is usually of more interest to conservation managers than absolute numbers of animals. In these cases, open population models whereby population growth can be estimated without relying on estimators of population abundance have now been developed (Pradel 1996).

Open population models are also normally used to estimate survival. Open population models are more complex than closed population models because extra parameters are needed to model recruitment, mortality and movements, and to distinguish between these properties and population survival rates.

A capture-mark-recapture study was initiated in 2012 to investigate the impact of chytrid on a wild population of Tasmanian tree frogs. Logistical and funding constrains meant that only two populations could be monitored: Lune River 4C and Melaleuca 6. Both populations were chytrid-free in 2012 but Lune River 4C was chosen because it occurred near a chytrid-positive frog population and was likely to become chytrid positive in the foreseeable future. Melaleuca 6 was chosen because it had high visitor use in an area remote from known chytrid areas and would act as an early warning for chytrid spread and would trigger a management response. The aim was to obtain long-term baseline trends in abundance of Tasmanian tree frogs and ecological data as well as disease status. The lack of replication was identified as a major limitation but the study could still provide insight into the response of a population to chytrid as well as survivorship of individuals. Obtaining population data for several years prior to possible infection was important given the lack of replication.

The first component was to estimate two population parameters (survival and population growth/decline) for Tasmanian tree frogs located in two different breeding ponds. Chytrid was recently detected in one of the ponds in 2013 (Lune River 4C). The other pond (Melaleuca 6) is currently chytrid-free (See section IIIB).

IID. Acoustic monitoring – assessing call activity

Auditory surveys of breeding frogs are a common tool used to verify distributions, investigate ecological relationships, and monitor population trends at various geographic and temporal scales. Frog call survey data are recorded typically on an integer scale of 1 to 3 (Weir, Royle et al. 2005, Weir, Fiske et al. 2009). Qualitatively, these scales indicate the number of frogs heard calling (one or two, a few, or many). However, based on the reasonable expectation that a larger number of frogs should generate a higher call activity rate, there has long been a desire to treat these numbers as quantitative indices to population size (Corn, Muths et al. 2011). Some authors have done that explicitly (Fahrig, Pedlar et al. 1995, Mazerolle 2005, Eigenbrod, Hecnar et al. 2008), but the more common approach is to treat these data conservatively as indicating whether a species was present or not, or to use frog call activity indices to compute metrics that in theory should be more indicative of population numbers (Shirose, Bishop et al. 1997, Corn, Muths et al. 2011).

There are several biotic and abiotic factors that may influence data collected with call surveys, including species and season (Crouch and Paton 2002, De Solla, Fernie et al. 2006), time of day (Bridges and Dorcas 2000, Oseen and Wassersug 2002), and weather, such as temperature and precipitation (Oseen and Wassersug 2002, Saenz, Fitzgerald et al. 2006). Research has also shown that observer bias can also impact frog call survey results, as different observers can often assign different index scores to the same chorus of frogs (Shirose, Bishop et al. 1997, Pierce and Gutzwiller 2007) or may disagree on which species are present (Lotz and Allen 2007, Pierce and Gutzwiller 2007).

To avoid the problem of intra-specific seasonal variation in activity, call surveys can be restricted to a species' maximum breeding activity (Corn, Muths et al. 2011). Conducting multiple surveys per pond per season can be used to minimise the impacts of other environmental influences on call activity rates, such as weather. Multiple surveys per pond, however, can be logistically difficult if there are a large number of ponds to be sampled during a single breeding season, or if the geographic area of the survey area is broad. A solution to this is to use remote automated recording systems (Peterson

and Dorcas 1994) which can collect data across large geographic scales at approximately the same time and in a relatively efficient cost-effective way.

The aim of the acoustic monitoring component is to determine whether chytrid will impact on the presence/activity of Tasmanian tree frogs should it spread into the TWWHA. Monitoring sites were chosen on the basis that Tasmanian tree frogs had been recorded there and that they were reasonably accessible by walking.

The second component was to assess and develop the utility of acoustic monitoring to survey frogs in the TWWHA. We did this by measuring the call activity of four frogs (Tasmanian tree frog, brown tree frog, common froglet, and Tasmanian froglet) from recorded sound files collected at 36 ponds across the TWWHA during the 2013 breeding season (1 July – 31 October). We tested whether different observers agreed on their call activity measures, provided a metric (the call saturation index) that can be generated from frog call data, and provided occupancy estimates where a particular species of frog was not present.

IIE. Chytrid update – Hartz Mountains and Birchs Inlet

Hartz Mountains National Park is a popular walking area on the eastern edge of the TWWHA. It was chosen for chytrid monitoring because it is one of the few accessible places where the endemic moss froglet (*Bryobatrachus nimbus*) occurs. Nothing is currently known of the putative impacts of Bd on moss froglet populations.

Hartz Mountains is also one of two Chytrid Exclusion Areas in Tasmania, the other being the Melaleuca/Port Davey area. Hartz Mountains is a priority area because Bd has been previously detected in roadside ponds leading to the start of the main trailhead, but has not been detected along the trail itself (Cashins, Philips et al. 2013).

Birchs Inlet is an important area as it occurs along the western edge of the TWWHA, and represents an area where human incursion and movement occur in a remote area of the TWWHA. The endemic Tasmanian tree frog and the Tasmanian froglet, along with the brown tree frog and the common froglet, occur here, in recent history in high abundance (M. Holdsworth pers. comm.). Surveys conducted in 2012 detected chytrid at Birchs Inlet for the first time (Cashins, Philips et al. 2013).

The third component was to provide updated information of chytrid status by capturing and swabbing frogs at both sites during 2014 surveys.

III. Methods: Capture-mark-recapture studies

IIIA. Tasmanian tree frog biology

Unfortunately, very little is documented concerning the basic biology and life history of Tasmanian tree frogs. Currently, there is only one scientific publication on the species (Zhang, Cashins et al. 2014). Thus, information provided in this section on Tasmanian tree frog life history is anecdotal and based on natural history observations (albeit generally expert observations). Peak breeding activity for Tasmanian tree frogs usually occurs during the final third of winter and runs until early spring (late July through the end of October). At the ponds sampled here for capture-mark-recapture study, males congregate at the breeding pond and begin to call sometime after sunset to attract females.

Wind speed as well as time of day affects male calling – males tend to call more when wind speed is lower (except in large populations, where wind has no effect), and there is a peak in male calling ~3 hours after sunset (Cashins, Philips et al. unpublished data). Nothing is known concerning Tasmanian tree frog movements, behavior, and activity outside of the times when males are congregated at breeding ponds. It is thought that breeding behavior (i.e. aggregating in ponds) can lead to increased contact amongst individuals and may facilitate Bd transmission. Bd also has motile zoospores which can move from infected to healthy frogs through the water (Carey, Bruzgul et al. 2006).

The number of females at breeding sites is probably much lower than the number of males, although females do not call, making them harder to locate and find. Because we captured many fewer females than males at our two sites across the three years of study (Lune River = 24 females, 274 males; Melaleuca = 5 females, 91 males), we use only males in this analysis.

IIIB. Sample sites

Two breeding ponds were initially chosen for study in 2012, one at Melaleuca 6 (MEL; 432197E, 5191852N) and one at Lune River 4C (LR; 488802E, 5188559N). The breeding pond at MEL was chosen because it is both deep within the TWWHA and has high human traffic. The breeding pond at LR was chosen because it is along the edge of the TWWHA, had high Tasmanian tree frog numbers, and was thought to serve as a good model of frog/Bd dynamics near roads. Both ponds were chytrid free in 2012; in 2013 chytrid was detected at LR (N = 38 swabs, 3 positive for Bd; prevalence = 7.9%) but not at MEL (N = 60 swabs/individuals), and in 2014 chytrid was again detected at LR (N = 202 swabs, 9 positive for Bd; prevalence = 4.4%) but not MEL (N = 21 swabs).

Other species of amphibians occur at both these sites (brown tree frog, common froglet and Tasmanian froglet; see Section VI). We did not capture these other species at LR; at MEL, some common froglet and brown tree frogs were captured and swabbed in 2014, but results have not been analysed and are not presented here.

Several environmental covariates could impact the results from a capture-mark-recapture study. For example, capture and recapture probabilities (and therefore survival estimates) could be impacted by temperature, recent disturbance, cloud cover, wind speed, current and recent rain patterns, pH, and water conductivity, to name a few (but see: Pilliod, Muths et al. 2010). While each of these environmental covariate measures were collected during the study, they are not included in the analyses because sample sizes were already below the minimum recommended for analytical models (see section IID), and addition of covariates would decrease estimate reliability further at this time.

IIIC. Data collection

From 2012 to 2014 each population was sampled during the breeding season (Table 1). Due to logistics, MEL was sampled once per breeding season, while LR was either sampled once (2012), twice (2013), or three times (2014) within the same breeding season. Each sample included multiple capture sessions/nights in which frogs were caught. Sampling effort for each capture session at each pond in 2012 and 2013 is unknown, but in 2014, for each capture session, one to four workers used headlamps to search the pond and its adjacent edges for frogs. Sampling began within two hours of sunset, and continued for a minimum of two hours. Sampling ended either at the end of two hours when all/most frogs were considered to have been captured, or in cases where there was high male

density, until 100 individuals were captured. Catch per unit effort estimates for each capture session, where unit effort is per person per minute, are given in Table 1.

Frogs were captured individually using clean vinyl gloves and new plastic bags. For each captured frog, we checked whether it had already been tagged with a microchip, and if it had not, we injected one into the dorsolateral subcutaneous tissue. All captured animals were measured (until early 2014 snout-vent length (SVL), thereafter left tibia length (TL), and weight for all frogs) and all individual frogs were swabbed once for Bd presence on each trip. Swabbing involved brushing a sterile swab across the ventral side of the torso, the inside of each of the front and back legs, and the pads of the hind and front feet (Hyatt, Boyle et al. 2007). Sterile swabs were brushed across each of these areas four times per frog. Each sample swab was then sealed in a plastic casing and sent to Tasmanian Animal Health Laboratories for analysis with polymerase chain reaction to detect Bd. To avoid potential contamination of the collected tissue and disease transmission among individuals, we adhered scrupulously to clean procedures in the field.

Table 1. Number of male Tasmanian tree frogs captured at two different sites from 2012 to 2014. CPUE = average catch per unit effort. In this case number of frogs per person per minute for a trip. CPUE estimate includes female frogs, whereas all other columns do not.

Year	Date	# sampling nights	# unique frogs	# new frogs	# recaptured frogs	# swabs tested for Bd	# swabs positive for Bd	Bd prevalence (%)	CPUE (95%CIs)
Lune River									
2012	21/8-22/8	2	50	50	8	36	0	0.0	Unknown
2013	20/8-22/8	2	35	30	10	34	0	0.0	Unknown
2013	25/9-27/9	3	4	1	8	4	3	75	Unknown
2014	5/8–7/8	2	161	152	38	109	3	2.7	0.53 (-0.59-1.64)
2014	20/8-22/8	3	10	3	18	10	0	0.0	0.05 (0.03-0.07)
2014	22/9-4/9	3	82	36	151	83	6	7.3	0.29 (0.23-0.35)
Melaleuca									
2012	10/9-2/9	3	39	39	24	39	0	0.0	Unknown
2013	28/8-30/8	3	59	41	44	59	0	0.0	Unknown
2014	29/8-2/9	5	19	13	45	19	0	0.0	0.03 (0.01-0.57)

IIID. Statistical analyses

We used program MARK (White and Burnham 1999) to analyse the capture- mark-recapture data. For each of the two ponds under analysis, we were interested in addressing the following questions:

- Is the population stable, increasing, or decreasing in size?
- What proportion of the population survives each year?

At one of our sites (LR) we were initially interested in whether Bd infection impacts on individual survival. However, given the very small number of individuals that were detected to be Bd positive at Lune River relative to the overall catch (Table 1), fitting Bd presence as a covariate in models was not performed, since heavily unbalanced models usually result in heavily biased and inaccurate estimates of parameters.

We analysed the two ponds separately, because there were different numbers of primary trips to each site, there were different numbers of secondary capture nights at each site, and the sites had different intervals of time between primary trips.

There is a wide array of models and approaches available for estimating animal abundance, survival and population growth (reviewed in: Amstrup, MacDonald et al. 2006). A typical capture-mark-recapture analysis involves model building and selection using a combination of closed population, open population, or robust design estimators. In robust design models (Pollock 1982) the abundance estimation feature of closed-population models is combined with the survival estimation component of open population models (Figure 1).

The minimum recommended sample size for open population models, which are required to estimate survival and population growth, is five capture sessions (Lebreton, Burnham et al. 1992). For example, in order to estimate survival from one breeding season to the next, one would need five years of data from each pond. Robust design models, which combine aspects of both closed and open models, recommended at a minimum three primary sessions (i.e. years) with at least five secondary sessions (i.e. trips to a site: Figure 1, Pollock 1982). Unfortunately, at both Melaleuca and Lune River these minimum sample requirements have yet to be met.

The simplest closed population models for estimating animal abundance require only two capture occasions (Lincoln 1930), but more capture occasions improves goodness-of-fit and reliability of closed population abundance estimates. Since closed population model estimates for abundance are notoriously imprecise, one alternative to estimating animal abundance is to instead estimate the observed rate of population growth between two samples, also called observed λ. Pradel models use a reverse-time, robust design mark-recapture model to estimate λ based on permanent emigration rather than absolute abundance. Pradel models also provide open population estimates of survival (Pradel 1996).

Given the structure of our data (Table 1) and considering that frogs are most likely leaving and sometimes returning to each annual breeding pond, we chose a robust design analysis to estimate survival and provide closed population models of abundance. Next, a Pradel model was chosen to estimate λ; estimates of survival from Pradel models were also used to compare against the estimates of survival obtained from robust design models. The modelling approach was as follows:

- For each site, first fit full time varying models using the Robust design with Huggins' p and c. These models include estimates of survival (Φ), temporary emigration (γ''), fidelity to remaining outside the sampling area (γ'), probability of first capture (p), and probability of recapture (c).
- Since we were interested primarily in Φ, we first used model selection techniques (i.e. AIC_c) to determine the structure of movement (γ' and γ'') and capture probabilities (p & c) that best fit the data. Once this was determined, Φ and $\check{N}$ (abundance) were estimated.
- A Pradel model was used on the same data to estimate λ. Φ estimates were also extracted for comparison with Φ estimates from Robust design models.

For Robust design models, three competing models of temporary emigration were tested: (i) a model with no temporary emigration (i.e $\gamma'' = \gamma' = 0$), (ii) a model with random temporary emigration (i.e. $\gamma'' = \gamma'$), and (iii) a model with Markovian temporary emigration (γ'' & γ' are allowed to be fully time varying). In Markovian movement, an individual's probability to remain inside/outside the sample area depends on its current location (i.e. whether it is present already or not in the sample area); in random movement, an individual's probability to remain is not dependent on its current location, and is instead random.

AIC_c values were used to determine models that provided the best fit to the data. When the difference in AIC between two models is <2, then we are reasonably safe in saying that both models have approximately equal weight in the data. If 2<AIC<7, then there is considerable support for a real difference between the models, and if AIC>7, then there is strong evidence to support the conclusion of differences between the models.

It is worth noting that there are currently no goodness-of-fit tests for Robust design or Pradel models. In other words, one can produce results from a best-fitting model relative to other models in the model set, but there is currently no statistical way that has been developed to test whether the 'best-fitting' model actually fits the data well. This, in combination with small sample sizes, requires careful inference from the current results.
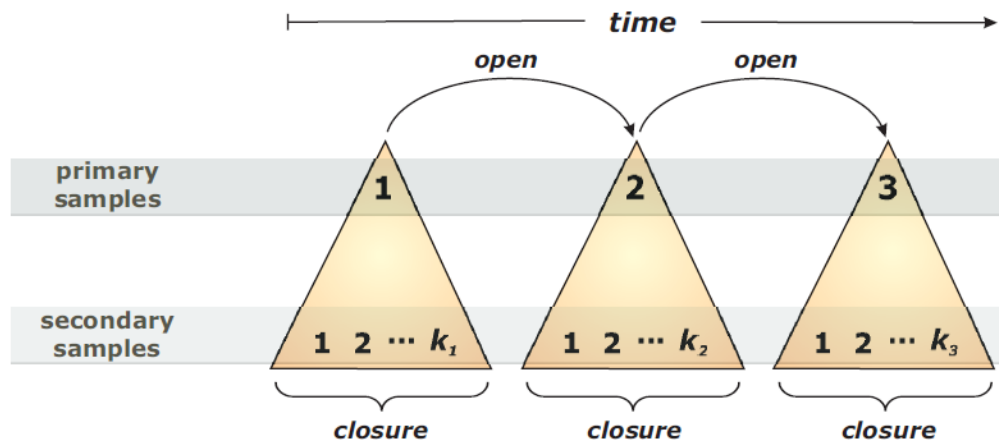
Figure 1. Basic structure of Pollock's Robust design. Primary samples are annual surveys taken at a pond, while secondary samples represent nights that frogs were trapped. Survival estimates were generated from the time intervals between primary samples (years) while abundance estimates were generated from groups of secondary samples (nights) within each year. The key difference between the robust design and standard Cormack-Jolly-Seber open population models is that instead of just one capture occasion between survival intervals, multiple (>1) capture occasions are used. In addition to providing estimates of abundance, the probability that an animal is captured at least once in a trapping session can be estimated from the data collected during the session using mark-recapture models developed for closed populations. This allows for subsequent estimation of survival, temporary emigration from the trapping area, and immigration of marked animals back to the trapping area based on the longer intervals between primary trapping sessions. Figure taken from Chapter 15 of the online book 'Program MARK: A gentle introduction' (http://www.phidot.org/software/mark/docs/).

IV. Results: Capture-mark-recapture studies

IVA. Melaleuca survival, abundance, and population growth

Bd was not detected in swabs taken at Melaleuca in 2014, confirming its ongoing chytrid-free status (Table 1). In 2012, 39 Tasmanian tree frogs were captured and marked in three nights; in 2013, 59 frogs were captured in three nights, 18 of which were recaptures from 2012. In 2014 there were five capture nights during which time 19 frogs were captured, six of them from previous years. Twenty frogs were caught in at least two different years, while three frogs were caught across all three annual trips.

The Robust design fully-parameterised time-varying model included 24 estimates/parameters, 19 of which could be estimated (model rank 3, Table 2). Allowing survival (Φ) to be different from 2012–13 and 2013–14 (i.e. time varying, $\Phi_{(t)}$), we first tested whether the probability of first capture (p) on each trip was equal to the probability of recapture (c) on each trip (model rank 2a versus model rank 3, Table 2). At Melaleuca, p and c could be modelled as equal. We then tested whether capture probabilities were time varying within a trip (model 2a, Table 2) or could be modelled by a single estimate per trip (model 4, Table 2). The best-fitting model for the structure of p and c indicated equality but also time variance in each parameter estimate ($p_{(t)} = c_{(t)}$; model ranks 1, 2a, & 2b, Table 2).

Using $p_{(t)} = c_{(t)}$ to model capture probability we next compared whether Markovian, random, or no movement was the best fit of the data. Models that included temporary emigration (model ranks 2a & 2b, Table 2) did not fit the data better than the model which did not account for temporary emigration (model rank 1, Table 2). Since the 'no movement' model was more parsimonious (i.e. fewer parameters are needed to fit different estimates for γ' and γ'') we retained a 'no movement' structure for γ'' and γ' to then estimate survival.

Annual survival was strongly time dependent (model rank 5, Table 2). Survival across 2012 to 2013 was low (0.13; SE = 0.20, Table 3) and the estimate included zero (L95%CI = -0.26; U95%CI = 0.51, Table 3); the estimate for survival across 2013 to 2014 was lower (-0.83; SE = 0.13; L95%CI = -1.09; U95%CI = -0.59, Table 3). Capture probabilities on each of the nights ranged from -0.49 to 0.89 (Table 3).

Closed population abundance estimates were also extracted using the best-fitting model found for Melaleuca (model rank 1, Table 2). Closed population estimates that included both time and capture heterogeneity resulted in abundance estimates with large standard errors compared to closed population estimates that only included capture heterogeneity. Abundance estimates of the population at Melaleuca in 2012, 2013 and 2014 are given in Table 4. Qualitative patterns of Table 4 indicate an upward trend in frog numbers from 2012 to 2013, and then a sharp decrease in numbers from 2013 to 2014.

Since the Robust design models for Melaleuca indicated the best structure for p and c was $p_{(t)} = c_{(t)}$, we retained this structure in Pradel models and first confirmed that Φ needed to be time varying (model ranks 1 and 2, Table 5). Next, we tested whether observed population growth λ was time varying as well, which it was (model ranks 1 and 3, Table 5). Quantitative estimates of population growth from Pradel models confirmed the small but positive population growth at Melaleuca across 2012 to 2013 (λ = 0.44; SE = 0.18; L95%CI = 0.08; U95%CI = 0.80), as well as the strong population decline across 2013 to 2014 (λ = -1.30; SE = 0.24; L95%CI = -1.78; U95%CI = -0.83). Survival estimates from Pradel models exactly matched those obtained from Robust design ones.

Table 2. Set of robust design models used to estimate Tasmanian tree frog survival and abundance across 2012–14 at Melaleuca. A period (.) indicates that a model parameter was fit as a constant (i.e. no time variance). Time variance is indicated by the subscript 't'. Φ is survival, while γ''= 0 γ' = 1 indicates 'no movement', $γ''_{(t)} ≠ γ'_{(t)}$ indicates Markovian movement, and $γ''_{(t)} = γ'_{(t)}$ equals 'random movement'. Robust design estimates of survival were extracted from a single model, the one with the best model rank (i.e. rank 1). The fully-parameterised time-varying model corresponds to the model below that was ranked 3rd. The Akaike information criterion (AIC) is a measure of the relative quality of a statistical model for a given set of data. As such, AIC provides a means for model selection. Changes in AIC values (ΔAIC$_c$) greater than 2 are usually indicative of a better fit.

	Rank	AIC$_c$	ΔAIC$_c$	AIC$_c$ weight	Model likelihood	Parameters	Deviance
$Φ_{(t)} γ''= 0 γ' = 1 p_{(t)} = c_{(t)}$	1	550.8	0.0	0.531	1.000	13	598.2
$Φ_{(t)} γ''_{(t)} ≠ γ'_{(t)} p_{(t)} = c_{(t)}$	2a	552.4	1.6	0.233	0.444	14	597.6
$Φ_{(t)} γ''_{(t)} = γ'_{(t)} p_{(t)} = c_{(t)}$	2b	552.4	1.6	0.233	0.444	14	597.6
$Φ_{(t)} γ''_{(t)} ≠ γ'_{(t)} p_{(t)} c_{(t)}$	3	561.5	10.7	0.002	0.005	19	594.7
$Φ_{(t)} γ''_{(t)} ≠ γ'_{(t)} p_{(.)} = c_{(.)}$	4	616.3	65.5	0.000	0.000	6	679.2
$Φ_{(.)} γ''= 0 γ' = 1 p_{(t)} c_{(t)}$	5	38621.9	38071.1	0.000	0.000	14	38667.0

Table 3. Robust design parameter estimates for Melaleuca extracted from a model with time variance in survival ($\Phi_{(t)}$), no movement ($\gamma' = 1$; $\gamma'' = 0$), and equal capture probabilities ($p_{(t)} = c_{(t)}$). Beta is the point estimate for the parameter of interest, SE = standard error of estimate, L95%CI = lower 95% confidence interval, U95%CI = upper 95% confidence interval.

Parameter	Beta	SE	L95%CI	U95%CI
$\Phi_{(2012–13)}$	0.13	0.20	-0.26	0.51
$\Phi_{(2013–14)}$	-0.84	0.13	-1.09	-0.59
γ'	1.00	N/A	N/A	N/A
γ''	0.00	N/A	N/A	N/A
p date 1	-0.49	0.16	-0.80	-0.18
p date 2	0.19	0.18	-0.16	0.54
p date 3	0.06	0.17	-0.28	0.40
p date 4	-0.88	0.12	-1.12	-0.64
p date 5	0.46	0.17	0.13	0.80
p date 6	-0.26	0.13	-0.52	-0.00
p date 7	0.15	0.23	-0.30	0.60
p date 8	0.26	0.23	-0.19	0.71
p date 9	0.89	0.23	0.43	1.35
p date 10	-0.06	0.23	-0.51	0.39
p date 11	-0.27	0.23	-0.72	0.18

Table 4. Closed population abundance estimates with capture heterogeneity for Melaleuca 2012–14.

Year	Population estimate	SE	L95%CI	U95%CI	Probability of capture
2012	53	8.35	44	80	0.40
2013	87	12.31	72	122	0.33
2014	20	1.77	20	29	0.56

Table 5. Set of Pradel open population models used to estimate observed population growth (λ) across 2012–14 in Tasmanian tree frogs at Melaleuca. A period (.) indicates that a model parameter was fit as a constant (i.e. no time variance, or t). Φ is survival, p is the probability of first capture, c is the probability of recapture. The Akaike information criterion (AIC) is a measure of the relative quality of a statistical model for a given set of data. As such, AIC provides a means for model selection. Changes in AIC values (ΔAIC_c) greater than 2 are usually indicative of a better fit.

	Rank	AIC_c	ΔAIC_c	AIC_c weight	Model likelihood	Parameters	Deviance
$\Phi_{(t)}\ \lambda_{(t)}\ p_{(t)} = c_{(t)}$	1	733.4	0.0	1.00	1.000	15	115.2
$\Phi_{(.)}\ \lambda_{(t)}\ p_{(t)} = c_{(t)}$	2	749.1	15.8	0.000	0.000	14	133.25
$\Phi_{(t)}\ \lambda_{(.)}\ p_{(t)} = c_{(t)}$	3	759.5	26.1	0.000	0.000	14	143.6

IVB. Lune River survival, abundance, and population growth

In 2012 at Lune River, 50 Tasmanian tree frogs were captured and marked in 2 nights; in 2013, 35 frogs were captured in 5 nights, 5 of which were recaptures from 2012. In 2014 there were 8 capture sessions during which time 203 frogs were captured, 12 of them recaptures from previous years. Ten frogs were caught across two years of the study; 2 frogs were captured across all three (Table 1).

Bd was first detected in swabs taken at Lune River in 2013 (7.9% prevalence), and was again confirmed in 2014 (4.4% prevalence), although prevalence of Bd remained low and the two years were not dissimilar (Table 1).

The Robust design fully-parameterised time-varying model at Lune River included 32 total estimates/parameters, not all of which could be estimated (Table 6). Allowing annual survival (Φ) to be different between 2012–13 and 2013–14 (i.e. time varying, $\Phi_{(t)}$), we first tested whether the probability of first capture (p) on each trip was equal to the probability of recapture (c) on each trip (model rank 1a versus model rank 3, Table 6), as well as whether p and c were equal and constant (model rank 6, Table 6), as well as constant but not equal (model rank 5, Table 6). At Lune River, the best-fitting model indicated that p and c should be modelled as time varying, and not equal (four models with the highest ranks: 1a, 1b, 2a, 2b, Table 6). This was the most fully-parameterized structure possible for p and c.

Using $p_{(t)}$ $c_{(t)}$ to model capture probability we next compared whether Markovian, random, or no movement was the best fit of the data. Markovian movement was identified as a slightly better fit than random or a no movement design (model 1a versus 2a and 2b, Table 6). We retained a Markovian movement structure for γ'' and γ' to then estimate survival.

Survival was not strongly time dependent (model 1a versus 1b, Table 6), and was estimated at 0.02 across all years (SE = 245.8; L95%CI = -481.7; U95%CI = 481.7, Table 6). The 2012 to 2013 estimate was 1.48 (SE = 0.00; U95%CI = 1.48; L95%CI = 1.48, Table 6), and the 2013 to 2014 estimate was -0.25 (SE = 0.00; U95%CI = -0.25; L95%CI = -0.25, Table 6). However, in all cases standard errors of survival estimates were either very large or zero, indicating that estimates were not reliable and that the model probably did not fit the data. Unfortunately, estimates of temporary emigration and return were also associated with standard errors so large to make point estimates meaningless.

Closed population abundance estimates were also extracted from the overall model using the best-fitting model design (model rank 1a, Table 6). Closed population estimates that included both time and capture heterogeneity would not converge, so closed population estimates that only included capture heterogeneity were generated.

Abundance estimates of the population at Lune River in 2012, 2013 and 2014 are given in Table 7. Abundance estimates were variable with large standard errors and associated confidence intervals, but went down from 2012 to 2013, and then up from 2013 to 2014.

Since the Robust design indicated the best fit structure for p and c was $p_{(t)}$ $c_{(t)}$, we retained this structure in Pradel models. Each combination of $\Phi_{(t)}$, $\Phi_{(.)}$, $\lambda_{(t)}$, and $\lambda_{(.)}$ fit the data equally well (model ranks 1a – 1d, Table 8). In other words, there was no strong evidence for temporal variation in survival or population growth. However, estimates of Φ and λ were not reliable from these models, partially due to the number of parameters needed to fit fully time varying p and c. Therefore, we set

$p_{(t)}$ = $c_{(t)}$ and then held Φ or λ constant to reduce the number of parameters and improve precision of estimates. We obtained estimates of both time-specific and time-general Φ and λ in this way (Table 9). Survival estimates were always negative, but across 2012–13 the estimate did include 0. Lambda indicated potential negative growth (loss) from 2012–13, strong positive growth in 2013–14, and overall positive growth that was different from 0. This combination of negative survival and positive population growth, if real, would have required a large number of immigrants and recruits into the population in 2014.

Table 6. Set of Robust design models used to estimate Tasmanian tree frog survival and abundance across 2012–14 at Lune River. A period (.) indicates that a model parameter was fit as a constant (i.e. no time variance). Time variance is indicated by the subscript 't'. Φ is survival, while γ''=0 γ' = 1 indicates 'no movement', $\gamma''_{(t)} \neq \gamma'_{(t)}$ indicates Markovian movement, and $\gamma''_{(t)} = \gamma'_{(t)}$ equals 'random movement'. Robust design estimates of survival were extracted from a single model, the one with the best model rank (i.e. rank 1). The fully-parameterised time-varying model corresponds to the model below that was ranked 1a. The Akaike information criterion (AIC) is a measure of the relative quality of a statistical model for a given set of data. As such, AIC provides a means for model selection. Changes in AIC values (ΔAIC_c) greater than 2 are usually indicative of a better fit.

	Rank	AIC_c	ΔAIC_c	AIC_c weight	Model likelihood	Parameters	Deviance
$\Phi_{(t)}$ $\gamma''_{(t)} \neq \gamma'_{(t)}$ $p_{(t)}$ $c_{(t)}$	1a	1789.7	0.0	0.594	1.000	26	2319.3
$\Phi_{(.)}$ $\gamma''_{(t)} \neq \gamma'_{(t)}$ $p_{(t)}$ $c_{(t)}$	1b	1789.7	0.0	0.594	1.000	26	2319.3
$\Phi_{(t)}$ γ''=0 γ' = 1 $p_{(t)}$ $c_{(t)}$	2a	1791.9	2.2	0.195	0.327	27	2319.3
$\Phi_{(t)}$ $\gamma''_{(t)} = \gamma'_{(t)}$ $p_{(t)}$ $c_{(t)}$	2b	1791.9	2.2	0.195	0.327	27	2319.3
$\Phi_{(t)}$ $\gamma''_{(t)} \neq \gamma'_{(t)}$ $p_{(t)} = c_{(t)}$	3	1798.2	8.5	0.008	0.014	18	2345.3
$\Phi_{(.)}$ $\gamma''_{(t)} \neq \gamma'_{(t)}$ $p_{(t)} = c_{(t)}$	4	1798.2	8.5	0.008	0.014	18	2345.3
$\Phi_{(t)}$ $\gamma''_{(t)} \neq \gamma'_{(t)}$ $p_{(.)}$ $c_{(.)}$	5	2052.6	262.9	0.000	0.000	9	2618.7
$\Phi_{(t)}$ $\gamma''_{(t)} \neq \gamma'_{(t)}$ $p_{(.)} = c_{(.)}$	6	2140.4	350.7	0.000	0.000	6	2712.7

Table 7. Closed population abundance estimates with capture heterogeneity for Lune River 2012–14.

Year	Population estimate	SE	L95%CI	U95%CI	Probability of capture
2012	160	52.8	96	318	0.18
2013	91	32.8	55	197	0.11
2014	384	48.3	312	505	0.13

Table 8. Set of Pradel open population models used to estimate observed population growth (λ) across 2012–14 in Tasmanian tree frogs at Lune River. A period (.) indicates that a model parameter was fit as a constant (i.e. no time variance). Φ is survival, p is the probability of first capture, c is the probability of recapture. The Akaike information criterion (AIC) is a measure of the relative quality of a statistical model for a given set of data. As such, AIC provides a means for model selection. Changes in AIC values (ΔAIC$_c$) greater than 2 are usually indicative of a better fit.

	Rank	AIC$_c$	ΔAIC$_c$	AIC$_c$ weight	Model likelihood	Parameters	Deviance
$\Phi_{(t)}$ $\lambda_{(t)}$ $p_{(t)}$ $c_{(t)}$	1a	2236.9	0.0	0.25	1.000	29	520.2
$\Phi_{(.)}$ $\lambda_{(t)}$ $p_{(t)}$ $c_{(t)}$	1b	2236.9	0.0	0.25	1.000	29	520.2
$\Phi_{(.)}$ $\lambda_{(.)}$ $p_{(t)}$ $c_{(t)}$	1c	2236.9	0.0	0.25	1.000	29	520.2
$\Phi_{(t)}$ $\lambda_{(.)}$ $p_{(t)}$ $c_{(t)}$	1d	2236.9	0.0	0.25	1.000	29	520.2
$\Phi_{(.)}$ $\lambda_{(t)}$ $p_{(t)} = c_{(t)}$	2	2248.2	11.3	0.00	0.004	18	555.7
$\Phi_{(.)}$ $\lambda_{(t)}$ $p_{(t)} = c_{(t)}$	3	2258.0	21.1	0.00	0.000	17	567.7
$\Phi_{(t)}$ $\lambda_{(.)}$ $p_{(t)} = c_{(t)}$	4	2259.1	22.2	0.00	0.000	18	566.7

Table 9. Pradel model Lune River parameter estimates extracted from models ranked 2, 3, & 4 from Table 8. Beta is the point estimate for the parameter of interest, SE = standard error of estimate, L95%CI = lower 95% confidence interval, U95%CI = upper 95% confidence interval.

Model	Parameter	Beta	SE	L95%CI	U95%CI
$\Phi_{(t)}\ \lambda_{(.)}\ p_{(t)} = c_{(t)}$	$\Phi_{(2012–13)}$	-0.25	0.25	-0.74	0.24
	$\Phi_{(2013–14)}$	-0.58	0.16	-0.90	-0.26
$\Phi_{(.)}\ \lambda_{(.)}\ p_{(t)} = c_{(t)}$	$\Phi_{(overall)}$	-0.46	0.12	-0.69	-0.22
$\Phi_{(.)}\ \lambda_{(t)}\ p_{(t)} = c_{(t)}$	$\lambda_{(2012–13)}$	-0.45	0.32	-1.08	0.18
	$\Lambda_{(2013–14)}$	1.30	0.20	0.90	1.70
$\Phi_{(.)}\ \lambda_{(.)}\ p_{(t)} = c_{(t)}$	$\Lambda_{(overall)}$	0.57	0.11	0.36	0.78

V. Discussion and recommendations: Capture-mark-recapture studies

Given the paucity of knowledge of frog population dynamics in Tasmania, simply understanding natural variation in frog population survival and growth is an important first step in any attempts to document impacts of chytrid in the TWWHA. In other words, establishing natural 'baselines' of population dynamics prior to any monitoring of populations with significant chytrid prevalence will allow for a more powerful inference of any observed population declines. Large natural variation in amphibian population numbers are well documented (Pechmann, Scott et al. 1991, Pechmann and Wilbur 1994), and characterise Tasmanian tree frog population dynamics thus far observed at Lune River, and to a lesser extent, Melaleuca. If there is a desire to document impacts of chytrid on individual and population survival, as well as population growth, continued annual capture-mark-recapture trips to Melaleuca and Lune River will be necessary.

Importantly, Melaleuca remains Bd-free, and further efforts should be made to ensure Melaleuca remains free from chytrid. This may be the most important work for any person or organisation involved with frog biodiversity conservation in Tasmania.

Melaleuca had more across-year recaptures than Lune River, and the numbers of nights trapping within each primary trip each year were more equal than the trapping effort at Lune River. Both of these aspects of the data contribute to the result that survival, abundance and population growth estimates from Melaleuca were more precise and reliable than those from Lune River. Adult male annual survival at both Lune River and Melaleuca was estimated to be low across breeding years. Population growth at the two populations was opposite – at Melaleuca, the most severe change in observed population growth was a drop in numbers from 2013 to 2014; at Lune River, this period coincided with the greatest observed population growth. At Melaleuca, we did not detect a significant effect of temporary immigration/emigration, while at Lune River, the pattern of survival and growth indicated that between 2013–14 a large number of individuals not present at the site in previous years had probably arrived. This large influx of males, along with the continued low prevalence of Bd at Lune River, means that if there were impacts of chytrid on population survival and abundance at Lune River these effects could have been masked by the concurrent high immigration of male frogs to the pond. Alternatively, the low prevalence of Bd in 2014 after chytrid incursion in 2013 at Lune River could be explained by chytrid dynamics; low Bd prevalence can precede high prevalence before an epizootic event, or can represent an endemic disease (Brem and Lips 2008). These results reinforce the idea of needing a longer-term annual capture-mark-recapture

study to further our understanding of frog movement, as well as to disentangle any effects of movement, chytrid, and naturally occurring frog population variability due to environmental effects.

For Lune River, an addition complication was that sampling effort in 2012 and 2013 was unknown. While all three years (2012, 2013 and 2014) were needed to estimate between-year survival, it could be that different sampling efforts in different years also contributed to the data from Lune River not fitting the statistical models very well. Finally, the large population increase at Lune River from 2013–14 relative to the lower number of frogs observed in 2012 and 2013 may have contributed to issues with fitting models and estimating parameters well. Future standardised sampling at Lune River should improve these modelling issues.

The merits of continued mark-recapture study are currently unclear, given biological (i.e. natural variability in frog population numbers) and management (i.e. funding) considerations. On the one hand, demonstrated impact of chytrid on Tasmanian tree frog populations is a critical first step in any management program, and the data collected thus far are a valuable start to any continued study at the two ponds of interest. Thus far, capture-mark-recapture models have identified population parameters that may be important drivers of frog numbers (lifespan and immigration/emigration), and in establishing natural variability in frog numbers at these ponds. On the other hand, capture-mark-recapture study is labour- and resource-intensive, and there may be other methods, such as manipulative experiments or sound recordings analysis, that could be used instead to contribute to demonstrating effects of chytrid on Tasmanian tree frog populations.

VA. Specific recommendations

1) It is unclear whether to continue with capture-mark-recapture study. However, if managers do decide to continue, it is worth noting that it is probably likely that given natural variation observed in frog populations, multiple years of annual capture-mark-recapture study are needed to determine any impacts of chytrid. Some results suggest at least seven years (Newell, Goldingay et al. 2013).

2) Given the variable number of frogs caught both within (Lune River) and across breeding seasons (Lune River and Melaleuca) any additional data should be collected in a standardised way. This includes attempting to collect data at both sites using the same design. Ideally, one would perform three trips to each pond (Lune River 4C & Melaleuca 6) each breeding season; each trip would include three nights of sampling. Standardising sampling at both ponds would be the most powerful way to gain information from current efforts, logistics notwithstanding for repeated visits to Melaleuca. Standardisation would represent a need for funding greater than that used in 2014. If funding continues at current levels, repeated sampling using the current design at both ponds in 2015 (three times during the breeding seasons at Lune River, once during the breeding season at Melaleuca) is warranted.

3) In an ideal world, with adequate funding, further sites should be included in the capture-mark-recapture study in order to improve replication. Logistics notwithstanding, replication of capture-mark-recapture methods on other ponds that are also either chytrid-free or chytrid-positive would allow for broader inference of any putative impacts. Clearly, capture-mark-recapture studies represent excellent methods to demonstrate impacts of chytrid on frog populations in Tasmania, but also are labour-intensive and require funding. These

trade-offs will need to be considered further by managers when further funding levels are known.

VI. Methods: Acoustic monitoring of frog call activity and detection probability estimation

VIA. Sound recording units

Thirty-six Wildlife Acoustics Model SM2+ SongMeter remote sound recording units were deployed from May – August 2013. Sound recording units were deployed within 3–5m of ponds where Tasmanian tree frogs were known or thought to occur. Units were programmed to record for 5 minutes at 3pm during the day, and for 5 minutes 2 hours after sunset daily. Daytime recordings were taken in order to better detect common and Tasmanian froglet.

VIB. Call activity indices, occupancy estimates, and inter-observer agreement

Auditory surveys of breeding frogs are a common tool used to verify distributions and monitor trends of populations at various geographic and temporal scales (Dorcas, Price et al. 2009). Call survey data are commonly recorded using a standard four-point Anuran Call Index (ACI: Weir and Mossman 2005): '0' for undetected species, '1' for individually identifiable frogs with no overlapping calls, '2' for individually identifiable frogs with some overlapping calls, and '3' for a full chorus of frogs with undistinguishable overlapping calls.

While many frog monitoring programs use a single ACI to represent the relative abundance at a site, this likely exacerbates problems with other sources of environmental variation that can have significant impacts on male calling (e.g. temperature and precipitation). Thus, we used the automated recording systems to generate multiple ACIs for each species of interest across a broader range of dates during the breeding season. Since there were 123 possible days between 1 July and 31 October 2013, and since this number of sound recordings for the number of ponds of interest was not logistically possible to analyse, we instead randomly selected 10 dates within the 1 July – 31 October date range using a random number generator for each pond, and then assigned sound files to one of five observers. Twenty-six ponds had the majority of sound recordings from the 1 July – 31 October date range available; an additional 10 ponds with more restricted date range of sound recording data that was available were also selected (Table 10). In total, 360 five-minute sound recordings were used to generate the 2013 sound recording results.

For each sound file we assigned an ACI value to each of the four species of frogs of interest: Tasmanian tree frog, brown tree frog, common froglet, and Tasmanian froglet. Multiple call activity indices were then used to compute call saturation indices (CSI) by summing the 10 ACI values observed at a site, and dividing by the maximum possible sum of index values (Corn, Muths et al. 2011). The CSI is the proportion (0–1) of total call saturation. In other words, if all recordings taken at a site have an ACI of 3, the CSI = 1. Depending on the number of sound recordings analysed per pond per year, the CSI should provide a more reliable estimate of true relative proportion within and between sites. In addition, the CSI is more directly comparable across studies.

However, because call survey data from 2011 and 2012 are not stored in a form that is currently amenable to generating CSI values, only single ACI values (i.e. maximum call activity observed) are

currently available from these previous years. Therefore, for 2013 sound recordings, in addition to estimating a single CSI per species per pond, we also report on the maximum ACI value observed.

If a species was not detected in a pond from the 2013 sound recordings, we estimated a probability of occupancy (MacKenzie, Nichols et al. 2003) for that species for that pond. We also report unconditional detection probabilities (i.e. with no covariates or environmental variables) for each of the four species that can be used in future work to determine the optimal number of sound recordings to listen to per pond.

To test whether individual observers were biased in assigning ACI values, 60 sound files randomly selected from the available pool were independently analysed by three listeners. We estimated a one-way absolute agreement 'intraclass correlation coefficient' (ICC: Shrout and Fleiss 1979, McGraw and Wong 1996). The ICC measured inter-observer absolute agreement of ACI values for each species of frog across all ponds.

Table 10. Thirty-six ponds that contributed to the sound recordings analysis from the 2013 frog breeding season.

Site	Pond	Number of days audio recorded from 1 Jul to 31 Oct 2013	Start date	End date
Birchs Inlet	5A	24	8-Oct-13	31-Oct-13
	5G	24	8-Oct-13	31-Oct-13
	5J	24	8-Oct-13	31-Oct-13
Lyell Hwy	2A	120	1-Jul-13	29-Oct-13
	2C	107	1-Jul-13	16-Oct-13
	2D	121	1-Jul-13	31-Oct-13
	2E	121	1-Jul-13	31-Oct-13
	2I	120	1-Jul-13	31-Oct-13
	2J	120	1-Jul-13	31-Oct-13
	2K	120	1-Jul-13	31-Oct-13
	2L	107	1-Jul-13	16-Oct-13
Lune River	4A	122	1-Jul-13	31-Oct-13
	4C	121	1-Jul-13	31-Oct-13
	4D	122	1-Jul-13	31-Oct-13
	4E	121	1-Jul-13	31-Oct-13
	4I	71	22-Aug-13	31-Oct-13
Melaleuca	1	71	22-Aug-13	31-Oct-13
	10	71	22-Aug-13	31-Oct-13
	14	71	22-Aug-13	31-Oct-13
	3	71	22-Aug-13	31-Oct-13
	6	122	1-Jul-13	31-Oct-13
	8	61	22-Aug-13	21-Oct-13
Northwest	1C	122	1-Jul-13	31-Oct-13
	1D	122	1-Jul-13	31-Oct-13
	1F	120	1-Jul-13	31-Oct-13
	1G	121	1-Jul-13	31-Oct-13
	1H	122	1-Jul-13	31-Oct-13
Strathgordon	3A	122	1-Jul-13	31-Oct-13
	3B	123	1-Jul-13	31-Oct-13
	3C	123	1-Jul-13	31-Oct-13
	3D	42	20-Sept-13	31-Oct-13
	3G	123	1-Jul-13	31-Oct-13
	3M	123	1-Jul-13	31-Oct-13
	3N	123	1-Jul-13	31-Oct-13
	3O	123	1-Jul-13	31-Oct-13
	3R	123	1-Jul-13	31-Oct-13

VIC. Reasoning for choosing 10 sound clips for each pond

For the four frog species of interest here, conditional detection probabilities (i.e. dependent on survey method, time of survey, and environmental variables) have been estimated for three of them (Cashins et al., unpublished data, Table 11). The sole species where detection probabilities (p) have not been estimated (brown tree frog) is at least as common as the other three species of interest; it is highly likely that choosing a survey method to detect the other three species is sufficient to also detect brown tree frogs. While detection probabilities are conditional upon a number of variables (i.e. there is no 'set' detection probability for a species), we used these previous detection

probabilities to help guide choices regarding the number of sound recordings to choose for the 2013 analysis.

There is a relationship between the number of surveys taken at each pond, the rarity of the species of interest, and the probability that the species will be detected in the sample. Let N be the number of sampling units/surveys, p be the probability of detection in a single sampling unit, and α be the probability or confidence that the species will be detected in the sample of N surveys. (1-p) is the probability of the species not appearing in a single survey, so $(1-p)^N$ is the probability of our overall study not detecting the species. Thus the probability of the species appearing in the sample is $\alpha = 1-(1-p)^N$.

For example, for the Tasmanian tree frog, if we sample from a pond 10 times over the study period, and the probability of detection in a single sample is 0.49, then the probability α of detecting Tasmanian tree frog in our study given that it is truly there is extremely high – indeed, almost certain ($\alpha = 1$).

With 10 surveys per pond, the range of α for each of the three species where detection probabilities are available was very high, 0.96–1 (Table 11). Ten surveys per pond were chosen because it was very likely – almost certain – that this protocol would detect all species of interest, given that they were truly present in a pond.

Table 11. Conditional detection probabilities (p) and confidence (α) that each species will be detected given 10 surveys/5-minute sound recordings per pond. Detection probabilities are taken from Cashins et al., unpublished data. Conditional detection probabilities from Cashins et al. are taken from models where 5-minute night time surveys were analysed and environmental variables were included (air and water temperature, relative humidity, cloud cover, wind speed, and whether it rained over the previous 24 hours). L95%CI = lower 95% confidence interval, U95%CI = upper 95% confidence interval.

Species	p	p: L95%CI	p: U95%CI	α	α: L95%CI	α: U95%CI
Common froglet	0.97	0.90	0.99	1	1	1
Tasmanian froglet	0.40	0.28	0.54	0.99	0.96	1
Tasmanian tree frog	0.66	0.49	0.80	1	1	1

VID. Detection probabilities estimations for Tasmanian tree frog, Tasmanian froglet, common froglet, brown tree frog

Cumulative detection probability was estimated using the formula $P=1-(1-p)^n$, where p is the nightly detection probability, and n is the number of surveys.

VII. Results: Acoustic monitoring of frog call activity and detection probability estimation

Table 12 gives the CSI estimates from each of the ponds for each of the four frog species. Brown tree frogs were observed in every pond sampled and, on average, a full chorus of brown tree frogs was observed in ponds in almost 70% of the samples (mean CSI = 0.69, standard deviation = 0.23). Calling activity was much lower and variable relative to the mean for the Tasmanian tree frog (mean CSI = 0.20, SD = 0.21), common froglet (mean CSI = 0.29, SD = 0.33) and the Tasmanian froglet (mean CSI = 0.25, SD = 0.32).

Tasmanian tree frogs were not observed at seven ponds, and an additional 10 ponds had CSIs < 0.10 (i.e. full choruses of Tasmanian tree frogs were observed in only 10% of samples). Six ponds had CSIs for Tasmanian tree frogs that were > 0.50 (i.e. at least half of the time one would visit this pond one would observe a full chorus). Ten ponds had CSIs for common froglet that were < 0.10, and 12 ponds had CSIs for common froglet that were > 0.50. Eight ponds had CSIs for Tasmanian froglet that were < 0.10, and nine ponds had CSIs for Tasmanian froglet that were > 0.50.

There were 26 cases in total where at least one of the four frog species did not occur (Table 12). In four ponds, two species were not observed. In each of these cases, the observed probability that a species of frog was actually present but not observed was very low, < 0.002.

Table 13 has the maximum calling ACI for each of the species across all ponds. The maximum observed ACI is given so that earlier years' data could be more comparable (Figures 4–9). In Table 13, the same species-specific occupancy probability estimates are given in each case where a frog species was not detected.

Inter-observer agreement coefficients for all four species' calls were quite high (> 0.70, Table 14), indicating that different listeners tended to assign ACIs to the four species of frogs in the same way. In other words, the 0–3 scale used by the observers appears to be a promising method to reliably measure frog calls.

The Tasmanian tree frog detection probability curves were lower than those for the Tasmanian froglet, common froglet, and brown tree frog. Figure 2 displays cumulative unconditional detection probability curves for all four species including lower and upper 95% confidence intervals.

Table 12. Call saturation index (CSI) for 36 ponds sampled across 1 July to 31 October 2013. For each pond, ten 5-minute sound recordings taken 2 hours after sunset were randomly selected across the entire date range and the four-point (0–3) amphibian call activity index for each of the species was given. The CSI was computed by summing all call activity indices and dividing by 30 (the max possible value, i.e. the proportion of samples where a full chorus of frogs of that species was observed). Asterisks (*) indicate ponds where a species was not detected. In these cases, a probability of occupancy given the species was not observed is given. The low probabilities all cases indicate sampling design was more than adequate to detect the four species of interest. For chytrid status, a '+', '-', or 'unknown' as well as a latest year of testing is given.

Pond	Species				Chytrid status
	Tasmanian tree frog	Brown tree frog	Common froglet	Tasmanian froglet	
Birchs Inlet 5A	0.10	0.83	0.53	0.17	+ 2014
Birchs Inlet 5G	0.23	0.70	0.53	0.27	+ 2014
Birchs Inlet 5J	0.20	0.97	0.70	0.03	+ 2013
Lyell Hwy 2A	0.30	0.47	0.03	0.97	+ 2013
Lyell Hwy 2C	0.27	0.63	0.001*	0.40	+2012
Lyell Hwy 2D	0.70	0.73	0.03	0.53	- 2014
Lyell Hwy 2E	0.07	0.77	0.001*	0.23	- 2014
Lyell Hwy 2I	0.01*	0.40	0.001*	0.60	+2014
Lyell Hwy 2J	0.17	0.97	0.001*	0.23	- 2012
Lyell Hwy 2K	0.07	0.87	0.001*	0.20	+2014
Lyell Hwy 2L	0.01*	0.90	0.001*	0.17	- 2014
Lune River 4A	0.40	0.67	0.67	0.03	- 2013
Lune River 4C	0.60	0.40	0.07	0.001*	+ 2014
Lune River 4D	0.47	0.67	0.77	0.001*	+ 2012
Lune River 4E	0.23	0.63	0.03	0.001*	- 2011
Lune River 4I	0.10	0.17	0.70	0.001*	Unknown
Melaleuca 1	0.33	0.93	0.90	0.03	- 2013
Melaleuca 10	0.10	0.90	0.80	0.001*	- 2013
Melaleuca 14	0.03	0.77	0.60	0.001*	- 2012
Melaleuca 3	0.23	0.87	0.63	0.13	Unknown
Melaleuca 6	0.47	0.60	0.50	0.03	- 2014
Melaleuca 8	0.13	0.93	0.93	0.001*	- 2011
Northwest 1C	0.07	0.13	0.03	0.83	+ 2013
Northwest 1D	0.01*	0.13	0.03	1.00	+ 2011
Northwest 1F	0.37	0.77	0.001*	0.50	- 2014
Northwest 1G	0.60	0.53	0.001*	0.87	+2013
Northwest 1H	0.07	0.57	0.03	0.73	+2014
Strathgordon 3A	0.10	0.80	0.03	0.03	- 2011
Strathgordon 3B	0.03	0.83	0.33	0.03	- 2012
Strathgordon 3C	0.01*	0.93	0.20	0.001*	- 2014
Strathgordon 3D	0.73	1.00	0.80	0.001*	+2013
Strathgordon 3G	0.01*	0.60	0.17	0.001*	- 2011
Strathgordon 3M	0.01*	0.90	0.07	0.17	- 2014
Strathgordon 3N	0.03	0.57	0.10	0.63	+2014
Strathgordon 3O	0.01*	0.67	0.10	0.10	Unknown
Strathgordon 3R	0.10	0.67	0.001*	0.03	- 2012

Table 13. Maximum call activity index for 36 ponds sampled across 1 July to 31 October 2013. For each pond, 10 five-minute sound recordings taken two hours after sunset were randomly selected across the entire date range and an amphibian call activity index for each of the species was given. The maximum value observed across the 10 nights is given below. Asterisks (*) indicate ponds where a species was not detected. In these cases, a probability of occupancy given the species was not observed is given. The low probabilities all cases indicate sampling design was more than adequate to detect the four species of interest.

Pond	Species				
	Tasmanian tree frog	Chytrid status	Common froglet	Tasmanian froglet	Chytrid status
Birchs Inlet 5A	1	+ 2014	3	1	+ 2014
Birchs Inlet 5G	1	+ 2014	3	2	+ 2014
Birchs Inlet 5J	2	+ 2013	3	1	+ 2013
Lyell Hwy 2A	2	+ 2013	1	3	+ 2013
Lyell Hwy 2C	3	+2012	0.001*	2	+2012
Lyell Hwy 2D	3	- 2014	1	3	- 2014
Lyell Hwy 2E	1	- 2014	0.001*	2	- 2014
Lyell Hwy 2I	0.01*	+2014	0.001*	3	+2014
Lyell Hwy 2J	2	- 2012	0.001*	2	- 2012
Lyell Hwy 2K	2	+2014	0.001*	1	+2014
Lyell Hwy 2L	0.01*	- 2014	0.001*	1	- 2014
Lune River 4A	2	- 2013	3	1	- 2013
Lune River 4C	3	+ 2014	1	0.001*	+ 2014
Lune River 4D	3	+ 2012	3	0.001*	+ 2012
Lune River 4E	2	- 2011	1	0.001*	- 2011
Lune River 4I	3	Unknown	3	0.001*	Unknown
Melaleuca 1	2	- 2013	3	1	- 2013
Melaleuca 10	1	- 2013	3	0.001*	- 2013
Melaleuca 14	1	- 2012	3	0.001*	- 2012
Melaleuca 3	2	Unknown	3	1	Unknown
Melaleuca 6	2	- 2014	3	1	- 2014
Melaleuca 8	1	- 2011	3	0.001*	- 2011
Northwest 1C	1	+ 2013	1	3	+ 2013
Northwest 1D	0.01*	+ 2011	1	3	+ 2011
Northwest 1F	3	- 2014	0.001*	3	- 2014
Northwest 1G	3	+2013	0.001*	3	+2013
Northwest 1H	1	+2014	1	3	+2014
Strathgordon 3A	3	- 2011	1	1	- 2011
Strathgordon 3B	1	- 2012	3	1	- 2012
Strathgordon 3C	0.01*	- 2014	3	0.001*	- 2014
Strathgordon 3D	3	+2013	3	0.001*	+2013
Strathgordon 3G	0.01*	- 2011	2	0.001*	- 2011
Strathgordon 3M	0.01*	- 2014	1	2	- 2014
Strathgordon 3N	1	+2014	1	3	+2014
Strathgordon 3O	0.01*	Unknown	2	1	Unknown
Strathgordon 3R	1	- 2012	0.001*	1	- 2012

Table 14. Inter-observer agreement coefficients for calling activity indices for four species of frogs at ponds located across 36 sites within the Tasmanian World Heritage Area. High coefficients (>0.70) indicate very good absolute agreement between different observers. ICC = intraclass correlation coefficient, L95%CI = lower 95% confidence interval, U95%CI = upper 95% confidence interval, $F_{(df)}$ = F value and degrees of freedom, P = probability of effect arising by chance alone.

Species	ICC	L95%CI	U95%CI	$F_{(df)}$	P
Tasmanian tree frog	0.79	0.68	0.88	12.6	<0.001
Brown tree frog	0.74	0.61	0.84	9.6	<0.001
Common froglet	0.79	0.68	0.88	12.4	<0.001
Tasmanian froglet	0.87	0.79	0.92	20.8	<0.001

Tasmanian tree frog

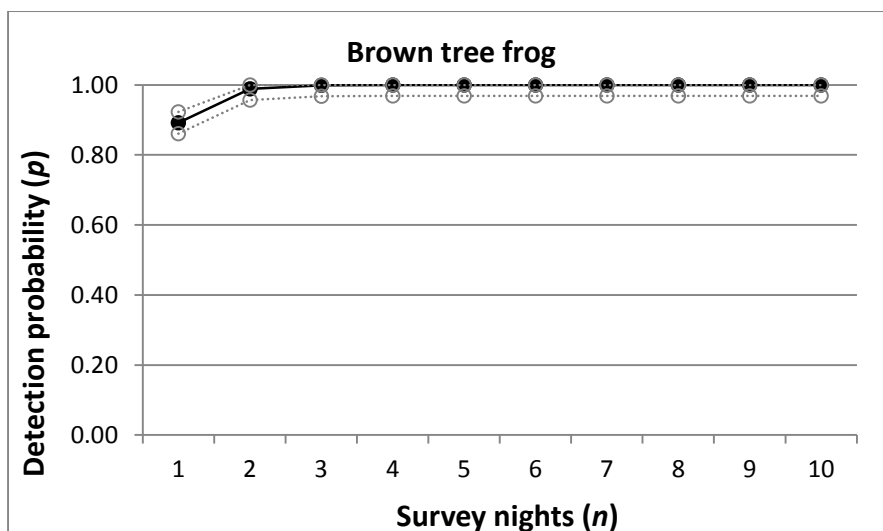Common froglet
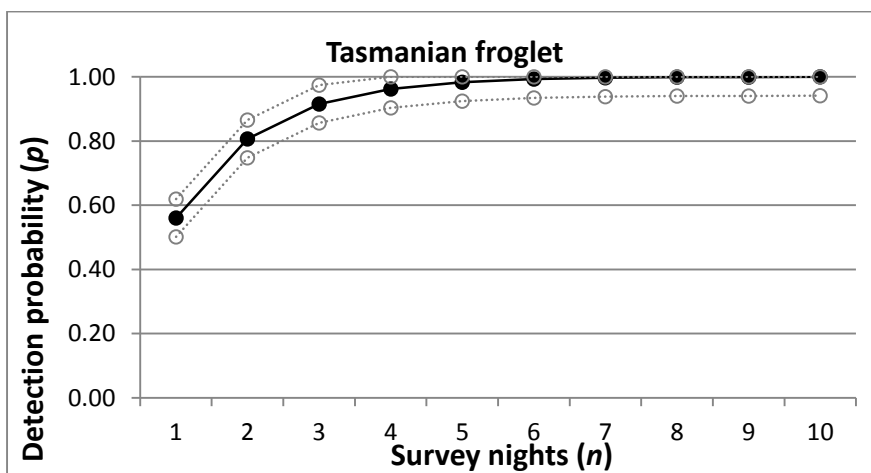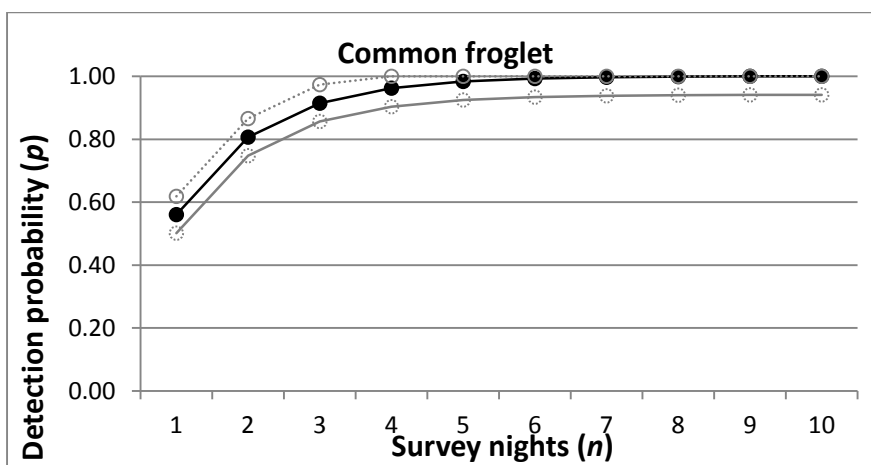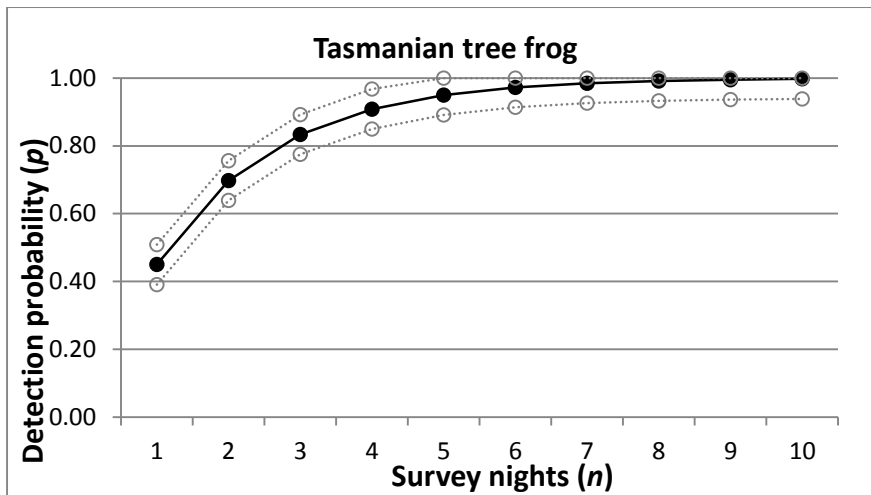
Tasmanian froglet

Figure 2. Cumulative unconditional detection probability curves for the Tasmanian tree frog, common froglet, Tasmanian froglet and brown tree frog from sound recording surveys conducted in 2012 and 2013. Closed black circles indicate detection probability; open grey circles indicate upper and lower 95% confidence intervals. Night 1 indicates the mean detection probability for each species, e.g. the probability of detecting a species on any given survey night, given that the species is present. Curves are interpreted as follows: e.g. the probability of detecting the common froglet during a two-night survey period, given the species is present, is 0.806; the probability of detecting the common froglet during a three-night survey period, given the species is present, increases to 0.915.

VIII. Discussion: Acoustic monitoring of frog call activity and detection probability estimation

Tracking frog populations using acoustic surveys could represent a cost-effective way to track several species of frogs across a wide geographical area. The 2013 analyses of sound recordings represent 'proof-of-demonstration' that remote sound recording units can be used to successfully record call activity rates of frogs in Tasmania. Indeed, different observers also agreed on the absolute call index value given to a particular sound recording sample, indicating that the method may be useful.

However, what metrics can be derived from sound recording units, and what those metrics represent, remains to be elucidated. For example, it is quite doubtful that a single ACI value will be a good indicator of frog population abundance (e.g. Table 13). On the other hand, even though not well studied, CSI values (e.g. Table 12) have been shown to be of limited value in a single study, only representing abundance well when populations were large, and not when they were small (Corn, Muths et al. 2011). Still, no findings have been replicated, and almost nothing is known concerning how CSI values might be interpreted in longer-term monitoring work. This report provides the first CSI values for a large number of ponds across Tasmania; future work should continue to monitor frog ponds using these methods, which will allow for statistical analysis of CSI trends in ponds with chytrid versus those without. CSI values may be a useful way to detect relative population trends, and be used as a signal for potential future management action.

Of course, confirmation of how CSI values covary with actual population numbers is unknown. Confirmation would require a multi-year study where capture-mark-recapture is combined with sound recordings analysis. This report provides the first reports of combining these types of

estimates at two ponds. At pond 4C in 2013 (Lune River) there was a CSI of 0.60 and an abundance estimate of 91; at Melaleuca, the 2013 CSI was 0.47 and abundance was 87. Further acoustic monitoring and further replication of capture-mark-recapture studies would be needed to provide any direct links between acoustic surveys and actual population abundance numbers in Tasmania.

As Tasmanian tree frog detection probability curves were lower than those of the Tasmanian froglet, common froglet, and brown tree frog, this species should dictate the period for future frog surveys. The probability of detecting the Tasmanian tree frog during a four or five night survey period, given the species is present, is 0.908 (upper and lower 95% confidence intervals of 0.850 and 0.967) and 0.950 (upper and lower 95% confidence intervals of 0.891 and 1.008), respectively (Figure 2). Surveys should be conducted over a four or five night period depending on desired confidence intervals. Detection probabilities for the other three species will be higher than necessary over a four or five night survey period but confidence in results will be very high.

VIIIA. Specific recommendations

1) Completing data entry and manipulation from 2011 & 2012 as well as analysis of the 2014 frog breeding season sound recordings awaits. Once this has been completed, multi-year examination of frog call data (2011–14) could be conducted and combined with knowledge concerning each pond's current and historical chytrid status. This would allow for an immediate test of whether CSI indices at ponds with chytrid are different than CSI indices at ponds without chytrid.
2) As with capture-mark-recapture studies, acoustic monitoring surveys should be considered long-term projects, and should be monitored acoustically annually for the foreseeable future, if funding and resources allow. Frog population dynamics are characterised by a high degree of natural variation, so any impacts of chytrid will require extensive baseline data on natural variation in frog calling activity.

IX. Methods, results, discussion, and recommendations: Chytrid update at Hartz Mountains and Birchs Inlet

Frogs at each pond were captured individually using clean vinyl gloves and new plastic bags during September (Birchs Inlet) and November 2014 (Hartz Mountains). All captured frogs were swabbed once for Bd presence on each trip. Swabbing involved brushing a sterile swab across the ventral side of the torso, the inside of each of the front and back legs, and the pads of the hind and front feet (Hyatt, Boyle et al. 2007). Sterile swabs were brushed across each of these areas four times per frog. Each sample swab was then sealed in a plastic casing and sent to Tasmanian Animal Health Laboratories for analysis with polymerase chain reaction to detect Bd. Swabs were analysed in batches and interpreted as an indicator of pond chytrid status (Table 15, Figure 3). To avoid potential contamination of the collected tissue and disease transmission among individuals, we adhered scrupulously to clean procedures in the field (following Allan and Gartenstein, 2010).

We detected Bd at Hartz Mountains for the first time since testing commenced in 2011, in a pond adjacent to the walking track past where a bootwash station has been installed (Table 15). This site was chosen as it is close to moss froglet habitat, and its proximity to the walking trail makes it a suitable indicator site for chytrid incursion. Longitudinal acoustic monitoring of the Hartz Mountains moss froglet population is now of particular importance to assess the impact of Bd.

At Birchs Inlet, two ponds were sampled for chytrid at locations where there were already existing acoustic monitoring sites (Table 14). Previously, one of these ponds (5A) was chytrid-free. Our results here suggest that both ponds that were surveyed (5A & 5G) are currently infected with chytrid. Surveys along the trail south to Low Rocky Point to determine spread south along the public-use track were not undertaken in 2014 due to weather and logistical constraints.

Ongoing biosecurity in both areas is important to continue to monitor any spread of chytrid into the TWWHA, as well as to prevent the introduction of new strains of Bd. In addition to annual chytrid testing, acoustic frog monitoring at both Hartz Mountains and Birchs Inlet will allow for assessing frog call activity at these ponds.

Table 15. Hartz Mountains and Birchs Inlet chytrid surveys 2014.

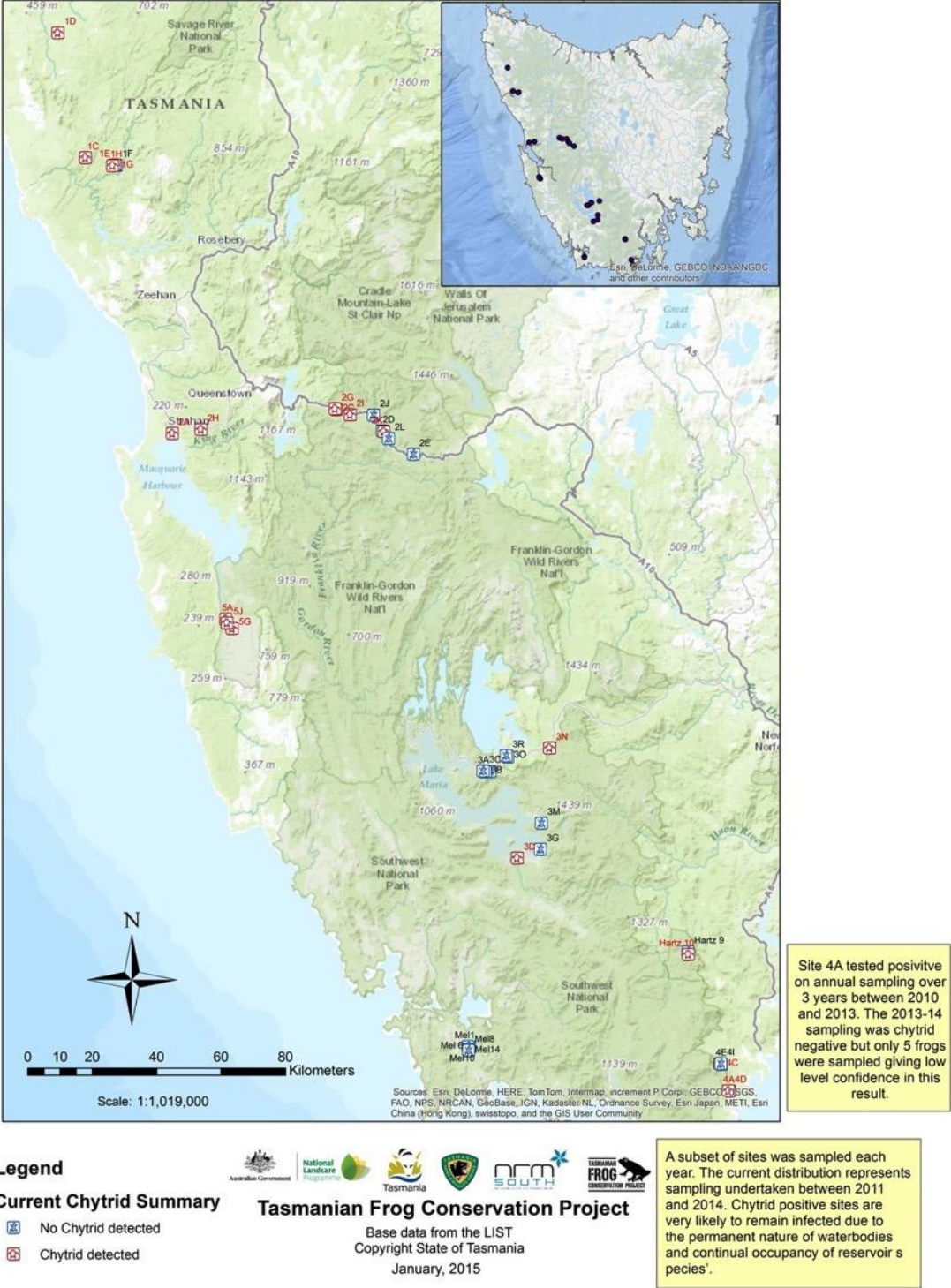| Site and Pond	Easting	Northing	Species swabbed and number of individuals swabbed	Chytrid status
Hartz 10	481556	5213119	*Crinia tasmaniensis* x 11	Positive (pooled sample)
Hartz 10	481556	5213119	*Litoria ewingi* x 1	Positive (pooled sample)
Birchs – 5A	375396	5288107	*Litoria ewingi* x 6	Positive (pooled sample)
Birchs – 5G	376921	5286014	*Crinia tasmaniensis* x 6	Positive (pooled sample)
Birchs – 5G	376921	5286014	*Litoria ewingi* x 4	Positive (pooled sample)
Birchs – 5G	376921	5286014	*Litoria burrowsae* x 1	Positive (pooled sample)

Current Chytrid Status

Site 4A tested posivitve on annual sampling over 3 years between 2010 and 2013. The 2013-14 sampling was chytrid negative but only 5 frogs were sampled giving low level confidence in this result.

A subset of sites was sampled each year. The current distribution represents sampling undertaken between 2011 and 2014. Chytrid positive sites are very likely to remain infected due to the permanent nature of waterbodies and continual occupancy of reservoir s pecies'.

Legend

Current Chytrid Summary

No Chytrid detected

Chytrid detected

Tasmanian Frog Conservation Project

Base data from the LIST
Copyright State of Tasmania
January, 2015

Figure 3. Chytrid status in south-west Tasmania 2014.
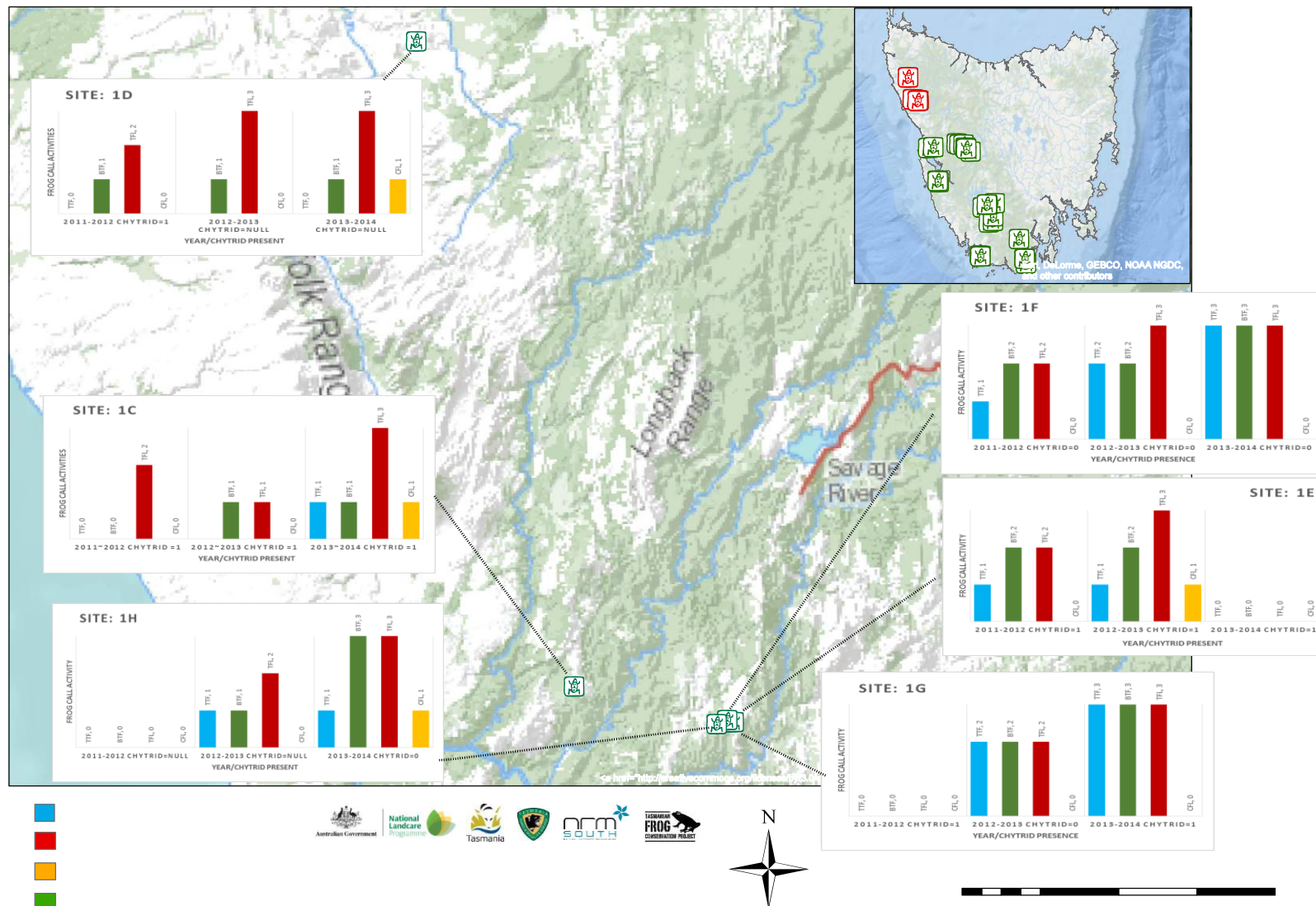
Figure 4. Maximum call activity (Anuran Call Index, ACI) and chytrid status at north-west region sites over 3 years 2011–12, 2012–13 and 2013–14.
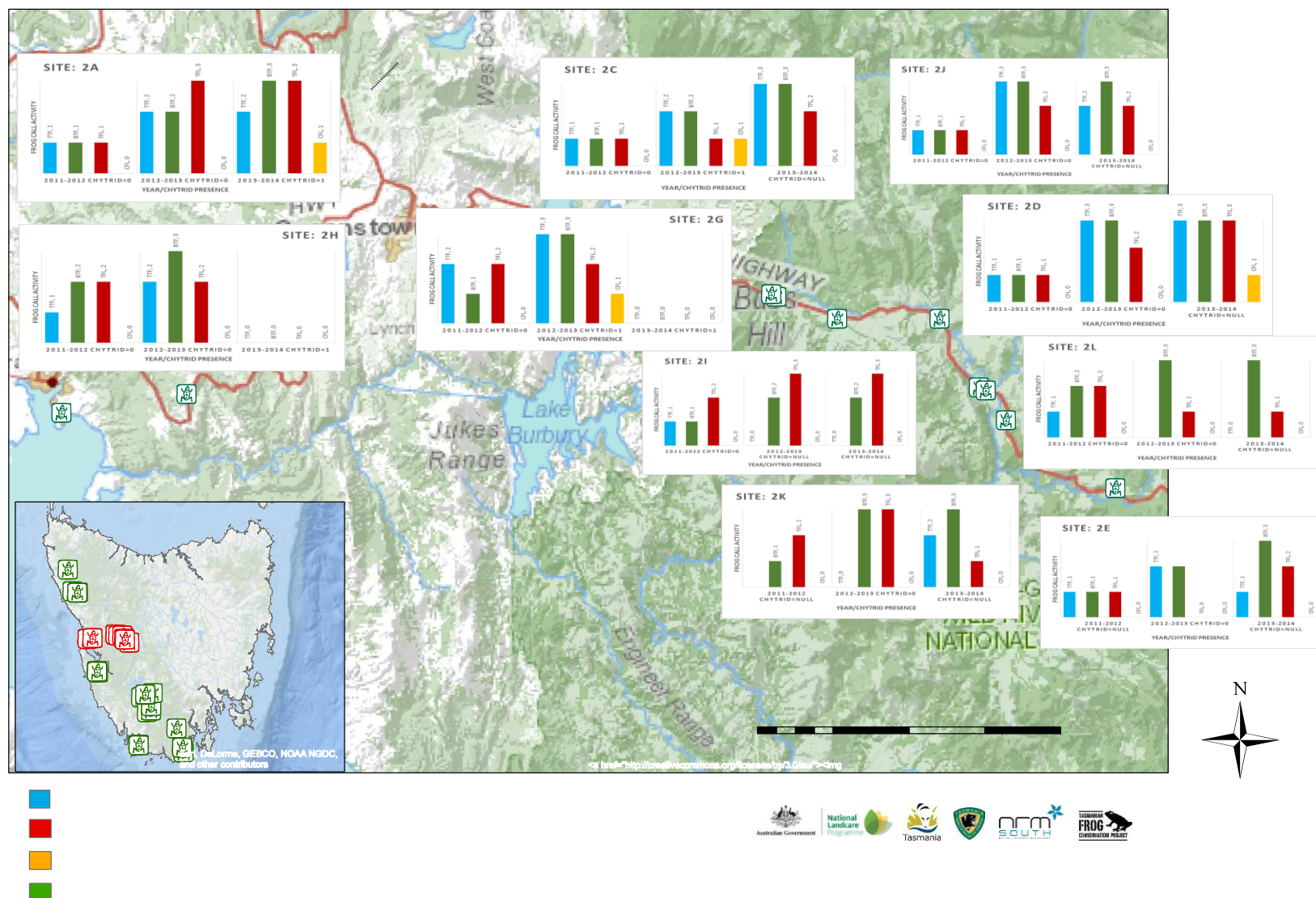
Figure 5. Maximum call activity (Anuran Call Index, ACI) and chytrid status at Strahan and Lyell Highway region sites over 3 years 2011–12, 2012–13 and 2013–14.
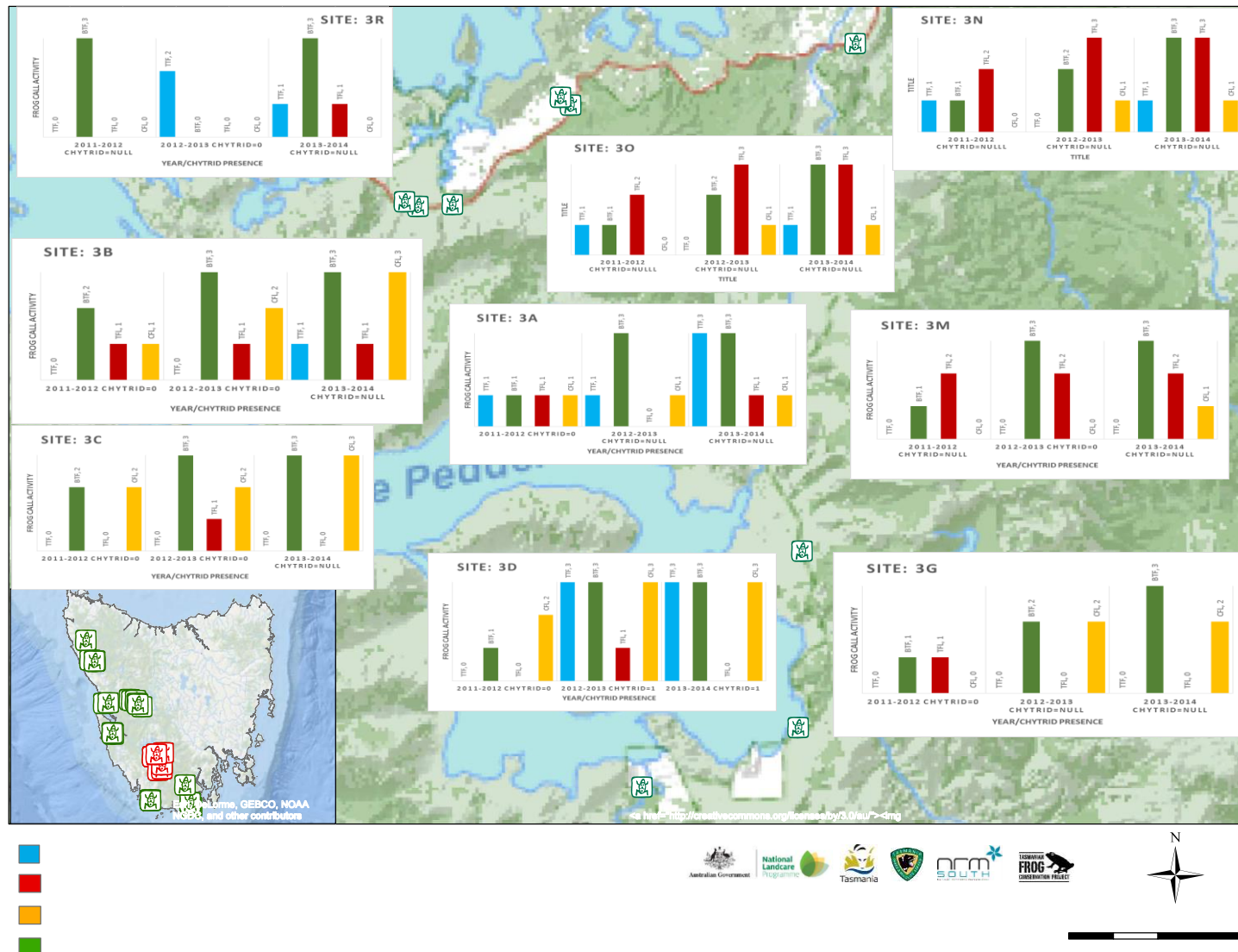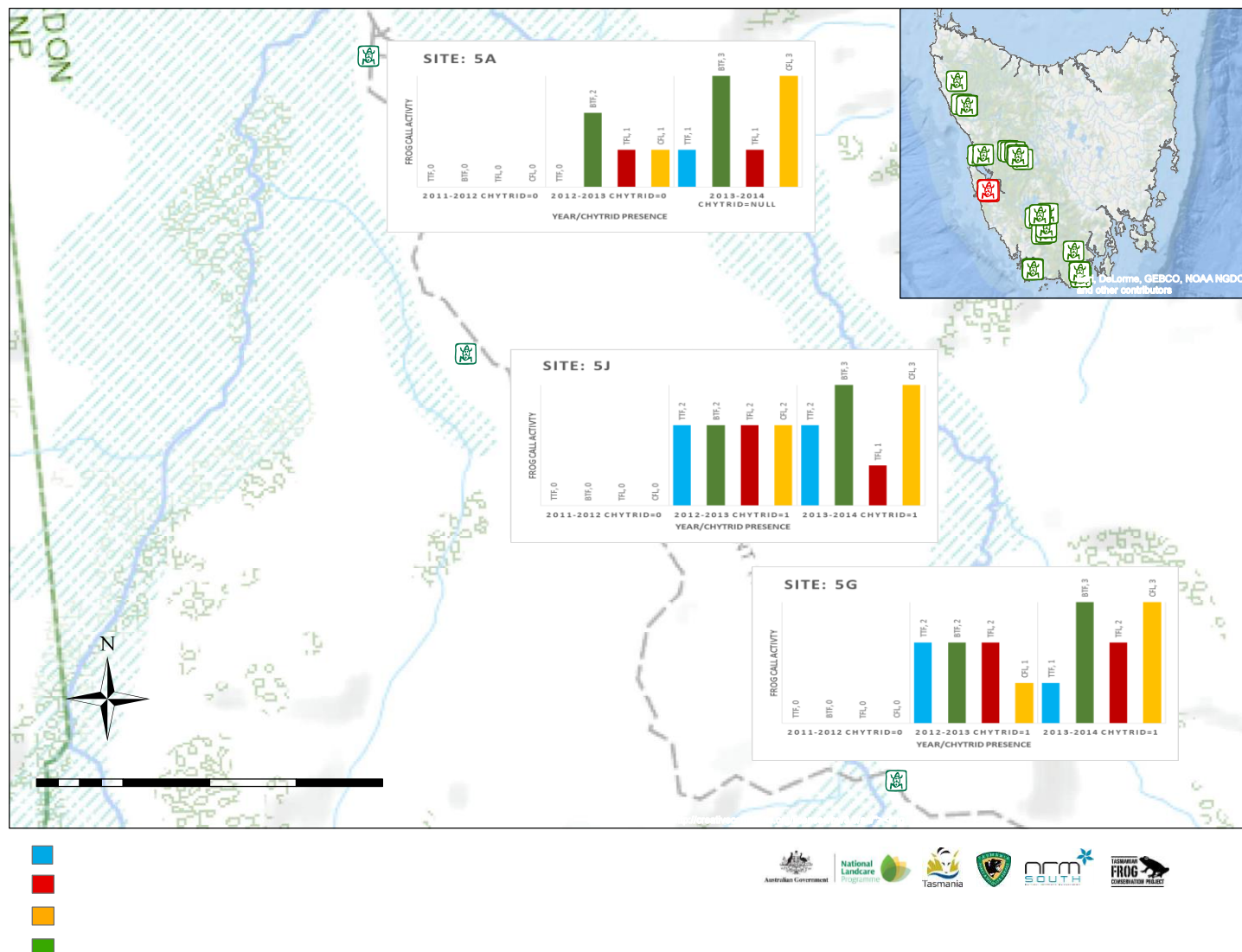
Figure 6. Maximum call activity (Anuran Call Index, ACI) and chytrid status at Strathgordon region sites over 3 years 2011–12, 2012–13 and 2013–14.

Figure 7. Maximum call activity (Anuran Call Index, ACI) and chytrid status at Lune River region sites over 3 years 2011–12, 2012–13 and 2013–14.

Figure 8. Maximum call activity (Anuran Call Index, ACI) and chytrid status at Birches Inlet region sites over 3 years 2011–12, 2012–13 and 2013–14.
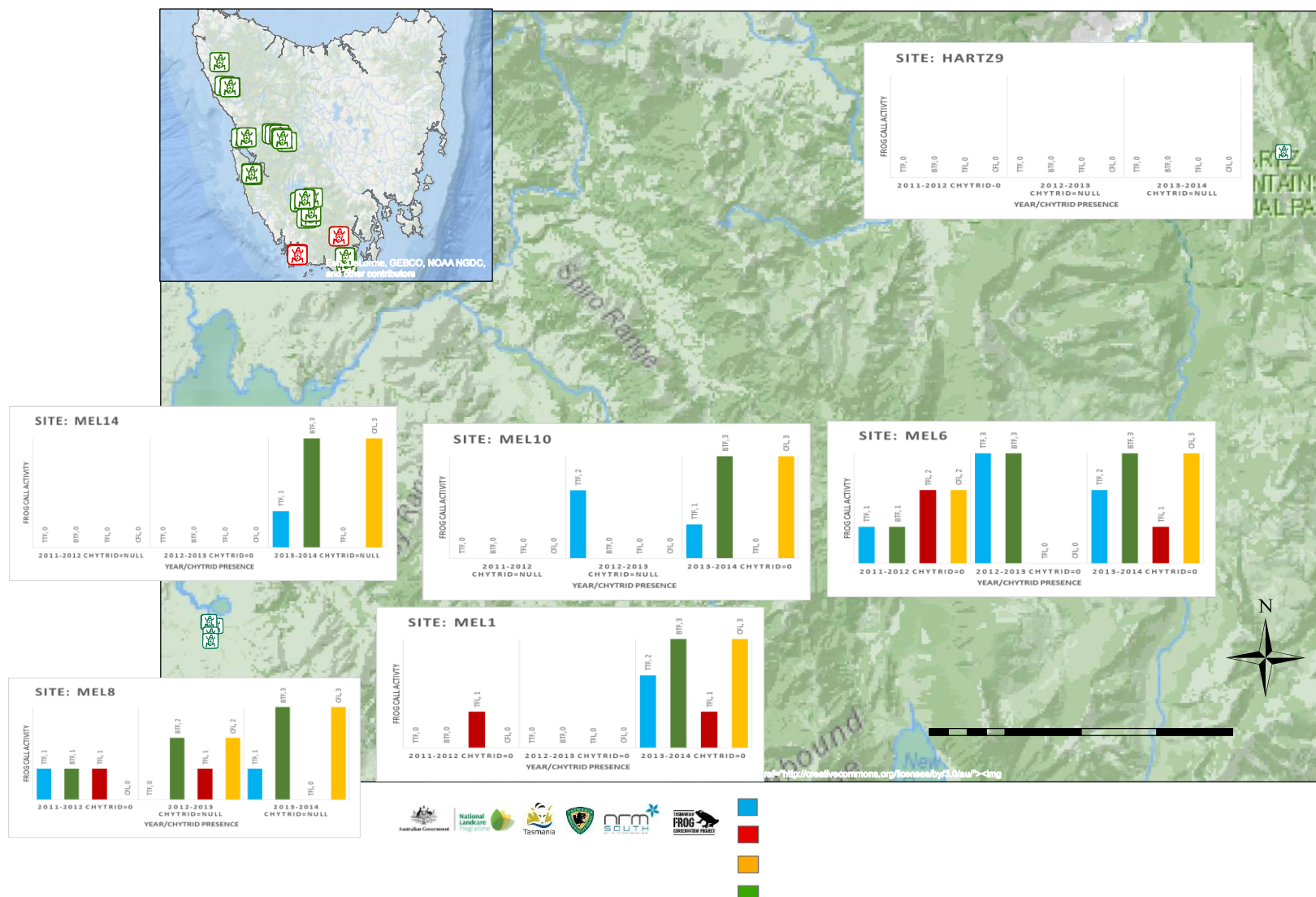
Figure 9. Maximum call activity (Anuran Call Index, ACI) and chytrid status at Melaleuca region sites over 3 years 2011–12, 2012–13 and 2013–14.

X. Overall suggestions for future research

- Chytrid disease has had devastating impacts on frog populations and often results in species extinction. Prevention, rather than managing the disease, is without a doubt the most powerful management tool currently available. Bootwash stations, along with an associated education program, currently represent the best management tool available that can be used to prevent chytrid movement into the TWWHA. Any actions that can be taken to deploy and manage more bootwash stations, along with actions designed to improve their use, seems to be the most powerful thing that could be done to prevent chytrid in the TWWHA.

- While Tasmanian tree frog were found to be highly susceptible in laboratory studies (Voyles, Philips et al. 2014), the susceptibility of other endemic species remains untested, and any potential impacts of chytrid on Tasmanian tree frog in the wild remains unknown. Capture-mark-recapture studies combined with acoustic monitoring aims to determine whether these endemic species are impacted by chytrid fungus in wild populations. Continued mark-recapture and acoustic monitoring surveys are needed to provide a robust demonstration of chytrid impact, and distinguish chytrid-related population trends from natural variability.

- Further research is needed to optimise chytrid sampling methods. The optimum combination of species, life stage and season to improve detectability of Bd needs to be refined.

- Continued chytrid surveys at Hartz Mountains and Birchs Inlet should be conducted. In both areas, implementation of further mitigation strategies (bootwash stations, public education) could be conducted.

- Manipulative experiments could be undertaken in order to demonstrate chytrid impacts on Tasmanian tree frogs. This could include studies of water chemistry and artificial pond use, which would allow for a future mitigation strategy to be used upon chytrid incursion.

- Movement studies of Tasmanian tree frog could be undertaken. This report shows data that indicate that immigration/recruitment may be important processes in frog population dynamics, but nothing is known about how much, where, and how individual frogs move. Frog movement could also contribute to chytrid spread in the TWWHA.

- Research into selective breeding for resistance in captive susceptible species should be undertaken to inform translocation of captive stock if required in the future.

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