

Article

Bioacoustic Monitoring Reveals the Calling Activity of an Endangered Mountaintop Frog (*Philoria kundagungan*) in Response to Environmental Conditions

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Abstract: Amphibians are the most endangered class of vertebrate on Earth. Knowledge of their ecology is crucial to their conservation; however, many species have received scant attention from researchers, particularly in regions that are difficult to access or when traditional monitoring methods are impractical. In recent years, technological advancements in environmental audio collection techniques and signal detection algorithms (i.e., call recognition) have created a new set of tools for examining the ecology of amphibians. This study utilises these recent technological advancements to examine the calling phenology of a poorly known Australian mountain frog (*Philoria kundagungan*). Audio recordings and meteorological data were collected from six localities across the species range, with recordings made every hour for ten minutes between July 2016 and March 2018. We developed an audio recognition algorithm that detected over 1.8 million *P. kundagungan* calls in 8760 h of audio recordings with a true positive rate of 95%. Our results suggest that calling activity was driven by substrate temperature and precipitation, which has potential consequences for the species as the climate warms and seasonal precipitation patterns shift under climate change. With this detailed knowledge of *P. kundagungan* calling phenology, this difficult-to-find species will now be more reliably detected, removing a barrier that has hindered efforts to study and conserve this species.

Keywords: bioacoustics; signal detection; automated recording; *Philoria kundagungan*; climate change



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1. Introduction

Amphibians are the most extinction-prone vertebrate group globally, with more than 30% of species facing a high to extreme risk of extinction in the wild [1]. Several key threats to this group have been identified and include habitat loss [2], the disease chytridiomycosis caused by the fungal pathogen, *Batrachochytrium dendrobatidis* [3,4] and, more recently, climate change [5,6]. Rising temperatures and changes to precipitation cycles generated by an accumulation of carbon dioxide in the atmosphere are predicted to become key drivers of amphibian population declines and extinctions over the coming decades [7]. However, it is now clear that global climate change is already affecting aspects of amphibian behaviour and ecology [8]. For example, extreme droughts have been associated with population declines due to desiccating conditions experienced by amphibians and their egg masses [9], and increasing temperatures have been found to cause physiological stress [10], alter range boundaries [11] and shift the timing of seasonal breeding cycles by up to 76 days [8]. Furthermore, extreme fire events have increased dramatically [12,13] and have started to affect ecosystems where fire has rarely been documented [14–16].

The Gondwana rainforests of eastern Australia, a network of World Heritage listed protected areas, is one example of an ecosystem whose amphibian inhabitants are beginning

to come under pressure from climate change [17]. Among the endangered species that occupy these forests are six of the seven species described within the genus *Philoria*. All are range-restricted species, have montane distributions, are listed as endangered by the International Union for Conservation of Nature and are considered at risk from climate change [1]. All six species occur on mountaintops in northern New South Wales and southeastern Queensland and occupy seepages or the boggy margins of headwater streams or drainage lines, mostly in upland rainforest [18–20]. They lay their eggs in small chambers excavated in mud, leaf litter or under rocks, and metamorphosis requires the constant moisture these microhabitats provide [18,21]. Adults are rarely encountered outside of their underground breeding chambers, and detection relies upon the advertisement call of males (from within burrows). Due to these traits and the remoteness of their habitats, all species are poorly studied [18,19,21]. Knowledge of their ecology is urgently required to develop effective conservation strategies to mitigate the effects of climate change.

Bioacoustics is a remote sensing technique that utilises audio recognition algorithms to identify the vocalisations of specified species within long-duration audio recordings from the field. Bioacoustics presents a new opportunity to improve traditional methods of studying the ecology of threatened species and can provide detailed insights into ecological parameters such as biodiversity indices, the density of individuals, presence or absence of a species and the timing and duration of seasonal breeding patterns [22,23]. For example, Hoffmann and Mitchell [24] employed bioacoustic techniques to reveal that *Geocrinia alba*, a critically endangered terrestrial-breeding frog in southwestern Australia, maintains a predictable breeding season that spans multiple months during the Austral spring. However, the study also revealed that declining winter rainfall, which impacts soil water potential and temperature, may lead to shortened breeding periods and fewer breeding opportunities for the species.

The chorusing behaviour of frogs makes obtaining audio recordings of vocalisations much more accessible than more mobile taxa, such as avian or mammalian species, which can vocalise over much larger home ranges. In most frogs, adult males use advertisement calls to attract a mate for breeding and generally call from a relatively small site, such as a pond, stream or swamp [25]. These traits provide an excellent opportunity to utilise bioacoustic methods to gain detailed knowledge of amphibian ecology not achievable with traditional methods.

Using bioacoustics, we examine the influence of environmental variables on the calling activity of *Philoria kundagungan* [26]. We had three specific aims: (i) develop a recognition algorithm capable of autonomously detecting *P. kundagungan* calls in audio recordings; (ii) determine the current seasonal and diurnal cycles of calling activity; (iii) determine the primary environmental variables driving calling activity. This study will allow future targeted surveys of this visually cryptic species to be conducted under conditions that induce calling activity and further understand the implications climate change may have on this endangered mountaintop frog.

2. Materials and Methods

2.1. Study Species

Philoria kundagungan occurs along high-elevation headwater streams and bogs in rainforest and adjacent wet sclerophyll in a small mountainous region of southeast Queensland and northeastern New South Wales, Australia (Figure 1). There are no data on diel, seasonal or inter-year variation in calling for this species, but it is thought to call diurnally from late August to mid-February [18], with males calling in small aggregations. The male *P. kundagungan* advertisement call comprises a short, pulsed note with most acoustic energy located between 300–1200 Hz. Call duration ranges between 0.2–0.3 s and calls are separated by 0.8–2 s when calling continuously (Figure 2).

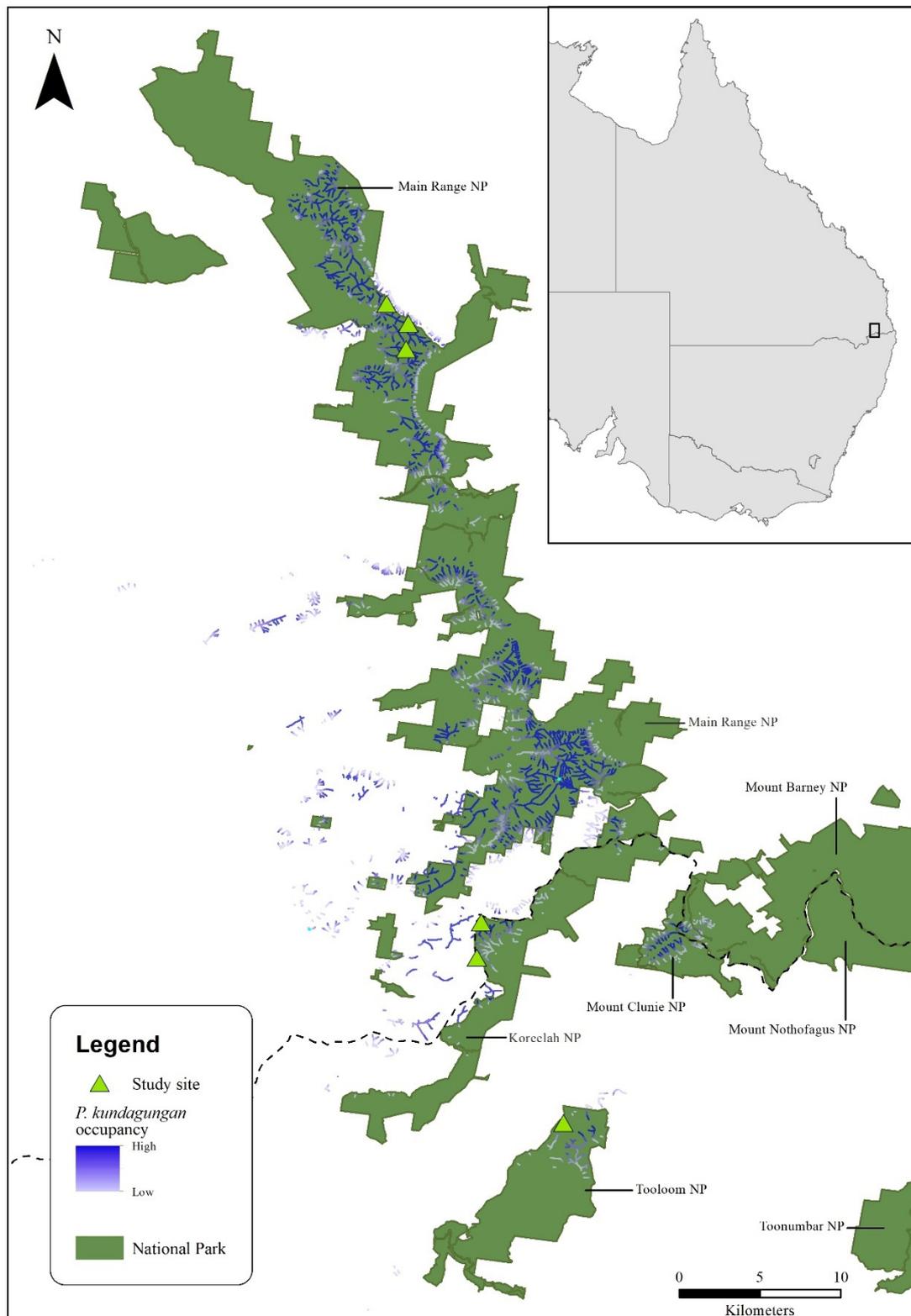


Figure 1. Map showing the location of the six study sites and *Philoria kundagungan* occupancy probability (adapted from Bolitho et al. [27]) in northeastern New South Wales and southeastern Queensland, Australia.

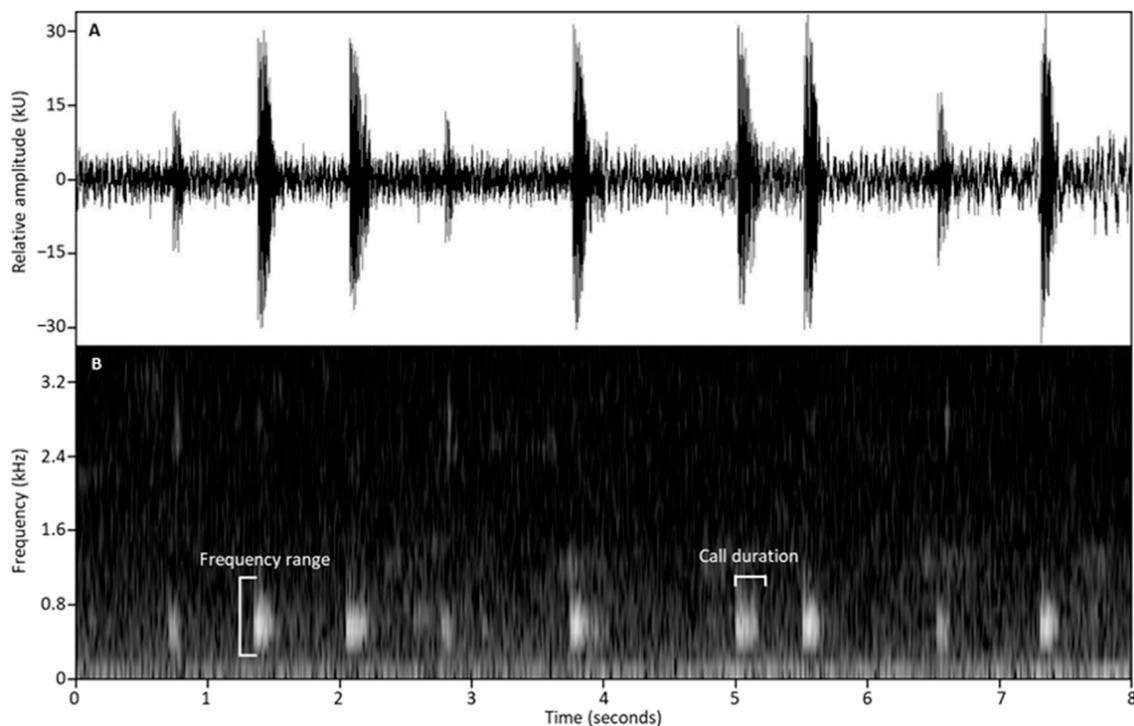


Figure 2. Oscillogram (A) and spectrogram (B) of nine typical male *P. kundagungan* advertisement calls.

2.2. Data Collection

Calling activity was examined at six known *P. kundagungan* breeding sites across the species range [27] (Figure 1). Audio recordings were collected at each site using a Song Meter v2 (Wildlife Acoustics®, Manyard, MA, USA) positioned at the height of 1 m above ground level. Song Meters were configured to record audio at a sample rate of 16,000 Hz for the first ten minutes of every hour, 24 h a day from July 2016 to March 2017 and from June 2017 to March 2018. However, due to equipment malfunctions, audio was not recorded on all days within the deployment period. Song Meters were powered with external 12 V, 28 Ah, lead-acid batteries which required changing every four weeks. The default setting was used for all other Song Meter configurations.

Hygrochron iButtons (DS1923, Maxim Integrated, San Jose, CA, USA) were used at each site to record hourly substrate and air temperature. The iButton for substrate temperature measurement was positioned approximately 10 cm below the soil surface and within one metre of a calling *P. kundagungan*. Air temperature iButtons were housed in 1-inch PVC pipes with holes drilled in them and placed 1 m above the ground. Subsequently, the recorded hourly temperature data was used to compute the daily mean and minimum temperature of substrate and air temperature at each site. Daily precipitation totals and the cumulative precipitation values for the preceding seven days were extracted for each site from a gridded dataset sourced from the Australian Bureau of Meteorology [28]. ArcMap 10.3.1 (ESRI) was used for the extraction and processing of all precipitation data. Cumulative rainfall was included so as to avoid any influence of reduced call detection during rainfall events due to noise. The daily day length was computed in hours using the ‘Suncalc’ package version 0.5.1 in R version 3.5.3.

2.3. Development of Automated Species-Specific Recogniser

An automated species-specific recogniser was developed using Kaleidoscope Pro 5.1 (Wildlife Acoustics®, Manyard, MA, USA) to identify male *P. kundagungan* advertising calls in raw audio recordings. Development and operation of this sound recognition algorithm involved three phases. In the first phase, a randomly selected subset of audio recordings

containing approximately 10,000 candidate vocalisations were selected as training data. We applied a broad filter to remove all biotic and non-biotic sounds that did not fall within the basic vocalisation characteristics of *P. kundagungan* (Figure 2). Specifically, all sounds outside the 200–1300 Hz frequency range and 0.1–0.4 s duration range were removed.

In the second phase, a sound recognition algorithm was trained to differentiate between *P. kundagungan* vocalisations and non-target signals that remained after filtering. Each candidate signal was converted into a multidimensional feature representing its unique audio signature. Each candidate signal was then manually listened to, and its representative multidimensional feature was categorised as either a *P. kundagungan* vocalisation or a non-target signal.

In the third phase, all raw audio recordings were filtered as in phase one and then passed through our final sound recognition algorithm trained in phase two. This process autonomously identified and time-stamped candidate *P. kundagungan* vocalisations and non-target signals in all audio recordings. These data were used to examine the relationships between daily totals of *P. kundagungan* vocalisations and measured environmental variables.

2.4. Recogniser Performance Assessment

We calculated the performance and error rate in our recogniser using a randomly selected subset of raw audio recordings from the primary analysis. Thirty-eight randomly selected ten-minute audio recordings (6.3 h) were analysed with our automated recogniser. Each *P. kundagungan* vocalisation identified by our automated recogniser was manually listened to and defined as either correctly or incorrectly identified. We also manually listened to the 6.3 h of audio to identify *P. kundagungan* vocalisations missed by our automated recogniser. A single observer (LB) completed all manual listening using Sennheiser HD4.40 headphones and was visually assisted through simultaneously viewing a spectrogram of the audio. From this, two metrics for recogniser performance were calculated: precision and recall [29]. *Precision* is the proportion of *P. kundagungan* vocalisations identified by our automated recogniser that are true and is calculated as follows:

$$Precision = \frac{tp}{tp + fp}$$

where *tp* is the number of correctly identified vocalisations and *fp* is the number of incorrectly identified vocalisations. *Recall* is the proportion of correctly identified vocalisations detected or missed by our automated recogniser and is calculated as follows:

$$Recall = \frac{tp}{tp + fn}$$

where *fn* is the number of missed *P. kundagungan* vocalisations [29].

2.5. Statistical Analysis

Generalised additive models (GAMs) were used to examine the relationships between daily totals of *P. kundagungan* vocalisations and measured environmental variables. Models were fit using the ‘mgcv’ package version 1.8 [30] in R version 3.5.3 [31]. All GAMs were developed using a Tweedie distribution ($p = 1.5$) with a log link function and included a random effect for both site and season. A first-order autoregressive structure for date was incorporated to account for temporal autocorrelation. Candidate models were assessed using Akaike’s Information Criterion (AIC) and Akaike’s weight of evidence (*wi*).

3. Results

A total of 8760 h of audio recordings were collected from six sites during season one (2016/2017) and five sites during season two (2017/2018). Equipment failures and logistical constraints prevented audio from being recorded for the full duration of the study period,

with 1672.8 recording days missed across all six sites over two years (Figure 3). A total of 5836 *P. kundagungan* vocalisations were used to train the sound recognition algorithm. The sound recognition algorithm detected 1,899,682 *P. kundagungan* vocalisations in these recordings. The recogniser performance assessment demonstrated that the algorithm had a precision of 0.95 and a recall of 0.22. The majority of missed calls occurred when multiple *P. kundagungan* were calling simultaneously or during intense chorusing events.

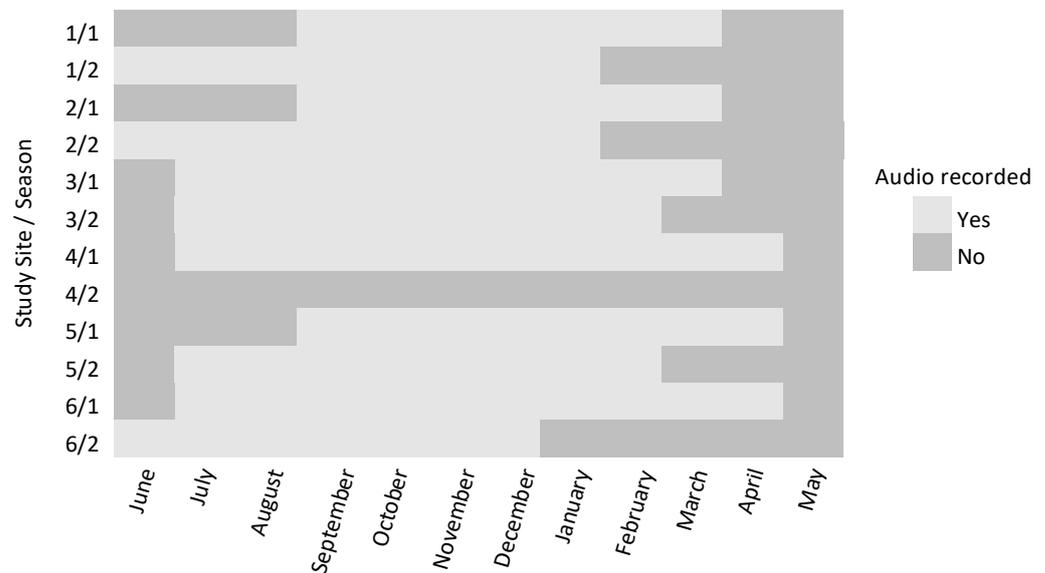


Figure 3. Months of the year audio was recorded at study sites over both breeding seasons. Season 1 = 2016/2017; Season 2 = 2017/2018.

3.1. Annual and Daily Calling Activity

There were strong diel and seasonal calling activity patterns for *P. kundagungan*. Monthly calling activity peaked in October with 44% of the total calls detected during this month (Figure 4). Calling activity was detected in all months that audio was recorded; however, there was a 47-fold increase in calling activity from August to September and a 71% decrease from November to December. Most calls (87%) occurred between the beginning of September and the end of November (Figure 4). A clear pattern of diurnal calling activity was observed from September through to January, with two distinct peaks. The first peak was approximately two hours after sunrise and the second was approximately two hours before sunset, with the first peak 14% larger than the second. Calling activity was detected during all hours of the day and night; however, calling activity was more common in daylight hours (Figure 4).

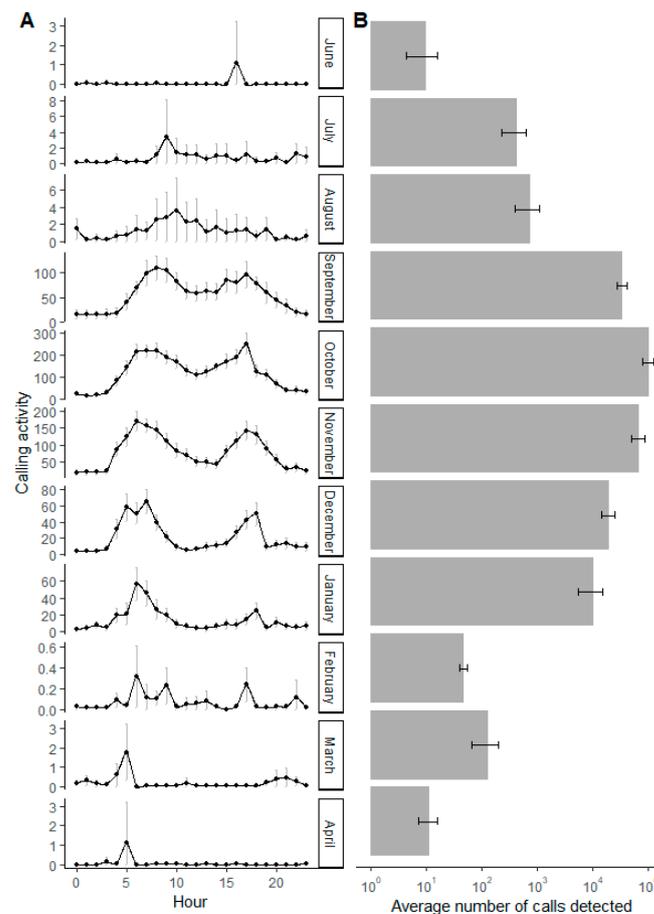


Figure 4. Diel and monthly calling activity in audio recordings made at six sites between July 2016 and March 2018. **(A)** The average number of *P. kundagungan* calls detected within a 10 min recording, per hour for each month (note the *y*-axis varies across months from a maximum of 0.6 to 300). **(B)** The average number of *P. kundagungan* calls detected per 10 min recording at each site for each month (note logarithmic scale of *x*-axis). The month of May was not sampled. Error bars indicate standard error in plots **(A,B)**.

3.2. The Influence of Environmental Variables on Calling Activity

Over the duration of this study, observed environmental variables ranged substantially. Daily rainfall ranged from 0.0–70.0 mm, rainfall in the previous seven days ranged from 0.0–94.5 mm, the daily average of substrate temperature ranged from 8.1–19.9 °C, the minimum daily substrate temperature ranged from 6.1–19.6 °C and day length ranged from 10.3–13.9 h.

There is a clear relationship between calling activity and substrate temperature (Table 1). The best-supported model suggested that the daily minimum substrate temperature and daily calling activity were correlated with peak calling activity occurring at 13.8 °C (Figure 5). The best-supported model explained 42.3% of the variance in calling activity. The next-best-supported model included the addition of rainfall.

Table 1. Summary of model selection statistics examining variables associated with mountain frog (*Philoria kundagungan*) calling activity. edf = effective degrees of freedom; AIC = Akaike's Information Criterion; Δ AIC = AIC – AIC of the top model; precipitation = daily rainfall; precipitation7 = rainfall in the preceding seven days; temperature = daily mean of substrate temperature; minimum temperature = daily minimum of substrate temperature. See Supplementary Material for model outputs.

Model	edf	AIC	Δ AIC
Minimum temperature	11.65	23,653.52	0.00
Minimum temperature + Precipitation	13.81	23,702.40	48.88
Minimum temperature + Precipitation7	13.21	23,766.99	113.47
Temperature	11.57	23,875.15	221.63
Precipitation7	8.20	23,912.72	259.20
Temperature + Precipitation	13.73	23,920.04	266.52
Precipitation	10.54	23,941.22	287.70
Temperature + Precipitation7	12.57	24,149.59	496.07
Day Length + Precipitation	13.10	24,165.91	512.39
Day Length	10.86	24,193.22	539.70
Constant	4.00	25,404.02	1750.50

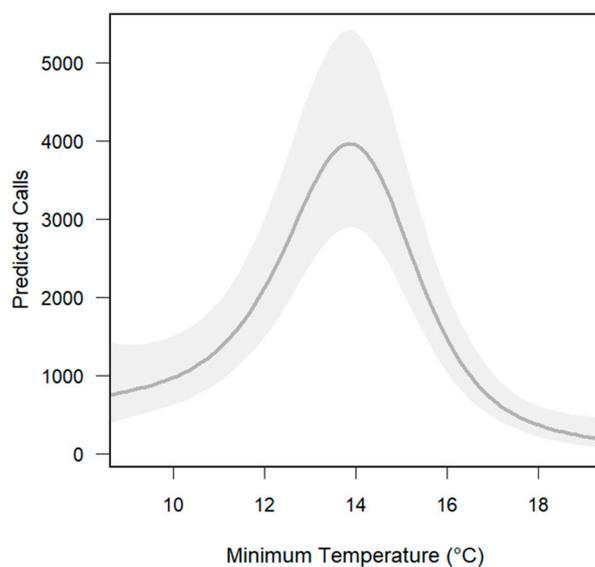


Figure 5. Modelled influence of daily minimum substrate temperature on daily *Philoria kundagungan* calling activity, with 95% confidence interval shaded grey.

4. Discussion

This study shows that the daily and seasonal calling activity of *P. kundagungan* is strongly related to the temperature of the substrate. We used a novel, automated approach that allowed insights that would not have been previously possible. The analysis of calling patterns is based on more than 1.8 million *P. kundagungan* calls, automatically detected in 8760 h of recorded audio across two seasons. Our recogniser had an impressive accuracy rate of 95% for correctly identifying *P. kundagungan* calls, surpassing previous research that employed the same software (e.g., [32,33]). Despite the low recall rate of 22%, most missed calls occurred when multiple *P. kundagungan* were calling simultaneously or during intense chorusing events. There may be potential to increase the recall rate of our recognizer to account for chorusing events; however, due to the high precision of the recognizer and the large dataset collected, the data was sufficient to analyse the calling phenology of *P. kundagungan*.

We provide a highly detailed baseline with which to detect changes in *P. kundagungan* calling phenology over time and in the face of climate change [17]. Our results also have important implications for further studies of *P. kundagungan* allowing targeted surveys

to be appropriately timed. Further, we demonstrate that using bioacoustics is a reliable method for examining the vocal behaviour in amphibians. Given that the vocalisations of *P. kundagungan* are indistinguishable from those of *P. loveridgei*, *P. pughi*, *P. richmondensis* and *P. knowlesi* [18,19], our algorithm and method could be utilised in multispecies calling phenology studies.

4.1. Diel and Seasonal Calling Activity

Calling is an energetically costly exercise for amphibians; as such, the timing of calling is synchronised to maximise reproductive effort. Understanding the factors that drive calling onset may provide insights into shifts associated with climate variability from year to year. From June through to August, *P. kundagungan* calling activity was minimal, primarily diurnal and did not occur in any regular pattern. During September (Austral spring), calling activity increased dramatically and formed a diurnal cycle comprising two distinct peaks of activity that lasted until calling activity decreased in January. While the onset of calling activity differed, a similar two-peak diurnal cycle was documented in a closely related species, *Phyloria richmondensis* [34]. The remaining *Phyloria* species are thought to also call diurnally [18,19,21]. This pattern is unusual in Australian amphibians as these are also the noisiest times of the day in many ecosystems, due to insect and avian vocalisations. Recent research suggests that diurnal calling patterns are evolutionarily conserved behaviours that are likely to have initially evolved with a species' ability to avoid diurnal predation, either via producing toxins or cryptic behaviour [35,36]. Accordingly, the similarities in diurnal calling behaviour observed within the *Phyloria* may have evolved alongside an ability to avoid diurnal predation through calling and breeding within concealed burrows.

While *P. kundagungan* diel calling patterns were similar to *P. richmondensis*, the timing of seasonal calling activity differed. Due to year-round detections of *P. kundagungan* calling activity, a specific date representing the onset of the core calling season cannot be attained. Instead, we attribute the 47-fold increase in calling activity observed in September as the beginning of the core calling season, which peaked in October and continued until the beginning of February, where we recorded an 80% decline in calling activity that occurred with increasing temperatures. This is consistent with assertions that *P. kundagungan* breeds in the spring [21]. The core calling season in *P. richmondensis* follows a similar pattern and is also similar in length; however, both the start date and peak date of *P. richmondensis* core calling season occur approximately one month earlier than in *P. kundagungan*, with calling activity intensifying in August, peaking in September and then slowly declining until the beginning of February [34]. Similarly, core seasonal calling activity in *P. loveridgei* is thought to begin in November [18,34,37]. Calling in anurans is generally limited to situations where environmental conditions are conducive to breeding and oviposition [25,38]. While *P. kundagungan*, *P. knowlesi*, *P. loveridgei* and *P. richmondensis* populations are all isolated from each other due to intervening lowland habitats (valleys and plains), they are all located on mountain tops in relatively close proximity, with very similar rainforest habitats and climatic conditions [18,27,34]. The variation in seasonal calling activity observed between these species is likely to be caused by each species requiring a slightly different set of environmental conditions to sustain breeding activity, differences in prevailing long-term climatic conditions at the times of the studies, or differences in substrate temperatures.

4.2. Environmental Factors

These results suggest that substrate temperature is responsible for triggering the onset of the core *P. kundagungan* calling season and, together with increasing substrate moisture, drives the peak in calling activity. While calling was recorded across a wide range of temperatures, rapid increases in calling activity in spring did not commence until the mean daily substrate temperature warmed to approximately 11 °C, and the most favourable substrate temperature for calling activity was 13.8 °C. Willacy et al. [34] found that air temperature was the primary driver of calling activity in the closely related species *P. richmondensis*; however, the study concluded that substrate temperature was likely to

be a more accurate predictor of calling but was not measured. Temperature is often a key variable affecting the calling and breeding activity in amphibians [39].

Rainfall was an important factor in two of the three best supported models. The increase in *P. kundagungan* calling activity during and after rainfall was likely the species' response to the increase in suitable oviposition sites made possible by additional moisture. The tail end of winter (August) is also the driest time of the year in the region, and as a result, the availability of suitable oviposition sites within the breeding habitat was likely to be at a seasonal minimum. The typical increase in rainfall in October and November likely increases the availability of suitable oviposition sites and, in turn, facilitates an increase and subsequent peak in *P. kundagungan* calling activity. Due to their general dependency on moisture, calling activity in frog species is commonly associated with rainfall [21].

4.3. Implications of Ongoing Climate Change

Climate change projections indicate that over the coming decades, eastern Australia will experience further increases in temperatures, with more frequent and longer-lasting heatwaves and further decreases in cool-season rainfall with more frequent periods of drought [40–42]. This region's long-term average seasonal conditions are characterised by cold and dry winters followed by spring, which sees a gradual increase in temperature and a slow increase in rainfall, including early-season storms. This is followed by warm and wet summers, then gradually returns to cold and dry weather patterns by late autumn. However, climate change is already altering these seasonal conditions [43], causing significant implications for *P. kundagungan*, and may ultimately challenge the species' persistence [14,17]. There are many reports of species shifting their distribution to higher latitudes or higher elevations in order to follow favourable thermal conditions [40,42,44]. However, *P. kundagungan* has a highly restricted and naturally fragmented montane distribution and has limited dispersal ability [27], so it has no opportunities to shift its distribution to higher latitudes or higher elevations as temperatures increase [17]. As temperature (substrate warming to 11 °C) is responsible for triggering the onset of the core breeding season in *P. kundagungan*, projected increases in average temperatures in June, July and August are likely to cause the core breeding season to start earlier in the dry season and, in turn, cause a mismatch between favourable thermal conditions and the start of the rainy season. With less water availability in streams, this scenario could result in an overall decrease in suitable oviposition sites and thus a reduced rate of recruitment.

Climate change has already caused a decrease in cool-season rainfall and more frequent droughts in eastern Australia [43]. These trends are projected to worsen over the coming decades [42,43] and have already been implicated in *P. kundagungan* population declines. Heard et al. [14] found that *P. kundagungan* was much more likely to call from streams with higher water availability and suggested that in addition to fire impacts in 2019/2020, a decrease in *P. kundagungan* occupancy and abundance observed in 2020/2021 may have been due to reduced breeding activity during drought. Our results provide additional evidence of the link between *P. kundagungan* calling activity and climate. Given that the species already has a low reproductive output [21], these potential consequences of ongoing climate change are likely to contribute to the population declines forecasted by Bolitho and Newell [17] over the next three decades.

5. Conclusions

This study shows that calling activity in *P. kundagungan* can be predicted based on the substrate temperature of the species' habitat and rainfall and adds to the growing literature concerning the effects of climate on breeding phenology. The results of this study show that temperature and rainfall affect calling activity (indicative of breeding) in *P. kundagungan* and given future climate change projections, there are clear implications for the species' long-term persistence. Although this study is focused on a single species, vocalisations of *P. kundagungan* vary little from *P. loveridgei*, *P. pughi*, *P. richmondensis* and *P. knowlesi* [18,19], allowing our algorithm to be used for multispecies bioacoustic monitoring

in future studies. Substrate temperature and moisture (rainfall) should be incorporated into future monitoring programs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15080931/s1>, Model output from Table 1.

Author Contributions: Conceptualisation, D.N., L.B. and H.H.; methodology, D.N. and L.B.; software, L.B. and D.N.; validation, L.B.; formal analysis, L.B.; investigation, L.B., D.N. and H.H.; resources, D.N., L.B. and H.H.; data curation, L.B., D.N. and H.H.; writing—original draft preparation, L.B.; writing—review and editing, L.B., D.N. and H.H.; visualisation, L.B.; supervision, D.N.; project administration, D.N. and H.H.; funding acquisition, D.N. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This research was undertaken in accordance with relevant state requirements. In New South Wales the study was approved by the Southern Cross University Animal Care and Ethics Committee (authority ARA-18-077) with fieldwork authorised by New South Wales Department of Environment Scientific Licence SL100492. In Queensland, Animal Care and Ethics approval was via the Department of Agriculture and Fisheries (authority SA 2016/ 08/565) and fieldwork was undertaken in collaboration with Queensland Parks and Wildlife Service (supervised by HBH).

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to concerns around disclosure of locations.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, interpretation of data; or in the writing of the manuscript.

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