The response of insectivorous bats to environmental water

Short-term response during inundation phase

L.F. Lumsden, A. Bush, P. Griffioen, L. Francis and P. Moloney

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Front cover photo: Environmental water in Lake Konardin, Southern Freetail Bat, full spectrum echolocation call (Photos: Lindy Lumsden).

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The response of insectivorous bats to environmental water

Short-term response during inundation phase

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Summary

Context:

The provision of environmental water is a key management tool for re-establishing water regimes which are more closely representative of historical natural flows across the Murray–Darling Basin, including the Hattah Lakes Icon Site. Environmental water has been shown to benefit a wide range of flora and fauna, including fish, frogs, waterbirds and plants. However, little is known of the response by insectivorous bats to environmental water. There could be short-term benefits in direct response to the availability of water through increased insect prey abundance, or over the medium to longer-term through increases in productivity and tree health. Short-term responses may be viewed in two phases: first, during inundation while water is present, and second, during drawdown as the water recedes. To address key knowledge gaps in the response of insectivorous bats to environmental water, the Mallee Catchment Management Authority (CMA) began a study in 2020 to quantify the change in activity and species richness of bats in the Hattah Lakes Icon Site, and collected baseline data on bat activity in 2020-21, prior to the delivery of environmental water.

Aims:

The aim of this project was to continue the study initiated by the Mallee CMA by:

- undertaking detector sampling in 2021-22 at the lakes sampled in the previous year, plus additional control sites, during a period of environmental water provision
- refining the new automated bat call analysis program through the collection and incorporation of additional reference calls, and further development of the program to improve identification accuracy rates
- analysing the bat calls collected in 2020-21 and 2021-22 to determine species richness, overall bat activity, and activity levels for each species for each lake
- assessing the short-term benefits of the provision of environmental water during the inundation phase, by comparing the species richness and activity levels in 2021-22 to that of 2020-21 when the lakes were dry.

Methods:

The original study design was based on a Before-After-Control-Impact approach. However, all except one of the lakes sampled in the first year received water in the second year, and so an additional five dry sites were incorporated to act as controls in the second year. Bat activity was measured by recording the bats' high frequency echolocation calls using Anabat Swift detectors. Detectors were set at 24 sites, operating from late November 2021 to March 2022, during the peak season of bat activity. In addition, bats were trapped using mist nets and harp traps to enable the collection of additional reference calls. Reference calls were sub-sampled into 0.75-second segments and incorporated into the deep learning one-dimensional convolutional neural network developed by the Arthur Rylah Institute (ARI) for bat call identification (now called 'BatLingo'). The predictive accuracy of the model was improved by providing an ensemble of networks, with a three-network ensemble model used to quantify accuracy rates of a withheld testing set of reference calls. A sixnetwork ensemble model was then used to analyse the field data from the Hattah Lakes sites.

To assess the benefits of environmental water, the number of bat calls recorded per night per site was modelled using a negative binomial mixed effects model. Comparisons were made between the number of calls recorded in the first year (before the provision of environmental water), and in the second year, for sites with water and dry control sites. The models were constructed in a Bayesian framework.

Results:

A total of 229 individuals of nine species were trapped during 2021-22. The reference calls collected from these individuals, plus additional reference calls sourced from elsewhere, considerably increased the number of reference calls available to train BatLingo – with 1,273 call sequences from 14 species, plus a large number of non-bat noise files, used to train the model. Iterative refinements were made to the model until accuracy rates were considered adequate for the purpose of analysing the field data. Accuracy rates varied between species, largely due to the number of reference calls available and the extent of overlap in call characteristics between species.

A total of 4.7 million files were collected (12.8 TB of data) in 2021-22, an increase from the 1.9 million files (2.7 TB) collected in 2020-21. The extensive nature of this dataset resulted in challenges for processing and manipulating the data. Forty-four percent of the 6.6 million files were successfully identified to species, with all 14 species represented, with the remaining identified as species complexes, unknown bat calls or as noise. There were low numbers of identified calls of Gould's Long-eared Bat (*Nyctophilus gouldi*), South-

eastern Long-eared Bat (*Nyctophilus corbeni*) and Large-footed Myotis (*Myotis macropus*) – three species that overlap in their call characteristics with each other and with the more common Lesser Long-eared Bat (*Nyctophilus geoffroyi*). As identification accuracy rates of these species are currently only moderate, there remains some uncertainty in these results. The sheer volume of data precluded extensive manual checking of the identifications within the time available.

All 14 species were identified in both the before and after sampling; however, the mean number of calls identified per night was higher in the second year than in the first (1347 *cf.* 722 calls/night). Modelling revealed a much higher level of total bat activity at the inundated sites compared to when sites were dry during the first year of sampling (87% higher). A similar pattern was found for 10 of the 14 species. Mean nightly species richness was also higher at sites with water (during the second year) compared to at the same sites when they were dry (during the first year), with the dry control sites from the second year midway in between.

Conclusions and implications:

A rich bat fauna was found within the Hattah Lakes Icon Site, with bats constituting a significant proportion of the native mammal fauna within the region. It is likely that the Icon Site acts as a refuge for bat species in this region due to its mesic environment within the surrounding semi-arid landscape. Some of the species recorded in this study are more typical of temperate regions, but can extend their range into the semi-arid environment by using the zone along the Murray River. The Hattah region is critically important for the Endangered South-eastern Long-eared Bat, with the main Victorian population located just to the west of the Hattah Lakes. This species occurs more typically in mallee habitats, but was recorded at the inundated lakes during this study, and individuals may expand their foraging ranges to take advantage of the additional foraging resources provided by the environmental water in the lake system.

To our knowledge, this is the first time that an Australian study has investigated the benefits of environmental water to insectivorous bats using an experimental approach with a Before-After-Control-Impact design, to enable causal effects to be explored. There appeared to be positive short-term benefits for insectivorous bats from the provision of environmental water, with higher levels of overall bat activity, activity of 10 species and species richness during the inundation phase. However, it is currently unknown if this pattern was due to a redistribution of bats from nearby areas to take advantage of the increased foraging resources associated with the water, or whether there had been changes in behaviour or increased survival rates. Therefore, the current findings only partly address if there is a short-term benefit, and further sampling would be required during the drawdown phase to determine whether there is a legacy effect of the environmental water, or whether the benefits are limited to just when water is present.

The other outcome of this study is that it has enabled the further development of the automated bat call identification program, including the addition of more reference calls, to increase accuracy rates. While progress has been significant, accuracy rates varied among species, and are likely to be lower for field-based calls than for the testing set of reference calls, and further refinement is required.

A fuller understanding of the benefits of environmental water would be obtained by:

- repeating the sampling over the summer of 2022-23 to provide information on activity levels and species richness in the period following inundation to investigate whether bat activity returns to the baseline levels recorded prior to the provision of water, or whether it remains elevated.
- investigating factors influencing the benefits of environmental water by exploring the drivers of these patterns. One key knowledge gap is how nocturnal, flying insects respond to environmental water and the relationship between changes in insect availability and bat activity.
- incorporating more reference calls into BatLingo, including the three additional species that may occur in the area, to ensure the full extent of call variability is included, and further develop BatLingo to improve the accuracy of the identification of field calls.
- analysing the third year's calls (once BatLingo is further refined and accuracy rates are optimised), and re-analysing the first two years' calls so that subsequent analysis is comparable.
- collecting data from other floodplains to investigate whether the patterns observed at the Hattah Lakes compare to those at other locations with different flooding attributes, landscapes and vegetation communities.
- investigating the response to environmental water by the most threatened bat species in the region, the South-eastern Long-eared Bat, as the current analysis suggests it may expand its foraging range to take advantage of the increased foraging resources during inundation periods. Recent advances in GPS radio-tracking technology could assist in investigating foraging ranges and patterns.

Such studies would provide a more comprehensive understanding of the benefits of environmental water for bats, including longer-lasting legacy effects, and not just short-term benefits during the period of inundation.

1 Introduction

The provision of environmental water is a key management tool for re-establishing water regimes that are more closely representative of historical natural flows across the Murray–Darling Basin, including in north-western Victoria. The aim of this approach is to increase connectivity along rivers and between rivers and their floodplains, and to improve the condition of wetlands and floodplains and their associated flora and fauna. Environmental water directly benefits many faunal groups; for example, by supporting an increase in the breeding success and abundance of waterbirds, enhancing connectivity for fish and improving their breeding success, and by facilitating an increase in the abundance of frogs (Murray–Darling Basin Authority 2019). It also provides benefits to the non-aquatic environment by improving the extent and condition of the vegetation, especially the iconic River Red Gum (*Eucalyptus camaldulensis*) and adjacent Black Box (*E. largiflorens*) woodlands, and benefits the associated woodland faunal species. Numerous studies have been undertaken investigating the benefits of environmental water to a wide range of flora and fauna (e.g., King et al. 2009; McGinness et al. 2014; Moxham et al. 2019).

Bats are one group for which little is known of the benefits of environmental water (Blakey et al. 2018). The majority of the species of bats occurring in north-western Victoria are small insectivorous bats, with only the occasional record of the larger flying-foxes (Lumsden and Bennett 1995). Insectivorous bats have three key habitat requirements: roost sites, invertebrate prey and free water for drinking. These bats typically have relatively large home ranges (Lumsden et al. 2002a), and so these habitat requirements can be spatially separated rather than needing to all be available in a single location. Each of these key habitat requirements are likely to benefit from environmental water, either in the short- or long-term.

All species of bats in this area are dependent on tree hollows for roosting during the day. Hollows provide shelter from temperature extremes and secure locations for breeding and raising young (Kunz and Lumsden 2003). Some species of bat prefer to roost in large dead trees (e.g., Lesser Long-eared Bat *Nyctophilus geoffroyi*) while others (e.g., Gould's Wattled Bat *Chalinolobus gouldii*) predominantly roost in dead spouts in large live trees (Lumsden et al. 2002b). Consequently, both live and dead hollow-bearing trees are required to support the full bat species complement in an area. In addition, high densities of hollow-bearing trees are required to support colonies, due to the behaviour of individuals regularly moving between roosts within a discrete roosting area, and to reduce competition for hollows with other fauna (Webala et al. 2010; Lumsden et al. 2020).

Insectivorous bats typically feed on a wide range of nocturnal, flying invertebrates including moths, beetles and bugs. Bats use water bodies as foraging sites, with the presence of water linked to increased foraging activity (Lumsden and Bennett 1995; Pierson et al. 2001). While many species are generalist feeders (Lumsden and Bennett 2005), others have more specialised diets, such as the Large-footed Myotis (*Myotis macropus*) which forages exclusively over water, taking aquatic insects and small fish (Campbell 2009). Free water is also required for drinking (Griffiths 2013), and this is particularly important over summer and when females are lactating (Adams and Hayes 2008).

Assessing the response of bats to environmental water requires considering a range of temporal and spatial scales. There may be short-term, local-scale responses during watering events, intermediate responses over several years, or longer-term responses that may only be apparent over decades or centuries (Sparrow et al. 2021). In the short term, the inundation of a site is likely to increase the abundance of insects (Bunn et al. 2006), and therefore provide more foraging resources for bats residing in the area (Fukui et al. 2006). Depending on the timing of the watering events, the increased access to open water for drinking could be beneficial particularly during warmer and drier times of the year and over the summer breeding period. However, there is unlikely to be an immediate, dramatic increase in the abundance of individuals (as may be seen for frogs, for example) because bats have a slow life history pattern, giving birth only once per year, and typically to a single young (Barclay and Harder 2003). As a result, it takes time to increase population numbers in response to a management intervention. The mechanism for an increase in abundance would likely be greater levels of prey availability improving breeding success, or increasing survival rates of adults and young. However, the evidence of these increases would take time to become fully apparent. In addition, short-term responses may be seen in two phases: first, during the inundation period when free water is available; and second, during or soon after the drawdown as the water recedes but the area is still benefiting directly from the provision of water.

The short-term response of bats to environmental water may also include spatial factors. Bats from surrounding areas may extend their foraging ranges to take advantage of increased prey availability when water is present. Insectivorous bats can forage considerable distances from their roost sites: for example, Lesser Long-eared Bats and Gould's Wattled Bats often forage up to 13 km from roost sites (Lumsden et al. 2002a). This gives them the capacity to readily expand their foraging ranges. The other consideration for

spatial scale is that there might be a spill-over effect whereby water in one location may also provide benefits to the nearby surrounding areas, increasing that area's prey availability and hence bat activity.

Over an intermediate timeframe, the increased vegetation productivity of the site could lead to a greater level of insect abundance during both the wet and dry phases. Within this timeframe, an increase in bat activity based on greater survival rates may become apparent. However, little is known of the response of nocturnal, flying invertebrates to environmental water and how this changes over time. Over the longer term the environmental watering would contribute to maintaining the health of the large canopy trees and assisting with germination of new ones. Healthy trees tend to grow larger and would in time provide more hollows (Bennett et al. 1994), and therefore more roosting habitat for bats.

Although it may be assumed that the effects of watering would be positive, there are very few studies that have investigated the relationship between bat species richness and abundance, and specific management actions. A comparative study of bats in the floodplain systems of the Murray–Darling Basin showed that floodplain habitats had greater value for bat communities compared to adjacent dry vegetation types (Blakey et al. 2017). Habitats that were flooded more frequently supported greater bat activity and species richness. Areas with open water and riparian habitats had five times the activity levels, 14 times the foraging activity and 1.5 times the species richness of dry habitats.

In the past, it was challenging to undertake detailed studies on bats due to the difficulty in obtaining comparable data between treatments. Data from trapping studies can be highly variable between sites and nights based on differences in vegetation density and weather conditions. As a result, this method is not suitable for use in before-after studies attempting to rigorously compare metrics of relative abundance. In contrast, bat detectors are an efficient way to collect data on relative activity levels, as they are a passive technique that consistently samples the same volume of air space. When bats are in flight, they emit high frequency echolocation calls, at up to approximately 12 pulses per second. Echolocation enables them to navigate, avoid obstacles and locate prey. As bats echolocate continually while in flight, recording their echolocation calls is an effective way to assess their activity patterns and habitat use. The traditional approach for collecting and analysing bat echolocation calls involves the Zero Crossing method, where a simplified version of the call is represented through a frequency/time output every time a sound wave crosses the 'zero-point'. This approach is limited by there being multiple species with overlapping call characteristics that cannot be reliably distinguished (Law et al. 2002, Pennay et al. 2004, Lumsden and Bennett 2005). A new approach using Full Spectrum recordings provides richer information on each pulse, and allows for the measurement and analysis of more call characteristics that may improve the ability to distinguish between similar species. There was previously no automated tool for identifying bat calls in Victoria using Full Spectrum; however, the Arthur Rylah Institute (ARI) is currently developing such an approach using Artificial Intelligence methods. As there is geographic variation in call parameters for some species, and different species assemblages across Victoria, regional keys need to be developed for this automated program. To develop a key for the Hattah Lakes area, a large number of locally-collected reference calls are needed for each species, with sufficient reference calls available to represent the full variation in call characteristics of each species, especially where there is significant overlap in call characteristics between species. The collection of reference calls involves trapping individuals, identifying them to species, and then recording their calls on release.

To address key knowledge gaps in the response of bats to environmental water, the Mallee Catchment Management Authority (CMA) commenced a study in 2020 to quantify the change in diversity and activity of insectivorous bats around wetlands in the Hattah Lakes Icon Site following the delivery of environmental water. The specific questions to be addressed were:

- 1. What insectivorous bat species inhabit the Hattah Lakes Icon Site?
- 2. Is there a difference in insectivorous bat species richness and activity between lakes?
- 3. Is there a difference in insectivorous bat species richness and activity before and after environmental water delivery?

The study was established as a Before-After-Control-Impact (BACI) study, with sampling before and after the addition of water to the lakes, with control sites that remained dry and impact sites that received water. Nineteen sites were sampled in 2020-21, with 18 of these dry at the time and only one lake (Lake Kramen) containing water. The collection of data at the other 18 lakes therefore provides a baseline dataset on the activity levels before environmental watering. Lakes that were unlikely to receive environmental water during the foreseeable future, were to be used as control sites. Sites were selected by the Mallee CMA, based on previously established vegetation monitoring sites. The Mallee CMA set Anabat Swift detectors at each lake and collected data on bat activity between November 2020 and March 2021. However, unfortunately there were issues with the operation of the detectors and the data were incomplete, with recordings patchy during this time and no nights where all 19 detectors were successfully operating (Lumsden et al. 2021a).

ARI was engaged to analyse the data that was collected from the detectors, using ARI's new Full Spectrum automated call analysis program (Lumsden et al. 2021a). Although significant progress was made on developing this new approach, the accuracy of species identifications was not suitable to analyse the data, as there were insufficient reference calls for some species to adequately train the model. This study indicated the need to collect significantly more reference calls for training and testing the model.

To progress the overall aims of the Mallee CMA's study, a follow-up study was instigated in 2021-22 led by ARI. The specific aims of this study were to:

- collect additional Full Spectrum reference calls to supplement the Mallee region Full Spectrum call library
- incorporate the new reference calls into the Full Spectrum call identification program currently being developed by ARI, to increase the accuracy of the identifications for the Hattah Lakes area
- undertake detector sampling at the 19 Hattah Lakes sites sampled in 2020-21, plus additional control sites, during a period of environmental water provision
- analyse the calls collected in 2020-21 and 2021-22 to determine species richness, overall bat activity, and activity levels for each species for each lake
- assess the short-term benefits of the provision of environmental water in 2021 during the inundation phase, by comparing the species richness and activity levels in 2021-22, to those of 2020-21 when the lakes were dry.

2 Methods

2.1 Study area and site selection

The study area is the Hattah Lakes Icon Site within the Hattah–Kulkyne National Park in north-western Victoria. This area is a complex system of semi-arid lakes and floodplains associated with the Murray River. The vegetation around the lakes is dominated by River Red Gum fringing the edge of the lake, with Black Box situated further from the lake edge. In the north of the Icon Site, Black Box is the dominant tree species at the lakes. Tree deaths are occurring at many of the lakes and within the surrounding woodlands, especially Black Box trees, reflecting altered flooding regimes in the past. Adjacent to the Black Box woodlands are open plains, with sparse dry woodlands and extensive areas of mallee habitats on sandy dunes.

Environmental flows were delivered to the Hattah Lakes Icon Site in 2021, with 27,207 ML provided in autumn (commencing 3 May 2021) and a further 48,138 ML delivered in spring (commencing 7 October 2021), filling all the targeted lakes. Water levels remained high for the duration of the sampling period during 2021-22, with only minimal drawdown occurring.

Nineteen lakes were selected for sampling bat activity by the Mallee CMA in 2020-21. In that year, Lake Kramen contained water resulting from environmental water delivered in spring 2019, while the remaining 18 lakes were dry. In 2021-22, Lake Kramen had recently drawn down of water, while most of the other lakes filled with water. Lake Kramen is in a different system to the other lakes and has a different watering regime. It was therefore considered separately in the analysis. With respect to the remaining lakes, the original BACI study design was for some of the 18 sites to act as control sites, i.e., remain dry throughout the entire study so that any changes between years, not associated with the watering regime, could be accounted for in the analysis. However, in 2021, 17 of these 18 lakes received environmental water, with only one (Lake Boolca). in the north of the Hattah Lakes Icon Site, remaining dry, and hence acting as a true control. As one site is insufficient to act as a control, five additional control sites were installed for the 2021-22 sampling. These control sites were selected to be as similar as possible to the watered sites, but were not part of the environmental watering regime. Two sites were selected in depressions that resembled dry lake beds to the west of Lake Lockie and Lake Little Hattah, two were selected in dry depressions near Chalka Creek, and one was selected in the north of the Icon site in Dry Lake (Table 1; Figures 1 and 2). These sites had not received environmental water in recent years and are unlikely to receive water in the near future at least; however, they do contain water for short periods after heavy rain.

2.2 Detector sampling

The sampling approach was the same in both 2020-21 and 2021-22 so that the resulting data were directly comparable. Anabat Swift detectors with omnidirectional microphones (Titley Scientific, Brendale, Queensland) were used to measure bat activity and species richness. These detectors record bat activity throughout the night, saving a sound file every time a bat flies past the detector (defined as a call sequence). The same detectors were used in both years, with the same settings (sensitivity 16, minimum frequency 10 kHz, trigger window 2 seconds, maximum file length 5 seconds). The maximum file length of 5 seconds is shorter than used in most other studies (normally it is set at 15 seconds), and this may reduce the identification success as longer calls are more readily identified. However, the same setting was used in 2021-22 to ensure that the measure of bat activity (number of call sequences) would be directly comparable between years. The one exception to using the same detector settings was the sample rate (which reflects the number of samples of sound that are taken per second to represent the call digitally). In the first year, this was set at 192 kHz; however, advice from the detector manufacturers was that the sample rate should be more than twice the highest frequency likely to be recorded, including harmonics. As the harmonics of some species in this region are higher than 96 kHz, it was decided to increase the sampling rate to 320 kHz (the next available setting) for the 2021-22 sampling. Although frequencies above 96 kHz are not recorded regularly, raising the sampling rate should reduce misidentifications when they are recorded. The increase in sampling rate does not affect the number of calls recorded (this was confirmed through paired testing prior to deployment). However, a consequence of this decision was that the files were twice the size, necessitating the use of larger memory cards in the detectors. In the first year, 64 GB cards were used, with two cards in each detector for the November 2020–January 2021 period, and one card for the February–March 2021 period. This led to the number of nights of sampling being limited at some sites. The survey period spanned 126 potential nights of sampling for each lake, however, the mean number of nights that detectors operated was 71, with the range 29 to 112 nights (Lumsden et al. 2021a). In 2021-22, larger memory cards were used (a 256 GB and a 128 GB in each detector) to account for the higher sampling rate and to extend the duration

of the recording. To facilitate longer operating times, high quality non-rechargeable batteries (Energiser Lithium) were used, as these out-perform rechargeable batteries. Battery life is influenced by the amount of bat activity, as well as the amount of high-frequency environmental noise (e.g., insects).

There were issues with units not recording as expected resulting in missing data during the first year of sampling, and so all 24 detectors were thoroughly checked prior to deployment in 2021-22. New firmware was uploaded, all settings were rechecked, and units were tested by running them concurrently over several nights – with the resulting number of files compared and the quality of the calls checked. Microphones were tested for sensitivity, and those that were found to have a lower sensitivity were replaced.

When the detectors were installed at the lake sites for the 2021-22 sampling, they were set on the same tree used by the Mallee CMA in the first year (identified from photographs) to ensure the same air space was sampled. The one exception was the site at Lake Hattah. This site had been set just 47 m from the site at Lake Bulla on the narrow bank between these two lakes, and hence would have been sampling the same bat activity. Therefore, it was decided to shift this site to the opposite side of Lake Hattah, resulting in it then being 750 m from the Lake Bulla site. The habitat features were similar at both Lake Hattah sites. This was considered preferable for the overall analysis; however, it would impact any direct comparisons of activity levels at this lake between the two years of sampling.

The detectors were positioned approximately 1.5 m above the ground, with the microphone pointing towards the lake, in the same direction in both years (Figure 3). There was considerable variation between sites in the distance to water during the second year at the lakes receiving environmental water. When the detectors were set in the first year, the site selection was presumably based on where it was thought water may reach under the proposed flooding regime. However, once the water had been delivered, some trees with detectors were within the flooded area (up to 15 m from dry land), while others were up to 50 m from the edge of the water at the time they were set in November 2021, and up to 145 m from water when they were retrieved in March 2022. This resulted in the sampling area for some sites being directly over water, while for others it was over dry land. The distance from which bats can be detected varies between species, but is typically in the range of 5–50 m. The dry, control sites were 280–5,600 m from large bodies of water (Table 2).



Figure 1. The sites sampled for bats in the Hattah Lakes Icon Site indicating those that were at lakes that contained water (wet, blue) and those that did not (dry, green) in 2021-22. Note that Lake Kramen (pink) contained water in 2020-21 but had dried several months before the sampling in 2021-22.

Table 1. Sites sampled for bats in the Hattah Lakes Icon Site in 2020-21 (19 sites) and 2021-22 (24sites), with details of environmental water at the time and broad habitat type.BB – Black Box.

Lake	2020-21	2021-22	Broad habitat type
Lake Arawak	Dry	Wet	Thin strip of mature RRGs along lake edge
Lake Bitterang	Dry	Wet	Narrow band of relatively small BB along edge of lake
Lake Boich	Dry	Wet	Narrow band of sparse large RRGs flanked by BB
Lake Boolca	Dry	Dry	Young BB with a few large trees and many dead trees
Lake Brockie	Dry	Wet	Mature RRGs flanked by BB back from lake edge
Lake Bulla	Dry	Wet	Mature RRGs along edge of lake
Lake Cantala	Dry	Wet	Lignum swamp with scattered RRGs and BB
Lake Hattah	Dry	Wet	Narrow band of sparse large RRGs
Lake Konardin	Dry	Wet	Medium sized RRGs along lake edge
Lake Lockie	Dry	Wet	Dense young RRGs, with several large RRGs
Lake Marramook	Dry	Wet	Predominantly BB with only a few RRGs
Lake Mournpall	Dry	Wet	Narrow band of large old RRGs
Lake Nip Nip	Dry	Wet	Narrow band of sparse large RRGs flanked by BB woodland
Lake Tullamook	Dry	Wet	Narrow band of large RRGs flanked by open BB woodland
Lake Woterap	Dry	Wet	BB woodland with many small dead trees
Lake Yelwell	Dry	Wet	Predominantly BB with a few RRGs
Lake Yerang	Dry	Wet	Young RRG regrowth with only a few large live trees
Little Lake Hattah	Dry	Wet	Mature RRGs along edge and up steep bank
Lake Kramen	Wet	Drawdown	Young BB and occasional RRG with some big dead trees
Chalka Ck Dry 1	_	Dry	Predominantly BB on dry creekbed
Chalka Ck Dry 2	-	Dry	Mature RRGs along edge of dry channel
Dry Lake 2	_	Dry	Mature BB with lots of big trees, some dying
Dry lake W Little Hattah	_	Dry	Mature RRGs with some very large trees
Dry lake W Lockie	_	Dry	Mixture of relatively young RRG and BB in dry channel

Table 2. Mean distance (± SD) detectors were set from water when initially deployed in November/December 2021 and when retrieved in March 2022, for all lakes excluding Lake Kramen*.

Water condition	No. sites	Mean distance to water at start of deployment (m)	Mean distance to water at end of deployment (m)
Wet	17	18 ± 16 (range 0–50 m)	44 ± 33 (range 7–145 m)
Dry	6	2,037 ± 2,272 (range 280–5,600 m)	2,054 ± 2,261 (range 280–5,600 m)

* Lake Kramen was excluded as it was wet in 2020-21, with 2021-22 reflecting conditions soon after drawdown.



Figure 2. Photographs of sites taken from standing next to the detector and hence showing the sampling area (except for a)) in November/December 2021: a) Detector positioned on tree within the water (Lake Mournpall – the arrow shows the location of the detector); b), detector positioned near the edge of the water (Lake Lockie); c) detector positioned some distance from water's edge (Lake Yelwell); and d) detector positioned at the edge of a depression (Dry Lake West of Little Hattah).



Figure 3. Anabat Swift detector with omnidirectional microphone, set approximately 1.5 m above the ground, pointing towards the lake.

2.3 Data collection

The data collection period was set to coincide with the season of peak bat activity (i.e., November–March). As there are high levels of variability in bat activity between nights depending on weather conditions (Scanlon and Petit 2008), the sampling period was run over several months to account for this variability. In 2020-21, detectors were set between 18 November 2020 and 23 March 2021, but sampling was patchy during this time and not all detectors were set at the same time (Lumsden et al. 2021a). Data collection was more consistent in 2021-22, with detectors set over two days (30 November–1 December 2021) so that all sites were at least initially sampled concurrently. Batteries and memory cards were replaced over two days (23–24 January 2022) again ensuring all lakes were sampled concurrently at the start of the second sampling period. Retrieval of the detectors was delayed until 22-26 March 2022 due to COVID-19 constraints. By this time, most of the memory cards were full or the batteries depleted. As only four sites (all control sites) recorded past 6 March 2022, to make the data more consistent between lakes in the second year, only data prior to 5 March 2022 was included in the analysis. On retrieval, data were downloaded from the memory cards on two 10 TB external hard drives, and then transferred to an 80 TB Synology drive.

The weather conditions during the two sampling years were broadly similar, although the mean maximum temperature was slightly higher in 2021-22, compared to in 2020-21 and the long-term average, while the mean minimum temperature was up to 3°C warmer in 2021-22 compared to in 2020-21 (Bureau of Meteorology data; Figure 4). The second year of sampling coincided with higher rainfall averages, with wetter conditions in November, February and March compared to those in the previous year (Figure 4).

2.4 Call analysis

2.4.1 Collection of additional reference calls

Additional reference calls were collected in 2021-22 to supplement those obtained during 2020-21, as it was found that these were insufficient to fully train the automated call identification program to provide accurate identifications (Lumsden et al. 2021a). As there is a high level of variability within the calls produced by individuals depending on where they are foraging (e.g., in cluttered environments, such as in and near trees and shrubs, compared to more open environments) and considerable overlap between species in their call characteristics, a large number of reference calls are required.

Bats were trapped during the field trips when detectors were installed, serviced, and retrieved, over a total of 14 nights. Trapping was undertaken using harp traps (Austbat P/L, Mount Taylor, Victoria) and monofilament mist nets. It was focused at waterbodies as bat activity is typically higher in these areas (Lumsden and Bennett 1995). However, activity may be dispersed at large bodies of water (i.e., full lakes), making it more difficult to trap the individuals that are present. Therefore, smaller bodies of water were typically selected for trapping, especially ones situated some distance from the main lakes.

Mist nets were monitored continuously, and harp traps were checked several times during the night. Trapped individuals were identified, weighed, measured, and their age and sex recorded. All bats were released at their point of capture on the same night they were trapped.

Prior to release, individuals were fitted with light tags, to enable them to be followed visually during the recording of reference calls. This ensured that the reference calls recorded, were from the identified individuals rather than from other bats (of any species) that may have been flying in the area at the same time. The light tags were attached to a small tuft of belly fur using sticky tape. Tags attached in this way typically fall off in less than an hour, and often much sooner, sometimes within minutes of release. However, this is usually long enough to obtain a reference call and so a longer attachment time is not necessary. The weight of the light tags was 0.2 g, which was considerably less than the recommended upper limit of 5% of body weight for most species so as to not impact their flight manoeuvrability (Aldridge and Brigham 1988). The illumination from the tags allowed bats to be seen from 50 m or more in open areas, but they were obscured quickly when they flew into the vegetation.

Individuals were released into a small clearing near the capture site, with monitoring prior to release to select a time when no other bats were flying within range, to facilitate obtaining a recording of only the target species. Two or more observers with handheld bat detectors (Echo Meter Touch and Echo Meter Touch 2 Pro from Wildlife Acoustics, Maynard, USA, and Anabat Walkabout and Swift from Titley Electronics) recorded each bat in flight after release. Bats were followed on foot in the dark where safe to do so, to record as long a sequence of pulses as possible. Details were recorded of the behaviour of the bat to aid in later interpretation of the call (e.g., the time of release, direction of flight and whether it returned overhead). The number of reference calls collected was maximised by recording throughout the night until just before dawn.



Figure 4. The mean monthly maximum and minimum temperature and rainfall over the sampling periods in 2020-21 and 2021-22, and long-term averages, from the Bureau of Meteorology weather station at Walpeup (50 km to the southwest; www.bom.gov.au).

Reference calls of rarer species that were either not caught or only caught in small numbers, were sought from other areas and other researchers, for those species that do not vary their calls in different geographic areas. For species with geographic variation, all reference calls were sourced from north-west Victoria.

2.4.2 Refinement of call identification program

The automated call identification program that was initially designed to identify frog calls (Howard et al. 2021), and later modified to include bat calls (Lumsden et al. 2021a) was refined further in 2021-22, and new reference calls were incorporated. This program has now been named 'BatLingo'. Reference calls were subsampled into 0.75 second sliding window segments (Figure 5). Although bat call sequences are typically longer than this, they repeat similar vocalisations with up to 12 pulses per second, and this length of time was considered long enough to provide call characteristics for each species. Using this approach also increased the number of reference call segments available to train the model. Figure 5 shows a typical reference call and illustrates the terminology used for different components of the call.



Figure 5. A reference call from a Little Forest Bat (*Vespadelus vulturnus*) to illustrate the terminology used to describe sections of reference calls. Time in seconds is on the horizonal axis and frequency in kHz is on the vertical axis. The teal squares delineate the 0.75 second overlapping windows ('segments') encapsulating approximately eight pulses within each segment, with the offset teal squares representing the overlapping, sliding windows. 'Observations' are a continuous string of pulses within the file. The full call 'sequence' equates to a file recording. The gap in the middle in this example is excluded because the time between pulses is greater than 0.75 seconds in length. This area could either be ignored or included as 'noise' if background noise typical of the site was present.

Spectrograms of all new reference calls were manually checked (using either Anabat Insight or Wildlife Acoustics Kaleidoscope) to ensure that the calls entered into BatLingo only contained a single bat of the target species, and that the pulses were clear and without background noise overpowering the bat pulses. Unsuitable or poorer quality segments were excluded based on the time stamp of these sections within individual files. Only good quality 'observations' of 0.75 seconds or longer were included as this was the minimum sample period used in the model development. Call segments recorded when the bat was held prior to release and call pulses just after release were also excluded as these are not typical of free flying calls. Background noises recorded during the collection of reference calls, and also from the field sampling at a number of lakes, were added as reference files to train the model to differentiate between bat calls and other site noises.

Pre-processing of each 0.75 second segment involved splitting the segment into 281 sub-samples of 2.67 milliseconds each, and sampling at a rate of 192 kHz. These sub-samples were analysed using Python packages pyAudioAnalysis and Librosa. These packages provide output values for 34 audio features designed for training sound classifiers, and 129 frequency magnitudes. The outputs when combined form a matrix containing 281 x (34+129) descriptive variables, and in conjunction with the species or noise label are the basis of the model. The model uses a deep learning one-dimensional convolutional neural network (CNN) that was developed with Keras within the Tensorflow software package (https://www.tensorflow.org/about/bib). Many variations of network design and controlling parameters were investigated with controlling parameters were

investigated with each iteration of network design being documented to determine which design changes provided improvements in rates of identification. Detailed information on the model design process is outlined in Howard et al. (2021). Approximately 70% of the available reference call data was used to train the model, with 30% set aside as an independent set of reference calls (test data) to test the accuracy of the model for identifying species. To ensure individual pulses were not included in both testing and training data, 'observations' (i.e., continuous strings of calls; Figure 5) were allocated to either the testing or training dataset. Ideally whole sequences would be used for either training or testing, but not both. However, for some species, the small number of reference calls precluded this option. This is unlikely to have caused significant issues unless there was marked intrinsic variation among individuals, rather than variation due to where that individual was flying at the time, which is more likely.

The accuracy of the model training and testing identifications were checked in several ways. All misidentifications of segments in the training or testing data were visually checked by inspecting individual plotted spectrograms of the misidentified 0.75-second segments, and updating their training label if required. This enabled checking whether there had been an error in the naming of the file (rare occurrence), or whether there were multiple species within the call sequence. In checking the data, the latter was found to be the case in some call segments where there were very faint pulses of a different species in the background of the target species' call. This revealed how sensitive the model was to detecting any pulses, and that even faint background pulses could lead to misidentifications. All such segments were subsequently removed from the training and testing data for the model. Although this reduced the number of available segments for some species, it resulted in cleaner, more accurate training data. Any segments that were correct, but had been misidentified, were retained in the training dataset to reinforce that calls of that type were part of individual species' call repertoire. Refinement of the model was an iterative process, with revisions occurring until adequate accuracies were obtained within the time available.

The predictive accuracy of models can be improved by providing an ensemble of networks (<u>https://en.wikipedia.org/wiki/Ensemble_learning</u>). This simple-to-implement system averages the predictions of several networks, which may have varied slightly in design or training data, to provide a more robust and accurate final result than is typically achieved with a single network design. To implement this approach for this project, two ensembles were developed: a three-network and a six-network ensemble.

The three-network ensemble consisted of three slightly varying network designs that used an identical 70% training and 30% test data set. The accuracy of the three-network ensemble model was assessed by calculating sensitivity and specificity values. Sensitivity is the true positive rate; that is, the proportion of a species' calls that are correctly identified as that species (i.e., species A is identified as species A). Specificity is the true negative rate; that is, the proportion of calls that are not of a particular species, that are correctly identified as not that species (i.e., if it is not species A then it does not get called species A). Some species may have a high score for one metric but a lower score for the other. For example, a species may have a low sensitivity score (i.e., the calls of species A are sometimes incorrectly identified as another species) but could have a high specificity score because other species are rarely misidentified as species A. The three-network ensemble was used to create the model statistics used within this report, based on the 30% withheld test data set (see Table 5).

The six-network ensemble approach included the three networks from the first ensemble and an additional three networks trained on all available data, with none withheld for testing. It was anticipated that this ensemble would have similar or likely better performance characteristics than the three-network ensemble due to the increased training data provided. However independent statistics on accuracy rates were no longer possible as all data had ultimately been provided to the model, leaving no withheld data on which to test the model. This six-network ensemble was used to process the field data from the Hattah Lakes sites.

The call identification program BatLingo generated a prediction of which bat species was calling in each 0.75-second segment within each call sequence. Each prediction had an associated probability (from 0 to 1) indicating the model's confidence in its prediction. Each segment identified by the model as a bat call (rather than environmental noise) was assigned a species label, which was based on the species with the highest probability of identification. The next step involved converting the identifications of each segment within a sequence into an identification for the whole sequence, as this was the metric used to represent bat activity (i.e., a recorded file). The simplest approach was to take the species with the most segment identifications, irrespective of the probability of correct identification of each segment (the 'mode' approach). A range of modelling approaches were tried using random forests and Bayesian ordinal regression to maximise the number of incorrect identifications. However, these did not markedly improve the sensitivity and specificity scores for each species, and in some cases made them worse. Although future testing and investigation of different approaches is warranted, the simple approach of using the mode (with a slight modification, see below) was used for the analysis in this project.

Where there was uncertainty in species identification due to the occurrence of overlapping call characteristics, calls were combined into three species complexes: 1) the three species of freetail bats (*Ozimops* spp.) combined with Gould's Wattled Bat and Inland Broad-nosed Bat (*Scotorepens balstoni*); 2)

the Chocolate Wattled Bat (*Chalinolobus morio*) with Little Forest Bat (*Vespadelus vulturnus*); and 3) the three species of long-eared bats (*Nyctophilus* spp.) with the Large-footed Myotis. This is a common approach in studies based on bat call identifications (e.g., Armstrong et al. 2020).

A number of rules were incorporated into the process for converting the identification of segments to the identification of a call sequence:

- If more than 51% of the segments were identified as a single species, the sequence was identified as that species (note this is similar to the mode approach but ensures that at least half of the segments are the same species).
- If no single species recorded more than 51% of the segment identifications, but all segments were within one of the species complexes, the sequence was identified as that species complex.
- If no single species recorded more than 51% of the segment identifications, with segments identified from different species complexes or any of the other species, the sequence was classed as an Unidentified Bat Call.
- Segments identified as noise were excluded from the above calculations.

This approach was found to reduce the number of incorrect identifications while maximising the number of true positive identifications. Although it meant that not all sequences could be identified, it is preferable to reduce the number of incorrect identifications, to reduce the number of times that species are recorded as being present when they are in fact not. Reducing the number of correct positive identifications is not believed to impact the overall interpretation of the data, due to the large amount of field data available for analysis. The calls identified as species complexes or unidentified bat calls contributed to the overall bat activity numbers.

2.4.3 Processing of field recordings

After refining BatLingo, all the field-recorded call sequences were processed using the six-network ensemble version (BatLingo version 51.3). Due to the large number of calls, a high-speed desktop computer was used to increase the efficiency of processing time. As the minimum identification unit was the 0.75-second segment, any complete call sequences that were shorter than 0.75 seconds were not processed. Where noise was present in the recordings, if all the segments within a sequence were identified as noise, a single entry of 'noise' was provided for that sequence. Where noise was interspersed within bat calls, the noise segments were ignored in the overall calculations. In addition, if only a small number of low-quality pulses were present, the segment was often recorded as noise as well.

2.5 Statistical analysis

Three metrics of bat activity were investigated:

- 1. Overall bat activity, based on the total number of call sequences identified as a bat call, including those identified as an individual species, species complexes and unidentified bat calls. This provided a measure of the total bat activity and was not influenced by whether or not the calls could be identified to species level.
- 2. Bat activity of each individual species, based on the number of call sequences identified as that species.
- 3. Species richness, based on the number of species identified at each lake, using the mean species richness value per night, rather than for the whole season. Using this approach, species recorded infrequently (some of which potentially could have been due to misidentifications) contributed in a commensurate way to their frequency of recording rather than dominating the species richness calculations.

The data from each sampling year were amalgamated with the core sampling metric being the number of call sequences identified as each species per lake per night. Comparisons were initially made between lakes that were dry and those that were inundated ('wet') during the 2021-22 sampling period using raw and summarised data. Statistical analyses were then undertaken to evaluate the data from both periods in relation to treatments (wet versus dry). As Lake Kramen received water in the first year and was dry in the second year, it was excluded from the statistical analysis and was considered as a separate single example of activity levels soon after drawdown.

The study largely followed the intended BACI design. However, due to the limited number of control sites sampled before and after the delivery of water (i.e., the 'intervention'), while the intervention sites could be compared before and after the provision of environmental water, five of the six control sites could only be assessed during the 'after' period (Lake Boolca was the only control site sampled during the 'before' period in the first year of the study). We assumed that the control sites had similar levels of activity as the intervention sites prior to the intervention, with no reason to believe that this was unjustified. Under this

assumption, the new control sites represented the dry treatment and could be directly compared against the wet (inundated) treatment to fulfil the BACI design

To model the number of bat calls per night per site, we used a negative binomial mixed effects model in a Bayesian framework (Lindén and Mäntyniemi 2011). This type of model is frequently used for count data where there are repeated measures at the same site (Irwin et al. 2013). Since we were using the number of bat calls per night per site, and the data were collected at the same sites over multiple consecutive nights, this was an appropriate model framework. The fixed variables of interest were the site categories that follow the modified BACI design, namely surveys in 2020-21 ('Before'), surveys at control sites in 2021-22 ('After: Dry'), and surveys at intervention sites in 2021-22 ('After: Wet'). To account for the potential effect of weather on bat activity, fixed effects for rain (did it rain during the 24-hour period that included the sampling night? Yes/No) and minimum and maximum temperature (as smoothers) were included in the model (source: Bureau of Meteorology weather station at Walpeup, 50 km to the southwest; www.bom.gov.au). To account for potential correlation at the site level, site was included in the model as a random effect. The number of calls for each species per night per site was modelled as well as the total number of bat calls. The total number of bat calls included any call sequence that was identified to the species or complex level by BatLingo, as well as those that were determined to be bat calls but were not identifiable to species or species complex levels. If a parameter estimate (fixed or random effect) had 95% credible intervals (CI) that excluded zero, it was considered sufficient evidence that the parameter impacted the number of bat calls.

The distance of the detector from the water's edge was also incorporated into the model as a fixed effect to investigate whether this covariate influenced the level of bat activity. For the lakes containing water in 2021-22, the distance from the detector to the lake's water edge was physically measured on three occasions: 1) when the detectors were installed in November–December 2021; 2) when the batteries and memory cards were replaced in January 2022; and 3) when the detectors were retrieved in March 2022. From these three data points for each lake, the distance to water was estimated for each day of the intervening time periods, with the assumption that the water level drawdown was linear during these periods. While this assumption may not always be valid, for the purposes of determining roughly where the water level may have been, it was considered that this simple approach was adequate. For the dry lakes, the distance to water was calculated using Google Earth, based on knowledge of where the closest water was at the time. For the first year of the study, before most of the environmental watering commenced, the distance to water was estimated based on the assumption that the closest water was in the Murray River or at Lake Kramen, with no water elsewhere on the floodplain or in lakes or creeks (E. Collins, Mallee CMA, pers. comm.).

The models were constructed in *STAN* (Stan Development Team 2022) through the interface 'brms' (Bürkner 2017) in the statistical program R (R Core Team 2022). Naïve priors were used in the model. Specifically, normal distributions with zero mean and a standard deviation of 100 were used for covariates. Model chains were run until the chains converged. Convergence was defined using Gelman and Rubin's convergence diagnostics with all potential scale reduction factors being less than 1.05 (Gelman et al. 2004). Note that in Bayesian modelling, the term 'significantly different' is not used. Therefore, the terminology used in this report to indicate a difference when making comparisons, is that 'there was evidence for a difference' or 'insufficient evidence for a difference'.

3 Results

3.1 Refinement of the call identification approach

3.1.1 Trapping results

A total of 229 individual bats of nine species were trapped during the 164 mist net hours and 240 harp trap hours undertaken during the field trips in 2021-22 (Table 3). For some species, it was not possible to confidently distinguish females as the diagnostic features are associated with male penile morphology (Eastern Freetail Bat *Ozimops ridei*, Inland Freetail Bat *O. petersi*, Southern Freetail Bat *O. planiceps*, Inland Forest Bat *Vespadelus baverstocki* and Southern Forest Bat *V. regulus*), and so these were recorded just at the generic level (Table 3). Where females were trapped and could not be definitively identified, they were released without the collection of reference calls, to exclude the risk of introducing incorrectly identified call sequences, as the call characteristics of the species within both genera overlap considerably. There is no evidence of differences between the sexes in call characteristics for these species.

Species	Scientific name	Number of individuals
Gould's Wattled Bat	Chalinolobus gouldii	68
Chocolate Wattled Bat	Chalinolobus morio	9
Lesser Long-eared Bat	Nyctophilus geoffroyi	53
Inland Freetail Bat	Ozimops petersi	17
Southern Freetail Bat	Ozimops planiceps	10
Freetail bat sp. (female)	Ozimops sp.	20
Inland Broad-nosed Bat	Scotorepens balstoni	9
Little Broad-nosed Bat	Scotorepens greyii	2
Inland Forest Bat	Vespadelus baverstocki	3
Little Forest Bat	Vespadelus vulturnus	37
Forest bat sp. (female)	Vespadelus sp.	1
TOTAL		229

Table 3. The number of individuals trapped in the Hattah Lakes area during 2021-22 for the collection of reference calls.

3.1.2 Expansion of the reference call library

The reference call library for north-western Victoria increased significantly through the collection of reference calls during this project. The number of reference call sequences available in 2021-22 was 1,273, almost three times that available in 2020-21 (489 reference calls) (Table 4). Fourteen species were included in the call library, which included two species we had been unable to source calls for in 2020-21.

For species with few reference calls from this region, calls from elsewhere in the species' range were included, where it is believed there is no significant geographical variation in their calls. Reference calls from near Melbourne were included for the Large-footed Myotis and Eastern Freetail Bat. Neither species have been trapped in Hattah–Kulkyne National Park. Nonetheless, their distribution is believed to include this area due to individuals being recorded elsewhere along the Murray River in north-western Victoria, both upstream and downstream of Hattah Lakes for the Large-footed Myotis (Australasian Bat Society 2022), or being trapped in the nearby Nowingi State Forest (Eastern Freetail Bat; Lumsden et al. 2008). Reference calls of the Little Broad-nosed Bat (*Scotorepens greyii*) were supplemented from interstate. White-striped Freetail Bat (*Austronomus australis*) reference calls were collected from along the Murray River near Echuca and from Melbourne. In addition, identified calls of the White-striped Freetail Bat from field recordings at Hattah Lakes were included, as this species has a distinctive echolocation call that does not overlap with any other species in the region. There is no known geographical variation in the calls of any of these species.

The Gould's Long-eared Bat (*Nyctophilus gouldi*) was included; however, the status of this species in Hattah–Kulkyne National Park remains unknown. The species primarily occurs in southern Victoria, with a range extension recorded in 2006 to Macredie Island north of Swan Hill (Lumsden et al. 2007). There is one confirmed record (a museum specimen) from near Mildura in 1998 (Gee 1999), so it is possible that this species extends further into north-western Victoria by following the mesic zone along the Murray River, although none have been trapped in the intervening areas. Reference calls for this species were sourced from southern Victoria and NSW.

There is one species that is known to occur in the area, but for which no reference calls are available: the Southern Forest Bat. A small number of calls were available in 2020-21 (Table 4; Lumsden et al. 2021a); however, the Southern and Inland Forest Bats are externally almost indistinguishable. The only reliable diagnostic feature, in this area, is the shape of a flap of skin on the side of the glans penis in males (Lumsden and Bennett 1995, Churchill 2008). This is best seen through a field microscope which was not available in 2020-21. In 2021-22, when a microscope was used, the three males caught were found to be Inland Forest Bats. The calls recorded from two bats identified in the first year as Southern Forest Bats were subsequently found to be indistinguishable from calls of Inland Forest Bats, and so it was decided to exclude these calls from the call library in case these bats had been misidentified. No Southern Forest Bats were caught in 2021-22, and as there is considerable geographic variation in the calls of this species (Law et al. 2002), reference calls could not be sourced from other regions. As a result, no reference calls are available for this species for inclusion in BatLingo.

Species	Scientific name	2020-21 sequences	2021-22 sequences
White-striped Freetail Bat	Austronomus australis	14	112
Gould's Wattled Bat	Chalinolobus gouldii	60	180
Chocolate Wattled Bat	Chalinolobus morio	27	64
Large-footed Myotis	Myotis macropus	3	194
South-eastern Long-eared Bat	Nyctophilus corbeni	46	62
Lesser Long-eared Bat	Nyctophilus geoffroyi	74	167
Gould's Long-eared Bat	Nyctophilus gouldi	53	53
Inland Freetail Bat	Ozimops petersi	56	121
Southern Freetail Bat	Ozimops planiceps	18	75
Eastern Freetail Bat	Ozimops ridei	0	20
Inland Broad-nosed Bat	Scotorepens balstoni	12	63
Little Broad-nosed Bat	Scotorepens greyii	0	28
Inland Forest Bat	Vespadelus baverstocki	5	25
Southern Forest Bat	Vespadelus regulus	6	0
Little Forest Bat	Vespadelus vulturnus	26	109
Noise		89	1,615
TOTAL BAT CALLS		489	1,273

Table 4. The number of reference call sequences of species known from the Hattah Lakes area available in 2020-21, and the increased number available in 2021-22 that were included in the automated bat call identifier, BatLingo.

3.1.3 Refining and testing BatLingo

There were 16,651 call segments (0.75 seconds in length) from the 1,273 bat call sequences, plus 14,022 noise segments available for training and testing BatLingo. Overall, the analysis compiled 30,673 segments across all species and noise samples, with approximately 21,661 (70%) used to train the model and 9,012 (30%) set aside as an independent set of calls to test the accuracy of the model for identifying species (Table 5).

Species	Train segments	Test segments	Total segments
White-striped Freetail Bat	641	333	974
Gould's Wattled Bat	2,007	671	2,678
Chocolate Wattled Bat	578	324	902
Large-footed Myotis	1,866	708	2,574
South-eastern Long-eared Bat	358	202	560
Lesser Long-eared Bat	783	426	1,209
Gould's Long-eared Bat	157	67	224
Inland Freetail Bat	1,919	736	2,655
Southern Freetail Bat	1,110	332	1,442
Eastern Freetail Bat	409	172	581
Inland Broad-nosed Bat	713	255	968
Little Broad-nosed Bat	217	156	373
Inland Forest Bat	230	126	356
Little Forest Bat	800	355	1,155
Noise	9,873	4,149	14,022
Total	21,661	9,012	30,673

Table 5. The number of 0.75-second segments from reference call sequences of species known from the Hattah Lakes area used to train and test BatLingo in 2021-22.

Iterative refinements were made to the model until the sensitivity and specificity figures were considered adequate for the purpose of analysing the field data within the time available. To quantify the accuracy rates of segment identifications, the sensitivity and specificity rates for each species were calculated based on the 30% of data set aside for testing. Sensitivity rates for the segment identifications (i.e., true positives, the proportion of that species' calls that were correctly identified) ranged from 52–98% (Table 6). Specificity for the segment identifications (i.e., true negatives) were all above 97% (Table 6). This disparity reflected the approach we took to prioritise minimising the number of incorrect identifications (the inverse of specificity) above maximising the number of correct identifications (sensitivity). In addition, specificity scores were typically higher than sensitivity scores, primarily because of the large number of noise files included in the model, which were mostly identified correctly and hence were not incorrectly identified as a bat species.

Once the segment identifications had been combined into identifications for the whole call sequence (using the rules outlined in section 2.4.2), the high specificity accuracy was maintained, while the sensitivity rates either increased or remained similar (Table 6). The increase in accuracy rates was at least partly attributed to the practice of ignoring the misidentification of a single segment if more than half of the segments were correctly identified. Sensitivity rates for full sequences ranged from 52–100%, while specificity rates were 99–100%.

Table 6. The accuracy of identifications of the call segments and the full call sequences for both sensitivity (true positives) and specificity (true negatives) based on the 30% withheld testing data (using BatLingo version 51.3 three ensemble model).

Species	Identification accuracy of segments (%) Sensitivity	Identification accuracy of segments (%) Specificity	Identification accuracy of full sequences (%) Sensitivity	Identification accuracy of full sequences (%) Specificity
White-striped Freetail Bat	82.9	99.9	100.0	99.9
Gould's Wattled Bat	89.6	98.7	94.8	99.7
Chocolate Wattled Bat	71.0	99.4	87.5	99.6
Large-footed Myotis	98.2	99.4	97.1	99.9
South-eastern Long-eared Bat	72.8	99.2	68.2	99.0
Lesser Long-eared Bat	82.2	99.0	80.3	99.2
Gould's Long-eared Bat	52.2	99.8	75.0	99.5
Inland Freetail Bat	73.8	98.2	81.0	98.8
Southern Freetail Bat	53.0	98.5	52.0	98.9
Eastern Freetail Bat	83.1	99.9	85.7	100.0
Inland Broad-nosed Bat	79.2	99.4	85.0	100.0
Little Broad-nosed Bat	94.2	100	90.9	100.0
Inland Forest Bat	63.5	99.9	85.7	100.0
Little Forest Bat	85.4	99.1	92.9	99.9
Noise	99.9	97.4	99.3	100.0

3.2 Influence of environmental water on bat activity and species richness

3.2.1 Field data from sampling at Hattah Lakes Icon Site

During the 2021-22 sampling period, 1,886 nights of detector sampling were successfully completed at 24 sites (Table 7). This was an increase on the 1,369 nights of sampling at 19 sites in 2020-21. Although the mean number of nights of sampling per lake was only marginally higher in the second year (78.6 vs 72.0 nights per lake), the data were more consistent, with sampling occurring simultaneously at all lakes for extended periods of time, in contrast to the data available from the first year when there were no nights when all detectors operated simultaneously (Table 7; Lumsden et al. 2021a).

Despite extensive testing of the detectors prior to deployment for the 2021-22 sampling to ensure they were all working consistently and reliably, there was an unexpected error that neither we nor the detector manufacturers were aware of. The Swift detectors have two card slots and are programmed to automatically switch to record on the second card when the first is full. However, in some instances, after switching to the second card the detector only recorded for the rest of that night, and did not resume recording on dusk the following night as programmed. This occurred on 10 of the 24 detectors set during the November–January period. As the problem was only discovered after all the units were reset in the field in January, the same issue occurred during the January–March period, when 11 of the 24 detectors did not fully record on the second card. This resulted in the overall recording period being shorter than initially planned. Fortunately, we always set the 256 GB card in the first card slot, and so recording was achieved on the larger-sized card before switching to record on the smaller (128 GB) card. As the detectors operated correctly up until that time, significant amounts of data were still able to be collected. The manufacturers have now located and fixed the problem, and developed a new firmware update, and so this issue should not occur in the future.

During the sampling in 2021-22, 4.7 million files were collected, equating to 12.8 TB of data (Table 7). In contrast, 1.9 million files were collected during 2020-21 (2.7 TB). The extensive nature of this dataset resulted in challenges for processing and manipulating the data. It took the high-speed computer more than one week of continuous computing time to process the data using the six-network ensemble BatLingo model. This resulted in 21.3 million segment identifications that were then converted into sequence identifications.

Table 7. Cor	mparison of data a	available from th	e first year of	f sampling iı	n 2020-21 (collected by the
Mallee CMA) and the second	year in 2021-22 (collected as	part of this p	oroject).	

Variable	2020-21	2021-22
Sites sampled	19	24
Total nights of sampling	1,369	1,886
Mean number of nights of sampling per lake (mean ± SD and range in brackets)	72.0 ± 25.9 (31-112)	78.6 ± 17.4 (42-106)
Number of nights when detectors worked at all sites concurrently	0	33
Number of files recorded	1,884,381	4,706,997
Mean number of files (± SD) recorded per night	1,568 ± 638	2,668 ± 925
Size of resulting data (TB)	2.7	12.8

Of the total 6,591,378 calls analysed from the two years combined, 2,927,962 (44.4%) were successfully identified to species (Table 8). A further 6.6% were identified to species complexes; however, as these showed broadly similar patterns to the individual species within each complex, these were combined with the unidentified bat calls (2.3%) to form the category of 'unknown bat calls' (Table 8). The unknown bat calls were incorporated into measures of overall bat activity (see section 3.2.2). Forty-seven percent of the recorded files were identified as noise. A manual check of a subset of these revealed that some files did contain bat call pulses, but typically they were too brief or of too poor quality for BatLingo to successfully identify any of the 0.75-second segments within the sequence, and hence the entire sequence was identified as noise. Although this may have underestimated the number of files with bat calls, these would not be of sufficient quality to identify to a species, so would have only contributed to the unknown bat calls category, and therefore would not significantly affect the interpretation of the species-specific results.

Fourteen species of bat were identified from the field recordings at the Hattah Lakes sites, which included all the species BatLingo had been trained on (Table 8). The sheer volume of the data precluded extensive manual checking of the identifications, and so for this analysis of short-term response to environmental water during the inundation phase, the results from BatLingo have been accepted without modification.

As there are differences in how readily species are both detected and identified due to the overlap of call characteristics, detailed comparisons between species should not be made, but some broad generalisations were possible. The Gould's Wattled Bat was the most recorded species, representing 26% of all identified bat calls (Table 8), and it was also the most frequently trapped species (Table 3). Other species that were both commonly identified in the bat call analysis and frequently caught included the Little Forest Bat, Southern Freetail Bat and Inland Freetail Bat. In contrast, the Lesser Long-eared Bat, a species with a comparatively quiet echolocation call, was trapped frequently, but identified relatively infrequently in the bat call data.

The three species recorded least frequently, each representing just 0.01–0.07% of the identified bat calls, were the Gould's Long-eared Bat, South-eastern Long-eared Bat (*Nyctophilus corbeni*) and Large-footed Myotis (Table 8). None of these were caught during the trapping component of this study, and their calls overlapped considerably in their characteristics, resulting in only moderate identification accuracies (Table 6). These recordings would have warranted manual checking had there been time; however, these species are difficult to distinguish manually and checking these 6,000 call files was not feasible. The lack of manual checking, coupled with the moderate identification accuracy, may have introduced some misidentifications into the dataset.

A higher number of calls were identified to species in the second year compared to in the first year (2,085,849 and 842,113, respectively). However, the relative proportion of each species was similar

(Table 8). The mean number of calls identified per night was also higher in the second year (1,347 *cf*. 722 calls/night), as was the number of noise files recorded (1,148 *cf*. 674) (Table 8).

Table 8. The species of bats identified from the Hattah Lakes in 2020-21 and 2021-22 (all sites
combined), with the total calls and mean number of calls recorded per night for each species.

Species	Total calls		Mean calls/night		
	2020-21	2021-22	2020-21	2021-22	
White-striped Freetail Bat	4,523	8,020	3.4	4.3	
Gould's Wattled Bat	183,399	740,951	136.0	392.9	
Chocolate Wattled Bat	64,696	332,490	48.0	176.3	
Large-footed Myotis	2,097	525	1.6	0.3	
South-eastern Long-eared Bat	484	2,132	0.4	1.1	
Lesser Long-eared Bat	5,073	10,660	3.8	5.7	
Gould's Long-eared Bat	16	562	0.01	0.3	
Inland Freetail Bat	187,332	201,395	138.9	106.8	
Southern Freetail Bat	106,486	194,438	78.9	103.1	
Eastern Freetail Bat	55,966	132,340	41.5	70.2	
Inland Broad-nosed Bat	22,480	143,597	16.7	76.1	
Little Broad-nosed Bat	7,518	26,136	5.6	13.9	
Inland Forest Bat	7,689	16,400	5.7	8.7	
Little Forest Bat	194,354	276,203	144.1	146.4	
Unknown bat call*	132,687	455,290	98.4	241.4	
Total bat calls	974,800	2,541,139	722.6	1347.4	
Noise	909,581	2,165,858	674.3	1148.4	

*Unknown bat calls consisted of calls identified as species complexes and those that were recognised as bat calls but the identification was too uncertain to identify as a single species or species complex.

3.2.2 Comparison of bat activity levels between lakes

To illustrate the difference in activity levels between lakes, the mean number of identified calls for each species has been plotted in Figure 6. The lakes that received environmental water in the second year of this study are shown first (the 'intervention' sites in Figure 6), split into the activity levels from 2020-21 before these lakes received water and in 2021-22 during the inundation period. The control sites are those that did not receive water in the second year. Only one of these lakes was sampled in both years (Lake Boolca), with the other five the additional sites that were included in the second year to provide more control sites for the study. Lake Kramen is provided at the end as it had a different watering regime to the other sites, being inundated in the first year and soon after drawdown in the second.

Overall, total bat activity was relatively consistent between lakes, although a small number of lakes (both intervention and control sites in the second year) had higher numbers of calls recorded per night than the others (Figure 6). A similar pattern was shown by many of the individual species, with more variability between lakes during the inundation phase than during the dry phase in the first year, although there were exceptions to this, such as for the Inland and Southern Freetail Bats where there was high variability between lakes during both phases (Figure 6). For many species, there was marked variability in the activity levels at the control sites in the second year, although the activity levels were remarkably similar for the Lake Boolca control site between the two years of sampling. For most species, and for total bat activity, there was a general pattern of higher levels of activity during the second year compared to the first year.



Figure 6. The mean number (with 95% confidence intervals) of calls per night for total bat activity and for each species across all lakes, for both years of the study. Intervention sites represent those that received environmental water and control sites represent those that did not, with the anomaly of Kramen provided at the end.



Figure 6 (continued). The mean number (with 95% confidence intervals) of calls per night for total bat activity and for each species across all lakes, for both years of the study. Intervention sites represent those that received environmental water and control sites represent those that did not, with the anomaly of Kramen provided at the end.



Figure 6 (continued). The mean number (with 95% confidence intervals) of calls per night for total bat activity and for each species across all lakes, for both years of the study. Intervention sites represent those that received environmental water and control sites represent those that did not, with the anomaly of Kramen provided at the end.



Figure 6 (continued). The mean number (with 95% confidence intervals) of calls per night for total bat activity and for each species across all lakes, for both years of the study. Intervention sites represent those that received environmental water and control sites represent those that did not, with the anomaly of Kramen provided at the end.



Figure 6 (continued). The mean number (with 95% confidence intervals) of calls per night for total bat activity and for each species across all lakes, for both years of the study. Intervention sites represent those that received environmental water and control sites represent those that did not, with the anomaly of Kramen provided at the end.

Short-term response of bats to environmental water

3.2.3 Bat activity in response to the provision of environmental water

Box plots of the mean number of calls per night are shown in Figure 7 to visualise bat activity after the provision of environmental water, with comparisons between the results from the first year of data ('Before') with those in the second year (split into the six sites that remained dry: 'After: Dry'; and the 17 lakes that received water: 'After: Wet'). These figures do not include Lake Kramen as it had a different watering cycle and is considered separately in section 3.2.5. For this comparison, there were a total of 3,454,625 sequences identified as bats over 3,138 sampling nights across lakes over the two survey periods. In the initial survey period ('Before'), there were 969,850 bat sequences identified across 1,338 survey nights across the lakes, compared to 2,484,775 identified bat sequences across 1,800 survey nights during the second year of sampling. All 14 species were identified in both the before and after time periods. Overall, there was a pattern of higher bat activity at the sites that received environmental water (Figure 7).



Figure 7. Box plots of the mean bat activity (identified calls/night/lake) for each species and total bat activity comparing before (2020-21) and after (2021-22) the provision of environmental water, and control sites that remained dry in the second year. The bottom and top of each 'box' indicate the 25th and 75th percentiles, respectively, and the black horizontal line indicates the median (50th percentile) reported value, with outliers as circles. Note that the scales on the y-axes are different, with species clustered into groups with similar levels of activity for clarity.



Figure 7 (continued). Box plots of the mean bat activity (identified calls/night/lake) for each species and total bat activity comparing before (2020-21) and after (2021-22) the provision of environmental water, and control sites that remained dry in the second year.

To investigate factors influencing the effect of environmental water using the BACI study design, models were constructed of the total number of bat calls, and the number of calls of individual species, per night. To explore if the effect of the environmental water was broader than just at the site level (i.e., was there a spill-over effect to nearby areas), the dry control sites were split into two categories: 1) sites where there was some water in the general vicinity (i.e., they were 'near' water, < 1 km); and 2) sites that did not have water nearby (i.e., they were 'distant' from water, > 1 km). Of the weather variables included in the model, the only influential variable was the amount of rain over the 24-hour period that incorporated the sampling night. Minimum and maximum temperatures were highly variable and were found to not be influential, and as a result are not included in the results presented below.

Total bat activity was influenced by the year of the survey, water nearby, and rain. Evidence for this conclusion can be seen in Table 9, which shows the percentage change in the modelled number of total bat

calls per night between each of the different model categories. If the estimate and upper and lower bounds of the 95% credible intervals (CI) are all positive, it is evidence of an increase (with the estimate showing the percentage difference) in calls per night for that category compared to the comparison category. Similarly, if all three values in a row are negative, it is evidence of a decrease. If the row has a mix of positive and negative values, the 95% CI includes zero, which means there is insufficient evidence to conclude that the two categories have different amounts of bat activity per night. For instance, taking the simplest comparison, the first line of Table 9 compares total bat activity of the 'Before' data in 2020-21 when all lakes were dry, with data from the second year at the sites that had received water ('After: Wet'). This shows that the number of calls recorded per night during 'After: Wet' was on average 87% greater than during 'Before' (i.e., almost double), and this difference was meaningful as the 95% CI was from 79% to 96% (both positive). In contrast illustrating where there was a negative effect, if it rained (last row of Total bat activity in Table 9), the modelled number of bat calls per night decreased by 14% compared to when there was no rain, and this difference was meaningful as the 95% CI was from -20% to -8% (both negative and hence not including zero). The other comparisons between categories are between either 'Before' or 'After: Wet' and the 'after' control sites that were either near or distant from water. There was insufficient evidence for a difference between the two types of control sites based on the availability of nearby water (i.e., 'After: Dry (near)' and 'After: Dry (distant)') as the 95% CI included zero (i.e., one was positive, and one was negative). There was, however, evidence for a difference between both 'After: Dry (distant)' and 'After: Dry (near)' with 'Before' (26% and 86% greater, respectively), and between 'After: Wet' and 'After: Dry (distant)' (49% greater). This indicates that for total bat activity, there was a positive effect of environmental water and that it had a spillover effect into sites without water but where there was water nearby. To illustrate these differences visually, Figure 8 shows the modelled total bat calls per night for each category, assuming there was no rain. Where the estimates and their associated 95% CIs are widely spaced, this suggests there is evidence for a difference, while if the 95% CIs overlap extensively, it indicates there is no evidence for a difference. Additional model parameters are provided in Appendix 1 for total bat activity and for each individual species.

The modelled number of White-striped Freetail Bat calls per night was influenced by the year of survey and rain (Table 9). The number of White-striped Freetail Bat calls per night was greater in the second year than in the first, both at sites with water ('After: Wet' 129%) and those without ('After: Dry (distant)' 230%, and 'After: Dry (near)', 473%) (Table 9; Figure 8). There was no clear difference between any other pairs of site categories. This indicates that there was a positive effect of environmental water, but there was insufficient evidence for a spill-over effect to nearby sites, at least at the scale measured. Rain had a negative effect on the number of White-striped Freetail Bat calls per night (Table 9).

The modelled number of Gould's Wattled Bat calls per night was influenced by the year of the survey, water nearby and rain (Table 9). The number of Gould's Wattled Bat calls per night was substantially higher in the second year compared to the first year, with 207% more calls at the inundated sites in the second year than the dry sites in the first year, and 84% and 236%, respectively more at control sites in the second year that were distant or near to water. In 2021-22, there was no evidence for a difference between sites with water and control sites near to water; however, there was a difference between sites with water and control sites further from water (Table 9; Figure 8). This indicates there was a strong positive effect of environmental water for Gould's Wattled Bats, and that there was a spill-over effect to nearby sites. Rain had a negative effect on the number of Gould's Wattled Bat calls per night (Table 9).

The modelled number of Chocolate Wattled Bat calls per night was influenced by the year of the survey and water nearby (Table 9). The number of calls per night was substantially higher in the second year than in the first year, with 297% more calls at the inundated sites than the dry sites in the first year. There were similar numbers of calls at the control sites near to water (i.e., 'After: Dry (near)') as at the inundated sites, but both of these were markedly higher than at the control sites far from water (Figure 8). This indicates a strong positive effect of environmental water and also a strong spill-over effect to nearby sites. Rain had a marginally negative effect on modelled Chocolate Wattled Bat calls per night, with the 95% CI just including zero (Table 9).

The modelled number of Large-footed Myotis calls per night was influenced by the year of the survey, water nearby and rain (Table 9). In contrast to the previous species, the number of calls per night were substantially greater in the first year than in the second (Figure 8). At inundated sites in 2021-22, the number of calls per night was similar to control sites close to water, but different from control sites distant from water. This suggests that there was not a benefit of environment water; however, as the sample sizes for this species were low, caution is required in drawing any conclusions. Rain had a negative effect on the number of Large-footed Myotis calls per night (Table 9).

For both the Lesser Long-eared Bat and South-eastern Long-eared Bat, the modelled number of calls per night was influenced by the year of the survey, water nearby and rain (Table 9). The number of calls per night was higher in the second year than in the first, with 70% and 212% more calls, respectively for the two species at the inundated sites than at the dry sites in the first year. In 2021-22, there was no difference in the

number of calls per night between the inundated sites and the control sites that were near to water, but there was a difference from the control sites distant from water (Figure 8). This indicates a benefit of environmental water, and a spill-over effect to nearby sites for both species of long-eared bats. Rain had a negative effect on the number of calls of both species (Table 9). There was insufficient data to model the number of calls of Gould's Long-eared Bat as it was only recorded on 6% of survey nights.

The patterns were less obvious for the three species of small freetail bats. All three species were influenced by the year of the survey, with 25% more calls in the second year at inundated sites than in the first year for Southern Freetail Bats, and 73% more calls for Eastern Freetail Bats (Table 9; Figure 8). In contrast, for the Inland Freetail Bat, the number of calls was 30% lower at the inundated sites in the second year, than at the dry sites in the first year (Table 9; Figure 8). There were no clear differences between other pairs of categories. Rain did not have a negative effect on the activity levels of any of these species (Table 9).

The modelled number of calls of both Inland and Little Broad-nosed Bats were influenced by the year of the survey, water nearby and rain (Table 9). There were substantially more calls per night at the inundated sites in the second year than at the dry sites in the first year, with 407% more for Inland Broad-nosed Bat and 186% more for Little Broad-nosed Bat (Figure 8). In the second year, there was no difference between the number of calls of the Inland Broad-nosed Bat at the inundated sites than at the dry sites with water nearby, but there was a difference between the inundated sites and dry sites distant from water. However, there was no difference between control sites close to or distant from water, and so the spill-over effect is unclear. This pattern is also unclear for the Little Broad-nosed Bat. There does, however, appear to be a strong benefit of environmental water for both species. Rain had a negative effect on the number of calls per night for both broad-nosed bat species (Table 9).

The modelled number of calls per night of the Inland Forest Bat was influenced by the year of the survey, water nearby and rain (Table 9; Figure 8). At inundated sites in 2021-22, the number of Inland Forest Bat calls per night was greater than at sites not near water (either dry sites in 2020-21 by 60%, or control sites without nearby water in 2021-22 by 40%). There was no clear difference between any other pairs of categories, nor was there a negative effect of rain although this result was marginal (Table 9). Therefore, there is some evidence for a benefit of environmental water for the Inland Forest Bat. In contrast, for the Little Forest Bat there was no evidence of any differences between years or proximity to water (Table 9; Figure 8). This suggests there was no benefit of environmental water for this species. Rain was the only variable to influence the number of calls, with activity reduced by 17% if there was rain (Table 9).

Table 9. Results from the comparison of the number of bat calls per night between levels in the model for total activity and individual species. All scores are percentage change from the comparison category to the initial category. LB and UB are the lower and upper bounds from the 95% credible interval respectively. Meaningful differences are indicated in bold. Dry (near) indicates control sites with water within 1 km. Dry (distant) indicates control sites far from water.

Category	Comparison category	Estimate	LB	UB
Total bat activity				
After: Wet	Before	87	79	96
After: Wet	After: Dry (distant)	49	26	77
After: Wet	After: Dry (near)	6	-34	65
After: Dry (distant)	Before	26	7	49
After: Dry (near)	Before	86	13	187
After: Dry (near)	After: Dry (distant)	48	-12	133
Rained: Yes	Rained: No	-14	-20	-8
White-striped Freetail Bat				
After: Wet	Before	129	95	167
After: Wet	After: Dry (distant)	-26	-58	22
After: Wet	After: Dry (near)	-49	-83	18
After: Dry (distant)	Before	230	92	436
After: Dry (near)	Before	473	94	1,241
After: Dry (near)	After: Dry (distant)	84	-45	358
Rained: Yes	Rained: No	-29	-43	-14
Gould's Wattled Bat				
After: Wet	Before	207	186	228
After: Wet	After: Dry (distant)	69	32	113
After: Wet	After: Dry (near)	6	-55	111
After: Dry (distant)	Before	84	45	131
After: Dry (near)	Before	236	44	580
After: Dry (near)	After: Dry (distant)	85	-23	281
Rained: Yes	Rained: No	-22	-29	-15
Chocolate Wattled Bat				
After: Wet	Before	297	268	327
After: Wet	After: Dry (distant)	217	142	309
After: Wet	After: Dry (near)	16	-50	130
After: Dry (distant)	Before	27	-2	62
After: Dry (near)	Before	297	72	693
After: Dry (near)	After: Dry (distant)	216	32	549
Rained: Yes	Rained: No	-9	-18	0
Large-footed Myotis				
After: Wet	Before	-84	-87	-81
After: Wet	After: Dry (distant)	410	97	1,104
After: Wet	After: Dry (near)	164	-35	640
After: Dry (distant)	Before	-96	-99	-92
After: Dry (near)	Before	-91	-98	-76
After: Dry (near)	After: Dry (distant)	179	-48	853
Rained: Yes	Rained: No	-40	-54	-24

Table 9 (continued). Results from the comparison of the number of bat calls per night between levels in the model for total activity and individual species.

Category	Comparison category	Estimate	LB	UB
Lesser Long-eared Bat				
After: Wet	Before	70	55	86
After: Wet	After: Dry (distant)	215	126	328
After: Wet	After: Dry (near)	39	-42	187
After: Dry (distant)	Before	-45	-60	-26
After: Dry (near)	Before	43	-41	195
After: Dry (near)	After: Dry (distant)	166	6	467
Rained: Yes	Rained: No	-22	-32	-11
South-eastern Long-eared				
Bat			1.50	
After: Wet	Before	212	159	271
After: Wet	After: Dry (distant)	262	80	551
After: Wet	After: Dry (near)	54	-45	256
After: Dry (distant)	Before	-5	-52	68
After: Dry (near)	Before	153	-12	471
After: Dry (near)	After: Dry (distant)	191	-18	642
Rained: Yes	Rained: No	-26	-43	-5
Inland Freetail Bat				
After: Wet	Before	-30	-35	-25
After: Wet	After: Dry (distant)	-16	-35	7
After: Wet	After: Dry (near)	-2	-45	60
After: Dry (distant)	Before	-15	-34	7
After: Dry (near)	Before	-23	-56	27
After: Dry (near)	After: Dry (distant)	-7	-50	57
Rained: Yes	Rained: No	-7	-15	3
Southern Freetail Bat				
After: Wet	Before	25	17	34
After: Wet	After: Dry (distant)	22	-5	54
After: Wet	After: Dry (near)	-21	-59	36
After: Dry (distant)	Before	4	-18	31
After: Dry (near)	Before	74	-7	201
After: Dry (near)	After: Dry (distant)	69	-15	201
Rained: Yes	Rained: No	-6	-15	3
Eastern Freetail Bat				
After: Wet	Before	73	61	85
After: Wet	After: Dry (distant)	44	11	84
After: Wet	After: Dry (near)	82	-8	224
After: Dry (distant)	Before	22	-5	55
After: Dry (near)	Before	5	-46	88
After: Dry (near)	After: Dry (distant)	-13	-58	59
Rained: Yes	Rained: No	0	-9	10

Table 9 (continued). Results from the comparison of the number of bat calls per night between levels in the model for total activity and individual species.

Category	Comparison category	Estimate	LB	UB
Inland Broad-nosed Bat				
After: Wet	Before	407	371	446
After: Wet	After: Dry (distant)	67	26	118
After: Wet	After: Dry (near)	16	-52	147
After: Dry (distant)	Before	209	135	299
After: Dry (near)	Before	420	106	962
After: Dry (near)	After: Dry (distant)	71	-36	262
Rained: Yes	Rained: No	-20	-28	-11
Little Broad-nosed Bat				
After: Wet	Before	186	160	215
After: Wet	After: Dry (distant)	15	-18	59
After: Wet	After: Dry (near)	96	4	239
After: Dry (distant)	Before	155	82	247
After: Dry (near)	Before	59	-15	176
After: Dry (near)	After: Dry (distant)	-36	-69	16
Rained: Yes	Rained: No	-33	-41	-23
Inland Forest Bat				
After: Wet	Before	60	46	74
After: Wet	After: Dry (distant)	40	4	84
After: Wet	After: Dry (near)	2	-59	114
After: Dry (distant)	Before	16	-13	52
After: Dry (near)	Before	85	-26	290
After: Dry (near)	After: Dry (distant)	62	-38	248
Rained: Yes	Rained: No	-11	-21	1
Little Forest Bat				
After: Wet	Before	2	-4	9
After: Wet	After: Dry (distant)	22	-3	51
After: Wet	After: Dry (near)	-4	-52	74
After: Dry (distant)	Before	-15	-32	5
After: Dry (near)	Before	18	-41	115
After: Dry (near)	After: Dry (distant)	40	-32	163
Rained: Yes	Rained: No	-17	-24	-10



Figure 8. The modelled number of total bat calls and calls for each individual species per night per lake, assuming no rain, comparing the dry sites in 2020-21 (Before), to those with water (After: Wet) and control sites (After: Dry) in 2021-22. The points are the median and the vertical lines represent the 95% credible intervals.



Figure 8 (continued). The modelled number of total bat calls and calls for each individual species per night per lake, assuming no rain, comparing the dry sites in 2020-21 (Before), to those with water (After: Wet) and control sites (After: Dry) in 2021-22. The points are the median and the vertical lines represent the 95% credible intervals.

3.2.4 The response of species richness to the provision of environmental water

The mean nightly species richness is shown in Figure 9, comparing the number of species recorded before and after the provision of environmental water, in the same way as shown for bat activity. Although the number of species recorded in each category appears similar (Figure 9), due to the large amounts of data available, even small differences could be detected, and modelling revealed some differences (Figure 10). Species richness was highest in the second year at the sites that contained water, and this was higher than the species richness at the sites that were dry in the first year of sampling (95% CI of the difference between the two excluded zero (0.02 to 0.06 on log scale) with the 95% credible intervals not overlapping in Figure 10). The species richness of the dry control sites in the second year was midway between these and did not differ from either of them (the 95% CI for the difference between the dry control sites and either dry first year sites or wet second year sites included zero (0.00 to 0.06 and -0.04 to 0.02, respectively, with the 95% CI overlapping with both in Figure 10). Recordings of zero species richness in the Before data resulted from nights with low numbers of sequences recorded and all these being identified as noise.



Figure 9. Box plot of the mean nightly species richness comparing before (in 2020-21) and after (in 2021-22) the provision of environmental water, and control sites that remained dry in the second **year.** The bottom and top of each 'box' indicate the 25th and 75th percentiles, respectively, and the black horizontal line indicates the median (50th percentile) reported value, with outliers as circles.



Figure 10. The modelled mean nightly species richness, comparing the dry sites in 2020-21 (Before), to those with water (After: Wet) and control sites (After: Dry) in 2021-22. The points are the median and the vertical lines represent the 95% credible intervals.

3.2.5 Lake Kramen

Since Lake Kramen did not follow the same watering pattern as the other lakes, it is considered here separately. The number of bat calls recorded per night was markedly higher in the second year when the lake was dry, soon after drawdown (655 calls/night) than during the first year when it held water (160 calls/night) (Table 10). Gould's Wattled Bat, Little Forest Bat and the Inland, Southern and Eastern Freetail Bats dominated the recordings in both years, with higher activity levels per night in the second year for each species.

In 2020-21, the majority (95%) of the files recorded were identified as noise, likely due to calling insects (Table 10; Figure 11). In comparison, in 2021-22 when the lake was dry, the percentage of noise files was lower, at 68%. A manual check of a subset of the noise files revealed the majority were exclusively noise (Figure 11a); however, some also included bat calls. The intense noise was in the same frequency range as many of the species of bats (i.e., 20–50 kHz) and so would likely have obscured any bat calls recorded at the same time within this frequency range (Figure 11b). However, if calls were higher or lower than this band of sound, some could be recorded and successfully identified (Figure 11c). This noise was virtually continuous when present, but varied between nights. Sometimes it continued for the whole night (resulting in no bat calls being detected); other times it ceased at about midnight; while on other nights only a small number of noise files were recorded (resulting in some calls detected). In 2021-22, the noise levels were considerably lower, enabling the bat calls to be visible and identifiable (Figure 11d).

Species	Total calls		Mean calls/night	
	2020-21	2021-22	2020-21	2021-22
White-striped Freetail Bat	9	357	0.3	4.2
Gould's Wattled Bat	1,034	15,472	33.4	179.9
Chocolate Wattled Bat	189	2,981	6.1	34.7
Large-footed Myotis	11	2	0.4	0.0
South-eastern Long-eared Bat	0	49	0.0	0.6
Lesser Long-eared Bat	85	336	2.7	3.9
Gould's Long-eared Bat	0	1	0.0	0.0
Inland Freetail Bat	1,281	6,646	41.3	77.3
Southern Freetail Bat	211	7,439	6.8	86.5
Eastern Freetail Bat	262	3,473	8.5	40.4
Inland Broad-nosed Bat	86	3,243	2.8	37.7
Little Broad-nosed Bat	33	1,105	1.1	12.8
Inland Forest Bat	69	310	2.2	3.6
Little Forest Bat	1,066	5,111	34.4	59.4
Unidentified bat call	614	9,839	21.2	114.4
Total bat calls	4,950	56,364	159.7	655.4
Noise	92,919	117,705	2,997.4	1,368.7

Table 10. The total number of calls and number of calls per night recorded at Lake Kramen in 2020-21 (n=31 nights) when the lake contained water, and in 2021-22 soon after drawdown (n=86 nights).



Figure 11. Examples of noise files from Lake Kramen in 2020-21: a) extensive noise likely from insects within the 20–50 kHz range; b) a bat call obscured by noise, which was identified as 'noise'; c) a Chocolate Wattled Bat calling higher than the noise, that was correctly identified; and d) a Chocolate Wattled Bat call recorded in 2021-22 when there was no water in the lake, showing minimal background noise. Call sequences are displayed using the Wildlife Acoustics Kaleidoscope program in uncompressed mode. The upper panel in each figure represents the intensity of the sounds.

4 Discussion

There are two significant outcomes from this investigation into the benefits of environmental water for insectivorous bats in the Hattah Lakes Icon Site. First, it has enabled the further development of the automated bat call analysis program using one dimensional convolutional neural networks, now called 'BatLingo', to the stage where it can be used to efficiently analyse huge numbers of remotely collected bat echolocation calls with a reasonable level of accuracy. This system is the first of its kind in Australia, and is one of the first internationally to develop such a sophisticated approach. In the UK, a similar approach was developed using convolutional neural networks; however, it only enabled the distinction of a bat call from other high frequency sound, and did not have the capability to identify individual species of bats (Mac Aodha et al. 2018). In the last two years, there has been an increased focus globally on developing automated approaches for identifying bat calls using artificial intelligence (e.g., Kobayashi et al. 2021; Paumen et al. 2021; Schwab et al. 2022; Tabak et al. 2022), and our approach will contribute to this expanding field, and introduce it into studies of the Australian environment.

Second, to our knowledge, this is the first time in Australia that a study has investigated the benefits of environmental water to insectivorous bats using an experimental approach with a BACI design. Previous studies investigating the benefits of water have used a comparative approach, revealing higher bat activity at sites with water, but have not been able to tease out before/after benefits (Blakey et al. 2017). This report outlines the first step of this investigation, by examining the short-term response during the inundation phase. The next step would be to investigate the response after drawdown has occurred and the water receded at all sites, to complete the investigation of the short-term response. Subsequent studies would be required to investigate the intermediate and long-term responses, resulting from increased productivity and tree health and associated increases in the survival rates and breeding success of bats.

4.1 Development of the automated call analysis approach

Significant progress was made during this project in refining the approach for automating bat call analysis. After the baseline data collection in 2020-21, the program was found to have inaccuracies because there were insufficient reference calls to fully train the model (Lumsden et al. 2021a). The threefold increase in reference calls collected and collated as part of the current project led to an improvement in the accuracy of identifications. An overall accuracy rate of 81% was achieved at the segment level on the testing component of the reference calls, although this was highly variable between species. The accuracy rate increased for most species when the segment identifications were converted to sequence identifications, while others remained at a similar level. The accuracy rate of sequence identifications is typically higher than that of the segments, as not all segments had to be correctly identified for a sequence to be deemed correctly identified. For example, as a bat homes in on an insect it goes into a feeding buzz where the pulses change shape and become more vertical (when viewed on a spectrogram), which can resemble the pulses of other species (particularly long-eared bats). As feeding buzzes are brief within the overall sequence, if there is a long enough sequence of pulses, one misidentified segment due to the presence a feeding buzz will not impact the overall identification.

It is likely that accuracy rates will have been lower for the field recorded files than for the testing component of the reference calls. Although there may have been increases in accuracy by using the six-network ensemble model (which made use of all the reference calls for training, rather than just the 70% used for the three-network ensemble model), it is not possible to quantify the size of such an increase. On the other hand, field recordings are often of a lower quality than reference calls, with some resulting from bats flying at the edge of the range of the detector (and hence recording fragmented calls), and others involving multiple species within the one recording, causing uncertainty in the model. Manual checking of a subset of field call identifications found that the accuracy rates were lower, sometimes markedly lower. However, as it was not possible to undertake extensive manual verification of the identifications, we could not quantify this reduced accuracy. Therefore, the accuracy rates provided in Table 6 based on the reference calls, should be treated with caution at this stage.

Although great progress has been made, developing a model such as this is an iterative process and further refinement is warranted to increase the accuracy of the identifications of the reference calls, and those of the unknown field recordings. A key requirement is to further increase the comprehensiveness of the reference call library. Bats vary their calls depending on where and how they are flying. Individuals of the same species flying in open environments have calls with markedly different characteristics to when they are flying in cluttered environments, because they require a different shaped call to distinguish insects from background clutter (e.g., trees and shrubs). When a bat detects an insect and homes in to catch it, the resultant feeding

buzz is very different to the search phase call (i.e., the type of call used for commuting or searching for prey). In addition, the call can appear different depending on how close the bat is to the microphone: when the bat is close the call will be intense and harmonics will be apparent, while if the bat is further from the microphone less of the call will be apparent. There are also differences due to the Doppler effect, when a bat is flying towards or away from the detector. Bats will also vary the frequency of their call depending on whether other individuals are flying nearby. They will also emit social calls that are distinct from the normal echolocation calls, but sometimes appear similar to the echolocation calls of a different species. Therefore, there is considerable variation in the structure and frequencies of calls within a species. This intra-species variability is compounded by the significant overlap between species in their call characteristics (frequency range and shape). The full range of such call variations recorded in field data is required to adequately train BatLingo. It became apparent in checking some of the field call identifications, that for some species the call library was not sufficiently comprehensive. For example, very flat calls at 24 kHz were seen in the field recordings; however, none of our reference calls had that shape and frequency (the closest were flat calls of the Southern Freetail Bat at 26 kHz). As there were no training data representing these calls, the model searched for the closest example, which appeared to be an upper harmonic of a White-striped Freetail Bat call where the fundamental frequency was at 11-12 kHz. As a result, the 24 kHz calls (which were likely to be Southern Freetail Bats) were misidentified as White-striped Freetail Bats. Further reference calls are therefore needed to capture the full repertoire of the Southern Freetail Bat to resolve this issue.

There are other species that also require more reference calls. It has traditionally been accepted that it is not possible to distinguish the calls of the three species of long-eared bats, and that it is difficult to distinguish these species from the Large-footed Myotis (Pennay et al. 2004: Richards et al. 2004). However, progress was made on distinguishing these species within BatLingo, with current accuracy rates of 80%, 75%, 68% and 97% for Lesser Long-eared Bat, Gould's Long-eared Bat, South-eastern Long-eared Bat and Large-footed Myotis, respectively. A large number of reference calls were available for Large-footed Myotis and this was the species with the highest accuracy. Gould's Long-eared Bat and South-eastern Long-eared Bat had the lowest accuracy rates, and also the lowest number of reference calls. Increasing the number of reference calls for all three long-eared bats, and undertaking further manual checking to confirm identifications, would most likely also increase the accuracy rate. If it was possible to accurately identify the three species of long-eared bats, this would be a breakthrough in call analysis in south-eastern Australia, as targeted bat call surveys have not been possible due to the inability to distinguish these species. This is especially important for the South-eastern Long-eared Bat for which surveys are urgently needed as this is a threatened species with an extremely small population size in Victoria (Lumsden et al. 2021b).

Ideally, all new reference calls would be obtained from north-western Victoria as there is regional variation for some species (e.g., forest bats *Vespadelus* spp. (Law et al. 2002)). It is not known whether other species also vary their calls regionally, as this has rarely been rigorously investigated. One species that does not appear to display regional variation is the Gould's Wattled Bat (Reinhold et al. 2001); however, as it is typically the most commonly caught species, we already have comparatively large numbers of reference calls for this species collected from this region. In contrast, for species not caught during this study, such as the Eastern Freetail Bat, we needed to rely on reference calls from outside the region. It is unknown whether there is regional variation for this species; however, if there is, this will have introduced error into the identifications. In addition, all the reference calls from this species were obtained from just three individuals. Therefore, it is important to collect more reference calls of this species, from more individuals, and from the north-west region if possible.

For some species, we had no reference calls at all. This was especially an issue for the Southern Forest Bat, which is difficult to identify both physically and from its calls. Two call frequencies have been recorded in Victoria: a high frequency form (with the characteristic frequency at approximately 54–55 kHz) and a low frequency form (at approximately 40–43 kHz) (Law et al. 2002; Pennay et al. 2004). It is thought that the high frequency form occurs in the northwest of Victoria, but it is possible that the low frequency form overlap extensively with those of the Inland Forest Bat, while the high frequency form overlaps with the Chocolate Wattled Bat and Little Forest Bat. Therefore, in this study, any Southern Forest Bats that were present would have likely been misidentified as one of these three species. Collecting reference calls from this species is a key priority for the future development of BatLingo for this area.

In addition to collecting more reference calls, further refinements could be made to BatLingo. A greater level of manual checking would enable the training data to be further refined. The efficiency of conducting manual checks could be increased significantly through the further development of the associated software that plots spectrograms for individual 0.75-second segments. In addition, the development of a reverse engineering process could facilitate locating the specific exemplar that has led to a misidentification so that it could be verified or excluded from the model. Another improvement relates to when multiple species are present within a call file. The current version of the model is trained on exemplars with just one species present. However, in field recordings there is often more than one individual of one or more species, recorded within a

file. At present if there were more pulses of one species, the identification would be based on that species; however, often the call sequence would be identified as an unidentified bat call or species complex. In future, it may be possible to incorporate into the model the ability to distinguish where two species are present, from where there is just a single bat but the call characteristics could be from a range of species. Another modification relates to how call sequences are identified, based on the identified call segments, as the approach taken in this year was relatively simple. Further manual checking and modelling, including incorporating the probability of each segment identification, may improve sensitivity and specificity rates. If improvements were made to BatLingo in future years, the existing data from the first two years of this study could be re-analysed and re-interpreted in combination with the analysis of new data.

4.2 Species recorded from the Hattah Lakes Icon Site

When interpreting studies undertaken using the analysis of echolocation calls, several key points need to be considered. First, the metric we use is 'relative activity' rather than abundance as we cannot determine the number of individuals making the calls. For example, if 10 call sequences are recorded, this may represent 10 individuals flying past the detector once, or one individual flying past 10 times (or any other combination of individuals and passes). This approach does, however, provide a useful measure of overall relative bat activity. Second, detectability varies between species (i.e., differences in the distance they can be recorded from a detector, based on whether their calls are quiet or loud), and differences in the ease of identification (based on accuracy rates of the reference calls and field data identifications). Therefore, the most valid comparisons are within species between treatments (e.g., between lakes, or before and after water) as their detectability and identifiability are likely to remain consistent. In contrast, while detailed comparisons between species should not be undertaken, broad generalisations on the species occurring within the region can be made.

A rich bat fauna was found within the Hattah Lakes Icon Site, with 14 species recorded. Bats constitute a significant proportion of the native mammal fauna within the region (Bennett et al. 2006). It is likely that the Icon Site acts as a refuge for bat species in this region due to its mesic environment within the surrounding semi-arid landscape. Additional species could also potentially be present, but these could not be included in this study due to the lack of reference calls, including for the Southern Forest Bat discussed above. The lack of captures of this species was surprising because it is known to occur in the region (Lumsden and Bennett 1995). As it is characteristic of temperate regions, it was expected to occur more commonly within the lakes system than the closely related Inland Forest Bat, which is distributed across inland arid and semi-arid environments and is at the southern limit of its distribution in this area (Lumsden and Bennett 1995). Further surveys are warranted to investigate the distribution of this species in this area, and to collect reference calls to clarify which call characteristic type occurs here.

Two other species have been recorded from north-western Victoria but were not able to be included in BatLingo due to a lack of reference calls. The Yellow-bellied Sheathtail Bat (*Saccolaimus flaviventris*) is an enigmatic species with an uncertain status in Victoria (Lumsden and Menkhorst 1995). There are old records from the region in 1934 and 1956 (Lumsden and Bennett 1995); however, it is unknown if there are current resident populations in north-western Victoria. It is a high, fast flying bat which is rarely caught in harp traps or mist nets set at ground level, so the lack of captures is not unexpected. When it is present, it is readily recorded by its echolocation call. Therefore, if reference calls can be sourced, it could be included in the next iteration of BatLingo, and then used to determine whether this species occurs within the Hattah Lakes Icon Site.

The other species known from north-western Victoria that was not included due to the lack of available reference calls was the Little Pied Bat (*Chalinolobus picatus*). The Little Pied Bat is a relatively new addition to the bat fauna of Victoria, having first been definitively recorded in this State in 2014, when it was trapped at Yarrara Flora and Fauna Reserve, 90 km to the northwest of Hattah in Belah (*Casuarina pauper*) woodland habitats (Bewsher et al. 2019a, 2019b). It has not been recorded from the Hattah–Kulkyne National Park; however, the inclusion of reference calls in BatLingo in the future could allow us to investigate its presence.

Of the species recorded from the area, based on the identifications from BatLingo, there were a few unanticipated findings. Some species were recorded less frequently than expected, while others were recorded more often than expected. The White-striped Freetail Bat is a common and widespread species throughout southern Australia (Churchill 2008). It is a high, fast flying species and so is rarely trapped; however, it has a distinctive, audible echolocation call (Pennay et al. 2004). Its call is the loudest of the species recorded during this study, and can be detected from approximately 50 m (authors pers. obs.); therefore it should be readily recorded on the bat detectors. However, it represented just 0.4% of the bat calls recorded. Although it is possible that some calls were obscured by insect noise within its frequency

range (10–15 kHz), a manual check of a subset of calls revealed a similar pattern to that of the BatLingo identifications. The reason for the low number of calls is unknown.

Another species that was identified by BatLingo less often than expected was the Lesser Long-eared Bat, which is common and widespread throughout all of Australia (Churchill 2008). In this study, however, it was represented by only 0.4% of the recorded bat calls. In contrast to the White-striped Freetail Bat, it has a faint echolocation call that is typically only detected within approximately 5 m of the detector. Therefore, it is not unexpected that there were a lower number of calls than for other species, but the numbers were considerably lower than anticipated, especially as the trapping results suggest it is a relatively common species in this area with 23% of all captures (Table 3). An earlier broadscale survey of north-west Victoria revealed similar capture rates (21% of captures; Lumsden and Bennett 1995). A manual check of a subset of the field recordings also revealed very low numbers of calls, supporting the BatLingo identification rates. It is unknown why there were such low numbers of calls, which was a consistent pattern across the majority of sites, with higher numbers at just three lakes (Figure 6).

The number of calls identified by BatLingo as the closely-related South-eastern Long-eared Bat and Gould's Long-eared Bat were lower still. The South-eastern Long-eared Bat is listed as an Endangered species in Victoria and Vulnerable nationally, with the main Victorian population occurring in the Nowingi State Forest and adjacent areas of Hattah–Kulkyne National Park (Lumsden et al. 2008, 2021b). It typically occurs in mallee habitat; however, there is a record from near the edge of Lake Mournpall in 1987 (Lumsden 1994). The extent to which River Red Gum forests are used is unknown. This species forages over large areas (Lumsden et al. 2008), and as a result, individuals could potentially incorporate inundated lakes into their foraging range if these lakes provided increased foraging opportunities. Recent studies suggest the population size in this area is extremely small, with potentially less than 50 individuals remaining (Lumsden et al. 2021b). Therefore, it is a species that warrants greater attention for monitoring and investigating responses to management actions, such as the provision of environmental water. Increasing the accuracy of identification rates in BatLingo is a key priority to facilitate expanding the tools that can be used to study this species.

Gould's Long-eared Bat was included in BatLingo as there are records upstream (from Macredie Island north of Swan Hill; Lumsden et al. 2007) and downstream (near Mildura; Gee 1999). A total of 578 sequences were identified by BatLingo as this species in this study. It was not possible to manually check all these files; however, this would be warranted in future analyses. As the call characteristics are very similar to Lesser Long-eared Bats, it is possible that these are misidentifications of that species, especially as there were often only one or two calls identified as this species per site, with larger numbers at just a few sites. Alternatively, it is possible that this species is present. Due to this uncertainty, conclusions about the presence of this species and its response to environmental water need to be considered with caution at this stage.

The distribution of the Large-footed Myotis in north-western Victoria is also poorly known. As a waterdependent species, within this region it is likely to be restricted to the Murray River corridor and its associated lake systems (Australasian Bat Society 2022). Prior to this study, it had not been recorded from the Hattah–Kulkyne National Park. Manual checking of a subset of the 2,622 calls identified by BatLingo as this species revealed at least some of these identifications were plausible; however, further interrogation of the BatLingo identifications would be warranted to confirm the presence of the Large-footed Myotis within this area.

One species that was identified by BatLingo more often than expected was the Eastern Freetail Bat. This species is more typical of temperate regions of south-eastern Australia, and in north-western Victoria occurs predominantly in the mesic zone associated with the Murray River (Australasian Bat Society 2022). It has only been trapped once in this region, in the nearby Nowingi area (Lumsden et al. 2008), but 5.4% of all BatLingo calls were identified as this species, only marginally lower than the other two related freetail bats that are frequently trapped in this area (Inland Freetail Bat 11.1%, Southern Freetail Bat 8.6%). Further interrogation of these calls is also warranted, to investigate this potential anomaly.

4.3 Benefits of environmental water for bats

This study has been a valuable first step in assessing the benefits of environmental water for insectivorous bats. It has enabled a comparison of bat activity before and during inundation. Future studies will be needed to investigate whether these benefits continue once the water has receded to complete the initial short-term assessment, and then subsequent studies required to investigate medium to long-term responses. There can be complex, interacting patterns between insect availability and bat activity at permanent or intermittent water, and as intermittent water dries up (Hagen and Sabo 2012). Therefore, the results of this study should be interpreted within the context that they represent just one part of the consideration on the benefits for insectivorous bats.

This study showed there were positive short-term benefits for insectivorous bats from the provision of environmental water at the Hattah Lakes Icon Sites while the water was present, with higher levels of overall bat activity, activity of 10 of the 14 species, and of species richness. This supports the findings of Blakey et al. (2017) that more frequently flooded habitats supported greater bat activity and species richness in a broadscale comparative study across the Murray–Darling Basin. There is good evidence that the responses in our study were due to the provision of water, as the sampling was undertaken in an identical way in both years, making the comparisons valid. While there was only one control site with data from the before and after periods, the number of calls per night recorded from this site (Lake Boolca) were similar in both years for the majority of the species (see Figure 6). Although this comparison is restricted to just one site, and stronger inferences could have been made had there been multiple before-after control sites (and ideally more than one year of baseline monitoring before the intervention), it does provide support to the overall findings.

The response of individual species to the provision of environmental water may be reflected in their biogeographic distributions, with the expectation that mesic-adapted species may be more reliant on, and respond more to, the provision of water compared to more arid-adapted species (Blakey et al. 2017, 2018). Species with a more mesic distribution that showed a positive response to environmental water included the Chocolate Wattled Bat and Eastern Freetail Bat, both of which are near the inland limit of their distribution within this area (Australasian Bat Society 2022). The Chocolate Wattled Bat was the species that showed the strongest relationship with floodplain habitats in the Murray–Darling Basin-wide study in Blakey et al. (2017). Other species showing a strong response to environmental water in our study were either inland species (Little Broad-nosed Bat, South-eastern Long-eared Bat) or widespread species (Inland Broad-nosed Bat, Gould's Wattled Bat, White-striped Freetail Bat and Lesser Long-eared Bat).

An unexpected finding was that the Little Forest Bat did not respond to the provision of environmental water, as it is more typical of mesic environments and extends into north-western Victoria largely within the zone along the Murray River and associated floodplains (Lumsden and Bennett 1995). The other surprising result was that the Large-footed Myotis did not appear to benefit from environmental water and had higher levels of activity in the first year before the provision of environmental water than during the inundation phase. Of all the species in north-western Victoria, this is the one with the highest dependence on water, as it feeds predominantly on aquatic insects and small fish, and selects roosts near water (Campbell 2009). In contrast, the Large-footed Myotis showed the strongest association with floodplain habitats in the Blakey et al. (2017) study. The relatively small number of identified calls may have influenced this result, and although there appears to be a high level of accuracy in the reference call identifications for this species, further investigations are required to confirm these, as more reference calls were available for this species, which may have positively biased the results. Increasing the number of reference calls of all long-eared bat species, which overlap in their call characteristics with the Large-footed Myotis, would be beneficial.

There are three possible mechanisms for an increase in the relative activity and species richness of insectivorous bats in response to the provision of environmental water, which involve changes in behaviour or increased survival rates. First, the increase in the number of calls recorded per night may be due to resident bats foraging for longer periods each night in response to greater food availability (Hałat et al. 2018), resulting in each individual being recorded more often on a detector. Typically, all individuals of insectivorous bats leave their roosts on dusk to forage, with peak activity occurring within the first few hours of dusk (Erkert 1982). Activity levels then usually decline during the middle of the night as temperatures and insect availability decline, before another peak in activity prior to returning to their roosts before dawn. It is not fully understood if the reduction in activity during the middle of the night is due to there being insufficient insects available for efficient foraging, as flight is very energetically expensive (Speakman and Thomas 2003), or if the bats have consumed sufficient food and then hang up to digest it before commencing another foraging bout. Assuming the abundance of nocturnal flying insects increases in response to water within the lake system, similar to when dryland rivers receive flow events (Bunn et al. 2006), bats could remain active for longer during the night, and hence consume greater quantities of prey, which would likely have survival benefits. Alternatively, if there was abundant prey available it might take less time to consume the optimal quantity of food, and so foraging time may be less. If food availability is not limiting (as suggested by Blakey et al. (2017) since they found no relationship between the abundance of insects and bat activity), activity patterns across the night would be expected to remain unchanged. As each file is date and time stamped, it may be possible to test these possibilities comparing the activity patterns throughout the night during the dry phase, inundation phase, and once the water had receded. Ideally, the abundance and diversity of nocturnal flying insects would be recorded concurrently, to explore causal links and fully interpret the findings. Although this mechanism could explain an increase in relative bat activity, it is less likely to explain the increase in species richness as the same resident species would be present.

Second, there could be a short-term increase in the survival rate and breeding success of the resident bats, leading to an increase in the number of individuals present, and hence the number of calls recorded per night. Breeding occurs over the summer months, with young typically born in November/December, which

commence flying in December/January (Lumsden and Bennett 1995). Little is known about breeding success or survival rates for tree hole roosting bats in this area. There may, however, be parallels to the Southern Bent-wing Bat (*Miniopterus orianae bassanii*) – a cave-roosting species in south-eastern South Australia and south-western Victoria. For this species, survival rates are relatively high throughout most of the year (linked to it being long lived – up to 22 years; Lumsden and Gray 2001), but are lowest in late summer-early autumn for lactating females and newly independent young (van Harten et al. 2022). If bats in the Hattah Lakes region showed a similar pattern, any increase in survival rates in response to the provision of environmental water may initially only be small, and it would only be reflected in the last two months of the study (i.e., February and March 2022). Therefore, this is unlikely to be the mechanism for the significant increase in activity (i.e., more than double) during the inundation phase. Increases would be more likely to be represented in future years if more individuals survived this season to breed the following year. This mechanism also does not explain the increase in species richness.

Third, there could be a redistribution of bats at a regional scale in response to the increase in food resources available during inundation, with individuals from surrounding areas expanding their foraging ranges to incorporate the lakes area (Gonsalves et al. 2013; Blakey et al. 2017). As foraging ranges of these species are typically large (e.g., 13 km; Lumsden et al. 2002a) it would be possible for individuals that normally forage in the surrounding mallee or dry woodland habitats, to extend their foraging ranges to incorporate the inundated areas. This could be reflected in increases in both relative activity and species richness. One species that may show this pattern is the South-eastern Long-eared Bat, which may extend its range from mallee habitats west of the lakes. It has been recorded flying up to 7 km per night in this area, which is likely an underestimate of distances moved as it was not possible to follow radio-tracked individuals all night (Lumsden et al. 2008). In northern NSW, when GPS tags were attached, individuals were recorded flying up to 21 km from roost sites (Gonsalves et al. 2021). As calls of the South-eastern Long-eared Bat were only infrequently recorded in the first year of this study, prior to the provision of water, the marked increase in calls per night in the second year may reflect movement into this area. It would be valuable to undertake further studies on habitat use and movement patterns of this Endangered species to determine how much it benefits from environmental water, using GPS technology. This pattern may also apply to Gould's Longeared Bat and Large-footed Myotis, which may disperse along the broader Murray floodplain corridor in response to improved conditions resulting from environmental water in the Hattah Lakes system; however, further investigation would be needed to explore these patterns. If bat species in this area do show a pattern of redistribution in response to water, sampling during the year after the floodwaters have receded would reveal if the benefits persisted and individuals continued to occupy these areas or returned to their former foraging areas.

At a smaller spatial scale, there may be spill-over effects with water in the lakes influencing activity patterns in the immediate surrounding areas. An apparent spill-over effect was found for total bat activity and four species (Gould's Wattled Bat, Chocolate Wattled Bat, South-eastern Long-eared Bat and Lesser Long-eared Bat), whereby the dry control sites that were within 1 km of an inundated lake had similar levels of activity to the lake sites, both of which were higher than at dry control sites more than 1 km from water. This finding should be seen as preliminary, as distance to nearby lake was not specifically included in the study design. There was not a consistent spread of distances from water for the control sites, with these either being close to (280–850 m) or a long way from water (4.2–5.6 km) and so it is not possible to determine if there is a threshold in how far the effect of the water extends. The mechanism for this spill-over effect may be an increase in insect numbers, resulting from insects dispersing away from the water to nearby dry areas. Little is known about insect dispersal patterns, but this warrants further investigation.

As outlined above, it is important to investigate how bats respond during the drawdown phase as the water recedes. During this study there was one site that reflected this situation, with Lake Kramen containing water in the first year which had only just receded by the second year of the sampling. However, it is not possible to draw conclusions from this site, first, because there is no replication, and second, because 95% of the files recorded in the first year were identified as noise. This noise, which was likely due to the calling of insects associated with the water, was so intense that it totally obscured most of the bat calls, so it is likely that the number of bat calls identified was an underestimate. When there is low level noise the bat calls are typically recorded in preference to the noise, but when the noise is intense it out-competes the bat calls. Some of these files may also have contained bat calls (see Figure 12); however, these were so obscured by the noise that BatLingo was unable to recognise them. Fortunately, this issue did not appear to occur to the same extent in the second year of sampling at the sites containing water (Figure 12a), and so future comparisons with data collected after drawdown should be valid. There were, however, isolated examples of excessive noise (e.g., Figure 12b) and these nights may need to be removed from any future analysis.





Figure 12. Examples of insect noise from inundated lakes during 2021-22: a) low frequency insect noise (below 20 kHz) recorded at Lake Konardin; and b) high intensity noise that obscured all frequencies of bat calls recorded at Lake Mournpall on the night of 25 January 2022, when 6,152 files were recorded with every file identified as 'noise' by BatLingo.

4.4 Future directions

This study has revealed preliminary insights into the benefits of environmental water for insectivorous bats during the inundation phase, and has provided a sound basis for future investigations. The following additional studies would provide a fuller understanding of the short-term benefits of environmental water by investigating the drawdown phase, as well as benefits over the medium and longer term.

• Continue the current study by repeating the sampling of bat activity over the summer of 2022-23, setting the Swift bat detectors in the same positions at the 24 sites sampled in 2021-22. This will provide information on bat activity and species richness in the year after inundation to investigate whether these return to the baseline levels recorded prior to the provision of water, or whether they remain elevated. This will demonstrate whether there is a legacy effect of the environmental water or whether the benefits are limited to just when water is present. In the planning for this sampling, determine whether any of the current control sites are scheduled to receive environmental water. If so, more control sites will need to be located, which may result in sampling less intervention sites given the current number of Swift detectors available. The results from Lake Kramen are inconclusive, and as there was no 'before' data, this site could be discontinued. After the third year of sampling, which would complete the immediate short-term response component, a decision could be made as to whether sampling should continue annually for several more years, or shift to a longer time interval to investigate the medium-term response. Alternatively, it may be possible to continue to collect the data annually, but analyse the calls for multiple years together, to make the analysis component more efficient.

- Prior to the next round of sampling, the detectors should be returned to the manufacturer to fix the issue discovered during this study that caused inconsistent recording on the second memory card. In addition, undertake the same level of checking the sensitivity of the microphones, and testing functionality and reliability of the detectors, as undertaken prior to last year's sampling (as outlined in Lumsden et al. 2021a), to ensure all detectors work optimally.
- Collect more reference calls from all species currently in the reference call library to ensure the full extent of call variability is included, to improve the accuracy of the identification of field calls.
- Expand the number of species incorporated into BatLingo by obtaining reference calls of species currently missing from the call library. This is especially important for the Southern Forest Bat that is known to occur in the area, but for which no reference calls were available. It would be beneficial to also incorporate reference calls of the Little Pied Bat and the Yellow-bellied Sheathtail Bat. Reference calls of the Little Pied Bat could be collected from Yarrara Flora and Fauna Reserve (90 km to the west), while calls of the Yellow-bellied Sheathtail Bat would need to be sourced from interstate.
- Further develop BatLingo through the incorporation of these additional reference calls, and undertake more manual checking of unknown calls to continue to refine the training algorithms. Develop tools to increase the efficacy of this process, including the 0.75-second segment spectrogram displays and reverse engineering the software to identify which exemplar segments are causing misidentifications. Undertake further exploration of the optimal approach for converting segment identifications into sequence identifications to maximise identification rates.
- Once BatLingo is further refined and accuracy rates optimised, use the revised version to analyse the third year's data, and re-analyse the first two years' calls so that subsequent analysis is comparable. Factor in more time to manually check a subset of identified calls, especially species that are rarely recorded, so that there is greater confidence in the results.
- When interpreting the data from all three years, consider checking and then deleting nights of data that contain continuous recordings of high intensity noise where this obscures bat calls, as the resulting data would not be representative of the bat activity at those sites on those nights.
- Investigate factors influencing the benefits of environmental water by exploring the drivers of these
 patterns, including testing which of the suggested mechanisms maybe operating. This may include
 investigating vegetation patterns that may affect the responses, including overstorey and
 understorey cover, and tree flowering patterns influencing invertebrate abundance. A key knowledge
 gap is how nocturnal insects respond to environmental water and the relationship between changes
 in insect availability and bat activity. In addition, nothing is known of the influence on bat activity of
 the timing of water, either seasonally or yearly, or the optimal frequency for the provision of water.
 Expanding the study to include additional floodplains would enable a comparison with other locations
 with different flooding attributes, landscapes and vegetation communities, which could help address
 investigating the driving patterns.
- Investigate the response to environmental water by the most threatened bat species in the region, the South-eastern Long-eared Bat, which may expand its foraging range to take advantage of the increased foraging resources during inundation periods. If further refinements of BatLingo can increase identification rates of this species and the other species with overlapping call characteristics, additional surveys could be undertaken using bat detectors sampling a range of habitats. However, it would also be worth considering undertaking GPS radio-tracking to investigate foraging ranges and patterns, as recently successfully conducted on this species in northern NSW (Gonsalves et al. 2021).

Undertaking these studies would provide a more comprehensive understanding of the benefits of environmental water for bats, including longer-lasting legacy effects, and not just short-term benefits during the period of inundation.

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Appendix

Table A1. Results from the models of the number of calls per night for total bat activity and individual species that relate to the covariates of interest.

LB and UB are the lower and upper bounds from the 95% credible intervals, respectively. The standard deviation and aggregation parameter are on the natural scale, all other estimates are on the logarithmic scale.

Parameter	Estimate	Estimated error	LB	UB
Total bat activity				
Intercept	6.624	0.099	6.431	6.822
After: Dry (distant)	0.231	0.084	0.064	0.398
After: Dry (near)	0.595	0.234	0.125	1.054
After: Wet	0.628	0.023	0.582	0.675
Rained: Yes	-0.153	0.033	-0.218	-0.088
Standard deviation for random effect	0.420	0.072	0.308	0.588
Aggregation	3.115	0.077	2.967	3.268
White-striped Freetail Bat				
Intercept	0.281	0.209	-0.131	0.693
After: Dry (distant)	1.162	0.259	0.652	1.679
After: Dry (near)	1.625	0.487	0.661	2.596
After: Wet	0.826	0.080	0.669	0.983
Rained: Yes	-0.353	0.106	-0.559	-0.145
Standard deviation for random effect	0.839	0.143	0.612	1.165
Aggregation	0.386	0.014	0.359	0.415
Gould's Wattled Bat				
Intercept	4.842	0.162	4.520	5.161
After: Dry (distant)	0.604	0.118	0.372	0.836
After: Dry (near)	1.136	0.389	0.366	1.917
After: Wet	1.120	0.035	1.052	1.188
Rained: Yes	-0.253	0.048	-0.347	-0.159
Standard deviation for random effect	0.693	0.117	0.506	0.955
Aggregation	1.532	0.037	1.461	1.606
Chocolate Wattled Bat				
Intercept	3.781	0.163	3.464	4.107
After: Dry (distant)	0.234	0.129	-0.022	0.484
After: Dry (near)	1.302	0.388	0.545	2.071
After: Wet	1.378	0.038	1.304	1.452
Rained: Yes	-0.098	0.051	-0.198	0.004
Standard deviation for random effect	0.677	0.114	0.493	0.940
Aggregation	1.312	0.032	1.250	1.375

Table A1 (continued). Results from the models of the number of calls per night for total bat activity and individual species that relate to the covariates of interest.

Parameter	Estimate	Estimated error	LB	UB
Large-footed Myotis				
Intercept	0.520	0.251	0.022	1.022
After: Dry (distant)	-3.374	0.449	-4.319	-2.548
After: Dry (near)	-2.632	0.613	-3.856	-1.425
After: Wet	-1.853	0.094	-2.037	-1.669
Rained: Yes	-0.526	0.129	-0.775	-0.275
Standard deviation for random effect	1.045	0.191	0.735	1.486
Aggregation	0.488	0.029	0.434	0.547
Lesser Long-eared Bat				
Intercept	1.195	0.167	0.861	1.526
After: Dry (distant)	-0.608	0.157	-0.913	-0.301
After: Dry (near)	0.278	0.406	-0.524	1.080
After: Wet	0.528	0.048	0.436	0.622
Rained: Yes	-0.252	0.069	-0.388	-0.117
Standard deviation for random effect	0.706	0.122	0.512	0.990
Aggregation	0.973	0.032	0.912	1.038
South-eastern Long-eared Bat				
Intercept	-1.147	0.206	-1.554	-0.739
After: Dry (distant)	-0.100	0.317	-0.733	0.518
After: Dry (near)	0.815	0.475	-0.130	1.742
After: Wet	1.132	0.092	0.952	1.310
Rained: Yes	-0.310	0.132	-0.568	-0.052
Standard deviation for random effect	0.816	0.145	0.583	1.153
Aggregation	0.397	0.024	0.352	0.447
Inland Freetail Bat				
Intercept	4.961	0.113	4.737	5.182
After: Dry (distant)	-0.175	0.123	-0.417	0.065
After: Dry (near)	-0.294	0.269	-0.825	0.240
After: Wet	-0.355	0.036	-0.425	-0.286
Rained: Yes	-0.069	0.051	-0.168	0.032
Standard deviation for random effect	0.475	0.083	0.343	0.666
Aggregation	1.397	0.034	1.331	1.465
Southern Freetail Bat				
Intercept	4.247	0.124	4.000	4.489
After: Dry (distant)	0.036	0.119	-0.197	0.273
After: Dry (near)	0.508	0.298	-0.077	1.103
After: Wet	0.226	0.035	0.158	0.295
Rained: Yes	-0.067	0.048	-0.162	0.028
Standard deviation for random effect	0.524	0.089	0.385	0.737
Aggregation	1.511	0.039	1.437	1.587

Table A1 (continued). Results from the models of the number of calls per night for total bat activity and individual species that relate to the covariates of interest.

Parameter	Estimate	Estimated error	LB	UB
Eastern Freetail Bat				
Intercept	3.690	0.133	3.424	3.957
After: Dry (distant)	0.195	0.126	-0.053	0.437
After: Dry (near)	-0.002	0.315	-0.625	0.630
After: Wet	0.548	0.035	0.479	0.616
Rained: Yes	-0.004	0.050	-0.100	0.095
Standard deviation for random effect	0.569	0.097	0.417	0.797
Aggregation	1.419	0.036	1.349	1.491
Inland Broad-nosed Bat				
Intercept	2.674	0.177	2.322	3.034
After: Dry (distant)	1.120	0.135	0.855	1.384
After: Dry (near)	1.563	0.416	0.724	2.363
After: Wet	1.623	0.038	1.549	1.697
Rained: Yes	-0.224	0.054	-0.329	-0.119
Standard deviation for random effect	0.744	0.126	0.547	1.038
Aggregation	1.292	0.033	1.228	1.359
Little Broad-nosed Bat				
Intercept	1.653	0.125	1.398	1.895
After: Dry (distant)	0.921	0.164	0.599	1.245
After: Dry (near)	0.423	0.296	-0.164	1.014
After: Wet	1.050	0.049	0.955	1.146
Rained: Yes	-0.399	0.067	-0.530	-0.264
Standard deviation for random effect	0.517	0.092	0.371	0.731
Aggregation	0.889	0.026	0.840	0.940
Inland Forest Bat				
Intercept	1.540	0.173	1.201	1.889
After: Dry (distant)	0.141	0.141	-0.135	0.419
After: Dry (near)	0.530	0.415	-0.297	1.362
After: Wet	0.466	0.044	0.380	0.552
Rained: Yes	-0.116	0.061	-0.235	0.006
Standard deviation for random effect	0.744	0.126	0.546	1.036
Aggregation	1.120	0.035	1.052	1.190
Little Forest Bat				
Intercept	4.979	0.141	4.691	5.262
After: Dry (distant)	-0.167	0.108	-0.380	0.044
After: Dry (near)	0.113	0.330	-0.533	0.766
After: Wet	0.022	0.031	-0.040	0.083
Rained: Yes	-0.186	0.043	-0.269	-0.100
Standard deviation for random effect	0.592	0.099	0.434	0.817
Aggregation	1.860	0.045	1.773	1.950

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