Pilbara Leaf-nosed Bat Research Plan

36-month monitoring report

FMG Iron Bridge (Aust) Pty Ltd

December 20, 2024

→ **The Power of Commitment**

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The Power of Commitment

Acknowledgement of Country

GHD acknowledges Aboriginal and Torres Strait Islander peoples as the Traditional Custodians of the land, water and sky throughout Australia. We recognise their strength, diversity, resilience and deep connections to Country.

Iron Bridge is located within the Traditional lands of the Nyamal people, and we acknowledge the ongoing connection to country of the various cultural and family groups.

We pay our respects to Elders of the past, present and emerging, as they hold the memories, knowledges and spirit of Australia. GHD is committed to learning from Aboriginal and Torres Strait Islander peoples in the work we do.

Executive Summary

FMG Iron Bridge (Aust) Pty Ltd (FMGIB) operates an iron ore mining operation located at North Star (Iron Bridge), approximately 110 kilometres (km) south of Port Hedland in the Pilbara region of Western Australia. The development of Iron Bridge is subject to ministerial conditions relating to the key environment factors identified during the assessment under Part IV of the *Environmental Protection Act 1986* (WA). One of these key factors is the clearing of fauna habitat, including critical roosting habitat for the Pilbara Leaf-nosed Bat (*Rhinonicteris aurantia* [Pilbara form]) (PLNB). A permanent diurnal roost has been identified at Chateau Cave, which is located within the North Star ore body and in close proximity to open cut mine and primary crushing facilities. To satisfy the objective of Ministerial Conditions related to the management of PLNB in the study area, FMGIB commissioned GHD Pty Ltd to implement the PLNB Habitat Survey and Research Plan (the Plan; GHD 2017) as approved by the CEO in accordance with Condition 10.9.

This 36-month report focuses on the implementation and results gathered from monitoring between March 2023 – January 2024. This report details and discusses the monitoring methods and results from this period and addresses the following key items:

- Detailed results for all surveys undertaken to date including bat call and visual activity data from long term monitoring sites.
- Detailed genetic analysis including the number of genotyped individuals recorded at each site and the movement of bats between roosts.
- Chateau Cave call activity and colony demography (based on multiple complimentary monitoring techniques).
- Dalton's Roost call activity and colony demography with continued evidence supporting its determination as a Category 1 maternity roost and a suitable alternative roost.

This report is subject to, and must be read in conjunction with, the limitations set out in section 1.4 and the assumptions and qualifications contained throughout the Report.

Genetic analysis

Genotyping of 17,217 scats, 69 wing biopsies and 31 dried wings collected over 36 months from Chateau Cave (8,340), Dalton's Roost (7,644) and eight surrounding locations (Python Cave, Cave 13, Joes Cave, Lalla Rookh, Abydos, Sanjiv Ridge, Mt Webber and Bow Bells) have produced a total of 16,627 successfully genotyped samples. This has allowed an estimate of a minimum colony size for these roosts and detected several movements of individuals between sampled locations within the 30km study area (surrounding Chateau Cave). The total number of individuals detected across all sites is 4,397, with the majority detected at Dalton's Roost (2,914 individuals on at least one occasion) followed by Chateau Cave (1,264 individuals on at least one occasion).

There was approximately three times as many females as males genotyped at Dalton's Roost and this was statistically significant. By contrast, there was a statistically significant bias towards males at Chateau Cave with approximately double the number of males detected overall, and in most months (with exception to February, and October to December). This data, combined with observations of pregnant female in December 2022, suggests Dalton's Roost is an important maternity roost, and it is likely that all parts of the breeding cycle occur there—mating, roosting by pregnant females, parturition and nursing young. In comparison, only two pups have been observed within Chateau Cave over three years, in addition to the male bias in colony demography, it is likely this roost is important as a mating site but is not regularly used by female PLNB during the birth and lactation period.

Out of the 4,397 individual PLNB detected through genotyping between April 2021 and January 2024, 65 (1.5%) were identified at more than one roost. Almost half of these individuals (n = 29) were observed moving between Chateau Cave and Dalton's Roost, which is 29.3 kilometres away. Some of these movements between Chateau Cave and Dalton's Roost occurred within a single sampling period – i.e. within 1-4 days.

While only a small proportion of scats could be attributed to between roost movements, this is a function of sampling only one or two nights per month and that not all scats can be successfully genotyped. PIT tagging data has since provided evidence that PLNB can move up to 60 km between roosts in a night. Also, these movements occur with regularity previously undocumented for PLNB. This demonstrates that movement between colonies occurs regularly and that this can occur in a very short period of time. Genetic analysis of scats and PIT tagging has been found to be complimentary in identifying individuals in the colonies of Chateau Cave, Daltons Roost and other permanent diurnal roosts and detecting movement between them.

Long-term monitoring of bat call activity data

A total of 15 in-situ (stationary) activity and/or presence sites were established across the study area using full spectrum ultrasonic detectors (Song Meter SM4BAT-FS, Wildlife Acoustics) in April and May 2021. PLNB detections from each site were analysed to determine ongoing PLNB presence and activity within the study area, and at key roost sites. The results of 33 months of monitoring show that only Chateau Cave and Dalton’s Roost recorded activity every night, and that activity at Dalton’s Roost was 1.5 times higher on average compared to the inside Chateau Cave detector (CC in) and 33 times higher than the outside Chateau Cave detector (CC out). This supports the categorisation of these roosts as permanent diurnal roosts (Category 1). There was significant variation in activity between all sites and monitoring periods. With all sites combined, activity was generally highest from May through to September and tended to be lowest between December and February although this varied from year to year with periods of low activity observed in January – February 2022, February 2023 and November – December 2023.

Activity at Dalton’s Roost previously contrasted with Chateau Cave by having sustained high levels of activity throughout the year. Recent activity at Dalton’s Roost has significantly departed from the previously recorded levels and is (as of January 2024) extremely low. Of interest is the observation the highest levels of activity at Dalton’s Roost also seem to coincide with cooler months (although this is variable between years) despite the cave functioning as a maternity roost between December – February (identified by levels of progesterone and presence of pregnant females, described in GHD 2022b and GHD 2023b). There is no evidenced based explanation for the observed reduction in activity at Dalton’s Roost in January 2024.

Visual monitoring of bat activity

Infrared (IR) CCTV cameras and IR Reconyx motion cameras were used to record the ingress/egress of bats at the entrance of the main chamber during the roost emergence period and to observe the behaviour of roosting bats within the main chamber. Data from visual monitoring was compared with ultrasonic call activity for the same recording period. From this, the estimated number of PLNB roosting within the main chamber ranges from approximately eight to 1,051. The highest count of all bats during the emergence period (all possible species) and highest counts of PLNB roosting within the main chamber occurred in May 2023 and is consistent with the high bat call activity levels reported for the same period. In contrast, the lowest counts were reported for February 2022, February, March and October of 2023.

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Abbreviations / Acronyms

Abbreviation / Acronym	Detail
AEC	GHD Animal Ethics Committee
AGRF	Australian Genome Research Facility
CASA	Civil Aviation Safety Authority
CDU	Charles Darwin University
Cth	Commonwealth
db	decibels
dBA	A-weighted decibels
DBCA	Department of Biodiversity Conservation and Attractions
DCCEEW	Department of Climate Change, Energy, the Environment and Water
DIWA	Drone Inspections Western Australia
DNA	Deoxyribonucleic acid
DWER	Department of Water and Environment Regulation
DVR	digital video recorder
eDNA	Environmental DNA
EMT	Echo-meter Touch (handheld bat detector)
EPA	Environmental Protection Authority
EPBC Act	*Environment Protection and Biodiversity Conservation Act 1999* (Cth)
FMG	Fortescue Ltd
FMGIB	FMG Iron Bridge Pty Ltd
GHD	GHD Pty Ltd
HA	Helicopter/foot assessment (detailed habitat assessment was not undertaken)
IR	Infrared
Iron Bridge	Stage 2 North Star Magnetite Project
kHz	kilohertz
km	kilometers
LiDAR	laser imaging, detection, and ranging
LRM	local relief model
MDE	North Star mining development and associated infrastructure envelope
MS	Mineral Statement
ms	millisecond
NATA	National Association of Testing Authorities
ng	nanogram
PLNB	Pilbara Leaf-nosed Bat (*Rhinonicteris aurantia* (Pilbara form))
OPF	Ore Processing Facility
the Plan	PLNB Habitat Survey and Research Plan
RHA	roost habitat assessment (detailed habitat assessment)
ROM	run-of-mine

Abbreviation / Acronym	Detail
SD	Standard deviation
SM4BAT-FS	Song Meter SM4BAT-FSFS, Wildlife Acoustics
SNP	Single nucleotide polymorphisms
TSSC	Threatened Species Scientific Committee
µL	Microlitre
1CCin	Location of ultrasonic detector within the main chamber of Chateau Cave
1CCout	Location of ultrasonic detector within two meters of the entrance of Chateau Cave
2JC	Joe’s Cave detector location
C13	Cave 13 detector location
4FP	Fig Pool detector location
5SSP	Mundagoora Pool detector location
6CP	Craig’s Pool detector location
7P12	Pool 12 detector location
8PC	Python Cave detector location
9CB, 9CD, 9CH	Abydos mine site, sites Cave B, Cave D and Cave H – detector locations
10CP	Camp Pool detector location
11WM	Wayne detector location
12WC	Wallaby Cave detector location
13CP	Cow Pool detector location

1. Introduction

1.1 Background

FMG Iron Bridge Mine Pty Ltd (FMGIB) operates the North Star Magnetite Mine and ore processing facility (OPF), approximately 110 kilometres (km) south of Port Hedland in the Pilbara region of Western Australia (Figure 1). FMGIB is a majority-owned subsidiary company of Fortescue Ltd (FMG).

Iron Bridge has been assessed and approved under Part IV of the *Environmental Protection Act 1986* (WA) (EP Act), subject to Ministerial Statement 993 (MS 993), and under the *Environment Protection and Biodiversity Conservation Act 1999* (Cth) (EPBC Act), subject to EPBC 2012/6689. The development of Iron Bridge is subject to conditions relating to the key environment factors identified during the assessment and approvals process. One of these key factors is the clearing of fauna habitat, including critical roosting habitat for the Pilbara Leaf-nosed Bat (*Rhinonicteris aurantia* [Pilbara form]).

The Environmental Protection Authority (EPA) imposed Condition 10 under MS 993 to mitigate the potential impacts to the Pilbara Leaf-nosed Bat (PLNB). The ministerial conditions refer specifically to the Cave 13 roost. The conditions are now accepted to refer to the PLNB colony at Chateau Cave, which has been substantiated as housing a permanent PLNB colony. Condition 10-1 states *"the proponent shall implement the proposal in a manner that maintains the viability of the population of PLNB from Cave 13"*. The intent of conditions 10-3 to 10-11 are to ensure the PLNB population is maintained through the relocation and establishment of a 'viable portion' of the colony at Cave 13 to an alternative (either natural or artificial) maternal roost site. In order to achieve this, a PLNB Habitat Survey and Research Plan (herein referred as 'the Plan') was developed and implemented.

Condition 10-7 outlines two objectives for the Plan:

i. *Provide evidence of alternative natural maternity roost site(s) for the PLNB existing within a 30 km radius of Cave 13 which could support a viable portion of the colony of PLNB from Cave 13*

ii. *Demonstrate to the satisfaction of the CEO that a viable portion of the colony of Pilbara Leaf-nosed Bat from Cave 13 has relocated and established itself in an alternative maternal roost site.*

To satisfy the objectives of Condition 10-7, FMGIB commissioned GHD Pty Ltd (GHD) to implement the provisions of the Plan as approved by the CEO in accordance with Condition 10-9.

1.2 Reporting requirements specified by the Plan

This report is the third annual monitoring report to support implementation of the Plan and is part of a broader body of work on PLNB within the study area. Eight (8) reports have been prepared to date (between August 2021 and August 2024) to support implementation of the Plan and supporting research objectives. Table 1 lists these reports and summarises the objectives and outcomes of each body of work.

Table 1 ***Reports prepared to date to support the Plan***

Report	Objectives and outcomes
Pilbara Leaf-nosed Bat Research Plan – Pilot study phase report (August 2021) (GHD 2021)	Pilot Study Phase including the completion of three months of bat activity, environmental variables and genetics data collection from Chateau Cave. A summary of results including identification of PLNB individuals in the Chateau Cave Colony after three months of scat analysis. The report also reviewed scat sampling and analysis for DNA, hormone collection and environmental DNA (eDNA), including suggestions for improvement and justification to continue/discontinue these techniques.
Pilbara Leaf-nosed Bat Research Plan – Six-month monitoring report (March 2022) (GHD 2022a)	The completion of six months of data, following completion of stage 1 and partial completion of stage 2 focusing on the implementation and results gathered from novel research techniques trialed during the Pilot Study Phase.

Report	Objectives and outcomes
Pilbara Leaf-nosed Bat Research Plan – 12-month monitoring report (August 2022) (GHD 2022b)	The completion of 12 months of bat activity, environmental variables and genetics data (April 2021 – March 2022) following the completion of stage 2 field surveys and monitoring. As recommended by DWER, the monitoring of Chateau Cave should continue until it has been demonstrated using the criteria discussed in Section 9 of GHD (2022b) that individual PLNB known from Chateau Cave have been recorded using the alternative roost site. Table 26 of GHD (2022b) detailed next steps regarding the ongoing monitoring according to the Plan.
Dalton's Roost technical memo – September 2023 (GHD 2023a)	A summary of the work conducted at Dalton's Roost since February 2022 and the evidence collected to date that the location should be considered a viable alternative roost. The memo includes a brief summary of work completed prior to February 2022 for historical context. The memorandum uses evidence collected by GHD to show that the Dalton's Roost meets the criteria to be classified a Category 1 maternity roost and Category 2 permanent diurnal roost (Bat Call WA 2021). The importance of Dalton's Roost as a regional site of biological importance is also discussed with reference to other documented Priority 1 Permanent Diurnal Roosts in the Pilbara (DCCEEW 2023).
Pilbara Leaf-nosed Bat Research Plan – 24-month monitoring report (November 2023) (GHD 2023b)	The completion of two years of bat activity, environmental variables and genetics data (April 2022 – March 2023) following the completion of stage 2 field surveys and monitoring.
Pilbara Leaf-nosed Bat RadiotrackingStudy (March 2024) (GHD 2024a)	Submitted in March 2024, this report details the results of a radio-tracking study on PLNB to record movement of individuals between roosts in the Study area. Also discusses the implications of bat movement within the study area in relation to the key project objective of identifying the location of alternate roost sites and demonstrating movement between key permanent diurnal roosts.
Pilbara Leaf-nosed Bat Research Plan – 36-month monitoring report (September 2024) (**this report**)	The completion of three years of bat activity, environmental variables and genetics data in January 2024 following the completion of stage 2 field surveys and monitoring. See section 1.3 below for more detail on the purpose of this report.
Pilbara Leaf-nosed Bat PIT Tagging Study (Phase 1) (September 2024) (GHD 2024b)	To be submitted in September 2024, this report details movement activity and site fidelity of tagged PLNB at both Chateau Cave and Dalton's Roost.
Pilbara Leaf-nosed Bat PIT Tagging Study Phase 2 summary – technical memo (pending issue)	To be submitted in late 2024, this memo will summarise project updates (number of bats tagged and readers installed) and key findings for movement of tagged PLNB between Chateau Cave and Dalton's Roost and other roosts in the region.

1.3 Report purpose

This report presents the results of the ongoing PLNB monitoring between March 2023 and January 2024, and consideration within the context of the previous data collected.

Key factors assessed in this report include:

– Results from all surveys undertaken to date including bat call and visual activity data from long term monitoring sites.
– Genetic analysis including individual occupancy rate as the proportion of total sampling dates where each individual was detected in Chateau Cave, Dalton's Roost and other sites.
– Results from monitoring activity of Dalton's Roost including an updated description of the occupying colony.

Unless there was a substantial departure from the methods used in the Plan or presented in the 12 and 24-month reports (GHD 2022b and GHD 2023b), this report presents a summary of the methods used to date to avoid repetition of text. For some data, the initial 24 months of monitoring results are provided for contextual purposes, rather than referencing the 12 and 24-month reports.

1.4 Limitations and assumptions

This report has been prepared by GHD for FMGIB and may only be used and relied on by FMGIB for the purpose agreed between GHD and the FMGIB as set out in section 1.3 of the document.

GHD otherwise disclaims responsibility to any person other than FMGIB arising in connection with this report. GHD also excludes implied warranties and conditions, to the extent legally permissible.

The services undertaken by GHD in connection with preparing this report were limited to those specifically detailed in this report and the Plan and are subject to the scope limitations set out in both documents.

The opinions, conclusions and any recommendations in this report are based on conditions encountered and information reviewed at the date of preparation of the report. GHD has no responsibility or obligation to update this report to account for events or changes occurring after the date that the report was prepared.

GHD has prepared this report on the basis of information provided by FMGIB and others who provided information to GHD (including Government authorities), which GHD has not independently verified or checked beyond the agreed scope of work. GHD does not accept liability in connection with such unverified information, including errors and omissions in this report which were caused by errors or omissions in that information.

The opinions, conclusions and any recommendations in this report are based on information obtained from, and testing undertaken at or in connection with, specific sample points. Site conditions at other parts of the site may be different from the site conditions found at the specific sample points.

Investigations undertaken in respect of this report are constrained by the particular site conditions, such as the remote location of sites, vegetation and blasting operations. As a result, not all relevant site features and conditions may have been identified in this report.

1.5 Definitions

Civil twilight – the period between sunset and full darkness and also the transition from darkness to sunrise. In this report civil twilight is only used to refer to the evening transition from day to night and times given for civil twilight are the time when civil twilight ends.

Colony - a set of individual bats that congregate in an underground diurnal roost at any one time. Colony membership may change—members of one colony may move to another colony, and most or all individuals of a particular colony might move from one roost site to another.

Maternity roost – a diurnal roosting site where any or all of the following occur: the congregation of pregnant females; birthing site; where young are raised before they become independent. This coincides with the terms in the Conservation Advice for the species (TSSC 2016) for permanent diurnal roost and non-permanent breeding roost.

Nocturnal refuge - occupied or entered at night for resting, feeding or other purposes, with perching not a requirement. Excludes overhangs. Not considered critical habitat but are important for persistence in a local area.

Non-permanent breeding roost - evidence of usage during some part of the 9-month breeding cycle (July–March), but not occupied year-round; considered as critical habitat that is essential for both the daily and long-term survival of the PLNB.

Permanent diurnal roost - occupied year-round and likely the focus for some part of the 9-month breeding cycle; considered as critical habitat that is essential for the daily survival of the PLNB.

Portion of a colony - a percentage of the colony in Chateau Cave that is demonstrated to persist elsewhere when Chateau Cave ceases to be available to the PLNB. This percentage can be based on an agreed number of genetically identified individuals, level of species-specific bat activity (e.g., passes in front of a bat detector; timing is also important), or number of females in breeding condition, at an agreed time (allowing for fluctuation across the year of any representation of membership or usage).

Relocated - the movement of PLNB individuals from one roost (e.g., Chateau Cave) to another (e.g., alternative diurnal roost). Can be either self-relocation, that is bats will move from the roost without physical assistance (e.g., capture and relocate to another roost); or assisted movement, when bats are progressively encouraged to vacate a roost using exclusion techniques (e.g., barriers and lighting).

Study area - the study area is the area within a 30-kilometre (km) radius of Cave 13 (geographic coordinates: latitude -21.2584, longitude 119.0562). Figure 1 displays the location of the study area and extent of the Iron Bridge mining development and associated infrastructure envelope (herein referred to as the MDE) within the study area. Chateau Cave is located approximately 500 m north of Cave 13.

Transitory diurnal roost - occupied for part of the year only, outside the breeding season (i.e., April–June), and which could facilitate long distance dispersal in the region; considered as critical habitat that is essential for both the daily and long-term survival of the PLNB.

1.6 Roosts and monitoring sites

Table 2 below provides a summary of the known roosts, monitoring locations and other study sites referenced within this report and relevant to the Plan. Each of these sites is also mapped in Figure 1.

Table 2 Roosts and monitoring sites relevant to the Plan

Site	Location (refer Figure 1)	Site type	Monitoring method(s); relevance to the Plan
Chateau Cave	Within Fortescue's Iron Bridge Mine	Category 1 Permanent diurnal roost, natural cave	One of only 2 known permanent diurnal roosts in the study area. Current monitoring: Scat collection, bat call activity, PIT antenna, visual observation, temperature and humidity, noise and vibration,
Dalton's Roost	29km southeast of Chateau Cave	Category 1 Permanent diurnal roost, natural cave	One of only 2 known permanent diurnal roosts in the study area Ongoing monitoring: Scat collection, bat call activity; visual observation, temperature and humidity, noise and vibration, PIT antenna
Cave 13	500 m from Chateau Cave within Fortescue's Iron Bridge Mine	Category 3 transitory/semi-permanent diurnal roost and/or Category 4 nocturnal refuge, natural cave	Condition 10 previously related to Cave 13, and now applies to Chateau Cave as the only permanent diurnal roost in the project footprint, and Cave 13 is considered a temporary diurnal roost. Current monitoring: Bat call activity Previous monitoring: Scat collection, bat call activity, temperature and humidity, noise and vibration
Python Cave	4km south of Chateau Cave	Category 3 transitory/semi-permanent diurnal roost and/or Category 4 nocturnal refuge, natural cave	Current monitoring: Bat call activity, visual monitoring, PIT antenna Previous monitoring: Scat collection, bat call activity
Joe's Cave	8 km to the west of Chateau Cave	Category 3 transitory/semi-permanent diurnal roost and Category 4 nocturnal refuge, natural cave	Ongoing monitoring: Bat call activity Previous monitoring: Scat collection
Lalla Rookh	32km northeast of Chateau Cave	Category 1/2 Permanent diurnal roost, historic mine working	Ongoing monitoring: Scat collection, bat call activity, PIT antenna
Sandjiv Ridge	67km east of Chateau Cave, Atlas tenement	Category 4 nocturnal refuge, natural cave	Opportunistic scat collection
Mt Webber	39km southeast of Chateau Cave and 10km southeast of Dalton's Roost Atlas tenement– MW-AN-27	Category 2 Permanent diurnal roost, natural cave	Opportunistic scat collection

Site	Location (refer Figure 1)	Site type	Monitoring method(s); relevance to the Plan
Abydos	Atlas tenement– sites Cave B, Cave D and Cave H	Category 3 transitory/semi-permanent diurnal roost and Category 4 nocturnal refuge, natural cave	Opportunistic scat collection
Bow Bells	83km east of Chateau Cave, Warrawoona Gold Mine,	Category 1 Permanent diurnal roost, historic mine working	Opportunistic scat collection
Mundagoora Pool, Camp Pool, Nicko's Gorge	Located within 2-10km of Chateau Cave	Priority 1 habitat; Foraging sites	Ongoing monitoring: bat call activity
Pool 12, Craigs Pool, Cow Pool, Fig Pool	Located within 2-10km of Chateau Cave	Priority 1 habitat; Foraging sites	Monitoring discontinued Previous monitoring: bat call activity
Wayne Manor	5km northeast of Chateau Cave	Category 4 nocturnal refuge, natural cave	Ongoing monitoring: bat call activity
Wallaby Cave	3km North of Chateau Cave	Category 4 nocturnal refuge, natural cave	Previous monitoring: Bat call activity

1.7 Acknowledgements

GHD respectfully acknowledges Aboriginal and Torres Strait Islander peoples as the Traditional Owners and custodians of the land and water on which all Australians rely. Iron Bridge is located within the Traditional lands of the Nyamal people, and we acknowledge the ongoing connection to country of the various cultural and family groups. We acknowledge the custodianship of cultural values of land, wildlife and waterways are shared by these local Aboriginal peoples. We pay our respects to their Elders past, present and emerging.

GHD and FMGIB acknowledge the assistance and information provided by the Atlas Iron Ore Environment team, particularly for access to the Abydos and Mt Webber sites and reports provided through a data sharing agreement. We also acknowledge assistance and site access provided by Calidus Resources and the team at Warrawoona Gold Project and historical data provided by Biologic's Environmental Survey of Dalton's Roost.

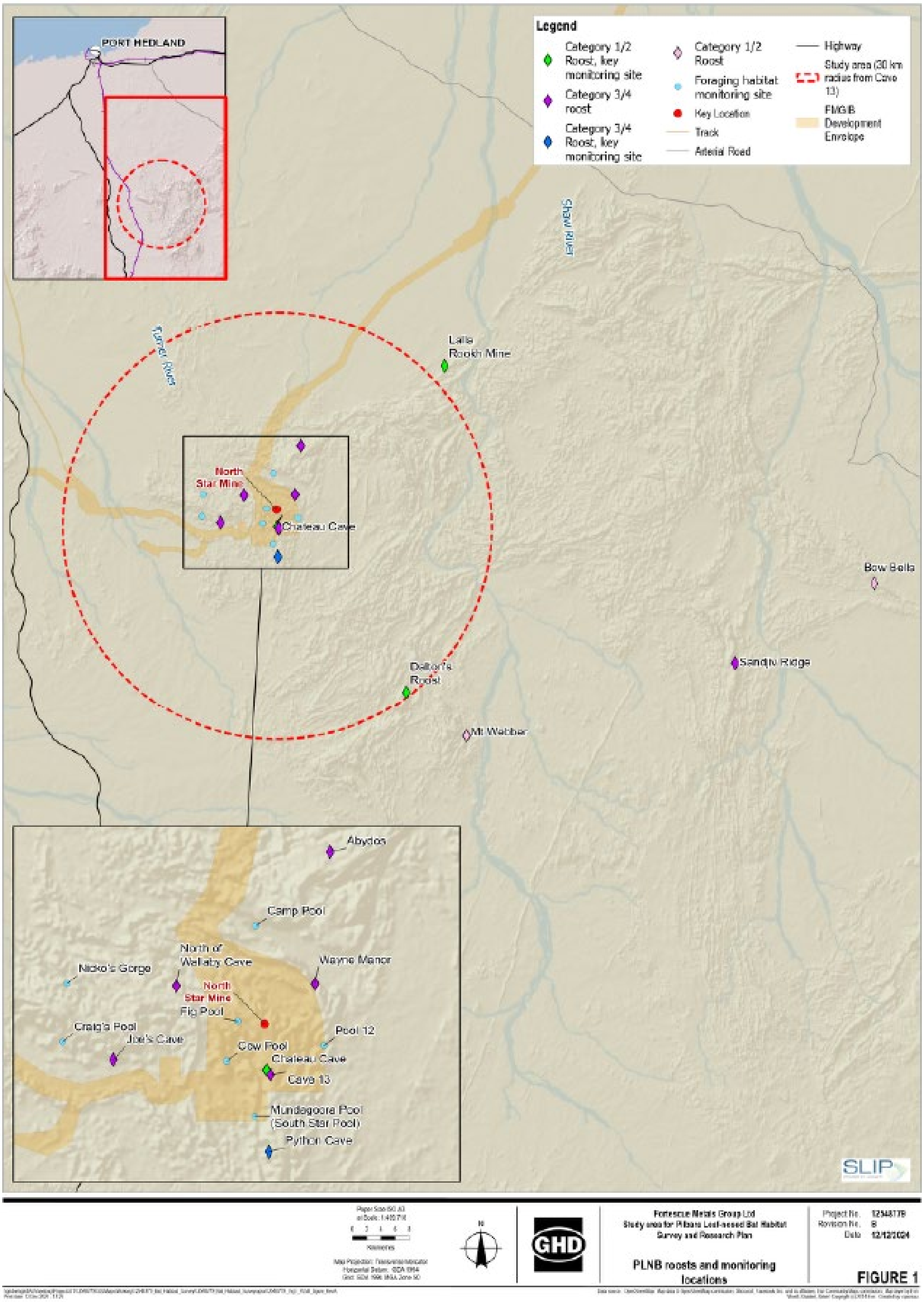


Figure 1 ***Study area and extent of the Iron Bridge mining development and associated infrastructure envelope***

2. Progress summary

2.1 Summary of progress against Plan stages

Table 3 provides a summary of the progress against the Plan to date. Survey methods, effort and results for data collected from the beginning of April 2021 to the end of January 2024 are provided in more detail in Sections 3 – 7.

Table 3 Summary of plan progress April 2021- January 2024

Plan Stage	Detail	Primary methods	Progress summary	Report and section
1,2,3	Primarily focussed on characterising the demography of PLNB in Chateau Cave (used as the most appropriate model colony based on surveys and research to date to inform the development of definitions around what a viable portion of the colony is) using novel research techniques including genetic and hormone analysis of PLNB scat.	– Monthly genetic analysis of DNA from scat samples to identify the number of individual PLNB bats occupying Chateau Cave – Monthly hormone analysis of PLNB scat in the first year of monitoring to determine timing and duration of pregnancy (based on progesterone) and quantify non-reproductive levels of progesterone – Trapping of PLNB to support the development of protocols for genetic analysis and hormone assays – Bat detectors installed at key sites including Chateau Cave, Cave 13, Joe's Cave, Python Cave and Dalton's Roost	– Monthly scat collection for genetic and hormone analysis completed for April 2021 – March 2023 – Bi-monthly scat collection for genetic analysis completed for March 2023 – January 2024 – Hormone analysis of scat completed for April 2021 – March 2023 – Genetic analysis of scat completed for April 2021 – January 2024 – 20 x PLNB trapped during April and August 2021 for tissue sampling – 47 x PLNB trapped during September 2023 for tissue sampling – Data collected each month from bat detector sites between April 2021 – January 2024	– Section 3 (collection and analysis of PLNB scat material) – Section 4 (long-term monitoring of bat call activity)

Plan Stage	Detail	Primary methods	Progress summary	Report and section
1,2,3	Commence long-term monitoring of the Chateau Cave colony and environmental variables within the Chateau Cave roost, and other selected key colonies.	– Using passive full spectrum ultrasonic detectors, infrared video (IR), temperature/ humidity and noise/vibration monitors. Bat activity (from acoustic and video data) will be analysed against environmental variables – Permanent multi camera IR video surveillance installed at Chateau Cave August 2021 – 5 x Temperature/ humidity loggers installed in Chateau Cave May 2021, additional 5 loggers installed in the main chamber in August 2021 – 2 x Noise/vibration loggers installed in Chateau Cave June 2021	– Data collected each month from at least 10 bat detector sites between April 2021 – January 2024 – Multi camera IR video surveillance (CCTV) suffered from technical issues however data collected was analysed and used to support colony estimates within Chateau Cave from January 2022 – January 2024) – Temperature and humidity data within Chateau Cave reported from May 2021 – January 2024 – Noise/vibration loggers installed in Chateau Cave suffered technical issues and have hindered data capture and analysis process (data reviewed for this report from May 2021 – January 2024)	– Section 4 (long term monitoring of bat call activity) – Section 5 (environmental variables) – Section 6 (visual monitoring of bat activity)
1,2	Examine remote sensing applications to create an inventory of cave sites within 30 km of Cave 13 (the study area) for the purpose of identifying alternative diurnal roost sites.	Use of LiDAR to locate cave-like structures within a 30 km radius of Chateau Cave. Use of thermal infrared imaging drones to efficiently determine if cave-like structures observed in LiDAR analysis are likely to be suitable for PLNB.	– LiDAR surveys completed for select known and potential roost areas including Chateau Cave, Cave 13, Python Cave, Joe's Cave and surrounding areas during June 2021 – Thermal drone survey completed for select known roost areas including Chateau Cave, Cave 13, Python Cave and surrounding areas during August 2021 and targeted search areas north and northeast of Joe's Cave October 2021 – Methods and results presented in six- and 12-month reports	– Desktop assessment of LiDAR and thermal drone surveys presented in six-month report. Detailed assessment in GHD (2022b) – No additional survey effort since October 2021

Plan Stage	Detail	Primary methods	Progress summary	Report and section
2, 3	Focus on locating natural alternative diurnal / maternal roosts and provision for the construction of artificial roosts.	– Scent detection dog will be trained to detect PLNB scat with a high level of accuracy such that it is able to distinguish between PLNB and other microbat species and be comfortable working in harsh conditions, wearing protective PPE and travelling to sites via helicopter as required – eDNA sampling will be used as a secondary method for contingency and validation for the scent dog to understand presence/ absence of PLNB in potential roost sites (eDNA sampling will include material samples from cave floor and swabs from possible roosting locations e.g., ceiling cavities) – The above survey techniques coupled with cave habitat assessments were used to categorise the roost habitats identified to date	– The dog and handler visited site in early August 2021 to undertake onsite training and familiarisation to assist with determining the dog's suitability for the work. Field surveys during June/July 2022 and September/October 2022 were completed to assist with determining alternative roost sites within study area – eDNA samples were taken from the floor and ceiling of Chateau Cave during June 2021 and additional sampling was undertaken in September and October 2021, January 2022, and February 2022 at other possible roost sites. The methods and results are presented in the 24 Month report (GHD 2023b) – Ongoing ground-truthing field surveys completed	– 24 Month report (GHD 2023b), section 7 presents the results for the assessment of alternative roost habitats including eDNA sampling, habitat assessments, scent dog detection and categorisation of roost types identified to date – No alternative permanent diurnal roost was located within the search area using these methods – Scent detection dog was not a suitable method, particularly in the harsh Pilbara environment – No additional survey effort since 2022
2, 3	Focus on locating natural alternative diurnal / maternal roosts	In addition to the stated Plan methods a PLNB movement study using radio-tracking and PIT tagging is being undertaken to assist with the location of alternate roosts and provide additional information about the movement of PLNB throughout the study area including movements between known diurnal roost sites	– Radio tracking study undertaken in Aug-Sep 2023 which identified areas of high commuting/foraging activity as well as between roost movements – 155 individuals PIT tagged over two trapping surveys (Sept 2023 and Jul 2024). Receivers installed at seven known roosts. Several between roost movements identified	– Pilbara Leaf-nosed Bat Radiotracking Study (March 2024) (GHD 2024a) – Pilbara Leaf-nosed Bat PIT Tagging Study (Phase 1) (September 2024) (GHD 2024b) – Pilbara Leaf-nosed Bat PIT Tagging Study Phase 2 summary – technical memo (pending issue - late 2024)

2.2 Permits

For the purpose of implementing the methods detailed in the Plan, an Animal Research Authority (ARA-12548779 - GHD Animal Ethics Committee) and Authorisation to Take or Disturb Threatened Species (TFA 2021-0081 – Department of Biodiversity Conservation and Attractions) under Section 40 of the *Biodiversity Conservation Act 2016* (WA) were obtained.

2.3 Field surveys

Field surveys have occurred monthly since April 2021, switching to bi-monthly from May 2023 – January 2024. The purpose of each month's survey during the current monitoring period are provided in Table 4 below. The May 2023 field survey collected environmental monitoring data from March 2023 onwards.

Table 4 Summary of the field survey activities associated with the delivery of the research plan March 2023 – January 2024

Survey dates	Purpose / Activities	Limitations / constraints
22-29 May 2023	– Collection of data from bat detector sites and other long-term monitoring equipment (CCTV, Reconyx cameras, temperature and humidity data loggers) – Scat collection at Chateau Cave, Dalton's Roost, Lalla Rookh and Wodgina Cave	– CCTV not working following collection of data. Limited data collected – SD card errors for 5 of the bat detector sites – Unable to access Cave 13 and collect data
24-29 July 2023	– Collection of data from bat detector sites and other long-term monitoring equipment (CCTV, Reconyx cameras, temperature and humidity data loggers) – Scat collection at Chateau Cave and Dalton's Roost	– CCTV not working – no data since previous collection – Almost all SM4 detectors had card errors and/or microphone issue
21-26 September 2023	– Collection of data from bat detector sites and other long-term monitoring equipment (CCTV, Reconyx cameras, temperature and humidity data loggers) – Scat collection at Chateau Cave and Dalton's Roost – 45 PLNBs were trapped and of these 28 of these bats were radio tagged and tracked for up to one month, results provided in the Radio tracking study report (GHD 2024a)	– Limited data collected for CCTV due to power issues – SD card errors or power issues for 5 of the bat detector sites
31 October - 6 November 2023	– Collection of data from bat detector sites and other long-term monitoring equipment (CCTV, Reconyx cameras, temperature and humidity data loggers) – Scat collection at Chateau Cave and Dalton's Roost	– Limited data collected for CCTV due to power issues – SD card errors for 3 of the bat detector sites
16-21 January 2024	– Collection of data from bat detector sites and other long-term monitoring equipment (CCTV, Reconyx cameras, temperature and humidity data loggers) – Scat collection at Chateau Cave and Dalton's Roost	– CCTV not working as solar panel had blown over – limited data collected – SD card errors for 6 of the bat detector sites

2.4 Opportunistic Observations

In addition to planned activities associated with the delivery of research plan objectives, field staff recorded opportunistic observations relevant to the PLNB Plan such as presence and estimated abundance of PLNB in addition to factors which could influence PLNB including natural and anthropogenic disturbance (including some observations from the PLNB Movement Study and other scopes of work completed within the monitoring period).

Records from the past monitoring period are listed in Table 5, records from previous monitoring periods are collated in the 24-month report.

Table 5 Opportunistic records of bats observed during field surveys

Date	Species	Site	Observation	Ecologists
25/03/2023	*R. aurantia, T. georgianus, V. finalysoni*	Chateau Cave	5-10 PLNB flying around main chamber when we entered to collect scats at 8 pm. Approximately 25-30 *T. georgianus* flying around main chamber and up one of the vertical chutes. *V. finlaysoni* calls detected flying around outside Chateau Cave between 7-8 pm.	Heather North, Erin Westerhuis
25/03/2023	*M. gigas*	Dalton's Roost	At least one Ghost Bat flying around outside the roost at night	Heather North, Erin Westerhuis
25/03/2023	*R. aurantia*	Dalton's Roost	Approximately 20 PLNBs seen flying to the back wall and appeared to go up into a chute/chamber that was not accessible for humans.	Heather North, Erin Westerhuis
26/03/2023	*R. aurantia, T. georgianus*	Chateau Cave	One PLNB and approximately 30 *T. georgianus* roosting in centre of the ceiling	Erin Westerhuis
26/03/2023	*V. finalysoni*	Cave 13	One roosting in one of the outside alcoves	Heather North, Erin Westerhuis
27/4/2023	*R. aurantia*	Chateau Cave	Approximately 80 PLNBs flying around, and lots were also hanging on ceiling at 7:20 PM	Dylan Goldspink
24/5/2023	*R. aurantia*	Chateau Cave	40+ PLNBs flying in main chamber at 7:50 pm	Erin Westerhuis
25/5/2023	*M. gigas*	Wodgina Cave	Two Ghost bats roosting from ceiling, and Ghost bat scats & Zebra finch feathers on ground	Heather North, Dylan Goldspink
25/7/2023	*R. aurantia*	Chateau Cave	Approximately 30 PLNBs flying around and roosting on back wall and in chute when we entered to set sheets at 11:45 pm	Heather North, Dylan Goldspink
26/7/2023	*R. aurantia*	Chateau Cave	Approximately 20 PLNBs within main chamber	Heather North, Dylan Goldspink
14/8/2023	*V. finalysoni, T. georgianus*	Matrix Cave	Two *V. finlaysoni* and one *T. georgianus* seen	Heather North
22/9/2023 & 22/9/2023	*R. aurantia*	Chateau Cave	100+ PLNBs recorded night 1 and 50+ PLNBs recorded night 2 during scat collection at Chateau Cave	Heather North
31/10/2023	*R. aurantia*	Chateau Cave	10 PLNBs observed flying around cave upon entry at 8:30 pm and then slowly left chamber	Jessie Moyses, Heather North
1/11/2023	*R. aurantia*	Chateau Cave	10 PLNBs observed flying around cave upon entry at 8:30 pm and then slowly left chamber	Jessie Moyses, Heather North
17/01/2024	*R. aurantia*	Chateau Cave	Approx 75 PLNBs roosting and flying in main chamber upon entry at 9:20 pm.	Jessie Moyses, Lucas Hurst
18/01/2024	*R. aurantia*	Chateau Cave	JM and LH went into Chateau 8:45 pm. Approx 20 bats in cave upon entry	Jessie Moyses, Lucas Hurst

3. Collection and genetic analyses of PLNB scat material

3.1 Purpose

The background to the genetic study to identify individual PLNB has been outlined in previous Specialised Zoological communications and reports including:

– Specialised Zoological (2023c) Iron Bridge Pilbara Leaf-nosed Bat project: Genetics-based identification of individuals from scat collections April 2021 to March 2023.
– Provided below is a summary of the results including the most recent monitoring period provided by Specialised Zoological (Specialised Zoological 2024; Appendix A).

Determining the population dynamics of the PLNB colony at Chateau Cave; such as number of individuals, turnover of individuals, proportion of males versus females and whether pregnant or lactating females are present, at Chateau cave is essential for informing management decisions. Under the Plan, analysis of scat samples was proposed as a non-invasive method to identify individual PLNB.

Advances in methods for obtaining genomic DNA from scats of free-ranging wild animals has greatly increased the potential for ecological monitoring of threatened species (Ottewell et al. 2020). Importantly, these methods also limit the disturbance to sensitive fauna (Zemanova 2020). Faecal samples can be collected from wild animals without requiring trapping or handling and are therefore a less invasive technique for obtaining DNA from the PLNB, especially where long term monitoring of all individuals in a colony was required. Multi-temporal analysis of faecal samples has been used to infer population dynamics and survival (Oyler-McCance et al. 2018) and is referred to as genetic mark-recapture.

Genotyping of scat collected monthly (and later bi-monthly) from roost locations (Table 2; Figure 2) was completed and used to detect movement of individuals between roosts in the study area (Figure 2). The aim of the genetic study was to determine the demography of individuals occurring within the Chateau Cave colony and other permanent diurnal roosts within the study area (Table 2; Figure 2). Measures of demography included identification of individuals and detection of movement of individuals between multiple roosts.

3.2 Methods

3.2.1 Collection of scats

From April 2021 through March 2023 scats were collected monthly from 10 locations. Results for this sampling period are provided in the 24-month report (GHD 2023b). See GHD 2022b for detailed description of field sampling collection methods. During the current sampling period, scats were collected approximately every two months. A summary of the number of scat samples collected up to January 2024 is provided below (Table 6).

Table 6 Total number of scat samples collected until January 2024

	Bow Bells	Cave 13	Chateau Cave	Dalton's Roost	Joe's Cave	Lalla Rookh Mine	Mt Webber Cave	Python Cave	Razorback Cave	Sanjiv Ridge Cave
Apr-21			70		*13*					
May-21		2	16					5		
Jun-21		9	42							
Jul-21			80		*14*			5		
Aug-21			170					7		
Sep-21		1	87					3		
Oct-21			127			23		18		
Nov-21			82			1				
Dec-21			143							
Jan-22			222			1				
Feb-22			96	44		2	2			
Mar-22			171							
Apr-22		7	172			1				
May-22			20	51	*2*	16		1		
Jun-22			7	168		27	83			38
Aug-22			384	81		12		19		
Sep-22			316	459		11				
Oct-22			651							
Nov-22			856	1,356		10				
Dec-22			479	143						
Jan-23			425	1,344			48			
Feb-23	20		31	506			66		47	43
Mar-23			2	680						
May-23			944	246		28				
Jul-23			667	1,045						
Sep-23			***1,109***	***768***						
Nov-23			591	607						
Jan-24			451	145						
Number of scat collection efforts	1	19	29	15	NA	11	4	19	1	2
Location of sheets	Outside entrance	Inside cave	Inside cave	Outside entrance	NA	Outside entrance	Inside cave	Inside cave	Inside cave	Inside cave

Table notes: This table does not indicate the total number of successfully genotyped samples. Grey shaded cells represent periods where sheets were set at a particular site. Bold italicised cells (Chateau, Joe's Cave) represent at least a subset of samples were collected when trapping bats.

3.2.2 Genetic analysis

3.2.2.1 Genotyping

Scats were submitted to the Australian Genome Research Facility (AGRF), to allow identification of individual bats based on a panel of genotypes from custom-designed genetic markers using a customised Agena Bioscience MassARRAY System.

The panel of markers designed by AGRF is based on 149 selected 70-base-pair DNA fragments (from Dr Kyle Armstrong's 'DArTseq' PLNB dataset) that have a single base position that varies amongst individuals. The patterns amongst the 149 markers are different for different individual bats. The methods for the marker development are described in detail within previous Specialised Zoological communications and reports (Specialised Zoological 2021 in litt. a,b, 2021, 2022, 2023a,b), also see Appendix A and GHD 2022b and 2023b for further detailed description of genotyping methods.

Testing of 17,217 scat samples and 100 tissue samples collected from the study area demonstrated that 134 of these markers are suitable to give genotypes. The different efficiency between markers (Specialised Zoological 2021) was accounted for in the pairwise similarity analyses that was undertaken based on the genotype panels, and also by quality filtering of the dataset (see below section 3.2.2.3).

The peer review by the Harry Butler Institute (2023) mentioned the amount of missing data (non-amplified loci) in the genotypes could affect how often the same individual could be detected. A detailed discussion has been provided for this in Appendix A.

3.2.2.2 Sex ratio

To determine the sex associated with scats collected and the ratio present at roosts two sex-linked markers were generated from as many samples as possible (12,581 unfiltered genotypes having a determination of either female or male) from collections May 2022 – January 2024. Samples collected earlier had insufficient remaining DNA extract for re-PCR of the sex-linked markers that were developed in February 2022

The number of females and males was counted at each site, and for each survey. This required the removal of repeat detections of the same individual from the same site and survey; but it allowed repeat detections of the same individual across sites and surveys. An overall and a series of 'per survey' Chi-squared tests was also undertaken to investigate the significance of the departure from an even sex ratio using 'per survey' totals of females and males

3.2.2.3 Genotype filtering and analysis

In order to process the genotyping results and provide data outputs a standardised {R} statistical computing language script was developed by Specialised Zoological for further analysis of AGRF results. The process is described in Specialised Zoological 2021 and 2022 and is summarised as follows:

1. Initial minimal quality filtering and analysis on the square matrix of pairwise similarity values amongst all successful samples provided by AGRF.
2. A run-through with raw genotypes provided by AGRF using the {R} package 'ScatMatch' recommended by DBCA.
3. A final run using some output metrics from the 'ScatMatch' procedure to help with quality filtering but with operations coded separately into a custom script.

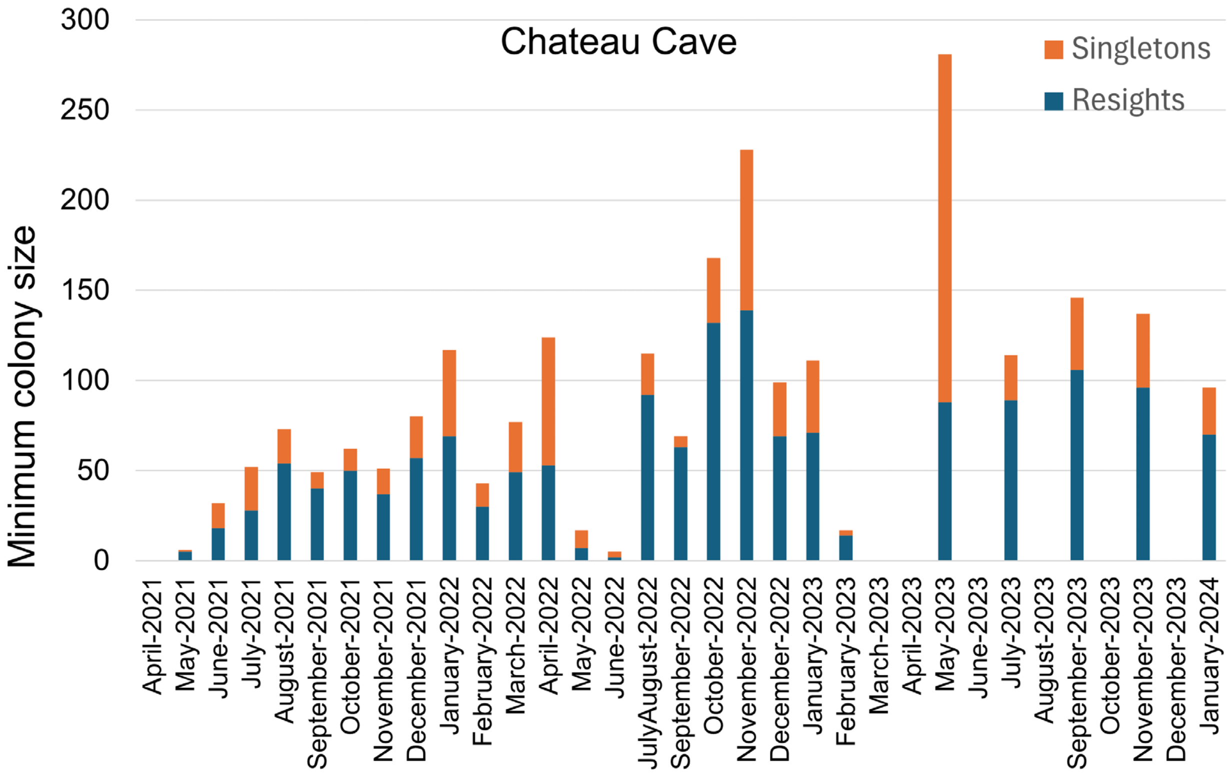
3.3 Results

Between April 2021 and January 2024, a total of 17,317 samples collected on 28 field visits were submitted to AGRF (https://www.agrf.org.au/) for DNA extraction and AGENA genotyping (see Table 1 Appendix A). This included 17,217 scats, 69 wing punch biopsies and 31 dried wings (from a cat predation event at Dalton's Roost). Samples were collected from a total of ten caves and mine adits, though most samples were from Chateau Cave (8,340) and Dalton's Roost (7,644).

There was an increased focus from May 2022 for identifying individual bats at roosts other than Chateau Cave, though mainly from Dalton's Roost (Table 2; Figure 1 Appendix A). Key findings to date include:

Individual IDs and total numbers detected

A total of 16,627 samples (scat or tissue) were successfully genotyped, identifying 4,397 individuals (detailed results provided in Appendix A). Among these, 2,182 individuals were identified from more than one sample, while 2,215 individuals were identified from only a single sample (singletons). The total number of individuals detected using Chateau Cave on at least one occasion was 1,264, and 2,914 individuals were detected using Dalton's Roost. The minimum colony size for each period was calculated by adding the number of resighted individuals to the number of singletons for each collection survey (Figure 2). These estimates are likely significant underestimates because not all individuals present during the time of scat collection would have been represented in the sample collection. However, they may be important indicators of the significance and general changes in colony size over time.



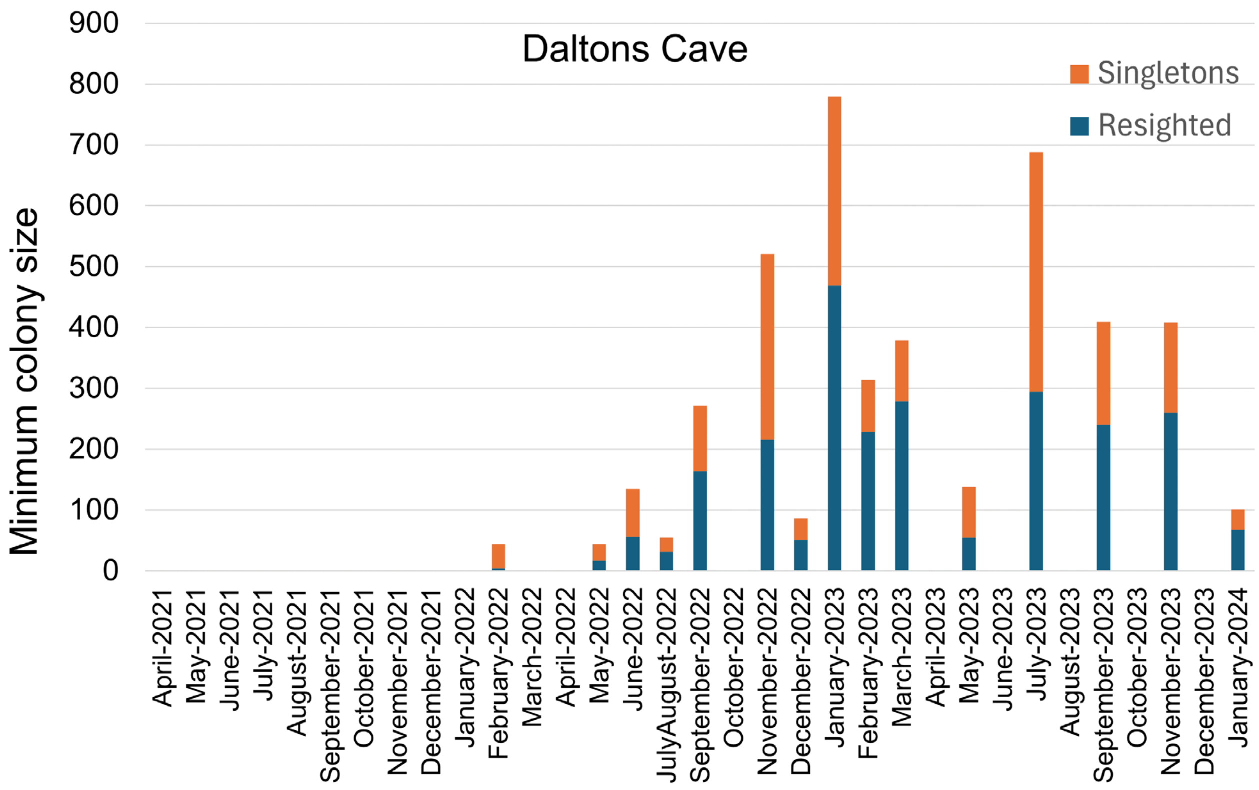


Figure 2 ***Minimum colony size for Chateau Cave (top) and Dalton's Roost (bottom), as calculated from the number of resighted individuals (those detected more than once) and singletons, for each collection survey.***

Sex ratio

- Only Chateau Cave and Dalton's Roost had sufficient sample size to give meaningful estimates of sex ratio (these results are provided in GHD 2023b).
- There was a bias towards males at Chateau Cave, with approximately double the number of males detected overall (65.6%; Table 7), and in most months except October to December (and February; Table 7; Figure 3).
- By contrast, at Dalton's Roost there were approximately three times as many females as males (74.5%; Table 7). There is a statistically significant bias towards female presence in all months except April – June (Figure 3).
- A highly significant Chi-squared test (p-value <0.001) from the overall test supported a bias towards males at Chateau Cave and towards females at Dalton's Roost (Figure 3).

Table 7 ***Summary of sex-linked data (derived from SZ 2024 Appendix A)***

Site	Female	Male	Dominant Sex	Female%	Male %	Total Samples
Chateau Cave	541	1,033	Male	34.4	65.6	1,574
Dalton's Roost	3,175	1,085	Female	74.5	25.5	4,260
Grand Total	**3,716**	**2,118**	**Female**	**68.6**	**31.4**	**5,834**

Note: The dominant sex identified overall at each site is indicated based on samples genotyped since May 2022.

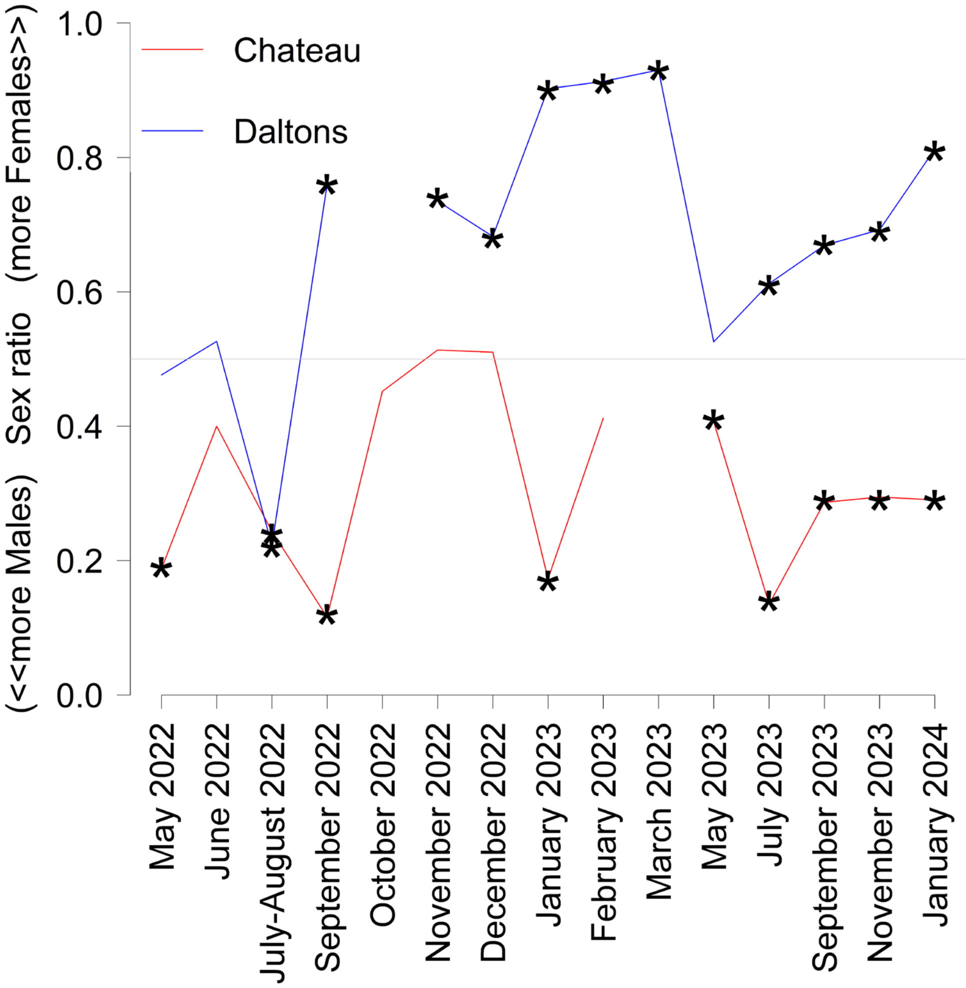


Figure 3 ***Changes in sex ratio on a 'per survey' basis from May 2022 to January 2024 at both Chateau Cave and Dalton's Roost. Asterisks indicate that the sampled sex ratio departed significantly from 0.5 at that time i.e. highly significant Chi-squared test p-value [<0.001])***

Movement of individuals

Detections of individuals from more than one sample fall into four possible situations:

1. More than one scat in a collection period (from a single night, or subsequent nights, at the same location for example (the collection of multiple scats from the same individual roosting above the collection sheet)
2. From scats collected on different nights on the same monthly survey but at different locations
3. From scats collected on different surveys at the same location
4. From scats collected on both different surveys and at more than one location

The latter three are of particular interest to the study because they show either movement amongst roosts on short or long-time scales, or persistence at one or more roosts over the longer term. Of the 2,182 individuals detected more than once, 3% (*n* = 65) were detected completing 76 inter-colony movements amongst seven different caves and mine adits between April 2021 and January 2024 (Table 8). There is evidence of 18 inter-colony movements within a single survey period (i.e. within the collection period of less than one week). This demonstrates short-term movement is possible, even at relatively long distances. Most individuals (*n* = 40) were only detected on two occasions, but the remainder (24) were detected between three and 13 times.

Table 8 ***The number of individuals that completed inter-colony movements between two or more roosts, as well as the number of actual movement events (some individuals were detected moving more than once), and the number of times an individual was detected moving within the same collection survey (calculated for a missingness value of 8).***

Roost 1	Roost 2	Roost 3	Number of individuals	Number of movements	Number of within-survey movements*
Chateau Cave	Cave 13		2	2	
Chateau Cave	Python Cave		8	10	
Chateau Cave	Lalla Rookh Mine		4	7	3*
Chateau Cave	Joes Cave		2	2	
Chateau Cave	Dalton’s Roost	Python Cave	1	1	
Chateau Cave	Dalton’s Roost		28	34	11*
Dalton’s Roost	Lalla Rookh Mine		2	2	
Dalton’s Roost	Mt Webber		18	18	4*
Total			**65**	**76**	**18***

* Indicating short term movement (i.e. within 1-3 nights)

3.4 Discussion

The purpose of the genetic analysis study was to determine the population dynamics of the PLNB colony at Chateau cave to inform management decisions. Each aspect of the Chateau Cave colony is discussed below, with Dalton’s Roost colony characteristics and other monitoring sites presented for comparison.

3.4.1 Number of individuals

Genetic mark-recapture sampling recorded 1,264 individuals at Chateau Cave and 2,914 individuals at Dalton’s Roost that have utilised the roost at some point between April 2021 and January 2024. Analysis of genetic data collected from all other regional roost locations combined (Table 2; Figure 2) recorded an additional 219 individuals from the surrounding region. The colony size estimates for both Chateau Cave and Dalton’s Roost have been validated during a study that estimated colony size using thermal video recordings in December 2022 which resulted in a combined estimate of c. 4,000 individuals (Specialised Zoological 2023c).

3.4.2 Movement of individuals

The success with which the genotyping has identified both a large number of singletons and an almost equal number of repeatedly detected PLNB has allowed the PLNB populations at both Chateau Cave and Dalton’s Roost to be estimated with a high degree of certainty. The identification of 2,215 singletons provides additional evidence of the variability in the PLNB population within the study area and the high number of potential transient individuals.

This dynamic roost use by Chateau Cave PLNB is supported by thermal data which shows the numbers of individuals that utilise this roost fluctuate significantly both within and across seasons. Minimum colony size data and recent thermal camera data (Specialised zoological; unpublished data 2024) indicates an average of 125 individuals utilise Chateau Cave at any one point, with maximum colony size recorded as 223. These findings not only validate the robustness of the genotyping approach, but also underscore the importance of employing multiple methods for accurate population assessments, thereby enhancing our understanding of the PLNB populations within the study area and dynamics at roost sites.

The recorded movement of 65 individuals amongst the roost caves surveyed provides clear evidence of the capacity for PLNBs to utilise multiple roost caves. Detections of individuals moving between Chateau Cave and other monitoring sites provides evidence for regular landscape scale movements of PLNB through the study area, both within and outside the mining project area limits. PLNB likely move between roosts as part of natural behavioural patterns, but also potentially in response to disturbance.

Of note is the ability for individual PLNB to reach alternative roosts in the study area within a very short period of time, even at relatively long distances of 30 km between Chateau Cave and Dalton’s Roost within the space of one night. PIT tagging monitoring methods has recorded a flight time of 3hrs 25min between Chateau Cave and Dalton’s Roost and 5hrs 18min from Chateau Cave to Wamerina (Fortescue, 2024 unpublished). It is important to note scat samples were collected once per month at the highest frequency, therefore it’s likely the rate of inter-colony movement is underestimated.

These inter colony movement findings have important implications for how to demonstrate the ‘viability’ of the colony at Chateau Cave. The Chateau Cave colony membership is part of a much larger population with individuals moving freely between roosts. This compliments findings from Bullen & Reiffer (2019) and Bullen & Reiffer (2022) in addition to the regional geneflow identified by Umbrello *et. al.* (2022) and Armstrong (2011b).

3.4.3 Sex Ratio and roost use for breeding

There is a bias towards males at Chateau Cave, with approximately double the number of males detected overall (Table 7) and in most months except October to December and February. The meaning of a male bias is not well understood but may be indicative of a site where mating takes place (similar to observations in the Northern Territory, except that there is no seasonal male exodus/relocation; Churchill 1995). Breeding activity has also been recorded at Chateau Cave (observation of young inside the rear chamber), but the number of females giving birth could be relatively low. By contrast, at Dalton’s Roost where there were approximately three times as many females as males (Table 7). This statistically significant bias towards female presence occurred in all months except April – June. Pregnant females have been captured from Dalton’s Roost, consequently this appears to be an important maternity site. At Dalton’s Roost, all parts of the breeding cycle are likely to occur—mating, roosting by pregnant females, parturition and nursing young.

4. Long-term monitoring of bat call activity

4.1 Purpose

Bat call activity surveys were undertaken using full spectrum ultrasonic recorders (bat detectors) to:

– Monitor bat call activity levels associated with known permanent diurnal roost sites including Chateau Cave and Dalton’s Roost (see section 4.2 of the Plan)
– Establish baseline bat call activity at long-term monitoring sites to assess the persistence of the PLNB in the North Star study area following the relocation of the Chateau Cave PLNB colony (see section 10 of the Plan).

4.2 Methods

4.2.1 Bat call activity monitoring

A total of 15 in-situ (stationary) activity and/or presence sites were established across the study area using full spectrum ultrasonic detectors (Song Meter SM4BAT-FS, Wildlife Acoustics) in April and May 2021. The Chateau Cave site was monitored using an SM4BAT-FS with the microphone positioned within the internal roosting chamber of the cave (Chateau Cave (CC) in) and a second recorder positioned outside the cave, 2 m from the entrance (CC out). The other sites including a second permanent diurnal roost (Dalton’s Roost), temporary diurnal roosts, other potential nocturnal refuge roosts and non-roost sites (e.g. permanent natural pools) were surveyed using one recorder located within 2 m of the entrance of the roost or edge of the water body.

As determined by the Plan, detector sites were divided into ‘Presence’ and ‘Activity’ monitoring sites. ‘Presence’ sites were used for the purpose of understanding the ongoing presence of the PLNB within the study area. ‘Activity’ detector monitoring sites were used for the purpose of understanding PLNB activity within the study area (e.g., within 20 mins +/- of civil twilight) and the potential presence of alternative roosting sites within the study area. The location of each ultrasonic detector and acoustic recorder site is provided in Table 9 and Figure 1.

Table 9 Type and category of long term in-situ bat detector monitoring sites

Site name – detector type	Types of monitoring site	Category of monitoring site
Permanent roost. Chateau Cave out = 1 x SM4BAT-FS outside cave at entrance (omni directional SMM-u2 microphone). Chateau Cave in = 1 x SM4BAT-FS inside main chamber (directional SMM-u1 microphone) Analysis presented in this report from April 2023– end of May 2023.	presence & activity	Impact
Joe’s Cave (transitory diurnal roost) 1 x SM4BAT-FS (SMM-u2) outside cave at entrance	presence & activity	Control
Cave 13 (transitory diurnal roost) 1 x SM4BAT-FS (SMM-u2)	presence & activity	Impact
Fig Pool (non-roost) 1 x SM4BAT-FS (SMM-u2)	presence & activity	Impact
Mundagoora Pool (non-roost) 1 x SM4BAT-FS (SMM-u2)	presence & activity	Impact
Craig’s Pool (non-roost) 1 x SM4BAT-FS (SMM-u2)	presence & activity	Control
Pool 12 (non-roost) 1 x SM4BAT-FS (SMM-u2)	presence	Impact
Python Cave (transitory diurnal roost) 1 x SM4BAT-FS (SMM-u1)	presence & activity	Impact

Site name – detector type	Types of monitoring site	Category of monitoring site
Abydos mine site, Atlas (transitory diurnal roost) – sites Cave B, Cave D and Cave H – Anabat Swift each site (omni directional microphone). Site discontinued – see results presented in 6- and 12-month reports	presence & activity	Control (to be determined).
Camp Pool (non-roost) 1 x SM4BAT-FS (SMM-u2)	presence & activity	Control
Wayne Manor (potential roost unknown type) 1 x SM4BAT-FS (SMM-u2)	presence & activity	Control
Wallaby Cave (potential roost unknown type) 1 x SM4BAT-FS (SMM-u2). Site discontinued as access no longer available – see results presented in 24-month reports	presence	Control
Cow Pool (non-roost) 1 x SM4BAT-FS (SMM-u2) Site discontinued as access no longer available – see results presented in 24-month reports	presence	Impact
Dalton's Roost (Permanent roost) 1 x SM4BAT-FS (SMM-u2)	presence and activity	Control
Nicko's Gorge (non-roost) 1 x SM4BAT-FS (SMM-u2)	presence	Control

Table notes: 'Presence' detector monitoring sites - for the purpose of understanding the ongoing presence of the PLNB within the study area. 'Activity' detector monitoring sites for the purpose of understanding PLNB activity within the study area (e.g., within 20 mins +/- of civil twilight) and the potential presence of alternative roosting sites within the study area. 'Control' sites are sites considered to be at the lowest or no risk of impact from the North Star mine development. 'Impact' sites are short term (e.g., 2-3 year) monitoring sites which may be directly or indirectly impacted due to the proximity of mining activity.

4.2.2 Bat call and statistical analysis

Bat call analysis

Bat call analysis was completed according to the methods presented in the 12 and 24-months reports (GHD 2022b and GHD 2023b). Bat call analysis of the acoustic recordings was completed by GHD up to and including June 2023. Analysis of acoustic recordings collected from July 2023 onwards was completed by Specialised Zoological.

Statistical analysis

To assess temporal trends in bat activity across multiple sites, the number of bat call detections (PLNB calls) recorded per night were plotted against the date. The data included all available records from sites including Cave 13, Python Cave, Joes Cave, Mundagoora Pool, Wayne Manor, Wallaby Cave, Pool 12, Nickos Pool, Fig Pool, Craigs Pool, Cow Pool, and Camp Pool. The dataset was initially investigated using R, and a trend analysis was conducted using the ggplot2 package. A smooth trend line was generated using geom_smooth with the Generalised Additive Model (GAM) regression model that allows for more flexibility than a standard linear regression by modelling the relationship between the dependent variable (y) and the independent variable (x) as a smooth, non-linear function. This method provides a non-parametric estimation of the trend in bat activity over time. The resulting figure (Figure 4) provides a visual summary of how bat activity has varied over the observation period across all sites combined, with confidence intervals illustrating the precision of the trend estimates.

To test differences in PLNB activity between the six primary monitoring sites (CCin/CCout, Dalton's Roost, Joes Cave, Cave 13, Mundagoora Pool and Python Cave) and between survey periods, PRIMER was used with PERMANOVA add-on (PRIMER E Limited version 7) for univariate analyses. PLNB daily activity per site was used as the dependent measure and site and survey period (month and year of survey) were used as explanatory factors. Non-parametric permutational analysis of variance (PERMANOVA) are well suited for unbalanced designs, for example where different days of data exist per month. Therefore, PERMANOVA was used to test for significance of the main temporal (survey) and spatial (site) factors in the experimental model. Pairwise tests of significance between each sampling period were also conducted for the purpose of determining if activity between permanent diurnal sites differed by month.

A square root (√) transformation was applied to the PLNB daily activity per site-by-month data (after examining the relationship between logged values of the standard deviation and mean). The transformed data was then used to construct a site-by-survey Bray-Curtis similarity matrix. Patterns in the data were examined in box plots.

The effect month had on PLNB activity at each permanent diurnal roost was tested in a one-factor PERMANOVA, with month nested in a year used as a fixed factor. Both internal and external SM4 data was used for analysis of Chateau Cave patterns. For the permutation method, permutations of residuals were unrestricted due to the single factor with type III sum of squares and 9999 permutations selected. Permutational P values were reported for results with more than 100 permutations. Significant results were examined using pair-wise tests.

Generalised linear mixed models (GLMMs) were fitted using the glmmTMB package in R to investigate the relationship between PLNB activity and a suite of environmental and anthropogenic predictors at the two permanent diurnal roosts in the study area – Chateau and Daltons. Given the count nature of the response variable and evidence of overdispersion, a negative binomial distribution was specified. Temporal autocorrelation (relationship of one day of activity to the next) was addressed by incorporating an autoregressive structure using ar1(dateAR1 + 0 | month_year). Several models were developed. The baseline model included only the AR1 structure to account for temporal patterns. The combined disturbance model included anthropogenic predictors such as cave entry, blasting, general mining activity within 500 metres of Chateau Cave and artificial light. The weather and climate model included scaled environmental variables such as mean temperature, mean and maximum wind, mean barometric pressure, and mean humidity, along with cumulative rainfall over the past 30 days. The fit of these models was evaluated using summary statistics, Akaike Information Criterion (AIC) comparisons, and likelihood ratio tests (via ANOVA) to compare nested models and assess the significance of individual predictors.

Diagnostics were conducted to ensure the appropriateness of the models and to identify potential issues. Pearson residuals were plotted against fitted values to check model assumptions, while autocorrelation in residuals was evaluated using autocorrelation function (ACF) plots. Predictions were also generated for significant predictors using the ggeffects package. The predicted effects of these variables were visualised to facilitate interpretation of their impact on bat activity. Statistical significance for predictors was evaluated using Wald chi-squared tests, with a significance level set at $\alpha = 0.05$. Results are presented as model estimates alongside their standard errors, confidence intervals, and p-values.

4.3 Results

4.3.1 Survey effort and overall patterns

During the 33 months of monitoring, 7,466 detector nights of data were recorded across 15 detector sites (Table 10). More than 14 million PLNB calls were detected across all sites and PLNB were detected every month at all sites where detectors were operational (Table 11).

Only Chateau Cave and Dalton's Roost recorded activity every night (excluding when equipment technical issues occurred). This is a characteristic of permanent diurnal roosts. Activity at Dalton's Roost was 33 times higher than the outside Chateau Cave detector (CC out).

All other detector sites had varying degrees of activity, but usually with at least some nights per month without PLNB activity. Across the study area, with all sites combined, a high degree of variation in activity was observed (Figure 4). Activity was highest overall in the dry season, generally from May through to September and tended to be lowest in the wet season months, although this varied from year to year with periods of low activity observed in January and February 2022 and 2023 and then November and December 2023. Sampling bias due to the larger datasets collected from Chateau Cave and Dalton's Roost was also explored, when these sites were excluded, the general pattern of higher activity during the months of June, July and August and lower activity in January and February is still observed, albeit at lower levels.

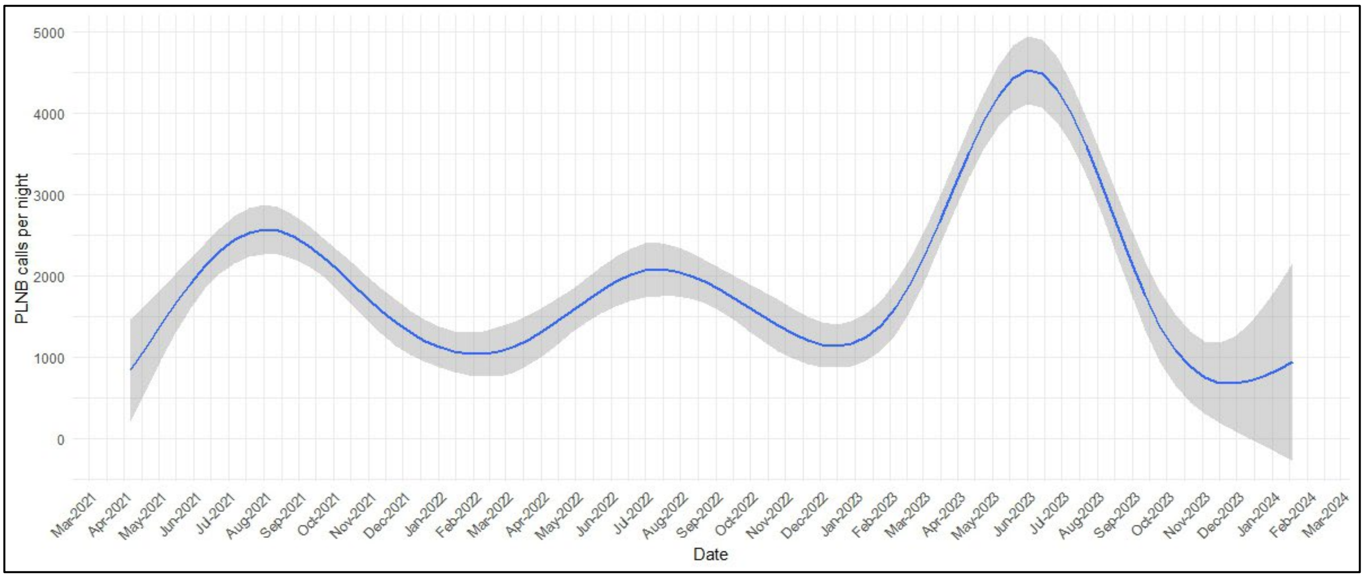


Figure 4 ***Temporal trends in bat activity for all 15 sites. The figure shows the number of bat call detections (PLNB calls) recorded per night plotted against the date.***

Table 10 *Number of nights PLNB recorded per month from April 2021 – January 2024*

Years	Month	Chateau Cave in	Chateau Cave out	Dalton's Roost	Cave 13	Mundagoora Pool	Python Cave	Fig Pool	Camp pool	Pool 12	Cow Pool	Craigs Pool	Joes Cave	Nicko's Gorge	Wallaby Cave	Wayne Manor	Total
2021	Apr	*17	*24	-	23	11	23	15	10	22	-	*	15	-	-	-	119
2021	May	*22	*13	-	30	22	29	27	10	23	-	19	*	-	13	20	193
2021	Jun	*11	*22	*28	24	21	24	24	20	20	-	23	23	-	15	22	216
2021	Jul	*22	*24	31	4	24	29	*	18	14	31	22	21	-	1	23	218
2021	Aug	*26	*27	31	26	17	29	23	25	25	31	31	6	-	27	29	300
2021	Sep	*2	30	30	29	21	31	23	20	10	22	30	21	-	20	10	297
2021	Oct	31	*30	31	30	12	31	27	3	31	6	27	30	-	*	21	280
2021	Nov	30	30	30	29	28	30	25	11	28	6	28	27	28	17	18	365
2021	Dec	31	31	*27	31	23	31	30	21	31	10	25	30	*	12	18	324
2022	Jan	31	31	*	31	18	30	16	22	16	*	23	11	*	2	29	260
2022	Feb	28	28	*	29	22	18	3	28	19	*	26	19	18	1	25	264
2022	Mar	*27	31	*	28	31	11	*	30	21	*	14	28	31	*	2	227
2022	Apr	*27	30	*29	11	30	20	*	9	30	7	16	8	30	*	18	209
2022	May	*21	*29	*31	*	24	9	*	12	28	7	25	25	24	9	29	192
2022	Jun	*2	30	*5	*	6	30	*	17	29	5	17	25	3	2	28	192
2022	Jul	31	31	*4	*	3	31	*	17	31	*	*	19	31	13	*	207
2022	Aug	31	31	31	29	12	31	*	24	29	17	2	26	31	7	28	329
2022	Sep	*4	*6	30	28	2	30	*	26	4	1	20	29	30	*	28	228
2022	Oct	31	*25	*27	22	29	31	*	16	27	9	*	31	30	6	16	248
2022	Nov	30	*8	*	*7	20	28	*	27	26	5	6	21	30	9	*	202
2022	Dec	*26	31	*15	31	31	20	*	25	31	12	24	3	31	15	*	254
2023	Jan	31	31	31	28	2	12	*	25	22	1	13	14	18	10	9	247
2023	Feb	28	*22	28	28	1	5	3	22	19	*	14	20	19	14	29	230
2023	Mar	*17	*13	*24	*23	3	15	26	29	15	2	2	18	22	*	24	156
2023	Apr	30	22	30	28	3	18	30	20	*	-	16	30	26	-	17	270
2023	May	24	23	21	16	6	26	24	8	6	-	7	15	7	-	6	189
2023	Jun	*	*	30	*	30	28	-	16	18	-	*	19	30	-	30	201
2023	Jul	^	^	31	7	31	30	-	10	6	-	7	7	25	-	25	179
2023	Aug	^	^	31	28	30	26	-	16	21	-	20	28	*	-	30	230
2023	Sep	^	^	30	21	7	29	-	7	8	-	11	16	11	-	27	167
2023	Oct	^	^	31	24	26	30	-	21	3	-	17	19	31	-	26	228
2023	Nov	^	^	1	*	30	22	-	26	9	-	5	11	30	-	1	135
2023	Dec	^	^	29	*	21	9	-	*	*	-	*	*	23	-	*	82
2024	Jan	^	^	17	*	8	3	-	*	*	-	*	*	*	-	*	28
	Total	387	410	524	615	605	799	296	591	622	172	490	615	559	193	588	7466

Table notes: Blank data does not necessarily indicate absences of PLNB, but rather linked to call logistical and operational issues with in-situ equipment. * Indicates operational issue with equipment resulting in loss of data during a night or entire date, - indicates detector had not been deployed/been discontinued. ^ Indicates data that will be collected and reported on by SuperSensory Technologies. Colour scale indicates the range of values with highest activity represented by red and lowest activity represented by green.

Table 11 Average nightly PLNB calls at all long-term monitoring sites

Years	Date	Chateau Cave in	Chateau Cave out	Dalton's Roost	Cave 13	Mundagoora Pool	Python Cave	Fig Pool	Camp pool	Pool 12	Cow Pool	Craigs Pool	Joe's Cave	Nicko's Gorge	Wallaby Cave	Wayne Manor	Average
2021	Apr	5905	327	-	69	149	121	37	15	17	-	-	641	-	-	-	809
2021	May	13623	552	-	129	314	371	25	8	12	-	25	*	-	4	38	1373
2021	Jun	12491	814	16607	258	242	263	52	6	12	-	27	432	-	6	42	2404
2021	Jul	12767	467	15326	142	136	485	*	5	5	21	28	176	-	6	27	2276
2021	Aug	14147	579	13644	47	311	531	30	9	9	25	42	138	-	3	24	2110
2021	Sep	4923	461	13232	41	345	181	17	6	17	24	24	164	-	2	23	1390
2021	Oct	6600	414	17142	36	318	49	7	2	11	2	5	180	-	*	4	1905
2021	Nov	6011	374	13101	9	12	7	15	4	12	2	6	72	106	4	3	1316
2021	Dec	7995	360	7771	29	15	27	20	8	15	1	10	140	*	1	4	1171
2022	Jan	7819	269	*	31	5	15	4	8	5	*	8	226	*	1	7	700
2022	Feb	3897	80	*	11	3	3	3	11	17	*	8	286	12	1	28	335
2022	Mar	8881	257	*	13	7	4	*	19	23	*	2	91	17	*	3	847
2022	Apr	12652	473	9977	42	13	9	*	11	20	2	12	79	27	*	31	1796
2022	May	8861	331	9846	*	9	13	*	22	9	3	10	866	41	3	53	1544
2022	Jun	12522	421	11350	*	1	106	*	6	6	2	4	511	20	2	24	1921
2022	Jul	13159	777	17387	*	3	401	*	5	9	*	*	288	54	3	*	3209
2022	Aug	11657	474	13842	143	4	165	*	20	14	2	3	187	65	2	48	1902
2022	Sep	4495	173	9568	40	2	107	*	20	8	1	4	98	26	*	20	1120
2022	Oct	5834	202	9322	75	110	58	*	18	24	1	*	133	19	6	29	1218
2022	Nov	6712	336	*	244	129	31	*	26	7	2	3	52	25	6	*	631
2022	Dec	7876	306	8606	139	74	4	*	28	6	2	5	27	27	5	*	1316
2023	Jan	4322	100	9215	156	5	2	*	28	4	1	2	23	15	8	34	994
2023	Feb	395	30	12488	48	2	2	2	21	3	*	6	38	4	8	63	936
2023	Mar	452	32	11397	39	1	5	26	25	4	1	3	26	19	-	37	862
2023	Apr	9282	657	15680	48	2	18	20	10	*	-	2	39	23	-	47	2152
2023	May	20259	1385	12867	9	227	40	170	12	38	-	3	127	794	-	168	2777
2023	Jun	*	*	13806	*	106	11	-	15	28	-	*	126	191	-	120	1800
2023	Jul	^	^	10196	213	64	13	-	10	16	-	15	216	131	-	77	1095
2023	Aug	^	^	10520	127	131	5	-	16	10	-	7	104	*	-	15	1215
2023	Sep	^	^	9107	38	292	6	-	23	8	-	4	65	194	-	4	974
2023	Oct	^	^	9625	131	234	11	-	10	3	-	4	56	397	-	5	1048
2023	Nov	^	^	2592	*	163	2	-	5	2	-	3	36	117	-	1	325
2023	Dec	^	^	1147	*	9	25	-	*	*	-	*	*	111	-	*	323
2024	Jan	^	^	791	*	2	1	-	*	*	-	*	*	*	-	*	265
Overall Average		8537	8598	10934	85	101	91	31	14	12	6	10	182	106	4	35	1355

Table notes: Blank data does not necessarily indicate absences of PLNB, but rather linked to call logistical and operational issues with in-situ equipment. * Indicates operational issue with equipment resulting in loss of data during a night or entire date, - indicates detector had not been deployed/been discontinued. ^ Indicates data that will be provided by SuperSensory Technologies but was not made available at the time of submission. This information will be integrated into this report when made available. Colour scale indicates the range of values with highest counts represented by red and lowest counts represented by green.

4.3.2 Chateau Cave

Acoustic data for CC "in" and "out" sites have been collected from April 2021 to the end of May 2023 by GHD. Analysis of this data has resulted in 5.3 million PLNB calls (1 second files) being identified from the CCin site and 270,888 PLNB calls (also 1 second files) identified from the CCout site. Activity based on the number of calls recorded has been highly variable month to month (Figure 5 and Figure 6), although some seasonal trends are evident. Activity has generally been highest at Chateau Cave during the cooler months from April – August, and lowest during the warmer months from October - March. Activity in May 2023 was significantly higher than any of the previous surveys for both inside and outside sites. February and March 2023 had significantly lower activity than all other periods.

Acoustic data for Chateau Cave from July 2023 onwards has been processed and analysed by Specialised Zoological, which was not available at the time of finalising this annual report. It is envisaged this information will be supplied by Specialised Zoological as an addendum to this report or made available for subsequent integration in the next annual report.

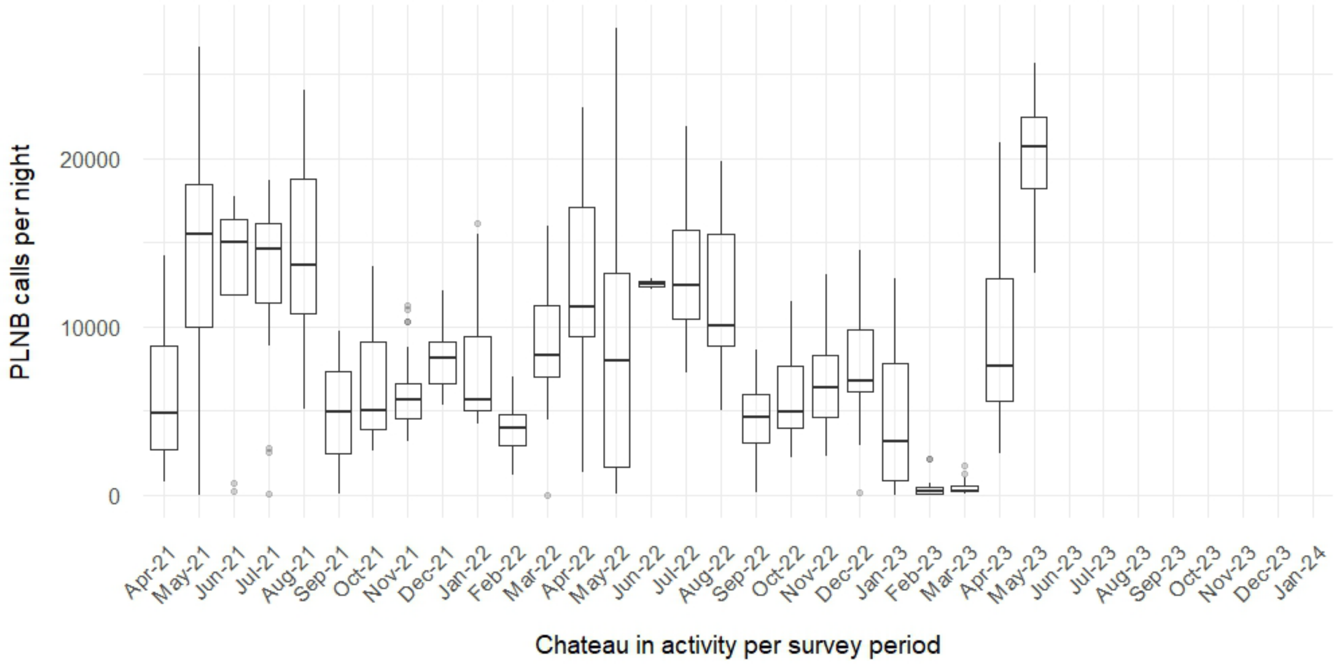


Figure 5 ***Boxplot of Chateau Cave in PLNB calls per night from April 2021 – May 2023***

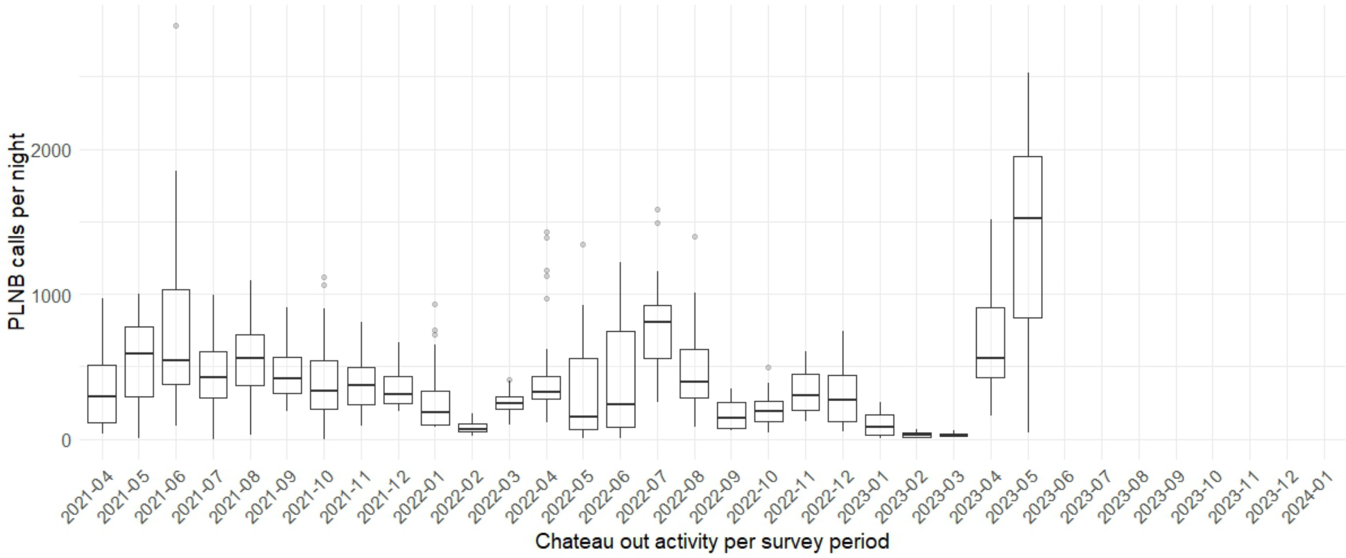


Figure 6 ***Boxplot of Chateau Cave out PLNB calls per night from April 2021 – Jan 2024***

4.3.2.1 Models of PLNB activity for CCin

Several generalised linear mixed models (GLMMs) were evaluated to assess the influence of environmental and temporal predictors on average nightly activity at CCin. The autoregressive (AR1) correlation structure had a high correlation coefficient of 0.97, indicating a strong degree of temporal autocorrelation in the data. Temporal autocorrelation refers to the similarity between observations across time, and in this case, the AR1 correlation of 0.97 indicates activity inside Chateau Cave on one night is closely related to activity levels on the following night. This high correlation indicates past activity levels are a strong predictor of current activity levels, suggesting that bat activity tends to follow stable patterns over short periods. By including this AR1 structure in the model, it accounted for these short-term dependencies, allowing other predictors to be assessed independently.

Model comparisons were primarily based on AIC values to identify the best-fitting model. The simplest model, containing only an intercept and the AR1 temporal structure, had an AIC of 10089. This model provided a baseline of nightly PLNB activity for the Chateau Cave roost detector but lacked environmental predictors, limiting its explanatory power.

Adding moonlight and cumulative rain significantly improved the model fit (AIC = 10084, p 0.01). Both predictors were statistically significant, with moonlight (estimate = -0.136, p = 0.0217) and cumulative rain (estimate = -0.278, p = 0.0151) indicating that lower moon illumination and cumulative rainfall over the past 30 days were associated with decreased activity. The improvement suggests these predictors substantially explain temporal variations in activity. Adding max daily wind speed and daily mean temperature did not significantly enhance model fit (AIC = 10087, p = 0.78), as neither predictor reached statistical significance. Similarly, a model which included factorial predictors of disturbance (cave entry and before/ after mining) along with daily mean barometric pressure and daily mean humidity, showed no significant improvement over simpler models (AIC = 10091.5, p = 1.00). In all models, most added predictors were non-significant, indicating limited contributions from these variables apart from moonlight and cumulative rainfall which both had a negative relationship with PLNB activity inside Chateau Cave. In other words, activity inside Chateau Cave tended to decrease as moonlight intensity increased and with increasing cumulative rainfall. Overall, these results indicated that moonlight and cumulative rainfall over the past 30 days are the strongest predictors of variation in average nightly activity inside Chateau Cave.

4.3.2.2 Models of PLNB activity for CCout

Several GLMMs were also evaluated to assess the influence of natural climatic variation in addition to disturbance from mining and cave entry as predictors for average nightly activity outside the entrance to Chateau Cave.

The baseline model, which only included the temporal autoregression without additional environmental or anthropogenic predictors, provided almost the highest AIC and BIC values, indicating a poorer fit than the other models. This suggested that survey period or temporality alone does not adequately explain the variability in PLNB activity. The best-fitting model (AIC = 6071.5, BIC = 6104.8), incorporated moon illumination, humidity, cumulative rainfall, and cave entry as predictors. This was a much stronger result than was observed in models of activity for the inside of Chateau Cave where only moon illumination and cumulative rainfall were significant in the most parsimonious model. The highly significant Chi-square value (Chi-sq = 61.0675, $p < 0.001$) indicates that this model offers the most robust fit.

This model indicates that, like for the detector inside Chateau Cave, lunar illumination causes a significant decline in PLNB activity patterns as does cumulative rainfall. Unlike the inside detector however, increasing humidity was also significant in explaining PLNB activity and caused a significant decline in activity. The relationship between cave entry and reduced bat activity was of note, as this was the only significant disturbance factor.

Other models incorporating variables such as artificial light, general mining disturbance and blasting along with combinations of wind, temperature, and barometric pressure did not significantly improve model fit compared to the baseline. These results imply that moonlight, humidity, and recent rainfall are the primary environmental drivers of bat activity, with cave entry also playing a notable role, while other predictors had less impact in this context. Because cave entry was required to collect scat samples and service recording devices every month, and this monthly visit tended to coincide with a new moon (when PLNB activity was higher), the effect of cave entry on PLNB activity may be larger than suggested by the model. However, the effect of cave entry was not significant for the PLNB activity within the roosting chamber of Chateau, i.e no decline in activity could be detected inside the cave, although a decline was observed outside.

4.3.3 Dalton’s Roost

Dalton’s Roost is a permanent diurnal roost located approximately 30 km southeast of Chateau Cave on the periphery of the study area. Consistently high levels of activity have been recorded from the Dalton’s Roost site to date with 8.1 million PLNB calls recorded. Data recorded from January – March 2022 and November 2022 was excluded from the analysis due to technical issues with the recorder. Between June 2021 and October 2023, 11,260 PLNB calls were recorded on average per night and in excess of 5,000 calls per night has been recorded on 92% of nights. A decrease in activity was recorded in the most recent data from November 2023 to January 2024.

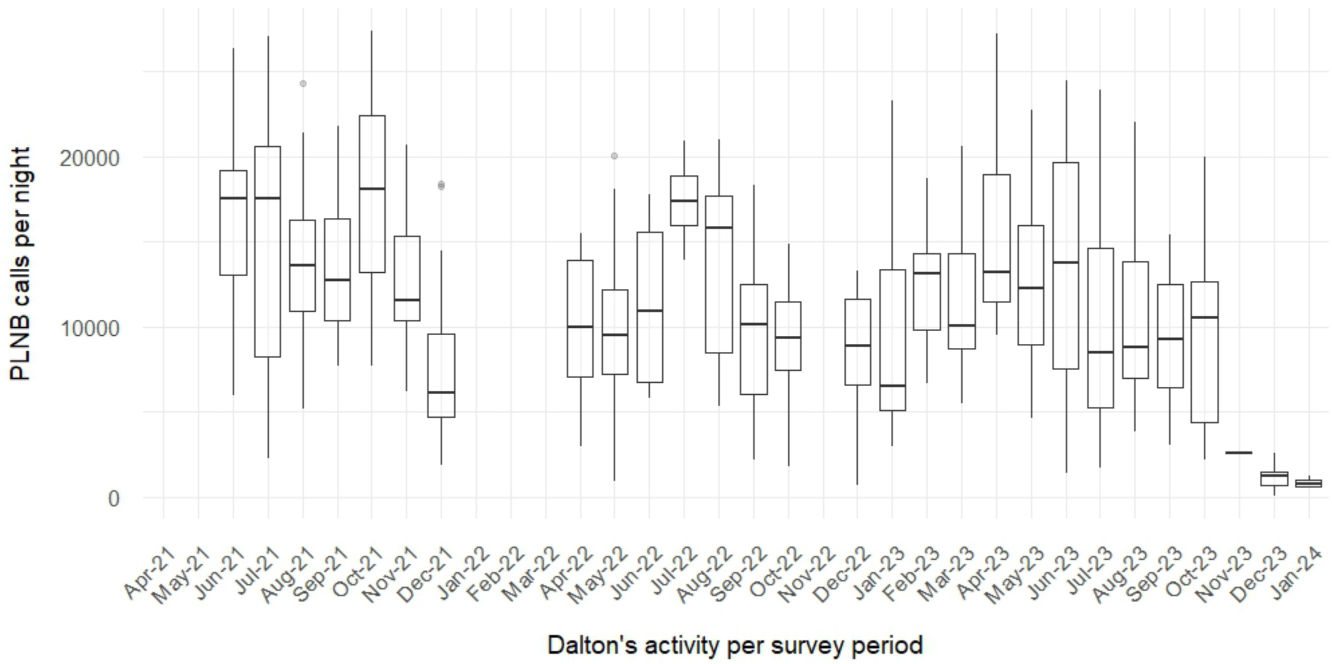


Figure 7 **Boxplot of Dalton’s Roost PLNB calls per night from June 2021 – January 2024**

4.3.4 Cave 13

Cave 13 is located 500 m from Chateau Cave, on the same ridgeline. Analysis of data collected between April 2021 and October 2023 has shown that Cave 13 generally exhibited low levels of activity (48,924 calls recorded) with higher activity in cooler months, consistent with activity seen at Chateau Cave. Average nightly activity was 76 PLNB calls per night, but large outliers of over 1,000 calls per night occurred in June 2021, December 2022 and January 2023 (Figure 8). The data collected over the last 36 months confirms that PLNB rarely roost in this cave, therefore it is considered that activity is primarily a result of visitation from Chateau Cave bats and that the cave is used as a temporary diurnal roost and/or nocturnal roost.

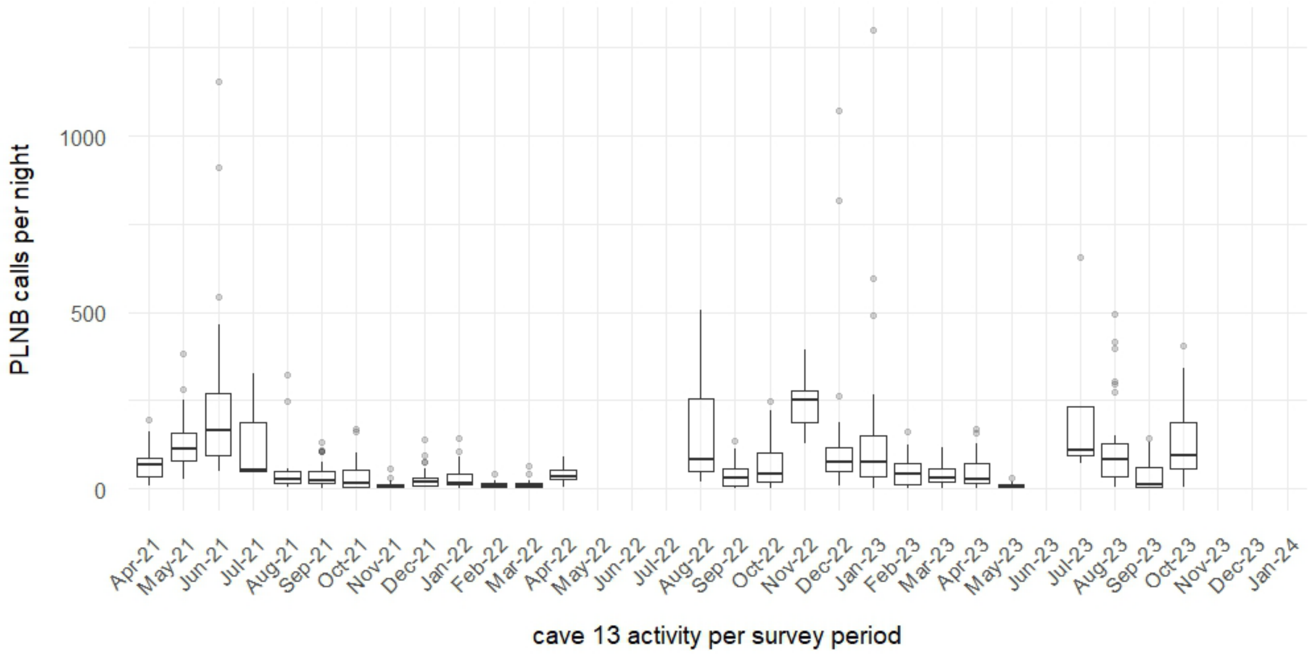


Figure 8 Boxplot of Cave 13 PLNB calls per night from April 2021 – January 2024

4.3.5 Python Cave

Python Cave is located 5 km from Chateau Cave and on the same ridgeline as both Chateau Cave and Cave 13. Python Cave is considered a temporary diurnal roost and few PLNB have been observed roosting in the cave, although it is regularly visited by foraging bats. Analysis of calls recorded between April 2021 to January 2024, have identified 86,592 PLNB calls. Activity at Python Cave was generally higher than Cave 13 but lower than Chateau Cave. Like Cave 13, average nightly call activity is low, but occasionally calls were in excess of 1,000 calls per night (Figure 9). Activity tended to be highest between 9pm and 2am, in contrast to the permanent diurnal roosts that had highest activity in the first few hours after sunset and just before sunrise. Patterns in activity were strongly associated with time of year with the highest activity occurring in the winter months. Activity also appeared to be lower overall in the third year of monitoring.

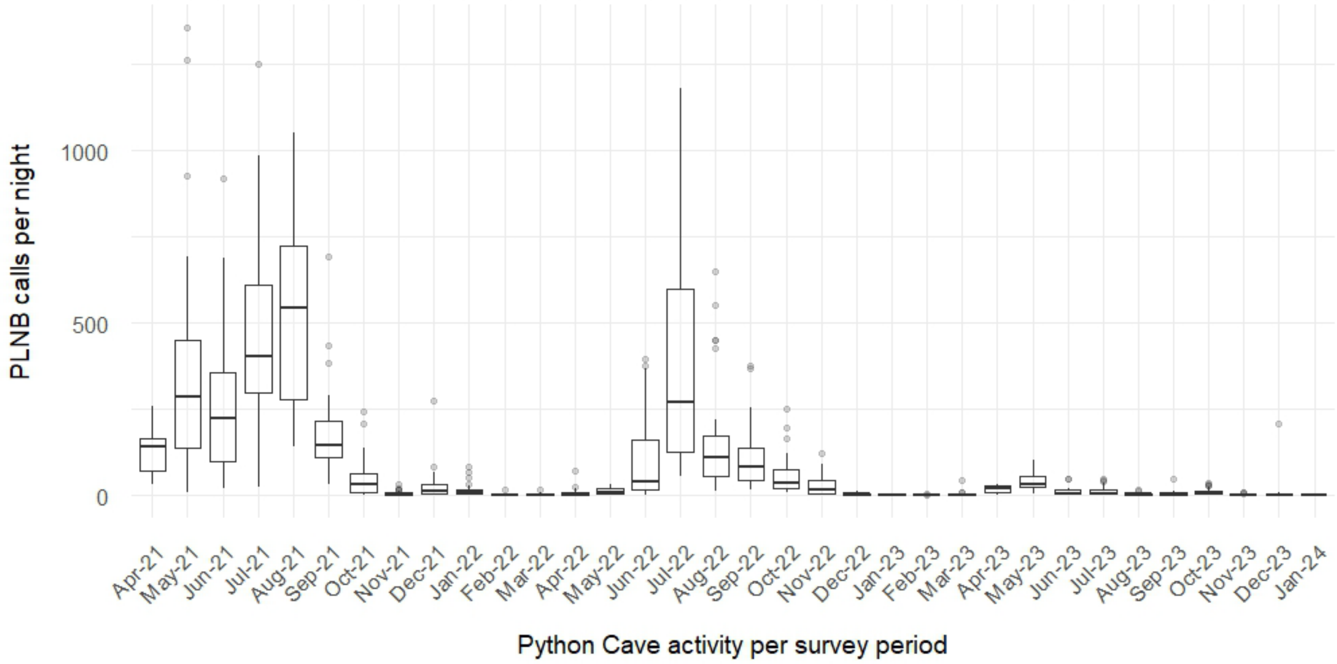


Figure 9 Boxplot of Python Cave PLNB calls per night from April 2021 – January 2024

4.3.6 Mundagoora Pool

Mundagoora Pool provides foraging habitat for PLNB and is located 2.5 km south of Chateau Cave and is the closest permanent natural water source to the cave that is outside the mining activities. From April 2021 to January 2024, 66,695 PLNB calls have been recorded at this site. Activity at Mundagoora Pool was above 100 calls per night initially, but then dropped substantially in November 2021. At this time the detector was moved to the other side of the pool (a distance of 20 metres) to reduce the risk of flood damage. The following reduction in activity was assumed to be associated with the adjustment to detector location, however activity increased again between October and December 2022 and then again more noticeably from May to November 2023 (Figure 10).

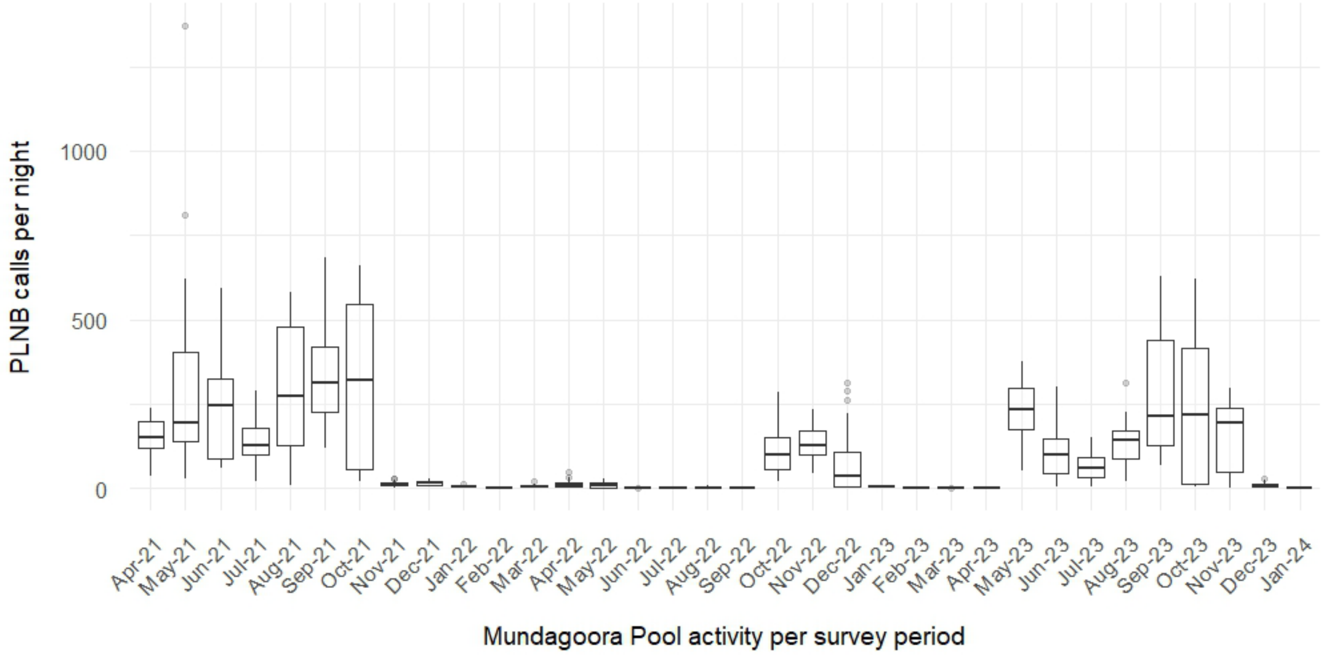


Figure 10 ***Boxplot of Mundagoora Pool PLNB calls per night from April 2021 – January 2024***

4.3.7 Joe's Cave

Joe's Cave is located 8 km to the west of Chateau Cave and is considered a transitory diurnal roost. To date there are no observations of PLNB roosting within the cave, however pre-civil twilight calls have been recorded from the cave (GHD 2021) suggesting may be very occasionally used as a transitory diurnal roost. To date, 117,395 PLNB calls have been recorded here. Activity at Joes Cave is extremely variable but tends to be highest in the cooler months of the year (Figure 11).

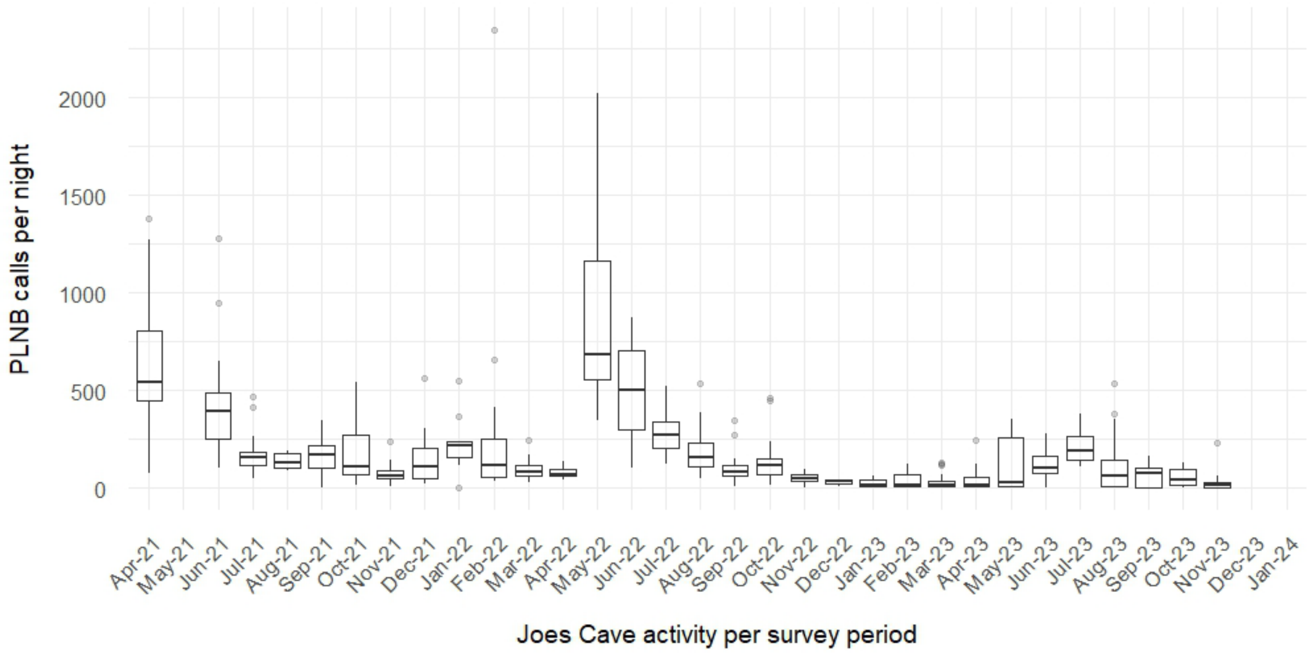


Figure 11 Boxplot Joe's Cave PLNB calls per night from April 2021 – January 2024

4.4 Discussion

The 33 months of data collected from long term monitoring sites has demonstrated that PLNB are present over much of the study area during all months of analysis. Although PLNB presence is reliably detected at both the temporal and spatial scales, there is significant variation in activity between all sites and monitoring periods, even permanent diurnal roosts such as Chateau Cave and Dalton's Roost. Although activity data from acoustic detectors cannot be used to derive estimates of abundance, the significant variation in activity recorded adds additional evidence to the results from genotyping and the visual monitoring (discussed in Section 6) that movement of individual PLNB is occurring at the landscape scale.

The modelling of PLNB at permanent diurnal roosts to environmental variables such as weather, fire, moon illumination and mining related disturbance indicates influences on activity vary between roosts (Chateau Cave and Dalton's Roost) as well as between detectors at the same roost (CCin versus CCout). While each permanent diurnal roost detector site had a unique set of predictor variables, the most parsimonious model for each site included moon illumination. This suggest moon illumination is consistently associated with PLNB activity, and activity declines as moon illumination increases. Sensitivity to moonlight (Lunar phobia) is widely reported for a range of bat species (Zeppelini et al. 2019) but bat species with tropical origins are reported to be most sensitive to moon light, as are bats with constant frequency echolocation. This confirms that PLNB would be sensitive to moon illumination as bats with CF echolocation and coming from a family of bats usually associated with tropical environments. The common observation of delayed emergence from cave roosts (usually not until after civil twilight) also points towards sensitivity to light (Bat Call WA 2021).

Cumulative rainfall was weakly predictive for reduced activity at Chateau Cave, this may be a characteristic of permanent diurnal roosts that do not function as a maternity roost as a similar relationship was not observed in Dalton's Roost models. Churchill (1991) proposed that *Rhinonicteris aurantia* abandons caves during the wet season in tropical savannahs because increased insect activity and humidity allows roosting in a greater variety of locations. This was based on Churchills observations that populations in accessible roosts dramatically decreased, and the colony membership became almost entirely male. This fits with observations at Chateau, however its more probable the female bats relocate to maternity roosts at this time, rather than occupying sub-optimal roosts elsewhere. Cave entry for scat collection and servicing of equipment once a month also had a marginal significant effect on PLNB activity, albeit only for CCout. The CCout model was the most complex, with humidity also a significant predictor of PLNB activity. All predictors were negatively correlated with PLNB activity, and so PLNB activity at Chateau Cave is predicted to be highest with low humidity, low levels of moon light, when no cave entry occurs and with increasing time since rainfall.

An important finding was the significance of fire in determining PLNB activity at Daltons Roost. Activity per night was higher before the fire in November 2021 occurred at this roost. The literature on bats response to fire is mixed but does tend towards reporting increased activity following fire. Insect abundance is often reported to be higher after fire (Doty et el. 20161). Foraging is also likely to be more successful in burnt habitat as insects have less cover to shield them from discovery. One possible explanation for why activity at Dalton's Roost declined following fire may relate to increased visitation from feral cats. Predation of PLNB by feral cats is a known issue at Dalton's Roost (Moyses et al 2024) and the removal of mature spinifex by hot fires has been correlated with increased cat activity in other parts of Australia (Moore et al. 2024). Further research focussed on determining if interactions between cats, PLNB and fire exist would be a valuable contribution to conservation efforts for PLNB in the Pilbara.

There is little long-term data for other PLNB roosts in the Pilbara in the public domain and is limited to annual counts (e.g. the Cane River Roost counts undertaken by Bat Call WA). In this study, the differences between activity patterns observed at temporary diurnal roosts and pool sites appear to be linked to proximity to a permanent diurnal roost, with sites close to Chateau Cave recording activity levels that reflect the patterns observed at Chateau Cave, however this has not been formally tested. In contrast, sites further away from Chateau Cave tend to have more variability in recorded activity.

The activity at Chateau Cave was highest in the cooler months. This pattern was also evident, though to a lesser extent, in activity recorded at Python Cave and Cave 13 which are located on the same ridgeline as Chateau Cave.

Activity at Dalton's Roost previously contrasted with Chateau Cave by having sustained high levels of activity throughout the year. Recent activity at Dalton's Roost has significantly departed from the previously recorded levels and is (as of January 2024) extremely low. The reason for this decline in activity is unknown but coincides with the assumed birth and lactation period and contrasts with the same period in the year before. Of interest is the observation that the highest levels of activity also seem to coincide with cooler months (although this is variable between years) despite the cave functioning as a maternity roost between December – February (identified by levels of progesterone and presence of pregnant females, described in GHD 2022b and GHD 2023b).

One explanation for the significant variation in activity between permanent diurnal roost sites like Chateau Cave and Dalton's Roost and between months at the same roost may be related to the extreme variability in rainfall and primary productivity both with and between years in the Pilbara (Morton et al. 2011). All sites monitored as part of this study are located close to the eastern boundary of the PLNB range. PLNB can travel up to 60 km in a night (FMG 2024 unpublished), and the population is known to be panmictic, with no genetic structuring in different regions of the Pilbara (Umbrello et al 2022). With a network of at least 22 permanent diurnal roosts across the Pilbara, and many more temporary diurnal roosts, highly mobile fauna such as PLNB have the potential to track resources, temporally and spatially.

Behaviour of individuals may also play a role in determining the activity measured at a roost. Little is known of the breeding biology of PLNB, and in particular the roost fidelity of individuals, however the first twelve months of PIT tag data from Chateau Cave and Dalton's Roost along with the genetic mark-recapture analysis conducted over nearly three years indicates that overall, there is low site fidelity and regular landscape scale movements between roosts by the majority of individuals while a small proportion (usually males) show a high level of site fidelity.

Viewing the acoustic data in this context, changes in activity patterns may be driven by individuals spending more time at roosts (i.e. during phases of the breeding cycle) or by individuals tracking resources in an unpredictable environment. Further research focussed on addressing knowledge gaps around the social behaviour of PLNB, such as site fidelity, group cohesion, behaviour during mating, birth and the maternity period as well as juvenile dispersal is required to fully understand patterns in PLNB activity. The observed variability in activity levels across sites and seasons also underscores the value of continuous monitoring. The observed variability in activity levels across sites and seasons underscores the need for continuous monitoring and adaptive management strategies.

5. Monitoring of Chateau Cave – environmental variables

5.1 Purpose

Ongoing monitoring for selected environmental parameters at Chateau Cave has been undertaken to:

– Describe and document the internal temperature and humidity to inform the construction of artificial roosts (see Section 4.1 of the Plan) in addition to providing long term data on microclimate to better understand the characteristics of PLNB permanent diurnal roosts.
– Increase baseline knowledge of the response of PLNB to natural variation in their roost environment, in addition to providing qualitative data to any response PLNBs may have to mining activities.

5.2 Methods

5.2.1 Climate of roost and surrounding area

Consistent with monitoring undertaken since 2021, Protech model QP6013 Dataloggers (Electus Distribution) were used to record temperature and humidity in Chateau Cave for 10 months (April 2023 – January 2024). Ten dataloggers were installed into crevices in the walls and ceiling of Chateau Cave with the sensor end of the datalogger clear of obstructions (Figure 12). Two additional loggers were installed into ceiling chutes within the main chamber for two days in September 2023. The loggers were all positioned so that they were touching the rock walls of the cave and therefore temperature readings should be consistent with what PLNB are exposed to when roosting. No data was collected between late July and mid-October 2023 due to technical issues with loggers.

External ambient conditions outside the cave were measured at the Nates Tower Weather Station approximately 800 m west of Chateau Cave. Temperature and humidity records were downloaded for dates corresponding to the datalogger deployment. From these values the median and quartile ranges were calculated to compare humidity and temperature records between locations within Chateau Cave and Nates Tower and presented as box plots consistent with reporting to date.

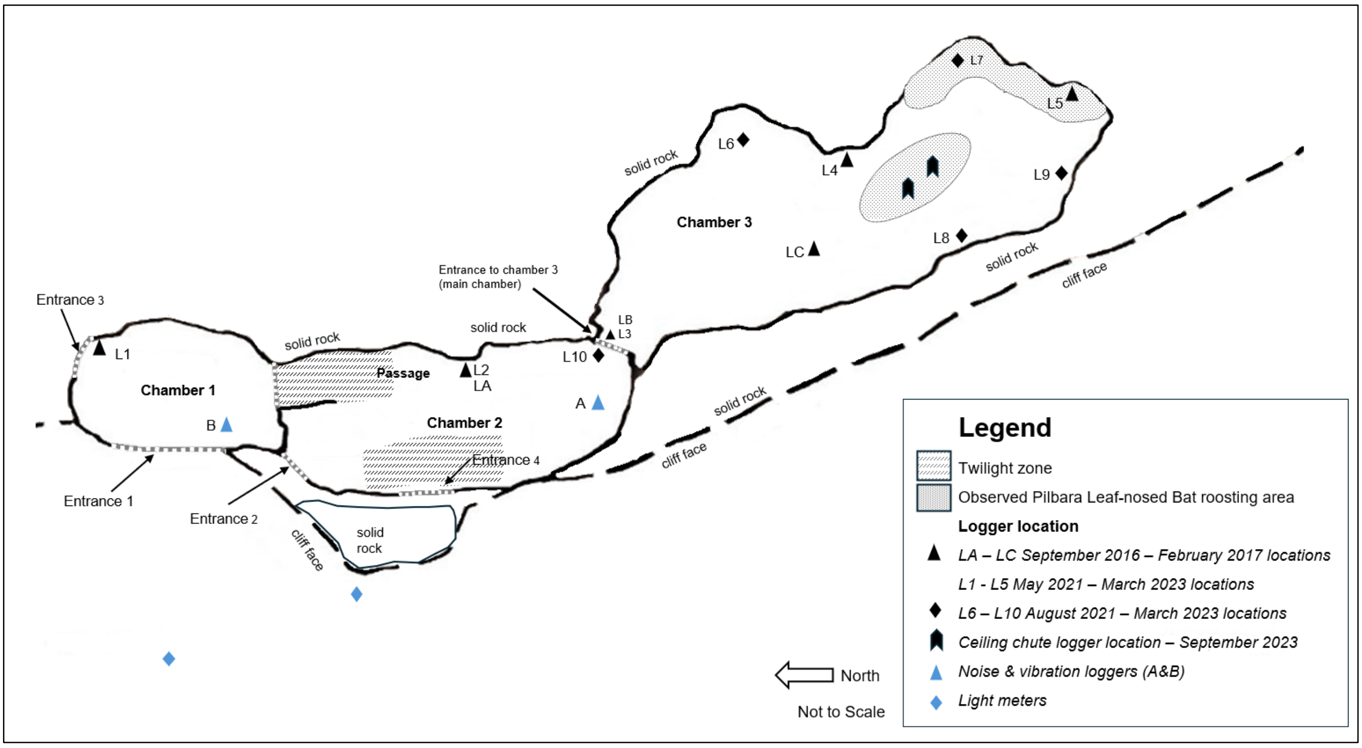


Figure 12 ***Location of the temperature and humidity loggers, noise and vibration monitors and light meters***

5.2.2 Noise and vibration monitoring

Two SVAN958A (SN 92867 (Monitor A, Chateau Cave Chamber 2) and SN 92873 (Monitor B, Chateau Cave main chamber entrance 1; Figure 12) noise and vibration monitoring systems were deployed within chamber 1 and chamber 2 (but outside the main roosting chamber) to concurrently record noise and vibration data at 15-minute intervals over a period of three years from 1 July 2021 to 30 Jun 2024.

The equipment settings were as follows:

- Channels 1 to 3: Tri-axial accelerometer, recording ground acceleration, converted to vibration velocity
- Channel 4: Microphone at a height of 0.3 m (Logger A) and 0.8 m (Logger B) above local ground, recording ambient noise parameters such as energy averaged, minimum and maximum noise levels. Noise levels were recorded with the linear (i.e. no weighting), A-weighting (weighting suitable for human hearing) and the C-weighting (weighting suitable for low-frequency noise)
- The equipment holds valid calibration certificates from a NATA accredited laboratory
- Summary noise and vibration results were extracted for every 15-minute period.

5.2.3 Light equipment monitoring

Two Extech Instrument SDL400 Real-Time Datalogger Light Meters were deployed to measure artificial light impacts at Chateau Cave. The two light meters have been continuously located at Chateau Cave Entrance 1 and Chateau Cave Side 2 for 11 months (1 March 2023 to 31 January 2024) (Figure 12).

The light meters were set to continuously log light levels in each location every 60-seconds, with data collected from instrument SD cards each month. Continuous data is not always available due to interruptions in logging, primarily due to issues with battery/solar power setups. Therefore, the recorded 60-second data was averaged to provide 15-minute average lux levels which are presented in this report.

5.2.4 Ambient environment

5.2.4.1 Ambient noise

The following metrics (summarised over 15-minute periods) have been analysed as part of the noise monitoring to quantify the ambient noise environment:

- L_{AF90} – The A-weighted statistical noise level that is exceeded for 90% of the measurement period. The L_{AF90} is often used as a descriptor of background noise in airborne noise assessments
- Maximum noise level (L_{max}): A-weighted, C-weighted and linear (no weighting)
- Energy averaged noise level (L_{eq}): A-weighted, C-weighted and linear (no weighting)
- Minimum noise level (L_{min}): A-weighted, C-weighted and linear (no weighting).

All three weightings (A, C and Lin) are examined as the A weighting was designed for the human auditory system and may not necessarily be relevant to understanding of noise impacts on bats. The A, C and linear (no weighting) curves are shown in Figure 13.

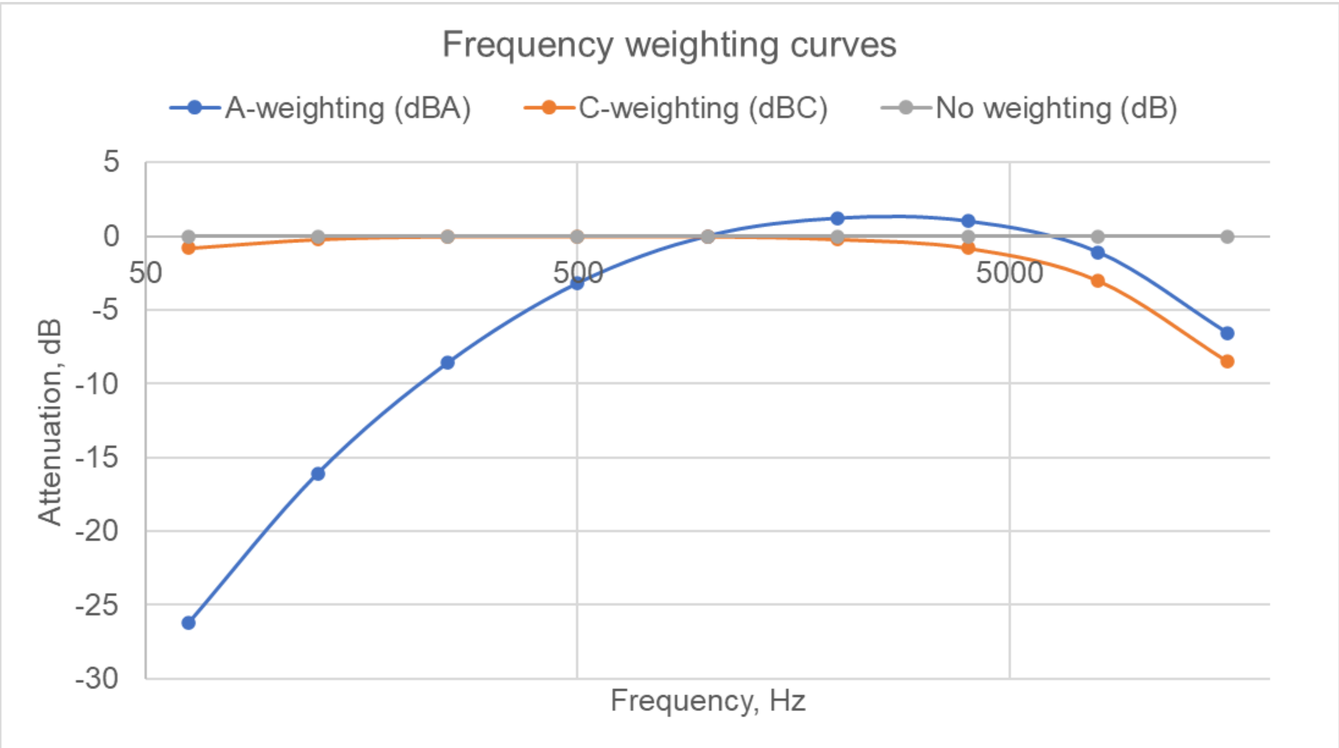


Figure 13 ***Frequency weighting curves***

The following metrics (summarised over 15-minute periods) have been analysed as part of the vibration monitoring to quantify the ambient vibration environment:

- Peak x, y and z vibration level in mm/s
- Root mean square (rms) x, y and z vibration level in mm/s
- Peak vibration velocity (mm/s) criteria are often used in structural vibration assessments while long term rms vibration levels can be used to quantify the background vibration environment.

5.2.4.2 Ambient light

Sources of abiotic data measured included when:

- Light towers within the active mining area were on or off
- Cave entry for scat collection (Section 3) by ecologists was undertaken
- Blasts occurred in the Active Mining Area as part of mining operations.

Ambient light levels over night (with moonlight) in remote areas without influence from artificial light are considered dark sky areas, with a lux level of <1. Light monitoring at Python Cave was undertaken to provide a reference, which was not impacted by artificial light demonstrates lux levels of <1 continuously during the night period, as represented in Figure 14.

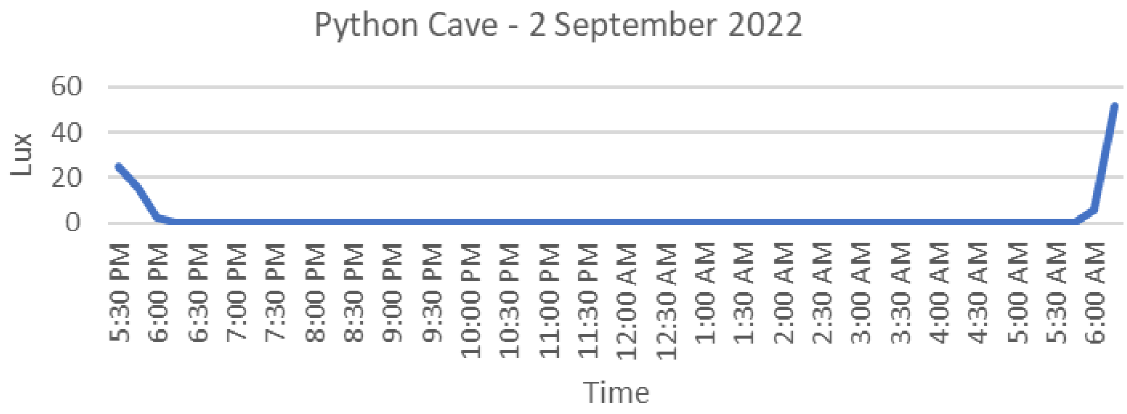


Figure 14 Measured lux levels at Python Cave on 2 September 2022

5.3 Results

5.3.1 Climate of roost and surrounding area

A summary of the temperature and humidity data recorded in Chateau Cave during the 2023-2024 monitoring period are presented in Figure 15 to Figure 18. Despite the data gaps, the results from this monitoring period are consistent with previous monitoring and demonstrate the temperature and humidity of Chateau Cave (particularly the main chamber) has remained stable throughout the current monitoring period, and throughout the three years of monitoring when compared to the climate of the surrounding environment.

The main chamber has maintained a consistent temperature with very low variation for the three years of monitoring (mean = 30.16 ºC, SD = 1.05, range = 6.5, min = 26.1 ºC, max = 32.6 ºC). Humidity within the main chamber displayed higher variation (mean = 58.48 RH%, SD = 11.11, range = 59.2, min = 36.7 RH%, max = 95.9 RH%) over time particularly during the 2023 – 2024 monitoring period (see Figure 18). The humidity within the main chamber is consistently higher and less variable than the humidity within other parts of the cave, and the surrounding environment (Figure 17 and Figure 18). The external air temperature and relative humidity (measured at Nates Tower) is more variable than inside Chateau Cave (Figure 15 to Figure 18).

Below mean annual rainfall (c. 232 mm) was recorded for the 2023-2024 monitoring period (83.2 mm for the entire period compared to 296 mm for 2022, source BOM - Marble Bar weather station) which may partially explain the overall lower and variable humidity levels (Figure 19). Humidity levels within the main chamber tended to correspond with the trend displayed for other locations being highly variable throughout the year. Humidity within the main chamber reduced from late April through mid-July 2023 then increased and steadied from late October through January, albeit gaps in data between late July – mid October 2023.

Data recorded by the two loggers located in the ceiling chutes during September 2023 did not provide a point of difference for temperature or humidity, with the measurements sitting within the range of data presented for other loggers within Chateau Cave (e.g. relative humidity: min = 26.7%, max = 57.3% and temperature: min = 28.1 ºC, max = 31.8 ºC). However, it should be noted these loggers were positioned approximately 2.5 m above the main chamber ceiling height, and did not reach the height of the area where bats have been observed to roost.

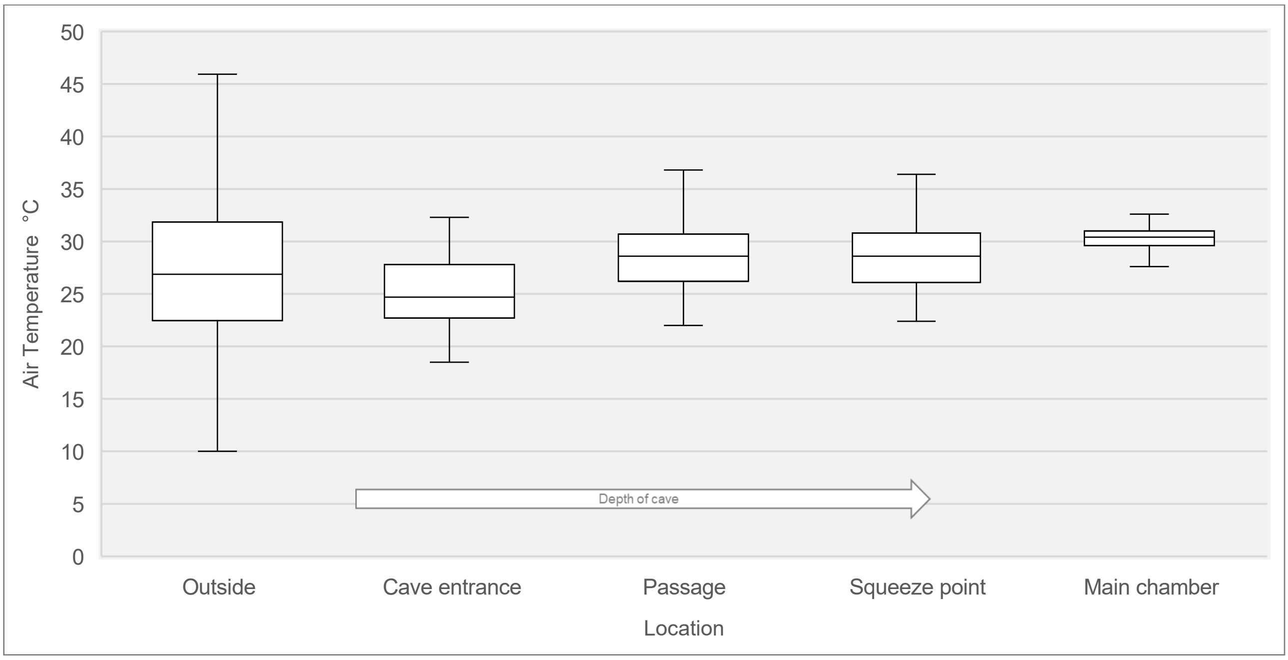


Figure 15 ***Temperature (ºC) of areas within Chateau Cave and the surrounding environment over 10 months of monitoring from April 2023 – January 2024. Box plots show the interquartile range of values for 5 simplified locations: Outside the cave (data from Nates Tower), cave entrance, the passageway leading from entrance to main chamber, the narrow squeeze point which separates the passageway from the main chamber and the main chamber where the PLNB roost.***

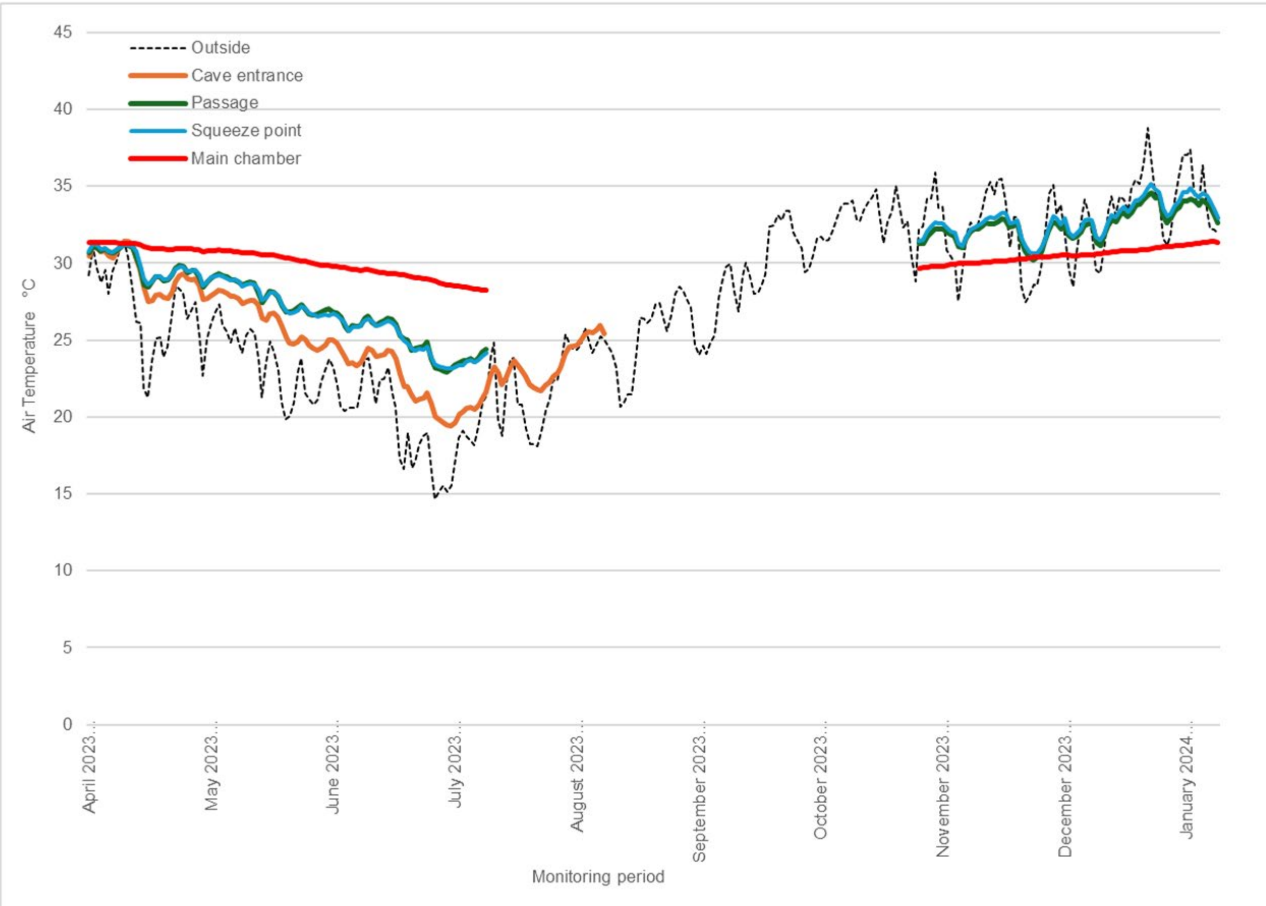


Figure 16 ***Average daily Temperature (ºC) of areas within Chateau Cave and the surrounding environment over 10 months of monitoring from April 2023 – January 2024 for five locations. Despite gaps in data the Main Chamber maintains a constant temperature with low variation and follows the trend corresponding with data from Nates Tower (Outside) and other locations within the cave. Data gaps = missing data due to technical issues with loggers (primarily between late July – mid October 2023)***

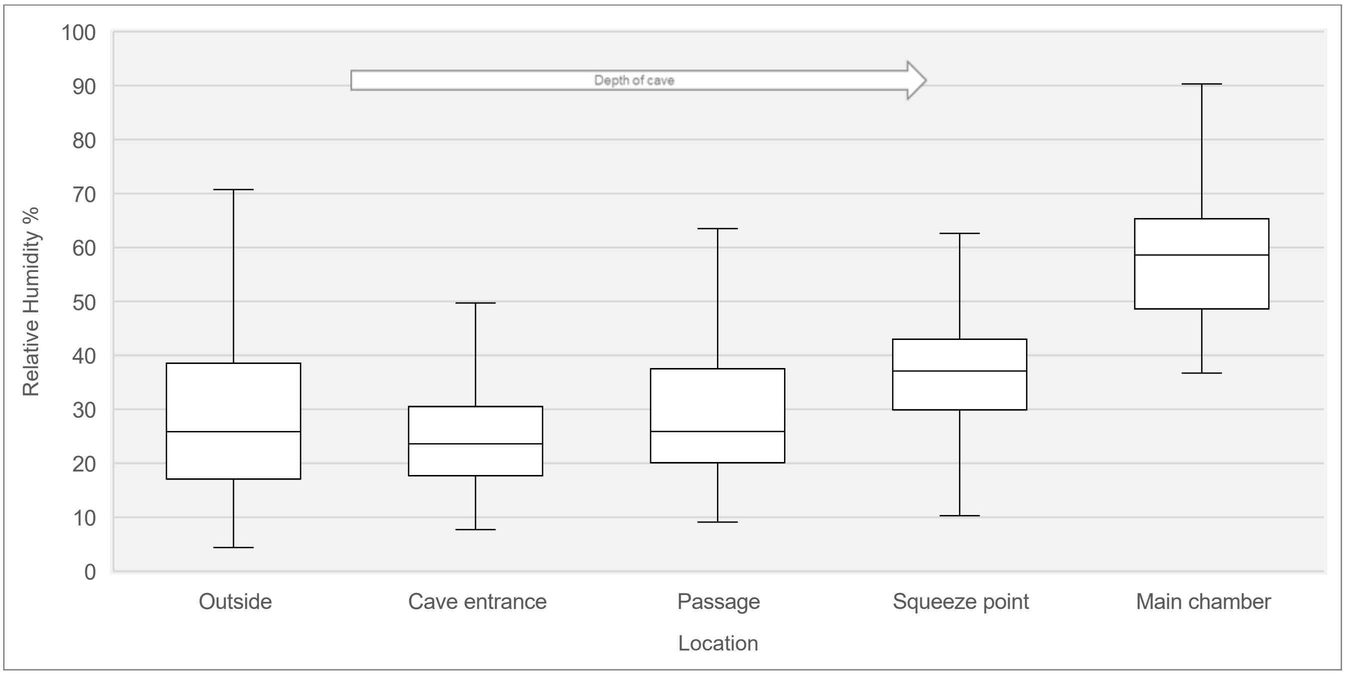


Figure 17 ***Relative humidity of areas within Chateau Cave and the surrounding environment over 10 months of monitoring from April 2023 – January 2024. Box plots show the interquartile range of values for five locations***

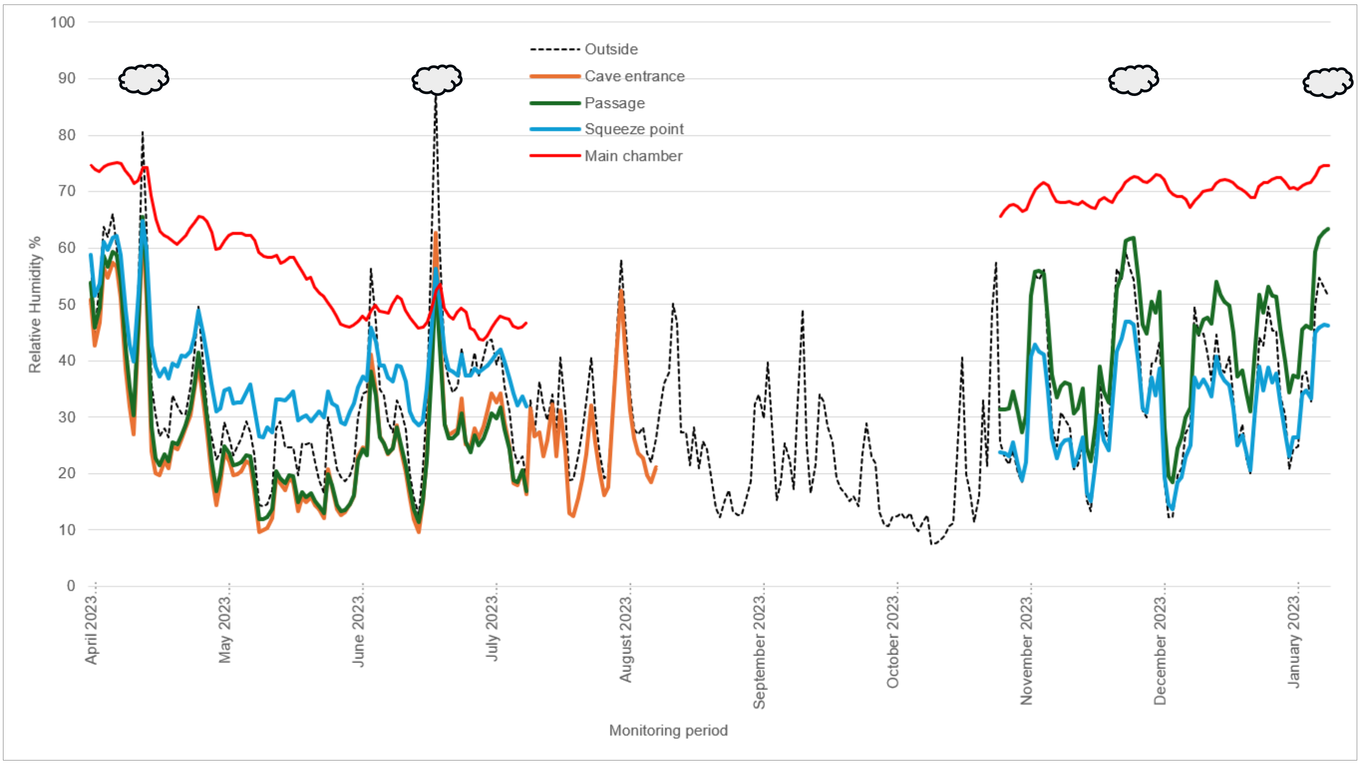


Figure 18 ***Average daily relative humidity for five locations within Chateau Cave and the surrounding environment over 10 months of monitoring from April 2023 – January 2024. Cloud symbols indicate higher periods of rainfall (per month - April 23 mm, June 17 mm, November 4.4 mm and January 3.2 mm) which correspond with elevated humidity levels for the outside monitoring site, squeeze point and passage monitoring points. Data gaps = missing data due to technical issues with loggers (primarily between late July – mid October 2023)***

5.3.2 Noise and Vibration

5.3.2.1 Noise

There was no significant relationship between PLNB activity and noise measured at Chateau Cave (Section 4.3.2). Noise monitoring data has been grouped by year and month and is presented in Figure 19 to Figure 21 for Location A (Chateau Cave Chamber 2) and Location B (Chateau Cave Entrance 1).

The presented metrics (LA90, Leq and Lfmax) generally show similar trends with steadily rising minimum noise levels over time at both the cave entrance and within chamber 2, noting the Leq and Lfmax levels are unweighted.

The increase in LA90 noise levels is attributed to a ramp up in mining activity outside the cave over time. LA90 and other A-weighted noise levels (not shown) at Location B are higher than at Location A, indicating additional shielding from the cave structures.

However, the unweighted levels at both locations (shown in Figure 20 and Figure 21) are similar. As the A-weighting significantly filters out the high and low frequencies to conform to the human auditory response, this indicates that significant noise exists in frequencies below 1000 Hz (as would be expected of typical construction noise sources) which is not significantly attenuated by the cave structures.

The highest maximum noise levels have generally remained consistent with time and are attributed mostly to blasting events. This is discussed further below, where blast times have been matched to 15-minute periods corresponding to the maximum noise events.

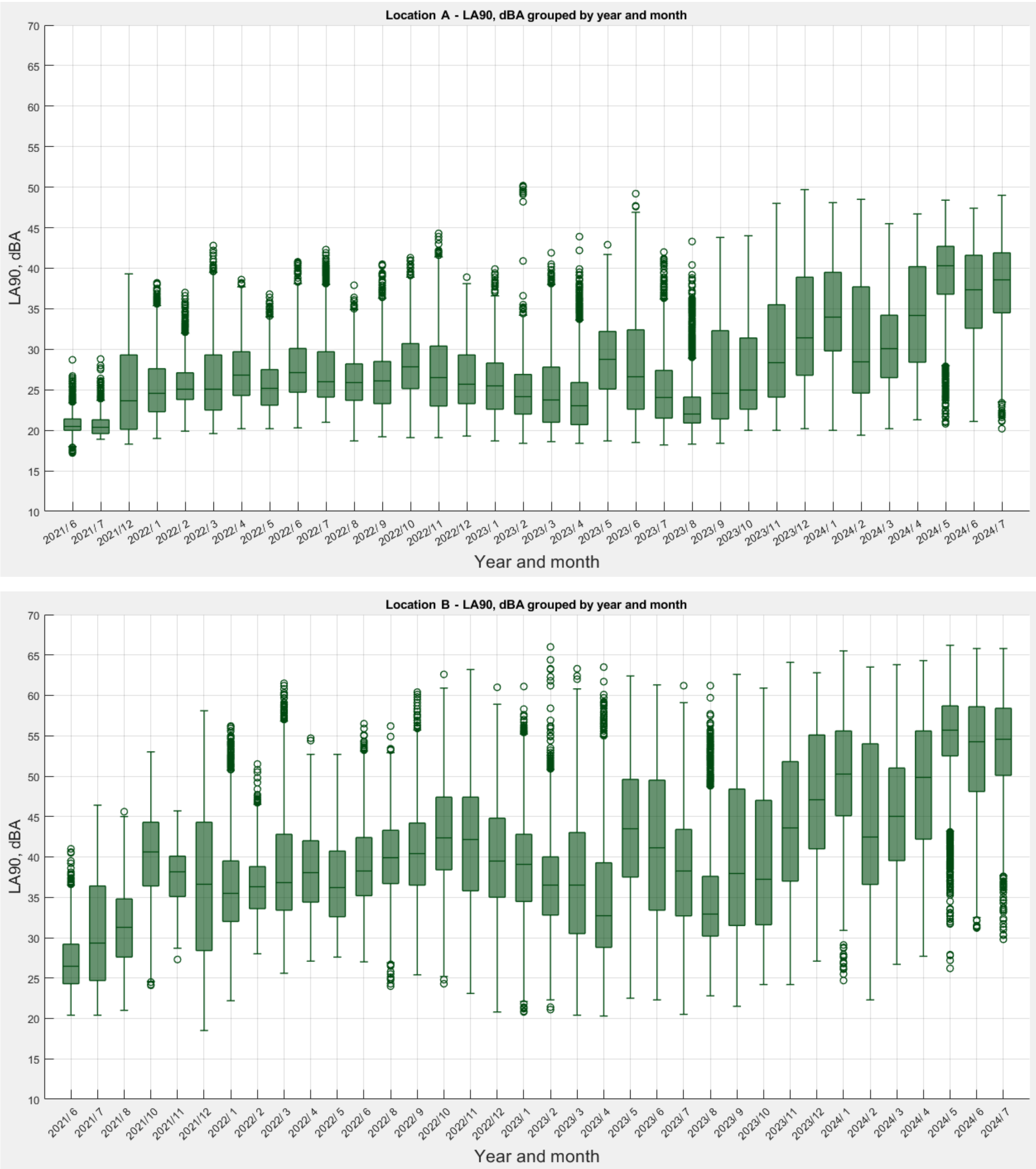
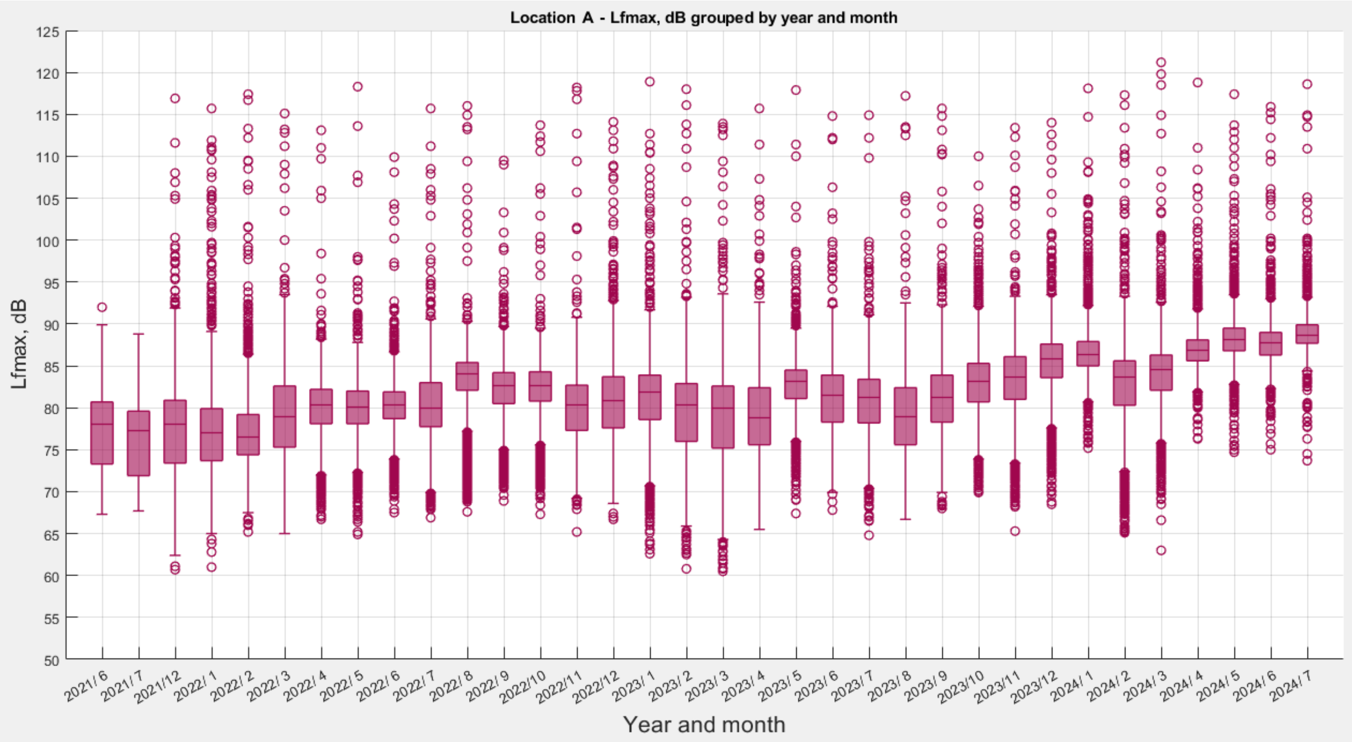


Figure 19 LA90, dBA at Location A (top) and Location B (bottom)

Figure 20 Leq, dB at Location A and Location B



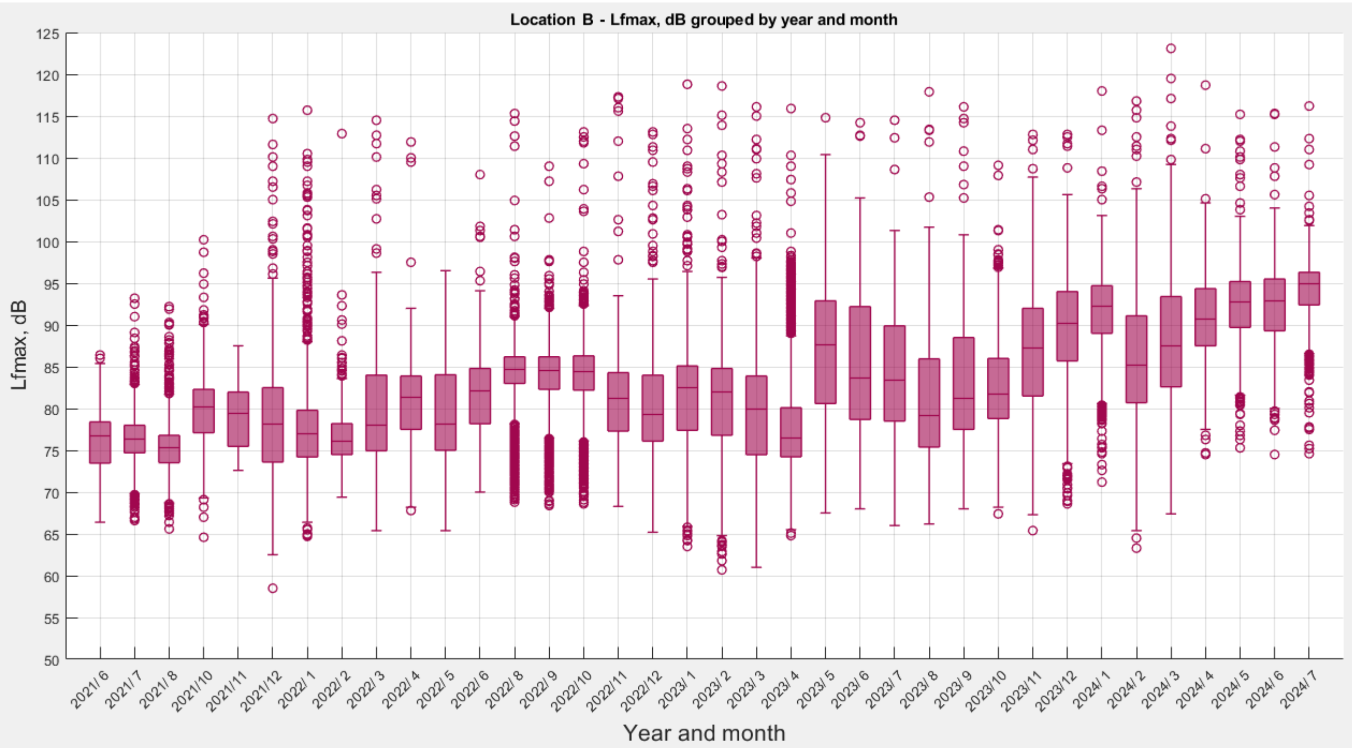


Figure 21 ***Lfmax, dB at Location A and Location B***

5.3.2.2 Vibration

Vibration monitoring data has been grouped by year and month and is presented in Figure 22 for Location A (Chateau Cave Chamber 2) and Location B (Chateau Cave Entrance 1). The figure is limited to the z-axis component of vibration as the other two axes show similar trends.

Vibration levels have generally remained consistent across the monitoring period, with higher levels typically corresponding to blasting events. This is further discussed below.

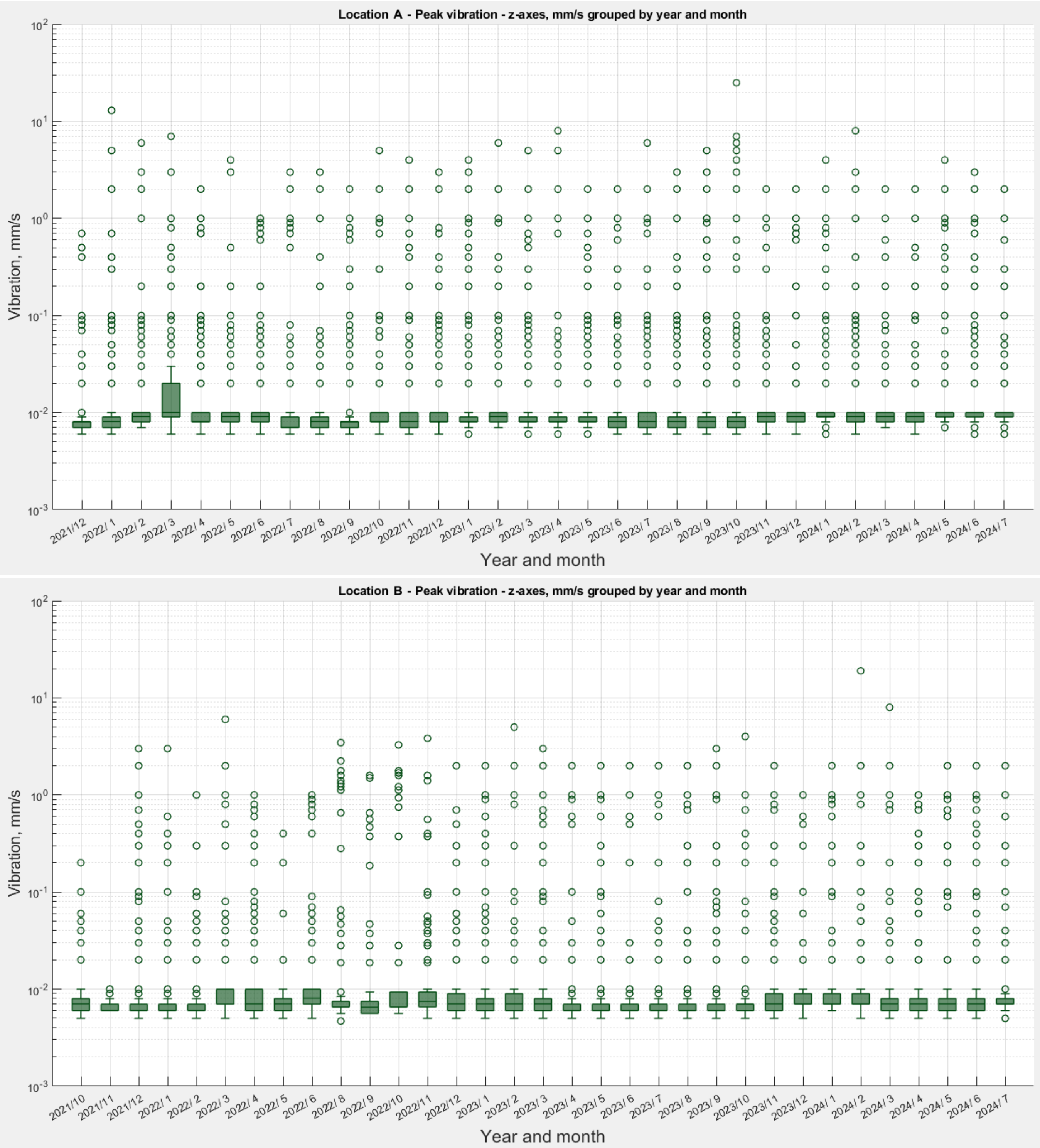


Figure 22 Peak vibration (z-axis), mm/s at Location A and Location B

5.3.2.3 Correlation with blast times

Unweighted maximum noise levels and peak vibration velocity levels[1] are plotted against distance in Figure 23. Maximum noise levels also show a similar scatter, with levels between 75-120 dB for blasts near the cave, decreasing with distance.

Vibration levels decay rapidly with distance, however there is considerable scatter, with levels typically below 6-8 mm/s. The site has adopted a best-practice vibration limit of 10 mm/s at environment sensitive receivers and with the exception of one outlier event, the peak vibration levels from all other blasts were found to be below this limit.

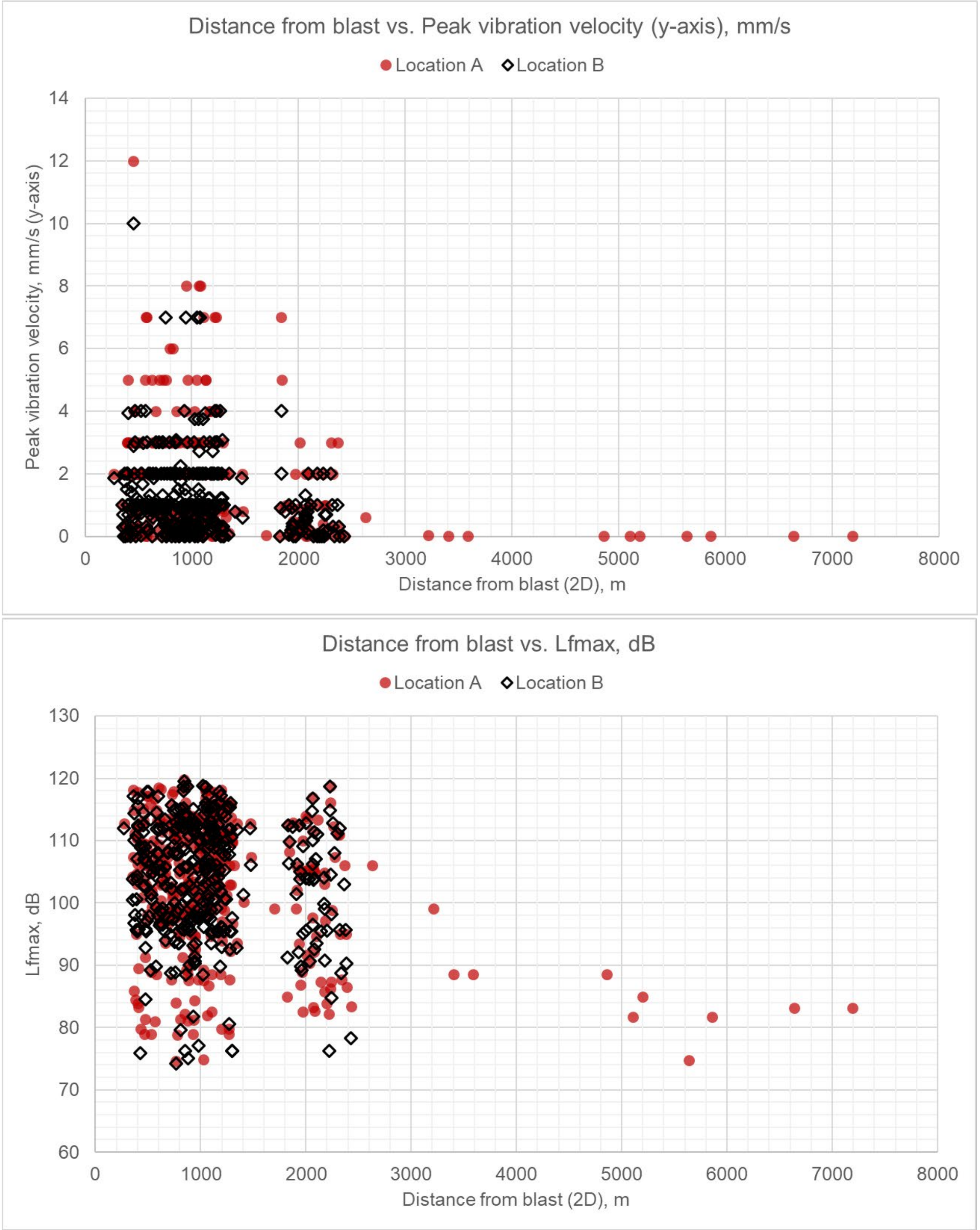


Figure 23 ***2-D distance from blast (m) vs. peak vibration velocity (mm/s) (above) and maximum unweighted noise level (below)***

[1] Note: The y-axis was selected for the vibration plot as the levels in the y-axis were found to be marginally higher than the other axes.

Noise and vibration data was mapped to blast time periods. The blast data has been overlaid as black diamonds on the long-term maximum noise and peak vibration data in Figure 24 for Location A (Chateau Cave Chamber 2). The overlay at Location B shows similar trends and has been omitted. The results indicate the highest maximum noise and vibration levels typically correspond to blasting events.

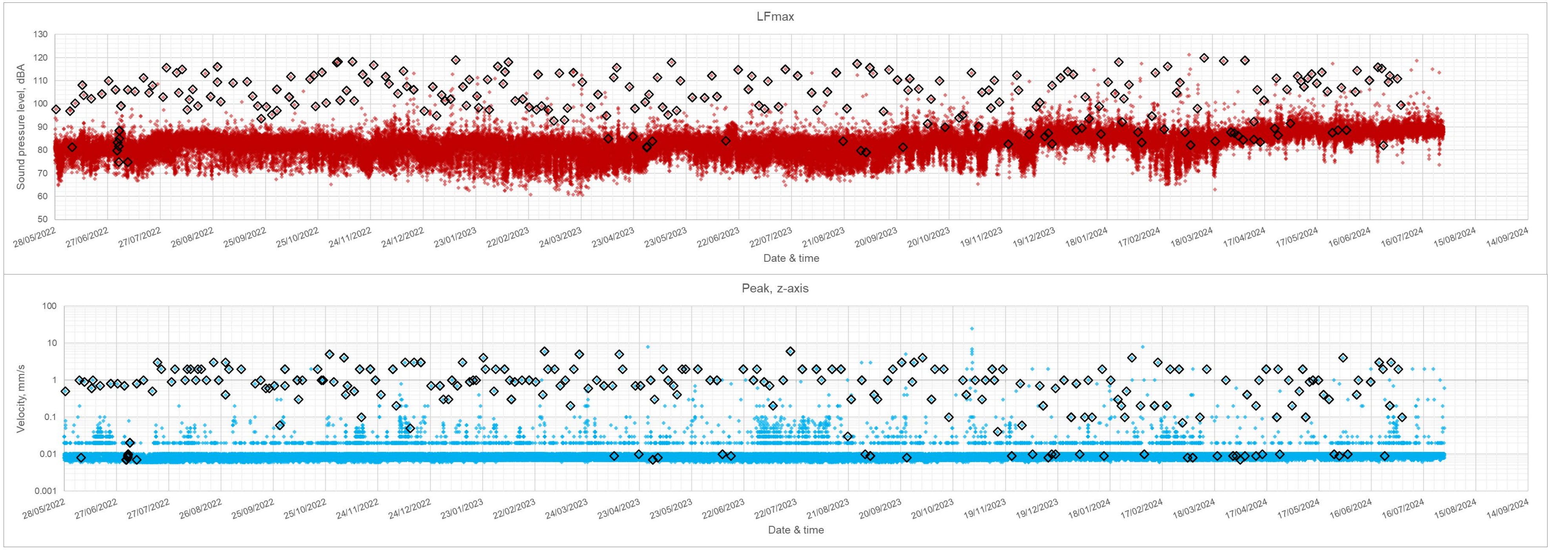


Figure 24 Blast time periods overlaid over long-term maximum noise levels and peak vibration levels (z-axis) at Location A

The number of blasts in a given month have been plotted against the number of peak vibration levels (z-axis) exceeding 0.5 mm/s at both locations in Figure 25. The figure clearly shows a linear increasing trend in the number of vibration peaks with the number of blast events in a month.

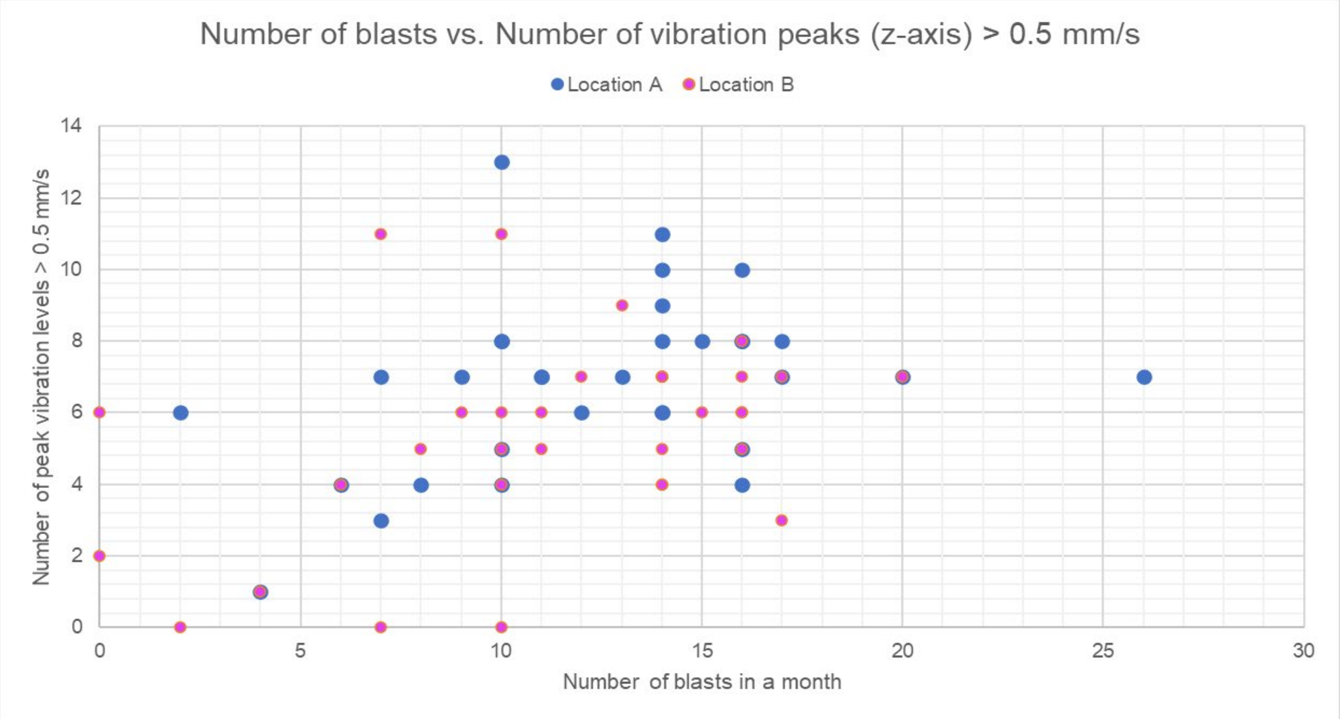


Figure 25 ***Number of monthly blasts vs. number of vibration peaks (z-axis) greater than 0.5 mm/s***

5.3.3 Light

Light monitoring data collected has demonstrated artificial light spills onto the entrance of Chateau Cave, primarily from permanent or temporary light towers and vehicle head lights on the haul road adjacent to Chateau Cave. Data from Python Cave was used to establish reference levels of light without anthropogenic influence. This demonstrated that light levels (lux value) greater than one are likely from light pollution.

Data from near the entrance to Chateau Cave (chamber 1) indicates lux values of up to 6 are occurring regularly during the night period and that the entrance to Chateau Cave is impacted by artificial light (Figure 26). Lux levels of 5 are equivalent to what may be observed in a suburban street, whereas lux level of 0 is indicative of night-time on a dark landscape (such as a remote area, for example Python Cave).

The available data indicates the Chateau Cave Entrance experiences higher measured lux levels compared to the light meter positioned along the access track (Chateau Cave Side 2; Figure 27). Although lux levels at Chateau Cave are higher than reference levels, there are only a few 15-minute periods of elevated lux levels present during each night. The frequency of light levels over five at Chateau Cave Entrance have decreased considerably from April 2024 onwards, potentially due to changed layout of the haul roads and stockpiles in proximity to the primary crusher, resulting in reduced light impacts from vehicles headlights. The new reduced/redirected lighting at the primary crusher was also installed 24 March which would also explain reduced light impacts at the entrance of Chateau Cave.

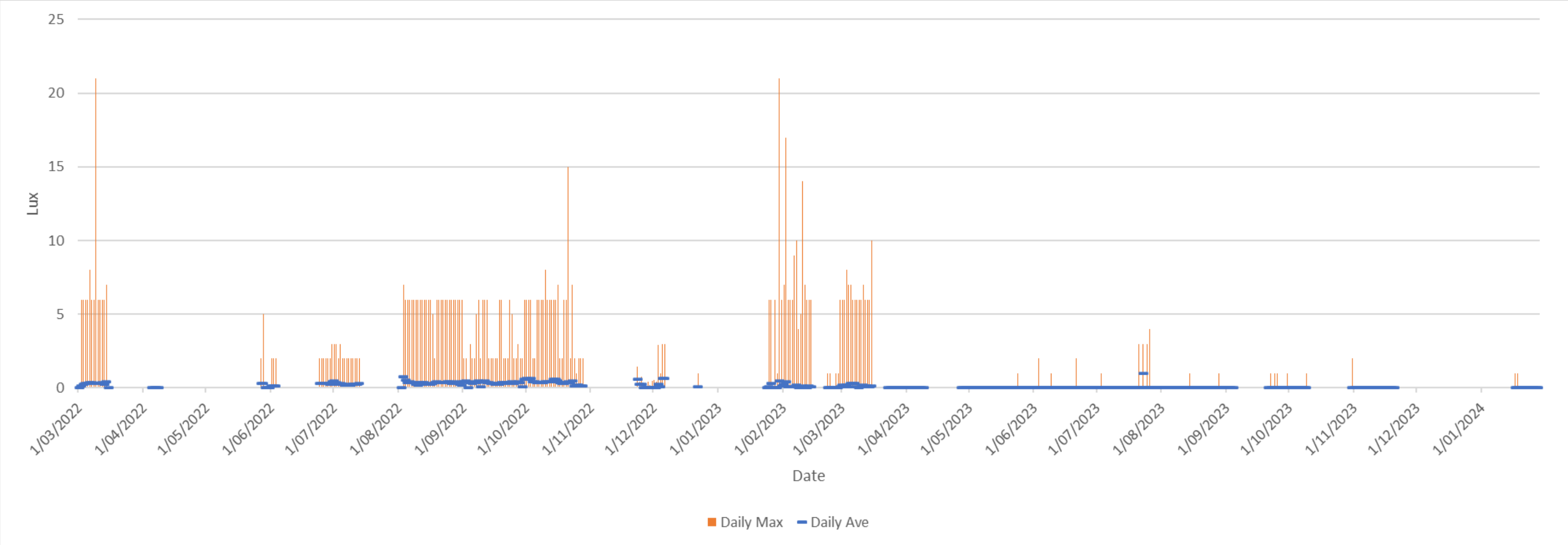


Figure 26 ***Chateau Cave Entrance 1 - Nightly average and maximum lux levels***

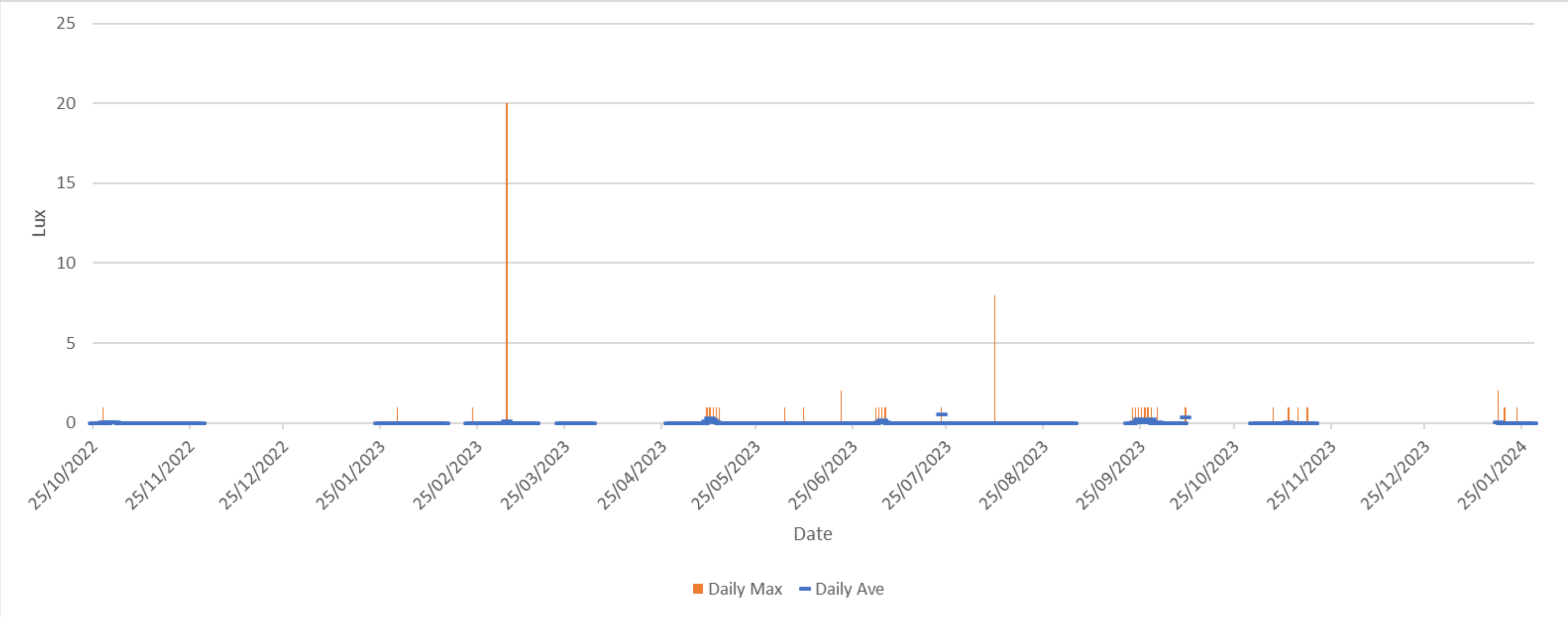


Figure 27 ***Chateau Cave Side 2 - Nightly average and maximum lux levels***

5.4 Discussion

Results are consistent with those reported in the 24-month report (GHD 2023b) which show significantly less temperature and relative humidity (RH) differential within the main chamber of Chateau Cave than other parts of the cave and ambient conditions, as previously reported. There is more variation in RH between the main chamber, other areas of Chateau Cave, and ambient conditions during summer months. Temperature varies more during winter months, though regardless of this variation, the main chamber has a significantly warmer and smaller temperature differential than other parts of the cave and outside conditions.

The RH results demonstrate that RH levels of a permanent diurnal roost can be lower than previously thought, with the main chamber presenting higher than expected RH variation and substantially lower mean (c. >20%) than the 85%-100% reported in D'Rozario (2022) and other studies (e.g., Bat Call WA (2021) and Armstrong (2000)). While less humid, the main chamber temperatures do fall within the ranges of those reported in other studies.

Studies of cave dwelling bats acknowledge the accumulated deposits of guano and bats themselves appear to be the primary modifiers of their own microclimate conditions (Baudinette et al. 1994). The ability of PLNB in Chateau Cave to utilise smaller sub-chambers (e.g. vertical ceiling chutes with narrow apertures) that are likely to have higher, consistent humidity may provide the stable microclimates facilitating year-round roosting within the overall roost complex.

6. Visual monitoring of bat activity within Chateau Cave

6.1 Purpose

Visual monitoring of the Chateau Cave roost using Infrared (IR) video and still image cameras was undertaken to:

- Assist with understanding the demography of the PLNB colony in Chateau Cave by estimating the colony size, particularly the number of PLNB that roost within the main chamber of Chateau Cave (Table 4 of the Plan).
- Assist with monitoring bat activity to understand the response of the PLNB in a known diurnal roost site to anthropogenic changes from mining activity (see Table 3, section 4.2 of the Plan).

In the first 12 months of monitoring, analysis mainly focused on counting bats roosting in the main chamber (locations 3 and 4) and the response of bats to mining activities, due to insufficient data from the passage to the main chamber (location 2). Reporting has since focused on the analysis of emergence data from location 2 (see Figure 28) as sufficient data was collected to support emergence counts. The results herein present data collected from January 2022 – January 2024 for the purpose of estimating the colony size of PLNB within the main chamber of Chateau Cave.

6.2 Methods

Since January 2022, 47 monitoring events were reviewed for bat count data from Location 2 (see Figure 28). Each monitoring event consisted of the following:

- Emergence period count from 5:30 to 9:00 PM each night (monitoring event) including the number of bats observed exiting (out counts) or entering (in counts) the entrance to the main chamber
- Review of a sub sample of the files for validation and contingency
- Review and comparison of acoustic data and bat count data to determine the timing and presence/absence of PLNB and other echolocating bat species and analyse the pattern of activity for PLNB and other bat species for the emergence period
- Presentation of bat count data for two periods: period prior to civil twilight (bc1) and the period from the end of civil twilight until the number of bats counted drastically reduced / or end of footage (bc2)[2]
- Estimate of the number of PLNB roosting in main chamber prior to civil twilight – calculated by bc2 bats out minus bc2 bats in (egress minus ingress)
- The collection, analysis and presentation of data is consistent with the methods presented in the 24-month report (see GHD 2023b Section 6.2).

[2] PLNB are known to emerge relatively late after sunset, usually not before the end of civil twilight

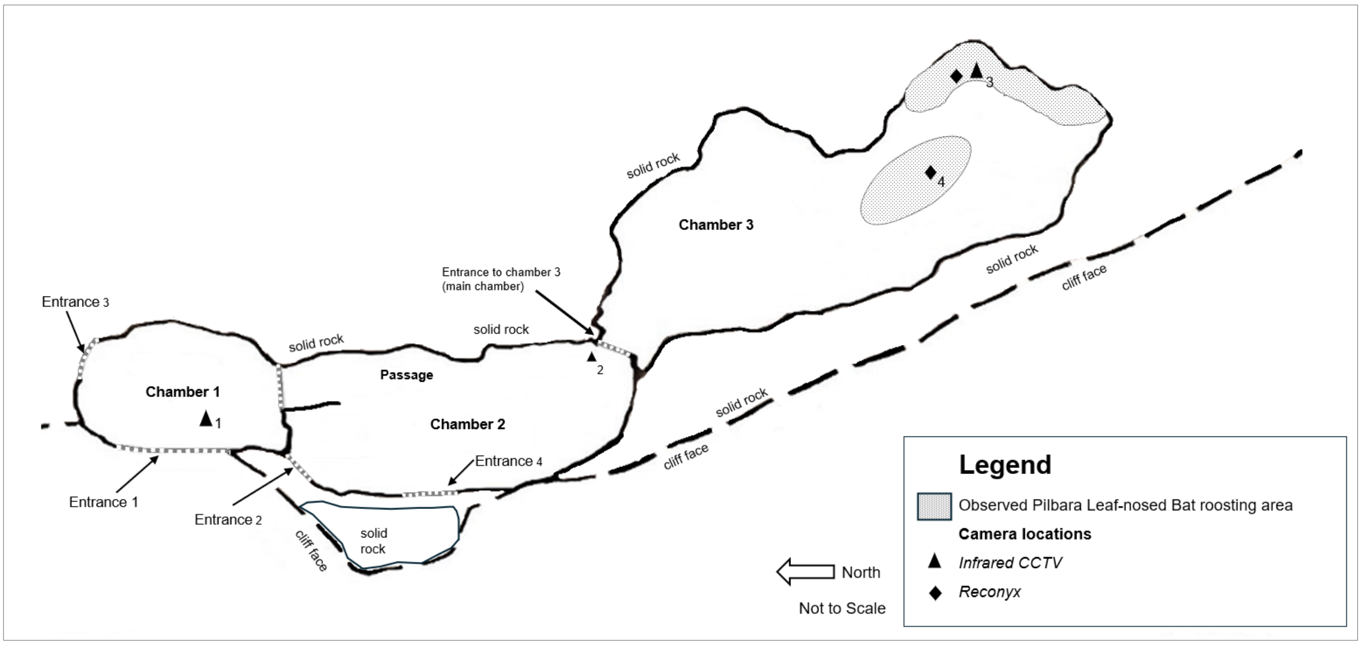


Figure 28 Location of infrared CCTV and Reconyx camera locations within Chateau Cave

6.2.1 Survey and data analysis limitations

The entrance to the main chamber from the passageway (Location 2) was selected as it is a narrow aperture, approximately 40 cm high and 30 cm wide, through which all PLNB entering and exiting the main chamber must pass through. All data collected to date has demonstrated the main chamber is the key roosting area for PLNB and it is the only location within the cave system PLNB congregate to roost. However, it cannot be definitively ruled out that a small number of individual PLNB roost within other areas of Chateau Cave (e.g., ceiling chutes).

Owing to the manual approach to counting bat emergence via video, the data analysed was limited to subsamples, ranging from one to three nights from each month. It is acknowledged that there may be within-month variation in the number of emerging bats due to weather or seasonal variation. As a result, long-term bat call activity data from within the main chamber and scat analysis was relied on as the primary means of the assessment of the colony presence, activity and size.

Due to the speed of bats entering and exiting the main chamber, bat counts were not always definitively attributed to PLNB, but all bats counted were small microbats (i.e., other than PLNB, *Vespadelus finlaysoni* and possibly *Scotorepens greyii* have been recorded in Chateau Cave). From the analysis of the long-term data these species are likely to exit the roost earlier, from just after sunset, whereas PLNB regularly begin emergence from civil twilight. *Taphozous georgianus* are also known to roost within the main chamber and passage of Chateau Cave but can be distinguished from PLNB or *Vespadelus finlaysoni* due to their larger size and slower flight speed.

Cameras were set to record continuously for three consecutive nights each month prior to and after each monthly site visit to Chateau Cave. The position and field of view of each camera changed both intentionally (e.g., to track the location of roosting bats in the main chamber) during the monitoring period but also unintentionally (e.g., knocked over by wallabies). Additionally, occasional equipment malfunctions (primarily power supply issues) and environmental factors (e.g., dust and guano on cameras) may have affected data quality on occasion but these limitations are not considered to have obscured long-term trends.

6.3 Results

Table 12 provides a summary of the bat counts and bat call activity including PLNB roost count estimates for monitoring events from April 2023 to January 2024 and Figure 29 displays the total egress/ingress counts for bc1 and PLNB roost count estimate for the monitoring events from 2022 – 2024. Figure 30 and Figure 31 display bat call activity data for nights with available data for bc1 from 2022 – 2024 including the number of PLNB calls recorded compared to other species.

Key findings from the emergence period counts include:

– From the available footage and review of bat call activity data, the peak emergence period for PLNB consistently occurred from civil twilight onwards for 1-2.5 hours. Furthermore, when over two hours of footage post-civil twilight was available, activity reduced substantially by the end of the sampling period for all sessions except two. This pattern of activity is consistent with bat call activity data within the main chamber (CC in) and from the detector placed alongside camera location.

– The number of bats (PLNB and other species) roosting within Chateau Cave is highly variable (Minimum count = 8, Maximum count = 1,051, Mean count = 262), (see Figures 5 to 7). More often, the bc1 total count is lower (27 of 47 night) than the mean count. Like the number of bats (all species) roosting within Chateau Cave, the estimated number of PLNB roosting within the main chamber is also highly variable (Minimum count = 0, Maximum count = 1,046, Mean count = 221).

– The lower numbers reported for February 2022, February, March and October of 2023 are also supported by the corresponding bat call activity levels (Section 4.3.2) and number of scats collected (Section 3.3).

– The highest emergence period count of all bats and highest estimate of PLNB roosting within the main chamber was during May 2023 (range of 3 days = 1,046; 975; and 858, respectively). This is consistent with the high bat call activity levels recorded at CCout detector for the same month and number of genotyped individuals reported for the same periods (Section 3.3).

– The counts undertaken in Feb, March, Oct and Nov 2023 are consistent with the bat call activity data, indicating low numbers of PLNB during these periods at CCin and CCout (Section 4.3.2).

Table 12 Summary of bat count observations (egress/ingress) and bat call activity at Chateau Cave location 2 – 2023 - 2024

Date	Period of analysis for bat count (Bc) 1 and bat cal activity	Civil Twilight (CT)	Bc1 OUT count / Bc1 IN count	Total emergence count (bats out - bats in) Total count	Period of analysis for bat count (Bc) 2 and bat call activity	Bat call activity summary	Bc2 OUT count / Bc2 IN count	BC2 (estimate of PLNB) count within main chamber prior to CT
28/04/2023	5:30 - 9:00 PM	6:02 PM	510 / 138	372	6:01 - 9:00 pm	Location 2 (main chamber entrance) – 2442 calls, >75% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus.* Post CT PLNB calls dominated activity, with <5% calls post CT attributed to other species.	454 / 118	336
29/04/2023	5:30 - 9:00 PM	6:02 PM	625 / 286	339	6:02 - 9:00 pm	Location 2 – 1967 calls, >69% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus.* Post CT PLNB calls dominated activity, with <5% calls post CT attributed to other species.	601 / 276	325
30/04/2023	5:30 - 9:00 PM	6:01 PM	759 / 371	388	6:02 - 9:00 pm	Location 2 – 1722 calls, >85% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus.* Post CT PLNB calls dominated activity, with <8% calls post CT attributed to other species.	715 / 353	362
14/05/2023	5:30 - 9:00 PM	5:54 PM	1386 / 335	1051	5:54 - 9:00 pm	No data	1347 / 301	1046
19/05/2023	5:30 - 9:00 PM	5:52 PM	1996 / 1012	984	5:52 - 9:00 pm	Location 1 (in main chamber) – 2699 calls, >64% of call activity was PLNB calls. Activity prior to CT was dominated by PLNB and other bats (c.28%). Post CT PLNB calls dominated activity, with <5% calls post CT attributed to other species.	1982 / 1007	975
20/05/2023	5:30 - 9:00 PM	5:52 PM	1312 / 437	875	5:52 - 9:00 pm	Location 1 – 2056 calls, >60% of call activity was PLNB calls. Activity prior to CT was dominated by PLNB and other bats (c.22%). Post CT PLNB calls dominated activity, with <3% calls post CT attributed to other species	1287 / 429	858
29/09/2023	5:30 - 9:00 PM	6:20 PM	1061 / 828	233	6:20 - 9:00 pm	Location 1 – 1697 calls, >87% of call activity was PLNB calls. Activity prior to CT was dominated by PLNB and other bats (c.12%). Post CT PLNB calls dominated activity, with <4% calls post CT attributed to other species	1048 / 815	233
30/09/2023	5:30 - 9:00 PM	6:21 PM	1028 / 724	304	6:21 - 9:00 pm	Location 1 – 1815 calls, >84% of call activity was PLNB calls. Activity prior to CT was dominated by PLNB and other bats (c.13%). Post CT PLNB calls dominated activity, with <8% calls post CT attributed to other species	1022 / 718	304
1/10/2023	5:30 - 9:00 PM	6:21 PM	1052 / 733	319	6:21 - 9:00 pm	Location 1 – 2230 calls, >82% of call activity was PLNB calls. Activity prior to CT was dominated by PLNB and other bats (c.11%). Post CT PLNB calls dominated activity, with <11% calls post CT attributed to other species	1048 / 730	318
27/10/2023	5:30 - 9:00 PM	6:31 PM	634 / 313	321	6:31 - 9:00 pm	Location 1 – 2825 calls, >73% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus* (c.12%). Post CT PLNB calls dominated activity, with <6% calls post CT attributed to other species	575 / 286	289

Date	Period of analysis for bat count (Bc) 1 and bat cal activity	Civil Twilight (CT)	Bc1 OUT count / Bc1 IN count	Total emergence count (bats out - bats in) Total count	Period of analysis for bat count (Bc) 2 and bat call activity	Bat call activity summary	Bc2 OUT count / Bc2 IN count	BC2 (estimate of PLNB) count within main chamber prior to CT
28/10/2023	5:30 - 9:00 PM	6:32 PM	62 / 12	50	6:32 - 9:00 pm	Location 1 – 1695 calls, >62% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus* (c.22%). Post CT PLNB calls dominated activity, with <10% calls post CT attributed to other species	49 / 8	41
29/10/2023	5:30 - 9:00 PM	6:32 PM	117 / 19	98	6:32 - 9:00 pm	Location 1 – 1681 calls, >79% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus* (c.9%). Post CT PLNB calls dominated activity, with <8% calls post CT attributed to other species	101 / 8	93
24/11/2023	5:30 - 9:00 PM	6:49 PM	218 / 188	30	6:49 - 9:00 pm	Location 1 – 1510 calls, >62% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus* (c.23%). Post CT PLNB calls dominated activity, with <10% calls post CT attributed to other species	101 / 96	5
25/11/2023	5:30 - 9:00 PM	6:49 PM	771 / 601	170	6:49 - 9:00 pm	Location 1 – 2587 calls, >64% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus* (c.21%). Post CT PLNB calls dominated activity, with <10% calls post CT attributed to other species	335 / 217	118
26/11/2023	5:30 - 9:00 PM	6:50 PM	919 / 728	191	6:50 - 9:00 pm	Location 1 – 1998 calls, >70% of call activity was PLNB calls. Activity prior to CT was dominated by *T. georgianus* (c.14%). Post CT PLNB calls dominated activity, with <10% calls post CT attributed to other species	363 / 272	91
22/12/2023	5:30 - 9:00 PM	7:07 PM	865 / 492	373	7:07 - 9:00 pm	No data	674 / 375	299
23/12/2023	5:30 - 9:00 PM	7:07 PM	484 / 87	397	7:07 - 9:00 pm	No data	367 / 29	338
24/12/2023	5:30 - 9:00 PM	7:08 PM	814 / 369	445	7:08 - 9:00 pm	No data	454 / 203	251
12/01/2024	5:30 - 9:00 PM	7:13 PM	570 / 209	361	7:13 - 9:00 pm	No data	328 / 68	260
13/01/2024	5:30 - 9:00 PM	7:13 PM	1537 / 1116	421	7:13 - 9:00 pm	No data	533 / 360	173
14/01/2024	5:30 - 9:00 PM	7:13 PM	440 / 81	359	7:13 - 9:00 pm	No data	154 / 47	107

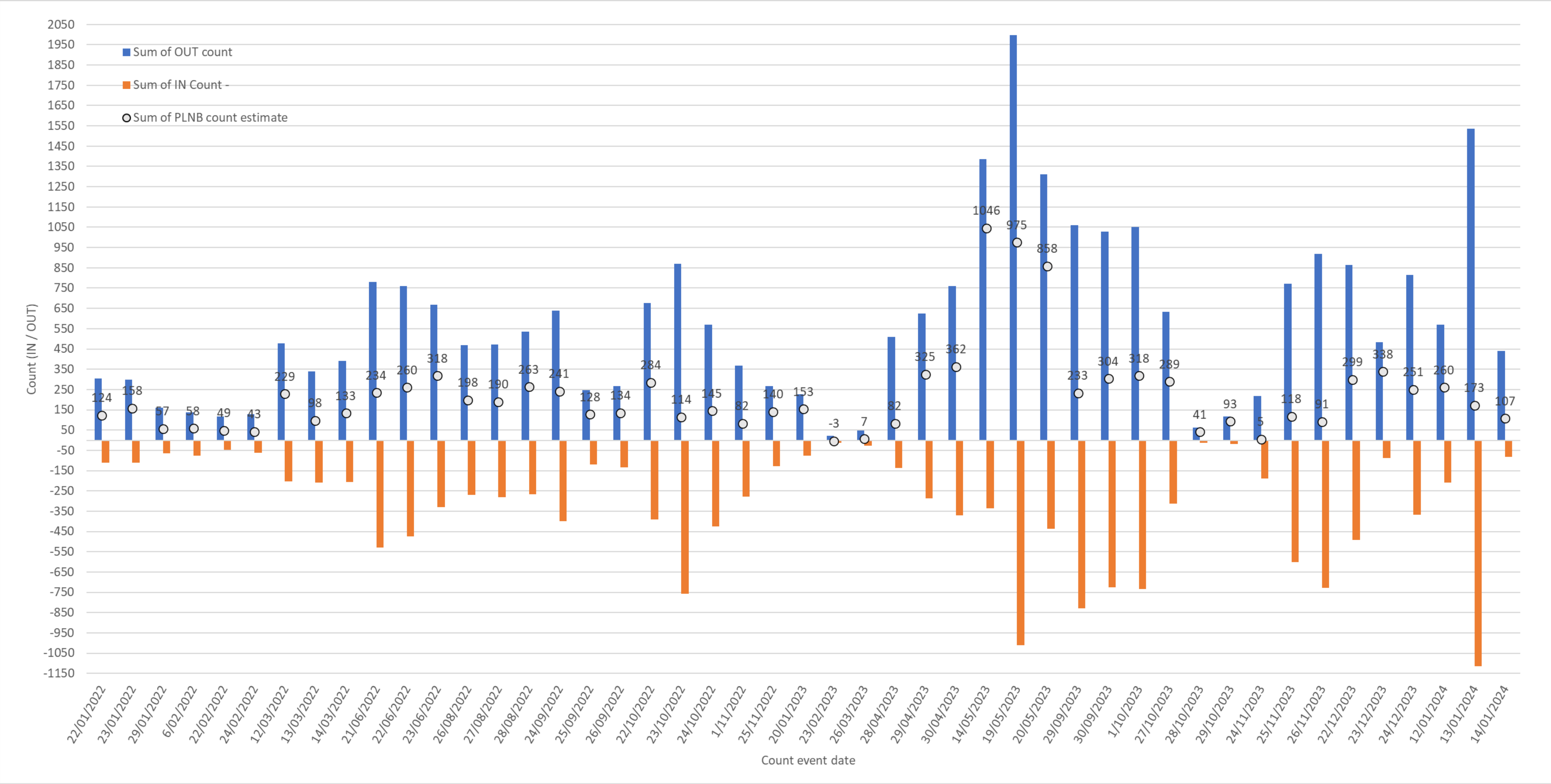


Figure 29 *Emergence count of camera location 2 by IR video analysis. Egress (out) and ingress (in) counts include all possible species each night of survey. PLNB roost count estimate calculated from out counts minus in counts following civil twilight supported by bat call activity data. (Note: data from the months of April, May, July, December 2022 and June, July, August 2023 not available due to technical issues)*

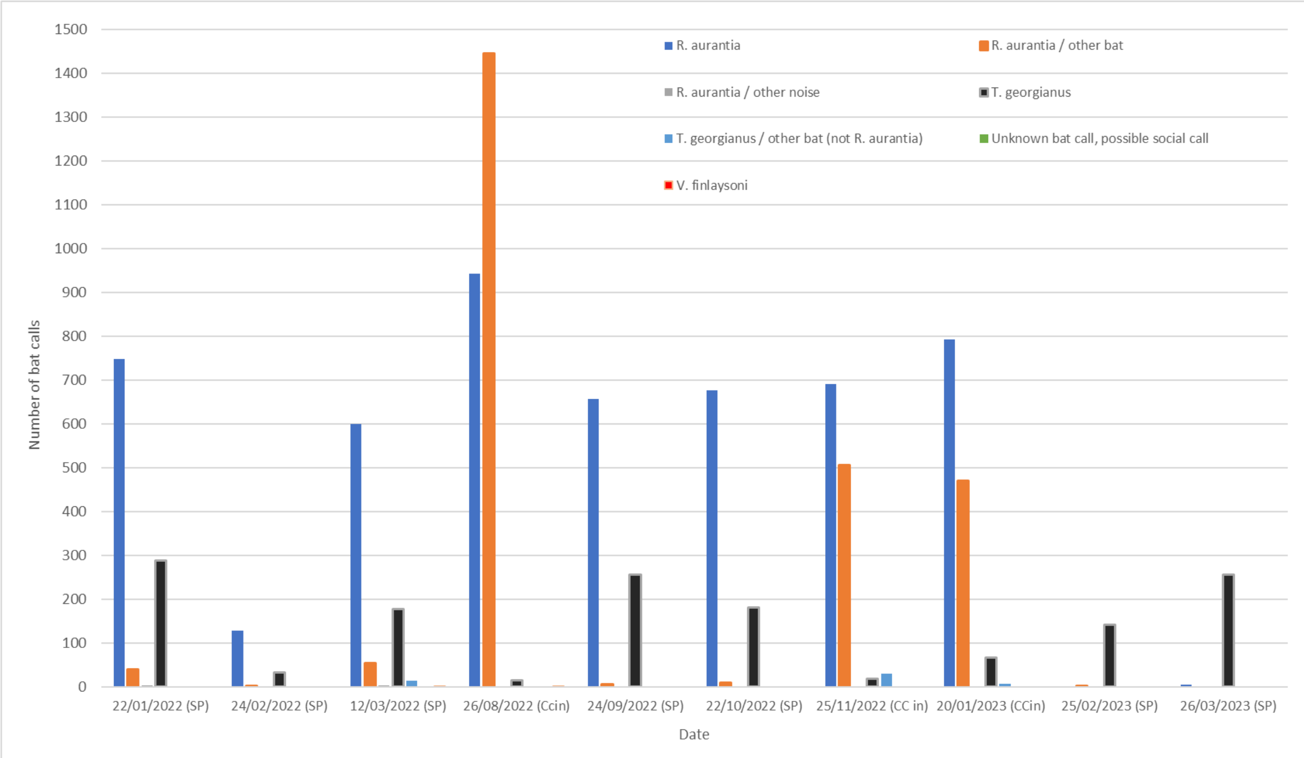


Figure 30 ***Summary of bat calls for each date of CCTV emergence period in location 1 (CCin) and 2 (Squeeze point) data. (Note: some bat call categories are combined to simplify the graph)***

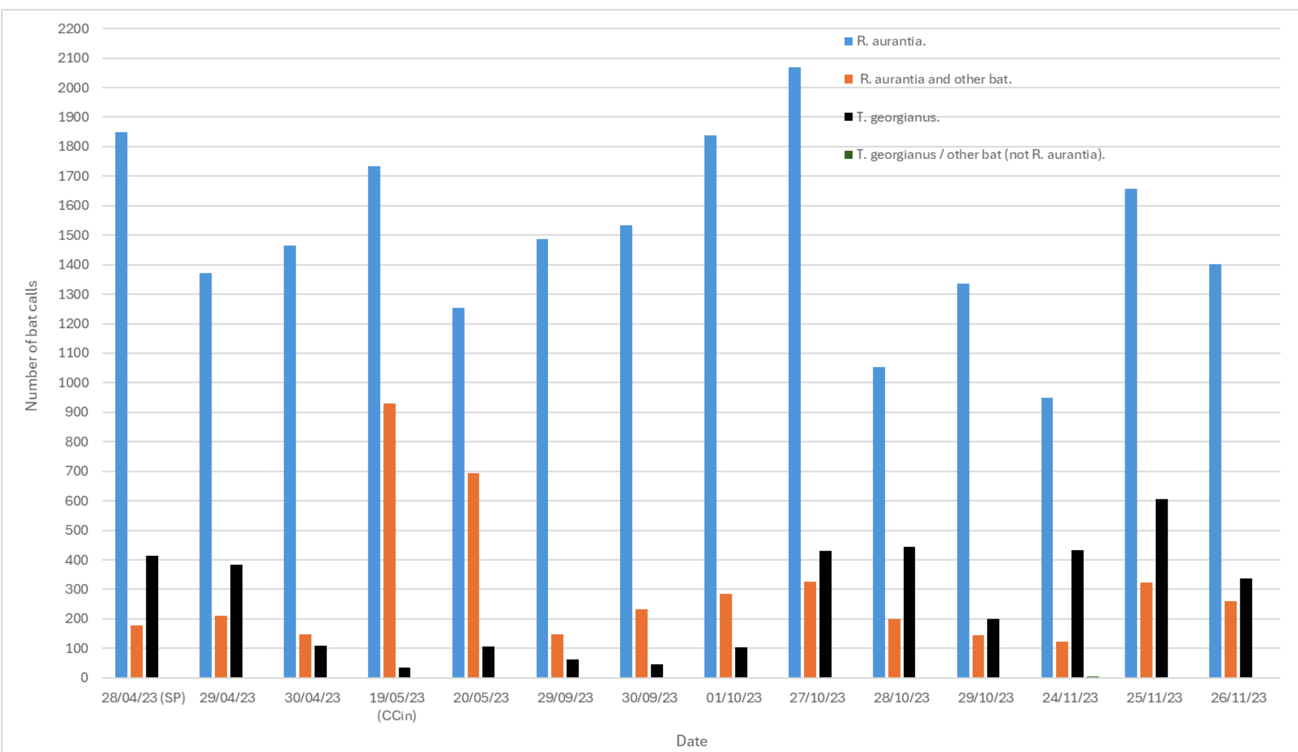


Figure 31 ***Summary of bat calls for each date of CCTV emergence period in location 1 (CCin) and location 2 (Squeeze point) from April 2023 – November 2023 (Note: data from the months of June, July, August, December 2023 and January 2024 not available due to technical issues).***

6.4 Discussion

Several important observational correlations were confirmed from the long-term visual monitoring of bat activity. The activity pattern, particularly the peak period of counts (in and out) is comparable with the activity pattern and peak period of bat calls recorded for PLNB by the ultrasonic recorders for the same period of time. There is an obvious correlation between PLNB visual count activity and PLNB call activity post-civil twilight. Although difficult to correlate an index of abundance, it is reasonable to assume the greater majority of movements (both in and out) recorded post civil twilight are PLNB as the majority of calls recorded were identified as PLNB.

All data collected to date has demonstrated the main chamber is the key roosting area for PLNB in Chateau Cave and it is the only location within the cave system PLNB congregate to roost and breed. However, it cannot be definitively ruled out that a small number of individual PLNB roost within other areas of Chateau Cave (e.g., ceiling chutes).

There is obvious night-to-night variation in the number of emerging bats with several outliers (e.g. zero PLNB counted in January 2023 and 1,046 counted in May 2023), however the visual count data is supported by other forms of corresponding data (e.g. bat call activity, number of genotyped individuals and scat collections). The fluctuating number of bats counted suggests the population is dynamic and probably responds to numerous environmental and anthropogenic factors within the study area.

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Appendices

Appendix A

DNA analysis report – Specialised Zoological

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The Power of Commitment